Immune Systems and Human Intrauterine Development

**Itzhak Zusman**, D.V.M., Ph.D., D.B.Sc.,
Professor
Koren School of Veterinary Medicine,
Faculty of Agricultural, Food and Environmental Quality Sciences,
The Hebrew University of Jerusalem, Rehovot,
Israel

**Pavel Gurevich**, M.D., Ph.D., D.M.Sc.,
Professor
Laboratory of Experimental Medicine,
Rabin Scientific Park, Rehovot,
Israel

**Herzel Ben-Hur**, M.D.,
Department of Gynecology and Obstetrics,
Assaf Harofeh Medical Center, Beit-Dagan;
Head, Laboratory of Experimental Medicine,
Rabin Scientific Park, Rehovot,
Israel
Contents

Preface

Introduction

Chapter 1. Morphological Characteristics and Functions of the Immune Systems
  1.1) Components of the nonspecific immune protection
  1.2) Components of the specific immune protection
      1.2.1) Cellular components of the common immune system
      1.2.2) Humoral components of the common immune system
      1.2.3) The secretory (mucosal) immune system
      1.2.4) The secretory immune system in the barrier structures

  2.1) Protection of the fertilization process
  2.2) Protection of preimplantation embryos
  2.3) Components of the secretory immune system in human embryos and early fetuses in the first trimester of pregnancy

Chapter 3. Immune Protection of Vitally Important Organs and Cells in Human Embryos and Early Fetuses
  3.1) Immune components in fetal endocrine glands and their precursors
  3.2) Immune components in the developing myocardium
  3.3) Immune protection of the developing brain
      3.3.1) Secretory immune components in the developing brain
      3.3.2) Types of barrier protection of the developing brain
  3.4) Immune protection of gonads and genital tracts
      3.4.1) Immune protection of gonads and genital tracts in adults
          3.4.1-1) Immunoprotective components in the female genital tract, ovaries and oocytes
          3.4.1-2) Immunoprotective components in the testes and male genital tract
      3.4.2) The secretory immune system in the developing gonads and genital tracts
          3.4.2-1) Secretory immune components in the developing genital tracts
          3.4.2-2) Secretory immune components in the developing female gonads
          3.4.2-3) Secretory immune components in the developing male gonads

Chapter 4. Immune Protection of Embryos and Fetuses in Normal Pregnancy and Under Pathological Conditions
  4.1) Mononuclear phagocytes in embryos and fetuses under normal and pathological conditions of gestation
  4.2) Immunoprotective role of the trophoblast
  4.3) Apoptosis in the embryonic cells and its consequences
  4.4) Pathology of the immune organs in growth-retarded or low-weight fetuses and newborns under antigen-induced influences
Chapter 5.  Mother-Embryo Immune Conflict as a Reason for Recurrent Spontaneous Early Abortions
  5.1) Possible reasons for the immune conflict
  5.2) Humoral and cellular mechanisms of the immune conflict
  5.3) Pathological changes in the placental barrier as a reason for spontaneous early abortions

Chapter 6.  The Fetal Immune Systems in Normal Development and under Pathological Effects
  6.1) The common immune system in fetuses
  6.1.1) Immune components in experimental fetal pathology
  6.1.2) The immune system in human fetal pathology
  6.2) The secretory immune system in human fetuses
  6.2.1) Lymphoid-epithelial components of the secretory immune system in self-protection of human fetuses
  6.2.2) Components of the secretory immune system in fetal membranes and decidua

Chapter 7.  The Secretory Immune System and the Placental Barrier
  7.1) The placental barrier: its morphology, function and pathology
  7.2) The role of the secretory component and J chain in maternal immunoglobulin transport through the placental barrier
  7.3) The secretory immune system as part of the placental barrier

Chapter 8.  Immune Systems in the Pathogenesis of RhD-Hemolytic Disease of Fetuses and Newborns (HDN)
  8.1) Immune response of fetuses and newborns in RhD-conflict
  8.2) Immune antigen-antibody complexes in RhD-HDN
  8.3) Mechanism of immune destruction of erythrocytes in RhD-HDN

Chapter 9.  Pathology of the Immune System in Fetuses and Newborns Affected by Infectious Diseases
  9.1) Nucleolar organizer regions in the lymphoid cells in fetuses and newborns with pneumonia and sepsis
  9.2) Intraorganic immunity of fetuses and newborns with pneumonia and sepsis
  9.3) Insufficiency of the immune system in fetuses and infants with sepsis

Chapter 10. Clinically Oriented Extrinsic and Intrinsic Factors in Etiology of Fetal Growth Restriction
  10.1) Geographic peculiarities of immunoglobulins in FGR
  10.2) Changes in the placenta as a reason for FGR
  10.2.1) Morphological aspects
  10.2.2) Biochemical aspects
  10.2.3) Transport across the trophoblast in FGR
  10.3) Maternal environment and FGR
  10.3.1) Effects of mother’s diet
  10.3.2) Effects of mother's diseases
10.3.3) Effects of mother's smoking
10.3.4) Effects of mother's alcohol consumption
10.3.5) Effects of drinking water
10.3.6) Effects of socioeconomic factors
10.5.7) Genetic predisposition to FGR
10.3.8) Racial effects on FGR
10.4) Effects of FGR on postnatal growth and development
Preface

In recent years it has come to be generally recognized that, directly or indirectly, immunology intrudes into nearly every aspect of human reproduction. The discovered importance of the natural transfer of immunity from mother to offspring, the ontogeny of the immune response, and the recognition of pregnancy as an example of the mother-embryo interaction, are only a few components of the multifaceted field known as the immunology of reproduction. In this book, we have compiled material, obtained in our laboratories and gleaned from the modern publications illustrating ongoing research in the field of reproductive immunology on the immune system in human intrauterine development. Many new approaches have been postulated and published in recent years, enough to warrant bringing them together in one place. In this book, we focus our attention mainly on the application of humoral and cellular changes that describe the role of immune systems in human intrauterine development. A substantial area of the reproductive immunology deals with the issue of the maternal-fetal conflict and the survival of the embryo/fetus as an allograft. Although much efforts has been invested in describing different aspects of this problem, relationships between the pregnant mother and her developing fetus are not yet fully understood from an immunological point of view. The role of the fetal immune system in its survival and in the maintenance of pregnancy have only been scanty studied, especially with respect to the secretory immune system. General skepticism has been developed regarding the diagnosis and treatment of recurrent spontaneous abortions. In our research, we have described changes in the secretory immune system of the embryo/fetus and mother as possible reasons for such abortions and we hope that these findings will yield a better understanding of the mechanism underlying this phenomenon.

For many years, our laboratories have been studying the pathology of fetuses and the immune response of their lymphoid systems to experimentally induced disorders in laboratory animals (mice and rats) and under some diseases in humans, such as hemolytic disease of fetuses and newborns, sepsis of fetuses, and others. In the last decade, we have concentrated our efforts on studying the immune protection of human embryos and fetuses under non-antigen and antigen-induced diseases, studying the secretory (mucosal) immune system. The changes in the different types of immune systems in the intrauterine-developing organism in normal pregnancy, under non-infectious pathology and under infectious effects as well as under mother-fetus immune conflict are discussed in this book. The protective role of mononuclear phagocytes and the non-villous infiltrating trophoblast is analyzed. The pathological morphology of some immune and inflammatory processes, including massive apoptosis of embryonic cells, is also discussed. We hope that the problems described in this book will be of interest to people with various professional orientations, such as physicians and researchers in the field of obstetrics and gynecology, reproductive immunology and intrauterine human development, as well as medical students. For the clinician and scientists in other areas of medicine, pregnancy provides an area in which many immunological ideas can be tested. This is particularly true now that the impact of pregnancy impacts on the maternal immune system is becoming better understood.
Introduction

One of the most intriguing phenomena in immunology is the exemption of the semi-allogenic embryo (or allogenic embryo in surrogate mothers) to be exempt from immunological rejection (1). Why a pregnancy sometimes runs successfully to full-term, while in other cases it is interrupted without any visible or recognizable cause, remains a mystery. The exact frequency of spontaneous abortions in the general population is unknown: it has only been suggested that pregnancy loss is common affecting 10% to 15% and even to 20% of all pregnancies (2,3). Our lack of knowledge in this field is reflected by the fact that the real causes of spontaneous early abortions (in humans, before week 9 of pregnancy, i.e., in the embryonic period of intrauterine development) are unknown in 50% of the cases (4).

One opinion holds that the uterus possesses some immune "privileges", that allows it to develop a tolerance to the implanting embryo and sustain its survival and normal development (5). But because some other organs, such the brain and eyes, have the similar immune privileges (6), it seems more plausible to suggest that the trophoblast cells which come into contact with the immunocompetent cells of the decidua do not contain the major histocompatibility complex (MHC) responsible for inducing the cell-rejection reaction. The immunological rejection of an embryo is also prevented also by the presence of immunosuppressive regulatory molecules in the placenta, such as progesterone (7), prostaglandin E$_2$ (8) and early pregnancy factor (9).

Some studies in the field of reproductive immunology have focused on the postulate of the mother-embryo conflict as a transplanting graft, a conception which was published in the 1950s by Dr. Peter Medavar. However, many aspects of immunological relationships between a mother and her developing fetus remain unclear, and some of them are discussed in this book. Molecules of human leukocytes antigens (HLA) expressed by trophoblast and placenta cells, play an important role in the immune relationships between the pregnant mother and her fetus. Promising are studies of the role of the cytokine cascade and regulation of cytokine gene expression in the processes of fertilization and implantation, as well as mechanisms regulating the invasion of the trophoblast into the endometrium, which as a result ensures adequate levels of fetoplacental complex development. The role of apoptosis process in fetal intrauterine development is especially interesting. Undoubtedly, studies into the molecular-cellular specificities of different fetal tissues in cases of habitual miscarriages, will yield important information.

One of the hypotheses that is analyzed in this book is that disturbances in maturation and activation of the fetal immune system are related to the onset of preterm labor (9). The question of whether activation of the fetal immune system and preterm labor occur as a consequence of the bi-directional trafficking of fetal and maternal cells is discussed. Increased fetal-maternal HLA compatibility may lead to interactions between maternal and fetal cells which in turn lead to activation of the fetal immune system and preterm labor (10).

Although many research efforts have been devoted to the detailed study of the early intrauterine development of human embryos, the role of the embryos and fetuses themselves and their immune system in normal and pathological development are less
known. For many years now we have studied the pathology of intrauterine human development in different diseases and the role of the fetal immune system in this process. Results of these studies are analyzed in this book and compared with up-to-date data from the literature. Our groups are studying the immune mechanisms underlying fetal protection against infection and other antigenic effects. Our main focus has been on evaluating the functions of mononuclear phagocytes in embryos and early fetuses and the protective role of the secretory immune system and its different variances.

This book describes the protective mechanisms of the secretory immune system and its components (secretory component (SC), joining (J) chain, immunoglobulins (Igs), Fc receptors, etc.) in normal development, under noninfectious pathologies, and antigen-induced infectious pathologies; the protective role of mononuclear phagocytes as well as of the non-villous trophoblast in early embryos; the pathological morphology of some immune and inflammatory processes, including the massive apoptosis of embryonic cells in response to the described pathogenic effects. Separate chapters in the book describe the role of mother-embryo immune conflict as a frequent reason for recurrent spontaneous early abortions; a role for the placental barrier in normal pregnancy and in those complicated by inflammation; a role of the secretory immune system in growth-retarded fetuses under antigen-induced influences. The last chapter analyzes the role of clinically oriented environmental factors as etiologies of restriction in the fetal growth.
References

Chapter 1.

Morphological and Functional Characteristics of the Immune Systems

Immune protection is an extraordinarily important function in an organism's prevention against the deleterious effects of microbes and viruses, parasites and antigens, etc. There are two types of protection: nonspecific and specific.

1.1) Components of the nonspecific immune protection

Non-specific types of protection include humoral, molecular, and cellular components. Humoral components include C-reactive protein, different types of interleukins (IL), as well as digestive enzymes, the high acidity of gastric juice, bile acids, etc. All of these are designed to demolish the useless and toxic substances, that accumulate in an organism. Proteins that mediate apoptosis (or controlling cellular death) are highly important in this process. Nonspecific cellular protection is carried out by cells that are able to perform phagocytosis. They include granulocytes and a group of mononuclear phagocytes, such as macrophages, monocytes, antigen-presenting cells for lymphocytes and other immunocompetent cells, and promonocytes, histiocytes, Kupffer's cells of the liver, osteoclasts, Hofbauer cells in the chorionic villi, the brain microglia, etc. These two types of nonspecific protection have one common characteristic: they act at early stages of the pathological process, before the specific immune response begins. The fertilization membrane provides as an example of nonspecific protection of early preimplantation embryos at stages of the zygote and morula stages against possible deleterious influences of the maternal environment.

1.2) Components of the specific immune protection

One characteristic of specific immune responses is that their protective activity has concrete targets and it is kept in memory cells for a long time, sometimes for the whole life. Upon reinfection with the same disease agent, these cells react more quickly than after the first infection. Cells of the specific immune response recognize their own cells and tolerate them. All types of specific immune responses are produced by different cells located in the lymphoid organs, such as the thymus, spleen, lymph nodes, tonsils, Peyer's nodules, etc. These cells are constantly mobile in the blood and lymph, and this provides common immune reactions and good contact between these cells and antigens.

Specific immune responses can be performed by: i) the common immune system which produces common immune reactions, cellular or humoral; or ii) the secretory immune system (SIS) which produces secretory (mucosal) immune response. The latter can be performed a) in the epithelium of mucosal membranes and in many organs and tissues that are in contact with the external environment, b) in an unusual form in vitally important organs and cells, and c) in some morphologically formed barrier structures.
1.2.1) Cellular components of the common immune system

Different immune-competent antigen-presenting cells participate in producing the common cellular immune reactions: mononuclear phagocytes appear in primary contact with an antigen, memory T cells are seen in recurrent contact with the same antigen, T lymphocytes (CD3) are responsible for the common response. There are a few types of lymphocytes, and each of them with its own function: T helpers (CD4) mediate the immune response, while cytotoxic and suppressor T lymphocytes (CD8) and natural killer (NK) cells (CD56 and CD8) produce this response.

The cell-specific immune response takes place in reaction to the appearance of foreign tissues containing MHC class 1. This can be seen, for example, in the incompatible transplantation of tissues or organs, the primary reaction of which occurs in two phases. The first, sensitization, is carried out for 7 to 11 days, from the first contact with the antigens. At this time, a large amount of specific cytotoxic T lymphocytes and NK cells is produced (1). During the second phase, T lymphocytes and phagocytes penetrate the graft tissue and infiltrate its vessels. The tissue is necrotized and torn. Different types of IL and cytokines have been described as major components of the T-cell immune response (2-4).

Human leukocyte antigens (HLA) play a crucial protective role in the process of implantation (5). During implantation, the uterine decidua is invaded by extravillous trophoblast cells whose function is to destroy the walls of the uterine spiral arteries in order to provide an adequate blood flow to the fetus. These cells express an unusual combination of HLA class I molecules, such as HLA-D, HLA-E and HLA-G (6,7), but not HLA-A or HLA-B which have not been found in the trophoblast (8). Recognition of HLA-G stimulates cytokine production (9) and regulates the development, growth and differentiation of the placenta (10).

1.2.2) Humoral components of the common immune system

The humoral immune reaction is trigged by the appearance of foreign antigens of MHC class II (HLA-DP, HLA-DQ, HLA-DR, HLA-G) or other incompatible antigens, such as rhesus-antigens causing the RhD-hemolytic disease of fetuses and newborns (11). After their first contact with foreign antigens, the mononuclear phagocytes transfer information to B lymphocytes which begin to multiply in the presence of T helpers and cytokines (period of sensitization). After about 7 days, a small amount of antigen-specific IgM appears in the blood, and after 2 weeks, a high amount of specific IgG antibodies are already present. The synthesis of antibodies takes place in the lymphoid tissue of many organs, such as the spleen, lymph nodes, tonsils, lymph nodules in the intestine and other organs. Immunoglobulins (Igs) are spread with the blood, lymph and intercellular fluids through all organs and tissue, where they come into contact with specific antigens and destroy them. Upon repeated contacts with the same antigens, this response takes only 3 to 4 days to develop.
1.2.3) The secretory (mucosal) immune system

The secretory (mucosal) immune system (SIS) participates in the induction and regulation of immune responses in both the mucosal and systemic compartments of an organism after antigen exposure. The significance of this system for the organism comes to light when one considers that the total internal surface of all organs which are in the permanent contact with the external environment amounts to many hundreds of square meters. The most important known function of the SIS is the immune protection of organs which are in close contact with this environment and therefore with "symbiotic" microbes and foreign antigens, such as the mucous membranes of the digestive, respiratory and urogenital tracts, and the eyes (12,13). The SIS consists of an integrated cross-communication pathway of lymphoid tissues made up of inductive and effector sites for the host protection against foreign antigens (14).

The SIS contains several protein components, such as Igs (IgG, IgA, IgM), polymeric Ig receptor (pIgR) also called transmembrane secretory component (SC), joining (J) chain, and antigen-presenting and immunocompetent cells, such as mononuclear phagocytes, B lymphocytes and plasma cells secreting Igs, particularly IgA (12,13,15). The simultaneous presence of SC, J chain and Igs in the same structure is recognized as morphological evidence of the functional SIS activity (15,16).

Despite its similarity to the common immune system, the SIS functions independently already at the stage of Ig synthesis. This was described at first by Dr. A. Besredka (1919), who found that, after introducing pathogens with food, antibodies appear at first in the intestine and only later in the blood (cited after ref.12). Production of Ig polymers in the mucosa-associated lymph-epithelial structures, particularly in the Peyer's patches in the small intestine, in the lymph nodes, and the tonsils, and in lymph follicles in other organs, is performed by B lymphocytes (17,18). The location of antigen-presenting cells allow them to begin synthesizing anti-pathogenic Igs very quickly. B cells migrate from these inductive sites as the memory cells to exocrine tissues all over the body.

Mucous membranes are thus furnished with secretory antibodies in an integrated way, ensuring a variety of specificities at every secretory effector site. Then the second stage in the SIS function begins: proteins ensure immunoglobulin transport through the mucous epithelium and secretion into intercellular spaces and then into the lumen of space-containing organs.

The trans-cellular transport of Igs through the epithelium of mucous membranes occurs in three phases: i) trapping of Igs on the basal-lateral surface of the epithelial cells (endocytosis or internalization), ii) transport of Igs throughout the cell cytoplasm (transcytosis), and iii) secretion of Igs on the apical surface of the epithelium (exocytosis). In the organ lumens, Igs come into contact with specific pathogenic antigens and destroy them. Ig transport is effected by two receptors: pIgR/SC and J chain. The third component of this transport is Igs themselves, represented mainly by IgA and, to a lesser extent, IgG and IgM in adults (19,20).

SC, which has been characterized as a glycoprotein, is the most important receptor of the SIS because it is responsible for the external transport of locally produced polymeric IgA and IgM (17,18). SC is expressed as a transmembrane protein in the secretory mucosal epithelial cells.
SC represents the soluble ectodomain of pIgR, a membrane protein that transfers mucosal antibodies across epithelial cells. In the protease-rich environment of the intestine, SC is thought to stabilize the associated IgA by as yet unestablished molecular mechanisms (23). In the mucosal immune protection, SC exerts its protective role in the soluble IgA by delaying cleavage in the hinge/Fc region of the alpha-chain, and by not holding together degraded fragments.

At the week 4 of gestation and during all subsequent human intrauterine development, SC and J chain are detected in the ectoderm- and endoderm-derived structures even when Ig-producing lymphocytes and lymphoid organs are absent (24,25). It appears that in embryos the whole SC is located inside the cells. This suggestion was proven by the finding that in the stroma of trophoblastic villi SC is not found during the excretion of Igs (26). The early presence of SC in normally and pathologically developed human embryos (fetus amorphous, anencephaly, etc.) suggests that SC is one of the earliest appearing proteins in the ectoderm- and endoderm-derived structures. In the evolution of life forms, SC has been described in vertebrates, mammalian species in particular (27).

J chain, a small (15 kDa) polypeptide, was first found outside of the SIS: in the bone marrow lymphocytes, thymocytes and in B lymphocyte-synthesized IgG and IgD (17). J chain was also discovered in IgA and IgM in some subclasses of B lymphocytes and in plasma cells (19). In the epithelium of the human intestinal mucosa and bile ducts, J chain is located together with Igs on the basolateral membrane and in the cytoplasmic villi, indicating its heterogenic origin and transport paths. In adult epithelial and other cells (except lymphoid cells), J chain presents exclusively in association with polymeric Igs (28). In secreted fluid, the J chain has been described as a part of the secretory Igs, in the form of sIgA and sIgM (29). J chain has not been found in non-lymphoid cells (30), but it has been detected in mucous cells of invertebrates which have neither Igs nor the lymphoid system (31).

J chain has been described in 3.5-4-week-old human embryos inside and outside the SIS (26), and in 16-week-old fetuses, in lymphoid cells of the spleen, thymus and bone marrow (32). The mature plasma cells, the main site of J chain and Igs synthesis in adults (32) are not formed in fetuses even under severe antigenic attacks, such as the RhD hemolytic disease of fetuses and newborns (11). In mouse, no J-chain expression was detected in embryonic tissues, but an expression of mu-heavy chain was detected in the fetal liver on day 17 (33). J-chain expression has been detected in the spleen on day 9 and in the intestine on day 15 after birth.

In epithelial cells of adults, J chain is involved in creating of the binding site for pIgR/SC in the Ig polymers, by determining the polymeric quaternary structure and interacting directly with the receptor protein (19,20). The main function of J chain is to form pIgA and pIgM by connecting two IgA molecules or five IgM molecules via Fc receptors (34). However, in early fetal development, it appears that J chain functions are not restricted to the formation of polymeric Igs.

Some researches believe that the whole process of Ig transport, including the stages endocytosis → transcytosis → exocytosis, is performed only by the SC. J chain
participates in the formation of polymeric Igs, helping with their transport in the form of endocytosis. According to an other opinion, Igs entrance into some epithelial cells is performed only by J chain without SC participation. This latter opinion was proven in studies with pIgR<sup>+/S<sup>-</sup> mice whose intestinal epithelium does not contain SC but does contain IgA (35). IgA was found in high concentrations in the blood and in low concentrations in the bile, intestinal content and faeces, indicating its low excretion. In healthy people with the normal cellular contents of SC, IgA has been found in high levels in the blood and in all excretions. These data suggest that SC participates in the IgA excretion.

Participation of J chain in Ig transport was confirmed in clinical observations of patients with IgA nephropathies complicated by the high blood levels of IgA. Excretion of IgA with the urine causes disorders in glomerular mesangium of the kidneys. Intestinal IgA concentration was extremely low in some of these patients due to the low content of J chain (36). Participation of SC and J chain in different phases of Ig transport has been proven by their different cellular localization: SC is located in the apical parts of cells while J chain is located in the basal parts (37). These data indicate that J chain participates mainly in endocytosis while SC participates in exocytosis.

It appears that in embryos, the SC is located inside the cells and does not going outside of their borders. This suggestion finds some confirmation in the fact that SC was not found in the stroma of the intestine or inside of its contents, even under an increased Ig secretion (26). Similar observations were made in the choroid plexuses and thyroid. In the thyroid gland, the follicular epithelium and in particular the colloid contain all three types of Igs, but SC and J chain are located only in the epithelium and not in the colloid (25,38). This means that upon Ig exocytosis, SC and J chain remain inside the cells. This conclusion was confirmed by observations of the intracellular localization of these receptors in other cells (39). It should be noted that the precise mechanism and time of the appearance of J chain in human embryos remain unclear: it is not known whether they are transported to the embryo together with maternal Igs or synthesized by cells of the embryo itself as it is described for invertebrates (31).

The described mechanism of Ig exocytosis without SC or J chain is characteristic of the merocrine (eccrine) secretion that takes place in the mucosal membranes, salivary and lachrymal glands and pancreas. In the apocrine secretion, the entire apical portion of the epithelial cells is excreted together with its organelles. SC as well as Igs are situated in the apical compartment and secreted into the lumen (37). Massive release of the free and conjugated SC takes place in holocrine secretion whereby the secretory cells are totally destroyed, and their contents released into the lumen.

Cellular defoliation may be significant under both normal and pathological conditions. The decrease and even disappearance of Igs in the bronchial, gastric, intestinal and pancreatic epithelium in embryos exposed to massive antigenic attacks show that exocytosis of Igs together with SC and J chain is not a universal characteristic (25,38). Igs disappear in the epithelium of the brain ventricles choroid plexuses in meningitis and sepsis. It appears that epithelial-cell excretion of Igs conjugated with SC and J chain is not the only method of exocytosis.
An unusual function of the SIS is characteristic for the protection of different organs and even separate cells that are of vital importance for the intrauterine-developing organism. A list of such organs includes the brain and ganglion neurons, the main endocrine glands, the myocardium, and the gametes (13,42,43). SIS functional activity is already seen in week 4 of gestation (25). A series of morphological and biochemical changes accompany this process. Morphological changes consist of transformation of epithelial layers into cellular clusters, and organs losing their lumens. Biochemically, such organs lose SC, the Ig-secreting receptor, and thus their ability to secrete Igs. However, J chain, a receptor that is able to protract Igs into the cellular cytoplasm, remains in the cytoplasm of newly differentiated organs. As a result of these changes, cells in these organs lose some characteristics of the SIS but acquire the ability to store Igs in their cytoplasm and thus to provide the 1 intracellular protection against pathogens.

These changes, i.e., the loss of SC and preservation of J chain, were not observed in all cells and, of course, not in all differentiated organs. For example, differentiation of neurons is accompanied by preservation of the J chain and intracellular storage of Igs, but in cells of the neuroglia, originated from the same precursors – neuroblasts, both J chain and Igs are absent. J chain and Igs are not only preserved in the neurons of the brain and spinal ganglions but also in cells of the large endocrine glands, the gametes and in the myocardium (for more details, see Chapter 3). In the myocardium, SC is absent and J chain appears only during this tissue differentiation.

It appears that the intracellular storage of Igs is of great significance to the maintenance of parenchyma cells in specific, strategically important organs: their loss in these organs in even the smallest amount of cells can cause very serious disorders. The described changes are not seen in tissues and organs which are able to intensively regenerate and proliferate. The intracellular storage of Igs may therefore be considered an example of the protection of cells against pathogens. Intracellular neutralization or destruction of many pathogens, such as the Sendai, influenza and hepatitis viruses, may serve as an additional example of such protection.

In vitally important organs or organs located in especially susceptible areas, there is combined functional activity of both known immune systems: common and secretory. Such a situation is seen in the female gametes, surrounded by follicular cells containing SC, in some endocrine glands, and in the brain. And each protective system has its own function. The common immune system protects the whole organism. The SIS protects separate organs and organ systems which are connected to the external environment and affected by massive pathogen attacks.

The above characterization can be useful for better understanding the pathogenesis of disorders caused by long-release viruses, such as human immune deficiency virus (HIV), herpes simplex, herpes zoster, hepatitis viruses B and C, oncogenic viruses of the female genital tract, etc. All of these diseases can be explained by the intracellular neutralization of viruses with the SIS. Aggravation of a disease can result from insufficient or decompensate functional activity in this system.
1.2.4) The secretory immune system in the barrier structures

In addition to the organs with mucous membranes and lumens into which Igs are secreted, there are some organs that do not have these features but nevertheless perform Ig transport by SC and J chain across anatomical barriers. Such barriers include the placental and periovular barriers, serous membranes of the body cavities, the hematooencephalitic barrier in the ependyma, and the choroid plexuses of the brain ventricles.

At week 3.5 to 4 of gestation and during the second trimester of gestation, both fetal and maternal parts of the human placenta already contain all of the typical components of the SIS (40). In the fetal part of the placenta, SC, J chain, IgA, IgM and IgG are found mainly in the cytotrophoblast and syncytiotrophoblast of the chorionic villi and in the epithelium of the amnion. Different subsets of lymphocytes are present in the corresponding stroma. In the maternal part of the placenta, the decidua, proteins of the SIS are found in the decidual cells, whereas macrophages and different subsets of lymphocytes are seen in the decidual stroma (40).

In addition to the mucous membranes and glands of human embryos and fetuses, SC is detected in the trophoblast, amnion, epidermis, mesothelium, thymus, ovary follicular cells, the ependyma of the brain choroid plexuses and some other structures which participate in the formation of the blood-tissue and tissue-tissue barriers (41-43). These data show that the Ig secretion takes place not only in the mucosal membranes and glands but also in the barrier structures via the same mechanism. Thus SIS activity is seen in areas others than the mucous membranes, and this implicates the presence of both mucosal and barrier SIS in the organism.

Functions of the barrier system are very similar to those of the mucosal SIS: they can be considered different types of the same process. Nevertheless, there are a few differences. Mainly IgG and, to a lesser extent, IgA and IgM are secreted in the barrier system, while in the mucosal SIS, IgA is the main secreted component. The mucosal SIS is spread over large areas, for example, in the intestine. The area of the barrier system is not as large, although in the placenta the active area of the chorion of 36- to 40-week-old fetuses can amount to 11.0±1.3 m² (44). The barrier system is usually located in small structures, such as ovarian follicular cells or follicles of the thyroid. Immune-competent cells have not been found in the barrier system, and it seems that this system uses Igs of the common immune system from the blood, lymph and intercellular fluid.

The barrier SIS should be distinguished from the tissue barriers that have no relation to immune reactions. Such tissue barriers are the air-hemolytic barrier in the lungs, the filtrate barrier of the primary urine in the kidneys, the perineural barrier, ovarian membranes as a barrier for sperm penetration, etc.
References


29. Krugmann, S., Pleass, R.J., Atkinson, J.D., and Woof, J.M., 1997, Structural requirements for assembly of dimeric IgA probed by site-directed mutagenesis of J chain and a cysteine residue of the α-chain CH2 domain, J. Immunol., 159, 244.


Chapter 2.

Immune Protection of the Process of Fertilization and Early Preimplantation Embryos.
Early Development of the Secretory Immune System

2.1) Protection of the fertilization process

Fertilization, or sperm-egg fusion, is a critical biological event that occurs in sexually reproducing organisms and is required for tissue organization during their further development. Any interruptions in this process may cause a disturbance in the genome of the newly developed organism, its malformations and even death.

The sperm interacts with three oocyte-associated structures during fertilization: i) the cumulus follicular cell layer surrounding the oocyte, ii) the egg extracellular matrix (the zona pellucida), and iii) the oocyte plasma membrane (1). Each of these interactions is mediated by the sperm head, through proteins both on the sperm surface and within the acrosome, a specialized secretory granule in the form of a small two-layer sack surrounding the head. In gamete fusion, attachment of the two membranes through cell-surface molecules is followed by a physical merging of the plasma-membrane lipids (2). The sperm-egg interaction is accompanied by the activity of different proteins, such as the complement regulator membrane cofactor protein which is synthesized in sperm (3).

Fertilization rate in humans has been shown to be associated with the secretion of seminal Ig antibodies. Sperm-bound IgA antibodies are associated with impaired sperm penetration but exert this impact only when their main location is on the head, while IgM affects fertilization rates when localized simultaneously in both the head and the tail (4,5). In patients with weak to moderate antibody levels, IgA antibodies can significantly reduce a fertilization rate but improve embryo-implantation rate (6). Some cases of man infertility have an immunological basis in sperm proteins: when the state of immune tolerance is disrupted, autoimmunization in the man can cause the appearance of antibodies directed against the sperm antigens with development of hypo-fertility (7). In some cases of autoimmune infertility, these impaired antibodies can result in sperm destruction (8) or premature acrosome loss (9,10).

Increased formation of antibodies against endometrial antigens has been seen in women with infertility caused by endometriosis (11). Such immune changes affect folliculogenesis, ovulation, oocyte quality, early embryonic development and implantation and may be related to alterations within the follicles or oocytes. Expression in unfertilized oocytes of several complement regulators, such as membrane cofactor protein, decay-accelerating factor and protectin, may represent a protective immune mechanism by which the human gametes escape from complement-mediated damage during their travel through the female genital tract (12,13).
2.2) Protection of preimplantation embryos

A series of processes occurs in the oocyte and surrounding membranes during and immediately after fertilization. These processes prevent the penetration of other sperms and create homeostasis for the newly formed organism, the zygote. First, a cortical reaction increases Ca concentration in the zona pellucida, which transforms it into a fertilization membrane, that protects the zygote and preimplantation embryo (conceptus – 14,15) from external (maternal) effects. This type of isolation occurs during the first 3 days after fertilization when the embryo passes through the oviduct. On the 4th day, the embryo enters the uterine cavity at the morula stage with 12 to 16 blastomers and is still surrounded by the zona pellucida. Day 4 is also characterized by the appearance of a cavity inside the embryo and its transformation to the blastocyst. The blastocyst contains a small group of cells (inner cell mass or embryoblast) located at one pole and one layer of flattened cells surrounding the blastocyst cavity and forming its epithelial wall. These cells are called the trophoblast. The zona pellucida is dissolved by uterine secretions and disappears. The embryo is now open to receiving water and nutrition from the uterus and at the same time, it is susceptible to external effects, including those from the mother.

The antigenic status of the preimplantation embryo is ill-defined and there are no clearly recognized maternal immune reactions against this early stage of development. Following implantation, the pregnant female shows evidence of immune recognition of her intrauterine-developing semi-allogeneic conceptus (16). The mammalian maternal immune system reacts to the presence of the conceptus by activating an immune response with its two constituents: a weak rejection reaction and a strong facilitation reaction (17). This immune deviation is modulated by the placenta, its secreted substances and their interaction with decidual cells.

The fate of the human embryo is partly determined by its alloantigenic status. The human preimplantation embryo expresses no MHC antigen, and is thus protected from direct attacks mediated by MHC-restricted T cells. Nevertheless, it may be vulnerable to the adverse effects of some antibodies and cytotoxicity by non-MHC-restricted effector cells (18). Such reactions involve the CD4+ helper T cells and CD8+ MHC-restricted cytolytic effectors and occur at the level of the antigen-presenting cells (APC). Both CD4+ and CD8+ MHC effector T cells can be triggered by the presentation of paternal or embryo-derived peptides by maternal APC. B cells can be triggered by soluble antigens.

There are three threats to the embryo: i) lysis or immobilization by anti-embryonic antibodies, ii) attack by non-MHC-restricted cells, and iii) an ongoing maternal immune response directed towards the implantation site.

In human reproduction, soluble human leukocyte antigen-G (sHLA-G) is considered a possible marker of developmental potential, particularly in embryo implantation (19,20). Its primarily location on the extravillous trophoblast makes this antigen a potential mediator of immune interactions at the maternal-fetal interface during gestation and one of the mechanisms involved in protecting the implanting embryo from rejection by the immunocompetent mother. The human preimplantation embryo
expresses several complement regulator proteins associated with the lack of MHC class I antigens that are considered a protective immune mechanism by which the embryo escapes from complement-mediated damage (12,13).

By the 8th day of development, the blastocyst is partially embedded in the endometrial stroma, and its outer layer, the trophoblast, has differentiated into two layers: i) an inner layer of mononucleated cells, the cytotrophoblast, and ii) an outer multinucleated layer, the syncytiotrophoblast, which effects invasion into the uterine wall. The main role in the mother's response to trophoblast invasion in early pregnancy belongs to local immune responses at the maternal-fetal interface (21). It has been hypothesized that specialized mechanisms exist to control access of maternal leukocyte subsets to the decidua and that these mechanisms are modulated during the course of pregnancy. Macrophages and uterine NK cells play different roles in this response (22). CD68+ macrophages, CD56+ lymphocytes and CD3+ T cells are present in the proliferative and secretory endometria. The number of macrophages and CD56+ lymphocytes is dramatically increased at implantation and remains high in the early pregnancy decidua. In contrast to macrophages, CD56+ lymphocytes are more evenly distributed throughout the decidua.

2.3) Components of the secretory immune system in human embryos and early fetuses in the first trimester of pregnancy

Protein components of the SIS (SC, J chain, and Igs) are present in different organs of the human embryo as early as week 4, and during the whole first trimester of pregnancy (23,24). In 4- to 6-week-old embryos, SC, J chain and IgG are highly reactive in the epithelium of most organs (Fig. 1). In 7- to 8-week-old embryos, SC, J chain, and different Ig subtypes appear in the epithelium of the developing thymus, salivary glands, and metanephric tubules. In 9- to 12-week-old fetuses, the location and rate of reactivity of SC, J chain, and Igs change slightly. In embryos or early fetuses with massive antigenic exposure due to chorioamnionitis, no changes were seen in the distribution or immunoreactivity of SC and J chain, compared to embryos not exposed to massive foreign antigenic attacks (24).

SC immune reactivity decreases or even disappears during development of the pituitary gland and thymus (Fig. 2). A similar picture has been seen in other organs and tissues (24). Matrix cells of the neural tube ependymal layer are SC-positive in 4- to 5-week-old embryos. At weeks 6 to 7, SC is detected in matrix cells in a narrow portion of the neural tube. After week 8 and during the second and third trimesters, SC is found only in the epithelium of the choroid plexuses in the brain ventricles. In the pancreas, 95% to 98% of ductal and acinar epithelial cells originating from the midgut epithelium are SC-immunopositive throughout intrauterine development. In contrast, only occasional Langerhans islet cells, deriving from the acinar epithelium and without acinar free lumen, display weak SC-positive staining up to week 20; thereafter all of them are SC-negative. All of these structures continue to be highly immunopositive for J chain and Igs, even when they become negative for SC (24).
Fig. 1. A 4-week-old human embryo.*

**A.** J chain-positive staining in myocardium, endocardium and epicardium (brown staining). The negative staining was seen in the liver. x100.

**B.** SC-positive staining in the liver (brown staining). SC-negative staining was seen in the myocardium. x200.

**C.** SC-positive staining in the epithelium of the stomach (s), pancreatic ducts (p), and gall bladder (g), in the coelomic mesothelium. x100.

**D.** SC-positive staining in hepatocytes (h) and the epithelium of mesonephric tubules (m). x100.

Avidin-biotin complexation and peroxidase technique with commercial markers were used to evaluate various components of the SIS.

* All color figures in this book have been prepared by Prof. P. Gurevich.
Fig. 2.

The number of SC-positive epithelial cells in the pituitary gland (A) and thymus (B) during human intrauterine development (% to the total number of epithelial cells on a slide) (After ref. 24)

Note the sharp reduction in the number of SC-positive epithelial cells in both organs which is going in parallel with progress in gestation.
In human embryos, SC is found almost only in the epithelial tissues: in the epidermis of the skin, in the epithelium of the mucosa, in the endocrine glands, and in the mesothelium (24-26). In the epithelium of the mucous layers of the bronchi, renal collecting ducts, and intestine and pancreatic ducts, SC is located mainly in the apical parts of the cells. In the multilayer epithelium of the skin and the renal pelvis, ureter and urinary bladder, SC is observed in superficial cells. Similar localization has been detected in the squamous epithelium of the skin, mouth, pharynx, esophagus, female genitalia (27) and some other organs (28). In the syncytiotrophoblast, SC is located in the superficial microvilli. In the cytotrophoblast, SC immunostaining was more intensive in its basal compartments (29,30), the site of IgG exocytosis (31). The localization of SC to different cellular compartments reflects its intracellular shift at different stages of its transport. This explanation is in agreement with results of immunoelectron microscopic localization of SC during its synthesis (32).

The main function of SC in human embryos and fetuses is Ig exocytosis, confirmed by the fact that SC has been found in cells that perform exocytosis (24,33), whereas in structures that lose the ability to perform Ig exocytosis during organogenesis, the intracellular concentration of SC is reduced or absent. The location of the SC in many structures is dependent on the direction of the Ig exocytosis: in the secretory columnar epithelium, SC is usually situated in the apical part of the cells; in the multilayer epithelium, it is found in the superficial layer.

Functions of the SIS in embryos, when their own Igs are not yet being synthesized, are different from those in adults. Maternal IgA and IgG pass via the placenta to the embryo (34). IgM and IgD do not pass throughout the placenta (35), and it is not clear whether IgE passes through this barrier (36,37). In fetuses, IgG, IgA and SC are detected in the fetal urine and in the amniotic fluid (38,39).

J chain is always found in cells containing SC, dispersed throughout the cytoplasm. In some organs, only J chain and Igs are seen, without SC. This is characteristic of the myocardium, endocardium, and capillaries of the lungs and stomach, the gonads, and many endocrine organs (25,26). Two types of relationship between SC and J chain have been exemplified. In some cases, SC disappears partially or completely from the epithelial tissue in organs such as the adenohypophysis and pancreatic islets, when they lose contact with mucous layers and do not secrete Igs. In other cases, SC is not present in cells from the beginning. This is characteristic of tissues of mesodermal origin, such as the adrenal glands, myocardium and endocardium, and the endothelium of the capillaries.

Igs exhibit different reactivities in cells with SC and/or J chain, the most common reason being the absence of Ig-synthesizing B lymphocytes in embryos. IgA- and IgM-synthesizing lymphocytes appeared in the liver, spleen, and lymph nodes in 9- to 11-week-old fetuses (40-42). Because only lymphocytes produce Igs and because they begin to function later in the fetal period (43,44), it has been suggested that Igs in early embryos are of maternal origin (34,45). Transfer of Igs across the syncytiotrophoblast
of the chorionic villi is mediated by the neonatal Fc receptors. Immune complexes are absorbed in the stroma of the villi, probably by FcγRI, FcγRII, and FcγRIII on placental macrophages. The mechanism of IgG transport across the endothelium of fetal capillaries is obscure. Endothelial cells in terminal villi express FcγRIIb. However, it is not known whether this receptor transfers IgG or prevents the transport of immune complexes to the fetus.

IgG is the major isotype in the amniotic fluid and contains mother-derived tetanus antitoxins (39). Specific IgG and IgA were found in the coelomic fluid of 6- to 12-week-old human embryos and fetuses whose mothers carried antibodies to rubella, Toxoplasma gondii, and cytomegalovirus (35). The levels of these Igs in the coelomic fluid of embryos were similar to those in the mother's blood, and this can be considered additional proof of the maternal origin of embryonic Igs. However, it is not true of all subclasses of Igs: the origin of IgM in early 4- to 5-week-old human embryos remains unclear. IgA and IgG were found in 73% to 98% of epithelial cells with SC and J chain of the digestive and respiratory tracts in 4- to 8-week-old embryos which had not undergone massive antigenic attacks (30). In fetuses in the second trimester of gestation, immune reactivity to IgA and IgG was seen in 62% to 97% of cells (33). This indicates that both types of Igs are located in the same cells. B lymphocytes synthesizing IgA and IgM appeared in fetal organs at weeks 8 to 9 of development (24).

A strong antigenic effect can explain a weak reactivity of Igs or their absence in cells with SC and/or J chain. Such an effect is seen in chorioamnionitis. The strongest decrease in the Ig reactivity has been seen in the epithelium and glands of the digestive and respiratory tracts, where a large amount of infected amniotic fluid enters. Massive antigenic attacks cause a sharp increase in the exocrine secretion (exocytosis) of Igs. This is manifested in a decrease in the number of Ig-containing cells. In cases of acute chorioamnionitis and massive aspiration and ingestion of infectious amniotic fluid, the number of Ig-containing epithelial cells in the mucous layers of the digestive and respiratory tracts decreases to 13% and 2%, compared to 93% and 61% in noninfected embryos and fetuses (33); moreover, the number of cells with all three types of Igs decreases sharply in glands of the digestive and respiratory tracts. The immune reactivity of J chain remains at the level found in cases without infection.

In septic fetuses with purulent inflammation of the brain ventricles, the number of epithelial cells in the choroid plexuses which are immunoreactive to IgA, IgM and IgG, decreases to 5% to 1%, compared to 93% to 64% in noninfected fetuses. As a result of exocytosis, Igs are also observed in fibrin clots in the bronchial lumens and the pancreatic ducts (26). Ependymal cells of the brain ventricles contain J chain and Igs but are free of SC in fetuses with sepsis and purulent inflammation. Immune reactivity to IgA, IgM and IgG is unchanged in these cells, indicating the absence of exocytosis. This is particularly distinct in comparison to the high exocytosis of Igs in the choroid plexus epithelium of the brain ventricles of the same fetuses containing the SC.

In cells with only J chain and Igs and no SC, Ig secretion is not seen, even under massive antigenic attack. This phenomenon was very distinct in the pancreas, where epithelial cells of the acini and ducts contain the whole protein complex of the SIS, including SC. Langerhans cells do not contain SC or contain it in extremely low amounts and show the immune reactivity to only J chain and Igs. In the pancreatic
acini and ducts, the number of cells containing IgA, IgG and IgM decreased from 96% and 73% in noninfected fetuses of the second and third trimesters, respectively, to 15% and 2% in fetuses in chorioamnionitis (24,33). The effect of antigenic attacks on the number of IgA- and IgM-positive lymphocytes in different fetal organs can be seen in Table I.

Macrophages are the only component of immune-competent cells during the embryonic period. At the end of the first trimester of pregnancy, the number of lymphocytes is still not high. Even chorioamnionitis does not accelerate the maturation process of different subsets of lymphocytes, which could be a reason for the earlier appearance of Ig-synthesizing lymphocytes. The accelerated rate of lymphocyte multiplication does not increase their amount. Perhaps the low number of lymphocytes is a result not only of “immaturity” of the lymph system, but also of its decompensation under acute antigenic effects (29,46).
Table I.
The number of IgA- and IgM-positive lymphocytes in different fetal organs (per 50,000 µm², mean ± SE) (After ref. 24,33)

<table>
<thead>
<tr>
<th>Organs studied</th>
<th>IgA</th>
<th></th>
<th>IgM</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
<td>Group II</td>
<td>Group I</td>
<td>Group II</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.8±0.3</td>
<td>2.3±0.5</td>
<td>3.0±0.6</td>
<td>5.4±0.9</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>1.1±0.3</td>
<td>2.6±0.6</td>
<td>2.3±0.5</td>
<td>5.2±1.2</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.2±0.1</td>
<td>0.9±0.3</td>
<td>0.3±0.1</td>
<td>1.6±0.5</td>
</tr>
<tr>
<td>Stomach, small intestine</td>
<td>0.4±0.2</td>
<td>1.8±0.5</td>
<td>1.9±0.7</td>
<td>6.9±1.8</td>
</tr>
<tr>
<td>Liver</td>
<td>1.2±0.4</td>
<td>2.4±0.7</td>
<td>1.9±0.4</td>
<td>4.8±1.2</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.2±0.1</td>
<td>0.8±0.4</td>
<td>0.6±0.4</td>
<td>2.5±0.8</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.2±0.2</td>
<td>0.6±0.3</td>
<td>1.3±0.7</td>
<td>1.9±0.8</td>
</tr>
<tr>
<td>Choroid plexus</td>
<td>0.3±0.2</td>
<td>0.6±0.3</td>
<td>1.3±0.7</td>
<td>1.9±0.8</td>
</tr>
</tbody>
</table>

\(^a\)Groups of fetuses: I, without antigenic attacks; II, with antigenic attacks.
\(^b\)Significantly different from Group I, \(p < 0.05\).

Note that in all organs antigenic effects increased the number of IgA- and IgM-positive lymphocytes and that in different organs a rate of this increase was significantly different.
References

(To 2.1 and 2.2)


3. Mizuno, M., Harris, C.L., Johnson, P.M., Morgan, B.P., 2004, Rat membrane cofactor protein (MCP; CD46) is expressed only in the acrosome of developing and mature spermatozoa and mediates binding to immobilized activated C3, Biol. Reprod., 71, 1374.


(To 2.3)


Chapter 3.

Immune Protection of Vitally Important Organs and Cells in Human Embryos and Early Fetuses

Whether early human embryos have immune protection remains an open question because they do not have immune competent cells (except monocytes) or immune (lymphoid) organs. The common immune system only starts to form at the very beginning of the fetal period. However, protein components of the SIS, such as SC, J chain and Igs, are already present in the epithelial structures of 3.5- to 4-week-old embryos (1). During organogenesis, the primary structure of some organs changes dramatically, and they lose their connection with the superficial epithelium and the organ lumen. As a result, such organs do not secrete Igs, and the SC disappears (2). However, in some of these organs, especially endocrine glands, neurons of the brain and spinal ganglions, gonads, the myocardium and adrenals, Igs and J chain have been described despite the absence from the start of the SC (3,4).

3.1) Immune components in fetal endocrine glands and their precursors

Endocrine gland function during intrauterine life is very important for fetal survival and development. Inadequate immune protection of the endocrine system during this period can result in serious disruption of fetal development (5,6). The presence of SIS components in endocrine gland cells and their ontological precursors has been described from as early as week 3.5- to 4 of embryonic development (3). In 3.5-4- to 7-week-old embryos which had not been exposed to massive antigenic influences, SC, J chain and IgG were observed in 82% to 98% of the cells from the epithelia of the oral cavity, pharyngeal gut, thyroglossal duct, Rathke’s and pharyngeal pouches, and pancreas, while the concentration of IgA and IgM was weak or nonexistent. The adrenal cells were positive for J chain, weakly positive for IgG and negative for SC, IgA and IgM. Lymphocytes were not present in the stroma of all these organs, except for the occasional single cells (Table II).

The anterior portion of the pituitary body (hypophysis) of 8-week-old embryos and 9-week-old fetuses has a tubular structure and is lined with multiple layers of epithelium (3). Of these cells, which are derived from Rathke’s pouch, 53% to 68% were found to be strongly positive for SC. In second-trimester fetuses, the SC was seen in 15% to 23% of these cells, and in the third trimester - in 5% to 8% of them. In glandular cells of the pituitary pars intermedia during the second and third trimesters of gestation, SC was seen in 68% to 88% of the cells, but no SC was present in the neurohypophysis. J chain, IgG and IgA were observed in all epithelial cells of the pars anterior and intermedia of the hypophysis, and only weakly in the neurohypophysis. In a few instances, a weak positive reaction to IgM was observed.
The functioning of the neuroendocrine system in fetuses is mediated by cytokines (7). Different interleukins (IL), tumor necrosis factor (TNF-α) and interferon (INF-γ) affect the secretion of hypothalamic and anterior pituitary hormones, and specific high-affinity receptors for IL-1, IL-2, and IL-6 have been identified in neuroendocrine tissues. IL-1 and IL-6 are present in the hypothalamus as well as in the anterior and intermediate lobes of the hypophysis indicating their paracrine role in the regulation of neuroendocrine functions.

System ontogenesis of endocrine organs begins with the appearance and histological development of the thyroid and pituitary glands followed by the development of the hypothalamus and the pituitary portal vascular system (8). The thyroid gland starts to develop during weeks 3 to 4 of gestation, and at weeks 7 to 9, consists of trabecular and alveolar groups of epithelial cells (3), 97% to 85% of which contain SC and J chain and are weakly positive for IgG, IgA and IgM. When its follicular structure becomes defined at 10 to 11 weeks, the follicular epithelium is positive for SC, J chain and Igs. The follicular colloid, however, is more immunopositive for IgG, IgA and IgM than the follicular epithelium, and negative for SC and J chain. Hypothalamic-pituitary control of thyroid function matures during the last half of human fetal development. The parathyroid glands, formed during weeks 4 to 7 of gestation from the endoderm of III and IV pharyngeal pouches, contain J chain and Igs in their chief cells, and a weak positive reaction was observed for SC in 43% of the cases studied (3).

From the time of their appearance during week 5 of gestation, epithelial cells of the pancreatic acini and ducts, are strongly positive for SC, J chain and Igs (3). Islets cells, which develop from acinar cells at the beginning of the second trimester of gestation, are negative for SC and positive for J chain and Igs. Both epithelial and mesenchymal pancreatic cells express chemokine receptors, suggesting their role in leukocyte recruitment and perhaps in early pancreatic development. Mature macrophages have been demonstrated in the pancreas of 6-week-old human embryos and 12-week-old fetuses (9,10).

The adrenal glands appear during week 6 of gestation in the form of clumps of coelomic epithelial cells of mesodermal origin. At week 8, these cells multiply intensively and form two zones of the cortex: a definite zone under the capsule and a more deeply located embryonic or fetal zone. J chain and Igs are seen in the embryonic zone. In the second and third trimesters, a weak positive reaction to J chain, IgA, IgG, and sometimes for IgM is seen (3). Reaction to SC is always negative.

An increasing number of immunocompetent cells can be seen in the various endocrine glands as development progresses. Lymphocytes secreting IgA and IgM appear after weeks 9 to 10 of pregnancy (Table II). Massive antigenic exposure causes little change in the distribution and immunoreactivity of SC and J chain in the endocrine cells. However, Ig immunoreactivity declines, especially if infection occurs during the second or third trimesters. A parallel decrease is observed in the number of Ig-positive endocrine cells. In the thyroid, for example, Ig-positive follicular cells amounted to less than 12% in fetuses exposed to infections, compared to 85% to 97% in unexposed fetuses (3). In the pancreas of infected fetuses, Igs were found in 45% to 59% of the islet cells and in 2% to 5% of the acinar cells, as compared 69% to 88% and 53% to 62%, respectively, in unaffected fetuses. Chorioamnionitis results in a reduced number
of different subsets of lymphocytes in the stroma of the endocrine glands. In contrast, a higher number of lymphocytes that secrete IgA and especially IgM are present in the regional (cervical and retroperitoneal) lymph nodes in infected fetuses than in their uninfected counterparts (2.8-3.9/50,000 µm² and 0.1-0.4/50,000 µm², respectively).

Not all SIS components appear to be at the same extent in different endocrine organs. J chain is constantly present in all endocrine cells of developing fetuses and in the precursor cells of embryos. The content and reactivity of Igs are changed with fetal maturation and with the establishment of the endocrine glands' functional status. Four to 7-week-old embryos do not have their own Ig-producing lymphocytes, and all endocrine gland precursors contain maternal IgG or IgA which has passed through the placental barrier (11), i.e. the embryonic SIS functions via maternal antibodies. In fetuses of the second and third trimesters, Ig-immunoreactivity decreases, especially in the adrenal glands.

The third protein component, SC, is present in some of the endocrine glands (thyroid, pars intermedia and sometimes the anterior lobe of the hypophysis, and the pancreatic islets) and consistently in cells of the endocrine gland precursors (3). The various manifestations of SC in different endocrine glands are closely related to changes in the organs’ cellular activity during intrauterine life. Cells of the pancreatic acini and ducts that perform excretory functions, including excretion of various Igs, consistently contain SC. Pancreatic islet cells perform only endocrine functions and contain no, or only trace amounts of SC. These cells have lost the ability to secrete Igs, storing them instead in the cytoplasm. This is very pronounced in cases in which massive antigenic stimulation causes large secretion of Igs from acinar and ductal cells containing SC, as witnessed by their negative reaction to Igs; however this event has no influence on the Ig content of the islet cells.

A similar SIS-component pattern is found during the transformation of the Rathke’s pouch epithelium into cells of the adenohypophysis. During this process, loss of cellular excretory function is accompanied by the loss of SC. Thus, the absence of SC and the storage of Igs in pancreatic islet and adenohypophysis cells are related events. However, other endocrine-organ structures, such as the epithelial cells of the pars intermedia of the hypophysis and of thyroid follicular cells, contain SC during their entire intrauterine life and still preserve the ability to secrete Igs. After secretion, these Igs are stored in the follicular colloid, which contains more of them than the follicular epithelium. These data reflect the close relationship between the presence of SC in endocrine cells and their capacity for exocrine secretion of Igs. In adrenals, the absence of SC is, perhaps, connected with the mesenchymal origin of the cortical cells.

Accumulation of Igs in cells of the main endocrine glands may act as a local protective mechanism against foreign antigens. The decreased Ig-immunoreactivity in endocrine cells following chorioamnionitis supports this assumption. Protein components of the SIS are detected as early as one month into intrauterine embryonic development, and are present within the endocrine organs for the remainder of the gestational period. Thus, the SIS in the endocrine organs appears and acquires functional activity much earlier
than the common (systemic) immune system in its organs (thymus, spleen, lymph nodes) (12).

*To summarize,* two main types of the immune protection of the endocrine glands can be recognized during their intrauterine development. The first is the SIS, the components of which are present at week 4 of gestation in the precursors of the endocrine glands. During subsequent development, this system remains only in the thyroid and in the intermediate lobe of the hypophysis. In the thyroid, SIS components may be related to the synthesis of hormones in this gland: they exocytose into the colloid, then by endocytosis (reabsorption) they re-enter the thyrocytes, and finally, via a second exocytotic event, enter into the inter-tissue fluid and progress in the capillaries through the basal parts of the thyrocytes.

The SIS does not produce its own Igs. Its functions during the embryonic period are performed by maternal Igs in the blood, and after weeks 9 to 11, by a small amount of B lymphocytes-synthesized IgM that appears in the stroma (Table II). One of the signs of functional SIS activity can be a decrease in the amount of intracellular Igs in endocrine cells under inflammation and antigenic attacks, as has been observed in the hypophysis at meningitis, and in the thyroid, pancreas and adrenals in common infections (3).

During organogenesis from weeks 5 to 8, changes in the SIS prepare it for the protection of parenchyma cells in the endocrine glands. The main changes in the SIS are as follows: SC is not produced and exocytosis of Igs does not occur, as they are stored instead in the parenchyma cells of the different glands. In the presence of infection, the concentration of Igs in these cells remains at high levels, while in cells of the SIS area in the same organs, Ig concentration decreases sharply as a result of exocytosis. This is especially clearly seen in the pancreas where, upon infection, Igs practically disappear in the epithelial cells of the acini and ducts (an area of the SIS), while in islets (an area of the changing SIS) the intracellular concentration of Igs remains unchanged.

The common immune system begins to form at weeks 9 to 10 of gestation when the first lymphocytes appear, particularly B lymphocyte-synthesized Igs. The functional activity of this system is manifested in the endocrine glands in the form of weak infiltration of immunocompetent cells, such as T lymphocytes, helpers and suppressors, and B lymphocytes (Table II). Monocytes appear earlier when of the yolk sac begins to function. It seems that the regional lymph nodes, such as the cervical for glands in this area or peritoneal for the pancreas and adrenals, are important organs of the common immune system.
Table II.  
The number of immunocompetent cells in 50,000 µm² of the endocrine glands stroma in human fetuses (mean±SE)  
(After ref. 3)

<table>
<thead>
<tr>
<th>Organs</th>
<th>I trimester</th>
<th>II-III trimester</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Macrophages</td>
<td>T Lymphocytes</td>
</tr>
<tr>
<td>Pituitary</td>
<td>1.3±0.4</td>
<td>single</td>
</tr>
<tr>
<td>Thyroid</td>
<td>1.5±0.4</td>
<td>single</td>
</tr>
<tr>
<td>Pancreas</td>
<td>3.8±0.7</td>
<td>single</td>
</tr>
<tr>
<td>Adrenal</td>
<td>1.9±0.6</td>
<td>single</td>
</tr>
</tbody>
</table>

Group I (without antigenic effects)

Group II (with antigenic effects)

<table>
<thead>
<tr>
<th>Organs</th>
<th>I trimester</th>
<th>II-III trimester</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Macrophages</td>
<td>T Lymphocytes</td>
</tr>
<tr>
<td>Pituitary</td>
<td>3.2±0.9</td>
<td>0.8±0.3</td>
</tr>
<tr>
<td>Thyroid</td>
<td>2.2±0.6</td>
<td>0.9±0.4</td>
</tr>
<tr>
<td>Pancreas</td>
<td>4.9±1.1</td>
<td>2.1±0.8</td>
</tr>
<tr>
<td>Adrenal</td>
<td>1.1±0.4</td>
<td>0.7±0.3</td>
</tr>
</tbody>
</table>

a Significantly different from the same group of trimester I, p<0.05-0.001.
b Significantly different from the group I of the same trimester, p<0.05.

Note that lymphocytes appear in an unaffected group at weeks 9 to 10 of gestation, and that their amount increases significantly during the second and third trimesters. Fetuses show a distinct reaction to antigen effects. Monocyte activity, including a clear response to antigen effects, is already seen in the first trimester of gestation, and this response increases to the end of pregnancy. In both compared groups, the clearest reaction to antigen effects was found in the pancreas, perhaps because of its location near the intestine, in an area with high pathogen effect. The reaction of T helpers is very weak in both groups of fetuses.
3.2) Immune components in the developing myocardium

The primordial heart tube is seen at week 3 of gestation as a part of a large cardio-craniofacial morphogenetic field, and at week 4 its wall consists of three layers: the epicardium, myocardium and pericardium (13,14). Heart differentiation is accompanied by programmed cell death (apoptosis) of the myocytes that proliferate rapidly during fetal life, mediate remodeling of the bulbus cordis and accompany the transition from a fetal to an adult circulatory system (15-17). These processes are mediated by a few specific proteins, such as the Fas death receptor CD95 and the TNF-type II membrane family protein, which can induce both cell death and proliferation/differentiation of the receptor-bearing cells (18). Shc proteins, participating in the control of apoptosis, are primarily expressed in the cardiovascular system during early embryogenesis and regulate heart and blood vessels development (19). Myocytes of the fetal heart undergo mitosis/apoptosis during gestation (16).

Some immune components are present in the developing heart. J chain, IgG and IgA are found in the endocardium and myocardium during the entire period of intrauterine development. On the other hand, SC is not observed. In the pericardium, all protein components of the SIS are present. In fetuses affected by antigenic attacks, there are no essential concentration changes in these components. In the myocardium, single CD3+ and CD20+ lymphocytes and a moderate number of monocytes (1.02±0.02/50,000 μm²) are observed (Gurevich, unpublished data). In infants with congenital heart disorders, reduced percentages of total T lymphocytes and T helper cells, and low levels of IgG and IgA, as well as of complements C3 and C4, have been found (21,22).

Some components of the common immune system are present in the fetal myocardium in the form of a small amount of lymphocytes and macrophages as well as of Igs entering with the blood. The presence of J chain and Igs in cardiomyocytes can be considered indications of immune protection of the heart. The presence of all protein components of the SIS (SC, J chain and Igs) in the epicardium and pericardium can be interpreted as a barrier type of the SIS in these areas.

3.3) Immune protection of the developing brain

The anatomical development of the central nervous system (CNS – the brain and its various parts) extends over a protracted period from week 4 of gestation to 20 months after birth (22). Its cellular elements arise from the primary and secondary neuroepithelium (23). Different proteins participate in the normal process of brain development. Apoptosis and bcl-2 proteins are important for CNS development at weeks 14 to 32 of gestation (24). The neural cell adhesion molecule L1 participates in the neural cell migration, axon elongation and axonal fasciculation (25). The microtubule-associated protein-5 is essential for the elongation and maturation as well as the function maintenance of axons and dendrites in the developing human brain (26).

Components of the common immune system, such as T and B lymphocytes, macrophages, etc., participate in protecting the developing brain. It is known that MHC class II molecules are known to be related to an early phase of immunological response. These molecules are responsible for the binding, transport, and presentation of
foreign antigens to T helper lymphocytes and determine the type of antibodies produced. They also stimulate the multiplication of specific B lymphocytes and participate in the elimination of autoreactive lymphocytes and the maturation of T lymphocytes. Cells expressing MHC II molecules on their surface have been observed in the frontal and temporal lobes of human fetal brain between gestational weeks 11 and 22 (27,28). MHC II expression was noted on the surface of the cerebral meninges cells, in the choroid plexuses of the lateral cerebral ventricles and blood vessel lumens, and in the microglia of the both cerebral hemispheres of human fetuses. The expression of MHC II on cells of the CNS already at as early as week 11 of gestation may constitute evidence not only of a capacity for early immune protection of the fetal nervous system, but also of a significant role that is potentially played by this system in normal embryogenesis.

Choroid plexus macrophages may contribute to an inflammatory cascade in the brain (29). Cytokines are signaling proteins that can be produced as a part of the inflammatory response to both ischemia and infection (30). Preterm infants with cerebral white matter injury had high levels of IL-6, IL-10, and TNF-α in the cerebrospinal fluid. The intrauterine environment can significantly affect fetal brain development, and a range of hemodynamic and metabolic compensations protects the fetal brain from the effects of different factors, such as acute hypoxia (31-33).

Components of the common immune system exhibit high activity during antigenic effects on the developing brain. Fetal T lymphocytes can be activated during fetal exposure to infection (34). These include specific recognition of bacterial antigens and autoantigens, polyclonal activation by Toll-like receptors, and bystander activation by cytokines. High concentrations of cytokines (TNF-α, and βIL1, IL6, and IL10) and CD45RO+ T lymphocytes in the umbilical blood were found in pre-term infants with cerebral lesions who were born at 23 to 29 weeks of gestation (35). Functional chemokine receptors and chemokines are expressed by microglial cells which may influence cellular function within the CNS (36). The CXCR3 chemokine receptor, expressed on activated T lymphocytes, is seen in the CNS in inflammatory conditions with a prominent T-cell response (37).

Fetal inflammatory response syndrome appears to be crucial to the association between intrauterine infection and a brain white matter disease in human preterm infants (38). Chronic exposure to intra-amniotic lipopolysaccharide-caused chorioamnionitis affects the ovine fetal brain, manifesting itself as a moderate to extensive infiltration of activated microglial macrophages in the subcortical white matter (39). The fetal inflammatory response that develops in response to intrauterine infection may contribute to the occurrence of brain damage (40,41). Components of the common immune system, such as cytokine or IL-6 elevation in the fetal plasma and neutrophil infiltrates in the umbilical cord, are significant risk factors for brain damage and/or cerebral palsy (42-45).
3.3.1) Secretory immune components in the developing brain

The SIS constitutes the first in the immune protection for the developing brain and it has been observed at week 4 of gestation (Gurevich, unpublished observations). At this stage, its function involves the transport of maternal Igs into fetus's cerebrospinal fluid (CSF). Protein components of the SIS, such as SC, J chain and IgG and to a lesser extent IgA, are located alongside the entire length of the neural tube in all cellular layers, from basal neuroepithelial cells to the superficial neuroblasts. IgG, IgM and IgA have been found in different parts of the rat fetal brain, such as the corpus callosum, cingulum and habenula (46).

Capillaries in the choroid plexuses form one of the hemo-encephalic barrier interfaces that control the brain's internal environment (47). The secretory function of capillaries consists of their ability to transfer Igs into the CSF. The state of the CSF has great significance in the protection of the developing brain. The brain fluid contains different salts, very little proteins, and starting from the second trimester of gestation, immunocompetent cells, including different lymphocyte subtypes. These cells pass into the CSF from the choroid plexuses of the brain ventricles and to a lesser extent from the capillaries and blood vessels in the brain membranes. A connection between the common blood stream and brain capillaries and choroids plexuses should be considered part of the immune system.

The number and distribution of choroid plexus epithelial cells, containing alpha-fetoprotein (AFP), IgG, IgA, and IgM, were recorded in the human fetal brain at different developmental stages (48). AFP and IgG were found in less than 40% of the cells, and this proportion declined later in gestation to only a few percents. In human fetuses at the end of the first trimester and the beginning of the second trimester of gestation, the concentration of SC in epithelial cells of the brain decreased and then disappeared, remaining only in narrow (undifferentiated) areas of the neural tube. The other protein components of the SIS, such as J chain, IgG and IgA, were still present in the neuroblasts alongside the neural tube. There were regional differences in the distribution of the SIS components. In the choroid plexus of the fourth brain ventricle, all mentioned protein components of the SIS were seen: SC, J chain, IgG and IgA. In neurons of the spinal ganglions from the caudal equine, J chain and Igs were found, but SC was not. In the second and third trimesters of gestation, neurons and dendrites in the brain and spinal cord, as well as neurons of the spinal ganglions, contained J chain, IgG, IgA, and sometimes IgM, but not SC. The whole protein complex of the SIS, including SC, was present in the choroid plexus epithelium and in the ependyma of the brain ventricles. Cells of the microglia did not contain any components of the SIS.

Pathological changes in the brain without inflammation have not been shown to develop in embryos that died as a result of birth canal infection and/or chorioamnionitis. The embryonic death in these cases comes very fast, and inflammation has no time to develop in the brain. At immune fetal-mother conflict during weeks 3 to 8 of gestation, the antigenic effect of maternal antibodies on the embryonic brain causes its early death during week 4. This is accompanied by massive apoptosis in the brain seen in 20% to 50% of the cells.
In cases with brain inflammation during the second and third trimesters of gestation, no essential changes were seen in the cellular distribution of SC, J chain or Igs, in the choroid plexus epithelium, ependyma of the brain ventricles and neurons of various ganglions. In cases with inflammation of the brain membranes and ventricles, a large accumulation of neutrophil leukocytes, monocytes, and different lymphocyte subtypes was seen in the CSF and the stroma of the choroid plexuses (Gurevich, unpublished data). Intracellular concentration of SC and J chain decreased, IgG and IgM disappeared, and IgA was sometimes present at weak concentration. In ganglion neurons of the medulla oblongata and cerebellum, intracellular concentration of the J chain did not change, while Ig concentration decreased.

3.3.2) Types of barrier protection of the developing brain

The mechanisms of barrier protection in the developing brain are distinguished from the hemo-encephalic barrier of the adult brain, and they can be non-immune (mechanical) or immune. An example of a non-immune barrier is the internal membrane of the brain (ependyma). In the adult brain, this is not merely an inert lining but may regulate the transport of ions, small molecules, and water between the CSF and neuropil (a feltwork of naked nerve fibers and neuroglial cells processes) and serve as an important barrier that protects neural tissue from potentially harmful substances. The fetal ependyma is believed to be secretory and plays a role in neurogenesis, neuronal differentiation/axonal guidance, transport, and support (49,50). Differentiation of the ependymal cells proceeds along particular regional and temporal gradients as does the expression of various cytoskeletal and secretory proteins (51).

The growth of the capillary net in the brain is another example of a non-immune barrier. Vessel endothelium growth factor (VEGF) affects capillary growth (47). This protein is expressed in high amounts during embryonic angiogenesis and is not produced in adults. VEGF is well correlated with the factor of vessel penetration, and both reflect the activity of the hemo-encephalic barrier. The immature brain contains a few non-immune barriers (52,53). The hemo-encephalic barrier comprises mechanisms that control the exchange of molecules between the internal environment of the brain and the rest of the body. The underlying morphological feature of this barrier is the presence of tight junctions between cerebral endothelial cells and choroid plexus epithelial cells (54,55). These junctions are present in the fetal brain's blood vessels and are effective in restricting the entry of proteins from the blood into the brain and CSF. In the immature brain, there are additional morphological barriers at the interface between the CSF and the brain tissue, such as strap junctions at the inner neuro-ependymal surface and intercellular membrane specializations at the outer pia-arachnoid surface. These barriers disappear later in development and are absent in adults.

The hemo-encephalic barrier and choroid plexuses are good examples of the immune barrier type of brain protection. SC, a protein component of the SIS, is detected in the ependyma of choroid plexuses and some other structures which participate in the formation of the blood-tissue and tissue-tissue barriers (47,56,57). The presence of SC in these structures suggest the possibility of Ig secretion by the same mechanism described in the mucosal membranes and endocrine glands. Moreover, the existence in
the barrier system of IgG as well as of IgA and IgM secretion may be considered additional proof of the presence in the developing brain of an immune-type protective barrier system.

In rat fetuses, choroid plexus tight junctions are impermeable to small molecules at day 15 of gestation or earlier, indicating that the blood-CSF barrier is morphologically and functionally mature early in the embryonic period of development (58). The transfer of proteins from the fetal blood to the CSF is selective: transfer of albumin is four- to fivefold greater in humans than in bovines. The number of choroid plexus epithelial cells that are immunopositive for endogenous plasma protein increases in parallel with an increases in the total protein content of the expanding ventricular system. These results suggest that different transcellular mechanisms are operating in the transfer of proteins and small molecules across the embryonic blood-CSF interface.

In summary, it should be emphasized that components designed to protect the brain and the entire CNS are present at the very beginning of the embryonic period. Protein components of the SIS, such as the SC, J chain, and maternal Igs, are seen in neuroepithelial cells and neuroblasts of the developing neural tube. These components remain in the choroid plexuses and ependyma of the brain ventricles. Neuroblasts that transform into microglia lose all of their SIS protein components, while the same neuroblasts transformed into neurons lose only their SC and retain their J chain, IgG, IgA and sometimes IgM, similar to that which occurs in the endocrine glands. These data suggest that during the brain’s development, the SIS undergoes some transformation that protects neurons of the brain and ganglions. Components of the common immune system are also seen in the fetal brain, and immunocompetent cells are present in the CSF. The developing brain has also protective barrier mechanisms that can be non-immune (mechanical) or immune.

### 3.4) Immune protection of gonads and genital tracts

#### 3.4.1) Immune protection of gonads and genital tracts in adults

Protection of the sexual cells' genome and thus of future generations starts long before fertilization and the appearance of the embryo itself. The immune system creates the protective background for the whole organism, and its protective elements have been found in the gonads, germ cells and genital tracts of both females and males.

#### 3.4.1-1) Immunoprotective components in the female genital tract, ovaries and oocytes

Different types of ILs and cytokines have been described as major components of the protective system in these organs. Factors produced by activated immune cells, including IL-1 and IL-2, play a role in the down regulation of these responses, and stimulation of T- and B-lymphocyte functions in females (59,60), and participate in the inflammation response (61). In the female reproductive tract, the immune system represents a defense mechanism that can act directly against pathogens and mediate an inflammatory response (62). The complement regulatory proteins are expressed
throughout the female genital tract, and play an important role in protecting the traversing sperm and implanting blastocyst from a complement-mediated damage (63). Endometrial cells are protected from complement attacks by membrane-bound complement regulatory proteins. The survival of these cells with some biochemical modifications enables the immune response.

The human female reproductive tract is an inductive site for immune responses, and cell-mediated immunity with all effector components of the SIS is present throughout the entire tract (64,65). B lymphocytes and plasma cells that secreted IgA (and in lower amounts also IgM and IgG) are found in the uterine endocervix and ectocervix, oviducts, and vagina. Epithelial cells lining the oviducts and endocervix express SC, which is required for the transepithelial transport of polymeric (p)IgA into external secretions. Secretory IgA (sIgA), which provides the first line of defense against invading pathogens, is produced locally in the female reproductive tract (64). Approximately two-thirds of the Ig-positive cells contain slgA and J chain, indicating that they produce (p)IgA. A local immune system functions in the human oviducts and may provide a first line of defense against ascending infection: T-suppressor cells, which participate in the induction of the immune tolerance, are found in the human oviducts (63).

Paracellular diffusion of serum-derived and locally produced IgG through the epithelia is an important part of humoral immunity in the female genital tract (66). The endometrium can perform external translocation of plgA. Uterine and cervical epithelial cells play a key regulatory role in the control of IgA transcytosis from the tissue into secretions, a manifestation of SIS functions (67). SC production by uterine epithelial cells is correlated with increased transepithelial resistance.

The lower reproductive tract in women is also immunocompetent as judged by the presence of different subtypes of T lymphocytes, macrophages, and dendritic cells in the endocervix and ectocervix, and in the vagina (68). The quantity and subclass distribution of IgA produced by the human uterine cervix have a significant impact on the defense against sexually transmitted diseases and even against oncological disorders (64,69). The female genital tract protects the host from pathogen challenges induced by such infections as herpes simplex viruses or Trichomonas fetus (70, 71).

The SIS appears to play an important role, additional to that of the common immune system in the protecting the female genital tract and gonads in relation to the peculiarities of the antigenic effects and the structure and function of target organs. As in the other organs, a protective mechanism consists first of Ig secretion on the surface of the mucous epithelium with of SC and J chain participation in this process (69). The presence of complex proteins characteristic of the SIS, such as SC, J chain and Igs, as well as of immunocompetent cells (macrophages, T and B lymphocytes, plasma cells), that participate in the synthesis of Igs, is highly specific to the SIS (72). Some of these cells (B lymphocytes and plasma cells) that participate in the synthesis of IgA and to a lesser extent IgG and IgM, are located in the subepithelial layer of the endocervix and ectocervix, as well as that of the oviducts, uterus, and vagina. The presence in the plasma cells of J chain and IgA indicates the synthesis of plgA which is typical of the SIS. Eighty percent of the IgA in the mucous membrane of the uterine cervix was
found to be polymeric, while in the vaginal fluid only 55% was (73). Infection of the genital tract causes a strong increase in the secretory activity of all components of the SIS (70,71).

Only some immunoprotective components have been described in the ovaries and oocytes in only a few publications. Oocytes, and follicular and thecal ovarian cells could represent sources and targets of ILs (74) which participate in local regulation of many reproductive functions (75). Different subclasses of lymphocytes that participate in the synthesis of Igs are found in the human ovaries (76). Ovulation has been found to be accompanied by the migrating macrophages into the follicle and the production of two macrophage-specific chemokines (77).

### 3.4.1-2) Immunoprotective components in the testes and male genital tract

In the epithelial lining of the testis, epididymis, and vas deferens, lymphoid cells are represented predominantly by T suppressor/cytotoxic cells. Elements of the SIS, such as SC and IgA, have been described in the male rodent's urogenital tract (78,79). Protective IgG antibody-mediated immunity in the male genital tract is provided by IgG and IgA derived from the systemic immunoglobulin pool and from local synthesis (73). Because genital tract tissues lack inductive mucosal sites analogous to the intestinal Peyer's patches, the local humoral and cellular immune responses stimulated by infections are weak or absent, and repeat local immunizations result in minimal responses (80,81).

The production of autoantibodies to spermatozoa in males is inhibited by both the physical isolation of the spermatozoa from the systemic immune system and by active immunosuppression mechanisms (63). In the testes, physiological protection from autoimmune attacks is provided by different ILs, particularly IL1 and IL18 (82-84). Testicular cytokines and growth factors, such as IL-1, IL-6, TNF, IFN-γ, etc., have been shown to affect germ-cell proliferation (85) and participate in pathologies such as orchitis, acute lymphoblastic leukemia of the testis, systemic inflammation and infertility disorders in men (86,87).

### 3.4.2) The secretory immune system in developing gonads and genital tracts

Development and differentiation of gonads and genital tracts are accompanied by the early appearance and functional activity of different component of the SIS.

#### 3.4.2-1) Secretory immune components in developing genital tracts

Both male and female embryos initially have two pair of genital ducts: i) mesonephric and ii) paramesonephric ducts. The mesonephric ducts represent a continuation of the mesonephros, whereas the paramesonephric duct arises on each side of the early embryo as a longitudinal invagination of the coelomic epithelium on the anterior-lateral surface of the primordial urogenital ridge. The mesonephric duct forms the genital tract in males while the paramesonephric duct develops into the genital tract in females.
In 3.5-4- to 6-week-old healthy embryos, the epithelium of the mesonephros tubules, the mesonephric ducts and the paramesonephric ducts, the proliferating coelomic epithelium and cells of the primitive sex cords of the genital ridge show high immunoreactivity to SC, J chain and IgG (4). Igs are secreted on the mucosa of these organs and have been observed in the lumen of the genital organs, fetal urine and amniotic fluid (88,89). IgA is present in embryos at weeks 5 to 6 of pregnancy, while IgM has been found in some of 6- to 7-week-old embryos. The epithelial cells of the uterine tubes and endometrium were found positive for SC, J chain and all Igs from week 9 until the end of pregnancy (4). In parts of the male genital tract, such as the deferent duct, epithelial cells are strongly reactive for SC, J chain, IgG and IgA, and weakly reactive for IgM. Massive antigenic stimulation in embryos with chorioamnionitis has no essential effect on SIS components, except for a decrease in Ig reactivity.
Fig. 3.
The ovary of a 22-week-old human fetus.
A. SC in the follicular cells (brown staining). Note that in oogonia SC is not present. x400.
B. J chain in oogonia and follicular cells. x400.
C. IgA in oogonia and follicular cells. x400.
The testis of a 22-week-old human fetus.
D. J chain in Leydig cells and spermatogonia. x1000.
3.4.2-2) Secretory immune components in developing female gonads

Although the sex of the embryo is determined during fertilization, in humans the gonads do not acquire sexual characteristics until week 7 of gestation. Components of the SIS exist in the gonads of 3.5- to 4-week-old embryos (4), i.e., long before these organs acquire their morphological organization. Initially, the gonads appear as a pair of longitudinal genital ridges that are formed by the proliferation of the coelomic epithelium and condensation of the underlying mesenchyme. The superficial epithelium (mesothelium) of the genital ridges contains SIS components, such as SC, J chain, IgG, and to a lesser extent IgA. Cells of the mesothelium penetrate into ovarian anlagen and during the second trimester of gestation form primordial follicles, which are surrounded by the follicular epithelium that exhibits positive reactions to SC, J chain, IgG, and later IgA. Oogonia display reactivity to J chain and IgG, and weak staining for IgA, but never any staining for SC (Fig. 3).

The immune system and mesenchymal-epithelial interactions play an important role in the regulation of ovarian function. Cytokines, for example, produced by mesenchymal cells can stimulate the development and regression of ovarian structures (90). Igs of the SIS have been found to participate in different functions of the ovaries: IgM binds to young luteal cells; in the corpus luteum of pregnancy, IgM binds only to luteal vessels; the regressing corpus luteum shows IgM binding to both luteal cells and vessels (90). Different subsets of lymphocytes have been found in human ovaries (91).

3.4.2-3) Secretory immune components in developing male gonads

SC has been found in the testes (92), and testicular macrophages have been shown to be involved in immune reactions (93-95). Similar functions have been attributed to the human epididymis (96). Epididymal tubules and rete testis are strongly reactive for J chain, IgG, IgA and weakly reactive for IgM. Interstitial (Leydig) cells and spermatogonia show a similar reaction (Fig. 3). Gonad interstitia contain a few macrophages, measured at 3.9±0.6/50,000 µm² (4). Scattered CD3+ lymphocytes are detected after week 7 of pregnancy, CD20+ lymphocytes after week 9, and CD4+ and CD8+ lymphocytes after week 11. The decrease in Ig reactivity is more pronounced in the epithelium and interstitial cells and less so in primordial germinal cells (oogonia and spermatogonia). The number of macrophages in the interstitium increases significantly (to 35.2±4.8/50,000 µm²) in embryos with chorioamnionitis relative to unaffected embryos (4). The number of lymphocytes varies from very low figures in some cases to very high ones in others.

In summary, it can be suggested that different humoral and cellular components of the SIS participate as a protective mechanism in the development and functional formation of gonads at the very beginning of the embryonic period. Macrophages are seen in the stroma of genital organs, beneath the mucosal epithelium and around the germ cells, in 3.5- to 4-week-old embryos (4). Different subsets of lymphocytes appear in 7- to 8-week-old embryos. As a whole, the SIS components and their major functions – exocrine secretion of Igs on the mucosal surface and in the lumens of organs – are similar to the structure and function of the SIS in adult genital organs (72).
Accumulation of Igs in the germ cells of embryos and fetuses perhaps may reflect a local immune response or they may be present as part of a cellular self-protection mechanism against foreign antigenic effects on these strategically important structures. It should be emphasized that the common immune system in embryos and even fetuses has “not yet matured” and is not fully functionally active, it is “immuno-incompetence”. The decreased reactivity of Igs in germ and interstitial cells in cases of chorioamnionitis can be considered a manifestation of cellular self-protection and reflects the functional participation of the SIS of genital organs and tracts in their immune response to antigen attacks.

There are two types of SIS. One of them is present in the genital tracts and in their precursors, i.e., in the mesonephric and paramesonephric ducts, as well in the genital organs that develop from them. The protein components of this type of SIS are represented by SC, J chain and Igs, located in the epithelium of the genital organs. Their stroma contains cellular components of the SIS: monocytes and, after weeks 9 to 10 of gestation, different subtypes of lymphocytes. The main function of this SIS type is the external secretion of Igs on the mucosal surface and in the lumens of the genital organs. This process is highly characteristic of SIS function in the digestive, respiratory and urogenital tracts of adults. The ovarian follicular cells have a similar function: they contain SC and are able to secrete Igs into the intercellular spaces of oogonia.

The other SIS type is present in the ovarian oogonia, and in many structures of the testes, such as the epithelium of the seminiferous and straight tubules, the Leyding and Sertoli cells, and the spermatogonia. Herein the SIS is represented only by J chain and Igs and does not contain any SC. Interstitial and germ cells of both sexes have no direct contact with the mucosal surface and do not participate in the exocrine secretion of Igs into the lumen of the genital organs: Igs are accumulated in the cytoplasm of the germ cells. There may be a link between this lack of secretory function in the germ and interstitial cells and the absence of SC (97). The latter precludes the possibility of Ig exocytosis. Therefore, these cells and structures cannot be considered a part of the SIS.

The SIS appears long before the structural formation of these organs, while the common immune system begins to develop after week 9 of gestation. The main characteristic of the latter system is the presence of the immunocompetent cells that produce their own Igs, such as IgM, IgA and others. Both of these systems protect mainly the genital tracts and surrounding tissues but not the gametes. Gamete development is accompanied by reorganization of part of the SIS. The SC gradually disappears from cells surrounding the gametes, and they therefore lose the ability for exocrine Ig secretion. It appears that the J chain brings Igs into the germ cells (oogonia in ovaries, and spermatogonia in testes), thereby conferring immune protection. Towards the middle of the second trimester of gestation, all types of immune systems are actively functioning.
References

(To 3.1)


(To 3.2)


(To 3.3)


(To 3.4)


60. Petroff, M.G., Petroff, B.K., and Pate, J.L., 1999, Expression of cytokine messenger ribonucleic acids in the bovine corpus luteum, Endocrinology, 140, 1018.


70. MasCasullo, V., Fam, E., Keller, M.J., and Herold, B.C., 2005, Role of mucosal immunity in preventing genital herpes infection, Viral Immunol., 18, 595.


Chapter 4.
Immune Protection of Embryos and Fetuses in Normal Pregnancy and under Pathological Effects

4.1) Mononuclear phagocytes in embryos and fetuses under normal and pathological conditions of gestation

The yolk sac and aorta-gonad-mesonephros region are well recognized as the principal sites of hematopoiesis in developing embryos, and the liver is the principal site of hematopoiesis in fetuses. Moreover, a significant number of committed and multipotent CD34+ progenitors with a capacity for expansion circulates in the fetal blood between weeks 7 and 19 of gestation (1). In normal pregnancy, the rate of fetal mononuclear-cell division rate decreases with gestational age from 1.8% at 18 weeks to 1% at 40 weeks (2). This rate is elevated in early pregnancy and in chromosomally abnormal fetuses, probably as a consequence of the higher number of circulating haematopoietic precursors. There is a significant association between cell division and the erythroblast count. The rates of both of these parameters increase in chromosomally abnormal fetuses, with an increase in the erythroblast count.

Both the fetal blood and liver provide a rich source of hematopoietic stem and progenitor cells (3), but the fetal liver provides a richer source of more primitive hematopoietic progenitor cells than does the fetal blood. The fetal red blood cells, white blood cells, and platelet counts all increase with gestation, from weeks 8 to 17, reflecting hematopoietic development. The number of normoblasts decreases dramatically with gestation. The number of circulating and hepatic T lymphocytes increases before week 13 of gestation, reflecting thymic maturation. The fetal liver contains fewer T lymphocytes than the fetal blood (2.5% vs. 18.6%) and more CD34+ hematopoietic stem and progenitor cells (17.5% vs. 4.3%). Fetal blood at an early (21-22 weeks) gestational age has a higher frequency of primitive hematopoietic progenitor cells (CD34+/CD38- cells) than does the umbilical cord blood at term (39-40 weeks) (4). In fetuses, the cord blood cytokine-receptor network, consisting of IL-1, IL-2, IL-12, IFN-γ and TNF-α, is biased towards anti-inflammatory activity (5). It should be noted that endothelial progenitor cells derive mainly from the monocyte/macrophage-containing CD34- mononuclear cells and only in part from the hematopoietic stem-cell-containing CD34+ mononuclear cells (6).

The high-affinity protein FcγRI plays a major role in the effector function of circulating monocytes and splenic mononuclear phagocytes, whereas FcγRIII, expressed strongly on the latter effectors, participates in target ingestion (7). Anti-FcγRII has no significant effect on the interaction of fetal spleen mononuclear phagocytes with the red blood cells, whereas anti-FcγRIII causes a significant (43%) inhibition of their phagocytosis. In the absence of any inhibitor, attachment and phagocytic indices of fetal monocytes are similar to those of their newborn and adult counterparts but markedly lower than those of mononuclear phagocytes from the fetal spleen.

CD95 is the most well-studied receptor mediating a signal for the cell death by apoptosis, and its inducible ligand has been demonstrated to mediate the death of
multiple types of CD95-expressing cells. This molecule has a crucial role in the homeostasis of haematopoietic cell populations in adults. Cord blood mononuclear cells enjoy some immune privilege due to their low level of CD95 expression (relative to adult peripheral blood lymphocytes) and due to expression of the CD95 ligand (8). An increase in IL-2γ receptor expression by the cord-blood mononuclear cells may significantly contribute to the prevention of neonatal infection (9). The blood mononuclear cells in newborns produce less IL-10 than adults, and the primary cells of origin and the regulatory mechanisms may differ from those observed in adults (10). Increased production of IL-6 and decreased production of IFNγ by the cord-blood mononuclear cells appear to be the hallmarks of newborns in the high-risk allergy population (11). The fetal peripheral blood mononuclear cells exhibit a proliferative responses to mitogenic and allergenic stimuli during gestation: fetal exposure in utero to allergens from around 22 weeks of gestation results in primary sensitization to those allergens, leading to the positive proliferative responses at birth (12).

Neonatal monocytes produce a different cytokine-expression profile than adult monocytes (13). After lipopolysaccharide exposure, fetal monocytes produce less TNF-α and more IL-8. In neonatal sepsis caused by *Streptococcus agalactiae*, a major cause of severe infection in newborns and pregnant females, different cytokine expression patterns (IL-6, IL-1 beta, and IL-12p40) have been found in the cord-blood mononuclear cells (14). In contrast to the response to *Escherichia coli* lipopolysaccharide, where TNFα, IL-1β, IL-6, and IL-8 appear almost simultaneously, the human monocyte response to *S. agalactiae* results in the production of TNF-α, but also in the delayed appearance of IL-1β, IL-6, and IL-8 (15). The lymphocyte response to *S. agalactiae* is manifested by IFNγ and IL-12 secreting, while the *E. coli* lipopolysaccharide fails to induce production of these critical cytokines. This suggests an important role for TNFα, IFNγ, and IL-12 in *S. agalactiae* pathogenesis and/or immunity.

Placental malaria in pregnant women is associated with up-regulation of macrophage migration inhibitory factor (MIF) in the intervillous blood. MIF may play a role in immune responses to malaria during pregnancy by virtue of its ability to activate macrophages and to overcome the immunosuppressive effect of glucocorticoids (16). The level of MIF in the intervillous blood plasma in placental malaria is higher than that in both the peripheral and cord plasma. The intervillous blood mononuclear cells produce significantly higher levels of MIF, than their peripheral blood counterparts. Placental malaria modulates MIF expression in different placental compartments. A consistent pattern of MIF expression in the syncytiotrophoblasts, extravillous trophoblasts, intervillous blood mononuclear cells, and amniotic epithelial cells, is found, irrespectively, of malaria infection status (17). Only the amniotic epithelial and intervillous blood mononuclear cells from infected placentas exhibit significantly higher levels of MIF expression than uninfected placentas.
4.2) Immune protective role of the trophoblast

Pregnancy is an immunological balancing act in which the mother's immune system has to remain tolerant of paternal major histocompatibility (MHC) antigens and yet maintain normal immune competence for defense against microorganisms (18). The placenta separates fetal and maternal blood and lymphatic systems, and it is the fetal trophoblast that plays the major role in evading recognition by the maternal immune system.

The trophoblast, the peripheral part of the mammalian conceptus, exerts a crucial role in implantation and placentation, and in the formation of the maternal-fetal interface. Both processes occur as a consequence of an intimate dialogue between fetal and maternal tissues, carried out by membrane ligands and receptors, as well as by the release of hormones and local factors (19). Chorionic or trophoblastic villi are the main functional units of the placenta within which the fetal blood is separated by only three or four cell layers (placental membrane) from the maternal blood in the surrounding intervillous space (20). After implantation, trophoblast cells proliferate and differentiate along two pathways described as villous and extravillous (21). Non-migratory, villous cytotrophoblast cells fuse to form the multinucleated syncytiotrophoblast, which forms the outer epithelial layer of the chorionic villi. It is at the terminal branches of the chorionic villi that the most of the fetal/maternal exchange occurs. Extravillous trophoblast cells migrate into the decidua and remodel uterine arteries. The physiology of pregnancy depends upon the orderly progress of structural and functional changes in villous and extravillous trophoblast, whereas a derangement of such processes can lead to different types of complications, including possible pregnancy loss and life-threatening maternal diseases (19).

Approximately one week after fertilization, the trophoblast participates in the contact with maternal cells that enables implantation, a process that quickly sequesters the human embryo within the uterine wall. Through an unusual differentiation process, trophoblastic acquire the properties of leukocytes and endothelial cells that enable many of their specialized functions (22). Further embryonic development requires the rapid assembly of the basic building blocks of the placenta: the floating and anchoring chorionic villi. The unique structure of the human maternal-fetal interface is established by differentiation of cytotrophoblasts into anchoring villi (23). These fetal cells form elaborate connections with maternal vessels, thereby diverting uterine blood flow to the placenta. Once the embryo is anchored in the uterine wall, the next major hurdle is rapid formation of extraembryonic lineages, a necessary prelude to assembly of the maternal-fetal interface. Formation of the placenta, the organ that feeds the fetus, involves a cooperation between maternal NK cells and fetal trophoblast cells that remodels the blood supply. This process and, consequently, human reproductive success, are influenced by polymorphic human leukocyte antigen (HLA)-C ligands and their killer cell immunoglobulin-like receptors (24).

In rhesus monkey, the number of macrophages and CD56+ lymphocytes increases dramatically at implantation and remains high in the early-pregnancy deciduas (25). Macrophages are conspicuously more numerous near the implantation site (decidua basalis) than in sites peripheral to the developing placenta (decidua parietalis), and are
found in close association with the cytotrophoblast adjacent to the decidua, as well as around arteries invaded by the extravillous cytotrophoblast. In contrast to macrophages, CD56+ lymphocytes are more evenly distributed throughout the decidua. A few CD3+ T cells were observed in pregnancy, scattered in the endometrial stroma with occasional aggregate formation.

HLA plays a crucial role in the process of implantation (26). During implantation, the uterine decidua is invaded by extravillous trophoblast cells whose function is to destroy the walls of the uterine spiral arteries in order to provide adequate blood flow to the fetus. These cells express an unusual combination of HLA class I molecules, such as HLA-C, HLA-E and HLA-G (27,28). NK cells from the decidua come into close contact with the invading extravillous trophoblasts and express a variety of receptors which are known to recognize HLA class I molecules. Interaction between these NK cells and extravillous trophoblast cells provides a regulatory influence on implantation. Recognition of HLA-G stimulates uterine NK cells to produce cytokine, via which intrauterine immunosuppression is established (29). Development, growth and differentiation of the placenta have been shown to be regulated by the produced cytokines (30).

Different types of HLA are expressed in different parts of the trophoblast and fetal membranes (31). Whereas HLA-A and -B class I genes are down-regulated in human trophoblast cells, nonpolymorphic class-I molecules, e.g., HLA-G class Ib, are expressed in the extravillous cytotrophoblast, in endothelial cells of fetal vessels in the chorionic villi, and in amnion cells and amniotic fluid. HLA-G presents antigens for gamma/delta T cells and at the same time defends the trophoblast from cytotoxic effector mechanisms.

Trophoblast invasion can be seen as a tightly regulated battle between the competing interests of the fetus survival and those of the mother. Successful pregnancy is dependent on the trophoblast invading the mother, attaching the pregnancy to the uterus and securing an adequate supply of oxygen and nutrient for the fetus (32). Trophoblast invasion and migration through the uterine wall is mediated by molecular and cellular interactions, controlled by the trophoblast and the maternal microenvironment (33). The process of migration/invasion of extravillous trophoblast cells is stringently regulated by many growth factors, their binding proteins, extracellular matrix components, and some adhesion molecules, in an autocrine/paracrine manner at the fetal-maternal interface in human pregnancy (34). For successful invasion to occur, the extravillous trophoblast has to perform a range of functions, such as transform the maternal spiral arteries, tolerate hypoxia, proliferate and die by apoptosis (programmed cell death), differentiate, adhere to and digest the extracellular matrix, and move and interact with the maternal immune system. Each of these functions has multiple overlapping control systems, such that trophoblast invasion is in essence a finely controlled balance of competing mechanisms (35).

Trophoblast invasion of the endometrium shares common features with the inflammatory response. This process is accompanied by the infiltration of uterine NK cells which interact with the nonpolymorphic HLA class-I antigens expressed by the
invading extravillous trophoblast (36). In humans, extension of trophoblast invasion beyond the decidual layer into the myometrium presents an additional challenge, which might be relevant in pregnancy complications such as pre-eclampsia.

The presence and distribution of different components of the SIS in the human chorion (trophoblast) and decidua from the first trimester of pregnancy has been described in normal human embryos and early fetuses as well as in those which have been exposed to acute antigenic effects (chorioamnionitis) (37). The SC, J chain, IgG, IgA, and macrophages are seen from 3.5-4 to 5 weeks of development and then during the whole first trimester of pregnancy in the syncytio- and cytotrophoblast, and decidual cells (Fig. 4, Tables III and IV). Macrophages with J chain, IgG and IgA are found in embryonic tissues on week 3.5 to 4, whereas lymphocytes, including those synthesizing IgA and IgM, appear only at the end of the first trimester of pregnancy. In the decidua, lymphocytes and macrophages are recognized throughout the entire period studied.
(Color Fig.)

Fig. 4. A 4-week-old normal human embryo.

A. The SC-positive trophoblast (brown staining) and SC-negative macrophages. x1000.
B. J chain-positive macrophages and the trophoblast. x1000.
C. IgG-positive monocytes (brown staining) in the trophoblast. x1000.
D. IgA in the syncytiotrophoblast and in the monocytes. x400.
Table III. The number of lymphocytes and macrophages in the chorionic villi (in 50,000 µm², mean±SD)
(After ref. 37)

<table>
<thead>
<tr>
<th>Groups of patients</th>
<th>Weeks of pregnancy</th>
<th>T lymphocytes</th>
<th>B cells</th>
<th>IgG-producing B lymphocytes</th>
<th>CD68+ Macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CD3+</td>
<td>CD20+</td>
<td>IgG+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD4+</td>
<td></td>
<td>IgA+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD8+</td>
<td></td>
<td>IgM+</td>
<td></td>
</tr>
<tr>
<td>Without infectious effect (I)</td>
<td>4 to 6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10.7±1.2</td>
</tr>
<tr>
<td></td>
<td>7 to 8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9.2±1.1</td>
</tr>
<tr>
<td></td>
<td>9 to 12</td>
<td>0.18±0.09</td>
<td>0.06±0.04</td>
<td>0.09±0.07</td>
<td>19.8±2.3 a,b</td>
</tr>
<tr>
<td>With infectious effect (II)</td>
<td>4 to 6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>17.4±1.9 a</td>
</tr>
<tr>
<td></td>
<td>7 to 8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>33.6±3.9 a,b</td>
</tr>
<tr>
<td></td>
<td>9 to 12</td>
<td>0.72±0.15 a</td>
<td>0.16±0.09</td>
<td>0.18±0.11</td>
<td>33.4±4.9 a</td>
</tr>
</tbody>
</table>

a Significant difference compared to similar parameter in the group I, p < 0.05-0.001.
b Significant difference compared to similar parameter of the previous age embryos in the same group, p < 0.05-0.001.
Table IV. The number of lymphocytes and macrophages in the decidua (in 50,000 µm², mean±SD) (After ref. 37)

<table>
<thead>
<tr>
<th>Groups of patients</th>
<th>Weeks of pregnancy</th>
<th>T lymphocytes CD3+</th>
<th>CD4+</th>
<th>CD8+</th>
<th>B cells CD20+</th>
<th>Igs-producing B lymphocytes and plasma cells</th>
<th>CD68+ Macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without infectious effect (I)</td>
<td>4 to 6</td>
<td>0.8±0.6</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
<td>0.5±0.5</td>
<td>0.6±0.3</td>
<td>3.6±0.6</td>
</tr>
<tr>
<td></td>
<td>7 to 8</td>
<td>0.9±0.4</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
<td>0.7±0.4</td>
<td>0.3±0.2</td>
<td>6.3±1.8</td>
</tr>
<tr>
<td></td>
<td>9 to 12</td>
<td>1.5±0.7</td>
<td>0.5±0.3</td>
<td>0.9±0.5</td>
<td>0.9±0.6</td>
<td>0.8±0.4</td>
<td>4.5±1.5</td>
</tr>
<tr>
<td>With infectious effect (II)</td>
<td>4 to 6</td>
<td>6.7±1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8±0.8</td>
<td>4.4±1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.8±2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.3±1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.1±1.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7 to 8</td>
<td>6.9±1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.1±0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7±0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.3±2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8±0.9</td>
<td>9.5±3.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>9 to 12</td>
<td>8.2±1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.8±0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5±1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.2±3.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.9±1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.3±2.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Uterine tube</td>
<td>4 to 6</td>
<td>4.4±0.8</td>
<td>1.3±0.6</td>
<td>3.2±0.9</td>
<td>2.9±0.8</td>
<td>2.2±0.9</td>
<td>0.8±0.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significant difference compared to similar parameter in the group I, p < 0.05-0.01.
In cases with chorioamnionitis, reactivity of IgG and IgA in the abovementioned fetal cells decreases sharply, while the rate of SC and J chain immunoreactivity as well as the number of T and B lymphocytes do not change (37). In the decidua, the number of immunoreactive cells increases significantly and the plasma cells appear. Lymphocytes were seen only after week 9 of pregnancy whereas macrophages were observed in high numbers as early as week 3.5 to 4. In the decidual tissue, the number of all types of immunocompetent cells, including Ig-synthesizing lymphocytes and plasma cells, increases sharply.

In fetuses of the second trimester of pregnancy, the Igs, SC and J chain are located in the syncytiotrophoblast of the chorion (38). The villous stroma contains a small amount of different subsets of lymphocytes. Macrophages account for up to 45% of the stromal cells of the villi and contain IgG and J-chain. In the maternal part of the placenta, the SIS proteins are in the decidual cells. Relatively few lymphocytes and macrophages are observed in the decidual stroma.

A different origin and composition of immunocompetent cells and a different course of immune reactions in fetal and maternal parts of the placenta have been shown (39). Two types of the SIS have been suggested to be present at the border between maternal and embryonic tissues (37,38). These systems are already in place at the beginning of the embryonic period, weeks 3.5-4 to 5, function during the entire first trimester of pregnancy and are the main immune mechanism underlying the barrier between these two organisms. For more details on this topic, see Chapter 1.

Trophoblastic villi have several protective mechanisms against the maternal immune system. Trophoblast cells fail to express MHC class I or class II molecules and the extravillous cytotrophoblast cells strongly express the non-classical MHC gene encoding HLA-G, which may downregulate NK cell function (18). Extravillous trophoblast cells selectively express the non-classical MHC class I molecules in the form of different types of HLA (HLA-B, HLA-C and possibly also HLA-G), which may play an important role in maintaining maternal immune tolerance of the semi-allogenic fetus and play a protective role during pregnancy (40-43).

The trophoblast expresses the complement regulatory proteins CD46, CD55, and CD59, which serve to protect the embryo (18). Moreover, uterine decidual and placental cells produce a huge array of cytokines which, in part, contribute to the deviation of the immune response from Th1 to Th2. This may leave the mother more open to infection whose control is Th1-dependent, but increased production of Th1 cytokines has been linked to spontaneous abortion and small-for-date babies. Th2-type cytokines appear to contribute to the maintenance of pregnancy by controlling the immune and endocrine systems and promoting the function of the trophoblast at the implantation site (44).

Cytokines released at the feto-maternal interface also play an important role in regulating embryo survival, controlling not only the maternal immune response but also angiogenesis and vascular remodeling (43). The delicate equilibrium established between the mother and her fetus can be compromised in pathological conditions of pregnancy as a result of the mother's humoral and/or cellular response against the trophoblast antigens, leading to spontaneous miscarriage. Cytotoxic cells and
antibodies to trophoblast and endothelial cells are frequently found in patients with recurrent spontaneous abortion (for details, see Chapter 5).

In cases with chorioamnionitis, the cellular composition is different in the embryonic and maternal parts of the gestational sac. In fetal membranes, only the macrophages were found to react to antigenic effects (37,38). The absence of a response by the lymphocytes showed that in 3.5-4- to 8-week-old embryos even a massive antigenic effect does not cause an acceleration in their maturation. A weak lymphocyte reaction in the trophoblast was seen only after 9 to 10 weeks of pregnancy. In the decidua, however, the number of lymphocytes, including those synthesizing IgG, IgA and IgM, plasma cells and macrophages increased significantly, even during the earliest embryonic period.

In patients with pre-eclampsia, a pregnancy complication with endothelial dysfunction, the sudden onset of maternal hypertension, proteinuria and edema, the cytotrophoblast fails to differentiate along the invasive pathway (45). The functional consequences of this abnormality negatively affect interstitial and endovascular invasion, thereby compromising blood flow to the maternal-fetal interface. In these cases, cytotrophoblast invasion is shallow and vascular transformation incomplete, resulting in abnormal placental production of vasculogenic/angiogenic substances that reach the maternal circulation (46). Pre-eclampsia has been shown to be associated with widespread apoptosis of the cytotrophoblast that invade the uterus. Moreover, the expression of HLA-G by extravillous trophoblast cells appears to be altered, resulting in activation of the maternal immune system (47). Intrauterine growth retardation in the context of pre-eclampsia is accompanied by reduced trophoblast numbers within smaller and more tortuous arteries and an increase in the proportion of CD56+ uterine NK cells and CD8+ T lymphocytes in the decidua (48). In the case of pre-eclampsia without fetal growth retardation, no increase in CD56+ uterine NK cells was seen, while CD8+ T lymphocytes were significantly increased compared to normal levels. The development of pathological pregnancies such as pre-eclampsia has been related to the differential expression of epidermal growth factor (EGF) receptor in the syncytiotrophoblast (49). In early-onset pre-eclampsia with intrauterine fetal growth restriction (FGR), trophoblast invasion into the placental bed is limited by increased apoptosis, resulting in narrower spiral arteries, which is in contrast to findings in anemia (50).

Insulin-dependent diabetes mellitus (Type I) affects the chorionic villi's development causing a significant increase compared to controls in placental volume, and in the volumes of the intervillous space and the trophoblast (51). A significant increase in the volume of the intermediate and terminal villi, the surface area of the villi and fetal capillaries, and the harmonic thickness of the villous membrane was found in the macrosomic subgroup compared to the controls. Morphological changes caused a significant reduction in the villous membrane's specific diffusing capacity in diabetic patients, and this may contribute to the fetal hypoxia and increased fetal and neonatal morbidity associated with diabetes.

Changes described herein in the placenta, and particularly in the trophoblast, of patients with FGR who died without antigenic effects are similar to those described previously in cases with anti-phospholipid syndrome (APS), especially those
associated with FGR (52,53). APS is characterized by recurrent fetal loss, vascular thrombosis and thrombocytopenia occurring in the presence of antiphospholipid antibodies (54,55). The incidence of APS increases from 5.3% in normal obstetrical patients to 20% in women with recurrent pregnancy loss, to 37% in women with systemic lupus erythematosus (56), and to 41% in cases of secondary recurrent APS cases (57). The mean age for APS is 35.6±7.2 years and the mean disease duration is 11.9±8.5 years (57,58).

The antiphospholipid antibodies are acquired antibodies against a phospholipid which has been associated with slow progressive thrombosis and infarction in the placenta (36). The incidence of extensive inflammation and infarction, decidual vasculopathy and vascular thrombosis, and perivillous fibrinoid changes are characteristic lesions of the placenta with APS (59-62). Although there are no specific histopathological placental abnormalities characteristic of APS patients, primary APS patients may be at increased risk of development of maternal floor infarction or massive perivillus fibrin deposition (63,64). Placental tissue shows large areas with infarctions, intravascular fibrin deposition, syncytial knot formation, and fibrosis.

APS causes aberrations in early trophoblast differentiation, predisposing the pregnancy to failure. These aberrations manifest themselves as decreased trophoblast area, a constant number of syncytiotrophoblast nuclei (in normal fetuses, this parameter is not constant), a decrease in the number of proliferating trophoblast, and a constant nuclear cytotrophoblast-to-syncytiotrophoblast ratio (65). These data suggest that abnormal trophoblast differentiation in early gestation may be due to the premature onset of maternal perfusion of the placenta and may be a likely antecedent for conditions associated with failure of placentation, such as recurrent miscarriage. Reduced placental growth and an increase in trophoblastic apoptosis were found in in vitro cultured human placental explants treated with antiphospholipid antibodies (66). Inflammatory mechanisms in the placental bed may contribute to APS-related pregnancy complications (67). APS biopsies show a high concentration of inflammatory cells, particularly macrophages, necrosis with hyperplastic vessels, and arterial thromboses.

APS may affect placental functions through several possible mechanisms. Phosphatidylserine is expressed on the trophoblast surface during differentiation and invasion of the extracellular matrix (68). The antiphospholipid against phosphatidylserine can directly affect trophoblast function by limiting the depth of decidual invasion and by concurrently creating a procoagulant surface on the trophoblast exposed to the maternal circulation. Interaction of antiphospholipid antibodies with cells involved in the coagulation cascade is thought to produce a procoagulant state. Upregulated expression of cell-adhesion molecules and subsequent stimulation of neutrophil and/or platelet activity within the placental villous tree is unlikely to be a mechanism by which an adverse pregnancy outcome arises in APS pregnancies (69).

The other mechanism contains the binding of antiphospholipid antibodies to proteins with an affinity for phospholipids, such as beta2-glycoprotein I (β2-GPI) (70). Following the attachment of β2-GPI to anionic phospholipids of the trophoblast, both molecules undergo conformational changes resulting in the exposure of cryptic
epitopes within the β2-GPI structure. This may allow the subsequent binding of antibodies, thereby affecting trophoblast functions directly. Moreover, anti-beta2-GPI antibodies induce the activation of endothelial cells, resulting in a proinflammatory state which favors prothrombotic diathesis syndrome. CD4+ and HLA class II-restricted T cells responsive to β2-GPI are involved in the production of antiphospholipid antibodies in APS patients (71,72). These cells preferentially recognize the antigenic peptide containing the major phospholipid-binding site and have the capacity to stimulate B cells to produce anti-β2-GPI antibodies through IL-6 expression and CD40 ligand engagement (73,74).

There are a series of immune components that are characteristic for APS. B lymphocytes are required for the initiation of antibody-associated disorders, including APS (75). Monoclonal antibodies to B cells, B-cell growth factors, complement proteins and integrin molecules, cell-surface complement regulator proteins or IL-3, all appear to play a role in the antibody-induced disease process (76). Primary APS is accompanied by stimulation of CD4+ and CD8+ T-cell production in patients (77).

4.3) Apoptosis of embryonic cells and its consequences

Programmed cell death, which since Kerr et al. (78) has been called apoptosis, is a common and reproducible feature in development of many mammalian tissues and organs. Apoptosis occurs during normal development and it is important in maintaining the correct balance between the loss of old, non-functional cells and the formation of new ones in different organs and tissues. The cell death associated with fusion of the neural folds and the removal of interdigital mesenchymal cells during digit formation represent two well-known examples of programmed cell death (79). Like normal development, abnormal development is also associated with increased cell death in tissues and organs that develop abnormally after exposure to a wide variety of teratogens. Apoptosis plays an important role in the processes of gamete maturation as well as in embryo development, contributing to the appropriate formation of various organs and structures, organ involution, and ageing, but may arise in pathology when the cell's genetic apparatus is damaged (80).

A series of proteins controlling cell death in mammals was identified in the 1990s, i.e., receptors/ligands, caspases, cytochrome c, Apaf-1, bcl-2 family proteins, etc. (79). Apoptosis is triggered by different cell-type-specific signals which involve several pathways, such as the intrinsic mitochondrial and extrinsic receptor-mediated pathways, resulting in caspase-cascade activation (81,82). Morphologically, apoptosis is characterized by pronounced cell shrinkage with subsequent fragmentation into apoptotic bodies surrounded by a membrane which are phagocytosed by macrophages without inflammatory reaction (83).

Apoptosis and its associated regulatory mechanisms constitute physiological events that are crucial to the maintenance of placental homeostasis; an imbalance in these processes, however, such as occurs in various pathological conditions, may compromise placental function and, consequently, the pregnancy's success. Increased apoptosis occurs in the placentas of pregnant women with several developmental disabilities, while increased bcl-2
expression is generally associated with pregnancy-associated tumors (84). Bcl-2 protein prolongs cell survival by blocking apoptosis. In human embryonic differentiation, a low apoptotic index value has been found to be mostly accompanied by the high expression of bcl-2, whereas bax expression was not proportionally related to the apoptotic index value (85). The apoptotic rate increases during pregnancy with gestational age. Apoptosis is stimulated in maternal peripheral blood during pregnancy, possibly accounting in part for the presence of free fetal DNA in the maternal serum (86).

Bcl-2 is widely expressed early in embryonic tissues derived from all three germ layers, and this expression becomes restricted as the tissues mature. In human embryos from the 4 to 12 weeks gestation, bcl -2 has been found in many organs of the gastrointestinal tract, in mesenchymal cells surrounding the primitive bronchial epithelium, and in the cells of the metanephric blastema and urethral bud (87). In human embryos and fetuses at 7 to 30 weeks of gestation, bcl -2 is involved in the regulation of apoptosis, and its effect is antiapoptotic. The highest bcl-2 expression has been demonstrated in metanephrogenic blastema cells and the lowest occurrence of bcl-2-positive cells was found in proximal tubules and in branches of the urethral bud (88). During development of human fetal heart, myocyte undergo apoptosis/mitosis, and CD95 and apoptotic/proliferative processes are present in the early gestation phase, and progressively fading thereafter (89).

Apoptosis mediated by the Fas/FasL system may also be associated with maternal immunotolerance to the fetus. The apoptosis, mainly through Fas-FasL or TRAIL-R-TRAIL signalling, may be a defense mechanism against rejection of the fetal allograft by the maternal immune system (81). Presented on trophoblastic cells CD95-L (Fas ligand) plays a part in establishing feto-placental tolerance by inducing apoptosis of immune-defense cells (90). Expression of FasL by the human trophoblast is accepted as a mechanism providing protection against activated decidual immune cells expressing Fas receptor (18,91). Trophoblast apoptosis increases in normal placentas as gestation proceeds, and a greater incidence of trophoblast apoptosis has been observed in pregnancies complicated by pre-eclampsia or FGR. Macrophages presented at the maternal-fetal interface may contribute to trophoblast survival by removing apoptotic cells and producing cytokines and growth factors that influence the progression of the apoptotic cascade (92).

In the placenta, as in other organs, the development and maintenance of the differentiated phenotype depend on a balance between cell proliferation, maturation, and death. Early on, cytotrophoblast cells express most of the important apoptotic proteins, which translocate into the syncytiotrophoblast with the fusion (81). This suggests that apoptosis has a central role in the villous trophoblast turnover. Taking together, the data suggest that regulation of apoptotic events is important in allowing the correct development, differentiation and functioning of the placenta throughout pregnancy and that an imbalance in this process leads to severe pathologies, such as pre-eclampsia and FGR.

Apoptosis plays a significant role in trophoblast development and differentiation. During the process of implantation, there are a large number of cells at the implantation site undergoing apoptosis, which has been suggested to play an important role in the regulation of endometrial decidulization and trophoblast invasion.
(93,94). The balance between trophoblast apoptosis, proliferation and expression of cyclins, inhibitors and cyclin-dependent kinases, may represent a mechanism to controlling normal trophoblast invasion (95). Trophoblast apoptosis may be caused by maternal cells such as macrophages but is highly regulated by the trophoblast itself, i.e. trophoblast cells need to be susceptible to be prone to apoptosis. The initial stages of the apoptotic cascade start within the cytotrophoblast, and the execution stages are seen in the syncytiotrophoblast (96).

During pregnancy, trophoblast cells are shed into the maternal blood from the placenta as they die via apoptosis. Trophoblasts are fetal cells and they are therefore immunologically foreign to the maternal immune system. The shedding of trophoblasts may not be simply a mechanism used by the fetus to dispose of aged trophoblasts, it may also provide a chronic source of tolerated paternally derived antigens in order to regulate maternal immune responses to the fetus (97).

Increased apoptosis occurs in the placenta of pregnant women with several developmental disabilities, including hyperglycemia, which may be a key factor evoking apoptosis in the placental trophoblast, and therefore, is relevant to diabetic placenta function (98). The increased rate of apoptosis seen in the placenta of pregnancies complicated by FGR may have an important compensatory role in transmitting nutrition to, and enabling earlier gas exchange easily with such fetuses (99).

The inflammatory cytokines TNFα and IFNγ stimulate villous cytotrophoblast apoptosis while EGF protects these cells. Bcl-2 is reported to be strongly expressed in villous syncytiotrophoblasts, whereas its expression levels are very similar in the first trimester and term cytotrophoblasts (100). Its expression is constitutive, and modulation of its expression levels does not mediate cytokine and growth-factor regulation of apoptosis in these cells. During the first trimester of pregnancy, endogenous expression of TNFα was detected in villous as well as in proliferating and invading extravillous trophoblasts, suggesting this protein's involvement in trophoblast differentiation (101). The high number of TNFα-positive cells in the first trimester of pregnancy resulted in the appearance of TUNEL-positive cells and an increase in caspase-3 enzyme activity, suggesting that the TNFα-dependent apoptotic cascade is executed in a portion of the early cytotrophoblast.

Apoptosis, which leads to phagocytosis by the mononuclear cells, represents the primary mechanism for removing neutrophils from inflamed tissues and minimizing injury (102). The activity of caspase 3 and expression of the proapoptotic proteins bax, bad, and bak are lower in neonatal than in adult neutrophils. Prolonged survival of neonatal neutrophils at injured sites is due, in part, to reduced responsiveness to FasL. This may be related to decreased expression of both FasR and bcl-2-family proteins which mediate neutrophil apoptosis. Apoptosis occurs in fetal nucleated erythrocytes that have crossed into the maternal circulation, which might explain the difference between the number of intact fetal cells and the amount of fetal DNA detectable in the maternal plasma (103). Apoptosis constitutes a mechanism for clearing fetal cells via the maternal circulation. For example, the p53-dependent apoptosis detected in p53+/+ knockout mouse embryos, is considered an immediate reaction detected mostly in the brain, whereas the p53-independent apoptosis is a
delayed reaction, with a prominent pattern being observed in the epithelial cells of most organs only in p53-deficient mice (104).

Severe hypoxia, which occurs in most preterm infants, also leads to cell death, which may be necrotic or apoptotic. Significant elevation of apoptotic activity may play a role in development of bronchopulmonary dysplasia, ischemic brain lesions, and renal failure in preterm infants who suffered from infant respiratory distress syndrome, cardiac failure, or periventricular leukomalacia (105). A high apoptotic ratio is detected in hypoxic injuries of the central nervous system of preterm infants. In the developing nervous system, sensory organs and orofacial regions of human embryos and fetuses, the expression of proliferative markers increases with age, whereas apoptosis is rare in these regions (106). Enhancement apoptosis was found in the fetal rat's central nervous system, craniofacial tissues and male reproductive organs immediately after the administration of ethylnitrosourea, a well-known DNA alkylating agent, that induces anomalies in different fetal organs (107,108). In chromosomally abnormal human fetuses, apoptosis was 2.5-fold higher than that found in pregnancies with normal embryos matched for gestational age (86).

4.4) Pathology of the immune organs in growth-retarded or low-weight fetuses and newborns under antigen-induced influences

The terms intrauterine or fetal growth retardation (FGR) and low birth weight (LBW) are assigned to newborns born with a birth weight and/or birth length below the tenth percentile for their gestational age. In mammals, size at birth is the outcome of length of gestation and rate of fetal growth (109). In the absence of premature delivery, fetal size within species is determined principally by the fetal growth rate which is dependent on both genetic and epigenetic factors. Failure of either of these mechanisms leads to FGR/LBW. In mammals, including human infants, FGR/LBW can occur naturally or pathologically. One major cause for natural FGR/LBW in animals is an increase in litter size. Parental genotype or antigenic differences between the mother and the developing conceptus may be potential causes. Pathological FGR/LBW is due to genetic causes (chromosomal abnormalities or inherited syndromes) or epigenetic causes (intrauterine infections, toxins and chemicals, maternal diseases of pregnancy affecting the placenta).

The underlying pathophysiological processes that occur at the cellular and molecular level in FGR/LBW are still little known. A reduction in the supply of substrates that are necessary for normal cellular function, and an alteration in mediator molecules that regulate cellular growth and differentiation, are important mechanisms. A decrease in growth-promoting factors or an increase in growth-inhibitory factors may lead to growth failure. Growth factors and their receptors are expressed in the developing embryo (at as early as the 1- to 2-cell stage), placenta and maternal uterine tissues, suggesting that these molecules play a role in regulating normal growth and differentiation of the conceptus as well as maternal reproductive tissues. Normal pregnancy is characterized by the transformation of about one half of all spiral arteries within the placental bed. FGR is associated with poor transformation of spiral arteries
and is characterized by an increase in uterine NK cells (110). Here we discuss some morphological and immune aspects of FGR/LBW.

Intrauterine mortality and morbidity and premature birth are acute problems in medicine. Infections hold great significance among the various reasons for fetal death and premature birth. The well-known susceptibility of FGR/LBW fetuses and premature neonates to infections derives from a deficiency in the immunological mechanisms that normally develop during the last trimester of gestation. It has been shown that maternal isoantibodies or other high-molecular-weight substances can enter the fetal bloodstream through the placenta and may act as antigens, causing an immune reaction in the fetuses (111,112). Infections and inflammatory processes resulting in FGR/LBW are considered to be one of the reasons for the associated high rates of pregnancy loss and child death (113,114).

Morphologically, the fetal immune organs are already formed at week 22 of gestation (115). In fetuses at 22 to 23 weeks without antigenic effects, the lymphoid organs are well developed and their differentiation is similar to that of full-term fetuses. In older unaffected fetuses (up to 32 weeks), a significant increase in size of the lymphoid organs and a rise in the rate of lymphoid-cell differentiation are observed. The morphology of the lymphoid organs in unaffected neonates can be regarded as a result of the early death of these newborns, 4 to 8 h after birth.

Different morphological changes are found in the lymphoid organs of fetuses that develop under antigenic influences (115,116). In 22- to 23-week-old LBW fetuses, differentiation of the lymphoid tissue resembles the normal picture. The only differences are manifested in the smaller weight and size of the lymphoid organs in LBW fetuses and the higher percentage of medium-size lymphocytes. Development of the lymphoid organs in older fetuses (24-32 weeks) is characterized by changes in some morphometric parameters, by the rate of maturation of the lymphoid cells, and by a marked increase in their number. As a result, the cortex of the thymus, the white pulp of the spleen, and the parenchyma of lymph nodes are all significantly larger in older fetuses. In parallel, maturation of the lymphoid cells is characterized by an increase in the percentage of mature small lymphocytes and a decrease in the number of lymphoblasts and medium-size lymphocytes (112,117).

In the thymus, the first phase of accidental involution can be detected under a light microscope (116). Apoptosis of thymocytes (118) and their concentration around macrophages and phagocytes is rarely identified. In fetuses severely affected by sepsis, with all neonates dying during the first 24 hours, the number and size of the thymic corpuscles showed an increase, with the formation of so-called pearls (Table V).

The immune reaction of the spleen was clearly recognizable in mildly affected fetuses suffering from bronchopneumonia and hyaline membrane disease (116). Relative to the unaffected group, the number of lymphoblasts increased sharply and their mitotic rate reached 0.75±0.01/10,000 μm². The number of macrophages rose. The number of lymphocytes remained high, and their ratio to the number of lymphoblasts was 1.6:1. Only about 75% of the follicles were found to react. The number of cells decreased in follicles and the red pulp. As a result of the cells'
transformation, the lymphocytes-to-lymphoblasts ratio decreased to 3.1:1 as compared to 42.9:1 in unaffected neonates (Table VI). This reflects not only the creation of a large number of lymphoblasts but also the high rate of lymphocyte loss.

The follicular area in the mildly affected spleen showed a tendency to increase in size. In the severely affected fetuses, the number of follicles and their areas decreased as a result of the sharp decrease in the number of lymphocytes. The decrease in follicular size was reflected in the appearance of so-called bare central arteries, which were not observed in unaffected or mildly affected fetuses. Where infection was very severe, the follicles disappeared.

The immune reaction of lymph nodes was clearly manifested in the mildly affected subgroup. Sinuses were open and contained many macrophages, lymphocytes, and erythrocytes. Phagocytosis was active. The parenchyma contained numerous lymphoblasts and macrophages (Table VI). In the severely affected subgroup, cell proliferation accompanied the processes of immuno-incompetence: the number of lymphoblasts increased significantly while the number of cells and the size of the parenchymal area decreased as compared to the unaffected group. The parenchymal area was markedly small, and there was a reduction in the number of lymphoid cells per unit of parenchymal area. In the most severe cases, the lymph nodes appeared to be devastated, containing only stromal cells and a few lymphocytes.

The presence of disease-related antigens appears to stimulate an immune reaction in fetuses, with accompanying changes in the morphology of the lymphoid organs (116). These changes are manifested in an increase in the number of macrophages and their phagocytic activity in the red pulp, in the follicles of the spleen, and in lymph nodes, accompanied by an increase in the number of lymphoblasts and their mitotic activity. The spleen and lymph nodes of normal fetuses and newborns are characterized by the presence of follicles with the reactive centers in those follicles and mature plasma cells playing a central role in the immune reaction in children and adults. In LBW fetuses affected by antigenic influences, neither the reactive centers nor mature plasma cells are found (115,116). No differences in the immune response are found between the younger (22-23 weeks) and older (up to 32 weeks) LBW fetuses. The immune reaction among such fetuses is generalized in all the lymphoid organs studied, although in regional lymph nodes (in the hilus of the lungs and the mediastinum) this reaction is stronger under pneumonia. We suggest that fetuses exposed to antigen-related diseases undergo morphological changes in the lymphoid organs presumably as a consequence of the primary fetal immune reaction.

A characteristic of the immune reaction in LBW fetuses is the rapid development of lymphoid-system decompensation. This phenomenon is manifested in a progressive decrease in the number of lymphoid cells, especially small lymphocytes (115,116). This was seen in severely affected fetuses where, as a result of the decrease in the number of lymphoid cells, the number and size of the follicles in the spleen decreased until they disappeared altogether. The number of lymphocytes in lymph nodes also decreased. Macrophages showed higher resistance (their number increased even in severely affected fetuses) and retained their phagocytotic activity for a prolonged period.
Table V.
Morphometric parameters of the thymus in different groups of infants (mean ± SE) (After ref. 115,116)

<table>
<thead>
<tr>
<th>Parameters studied</th>
<th>Groups of infants</th>
<th>Without antigenic effects</th>
<th>With antigenic effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mild</td>
<td>Severe</td>
</tr>
<tr>
<td>Area of cortex a</td>
<td>61.2±1.3</td>
<td>57.9±1.3</td>
<td>27.5±3.6 b,c</td>
</tr>
<tr>
<td>Area of medulla a</td>
<td>23.1±3.0</td>
<td>27.8±3.0</td>
<td>53.0±2.8 b,c</td>
</tr>
<tr>
<td>Thymus corpuscles a</td>
<td>2.4±0.3</td>
<td>2.9±0.3</td>
<td>6.1±1.1 b,c</td>
</tr>
<tr>
<td>Trabeculae a</td>
<td>15.7±1.8</td>
<td>14.3±1.1</td>
<td>19.5±1.8 b</td>
</tr>
</tbody>
</table>

a As a percentage of the whole square of the organ on a slide.
b Significantly different from unaffected group (p<0.05-0.01)
c Significantly different from mildly group (p<0.05-0.01)

Table VI.
The lymphocytes-to-lymphoblasts ratio in the follicles of the spleen and lymph nodes in infants of different ages and groups (After ref. 115,116)

<table>
<thead>
<tr>
<th>Organs</th>
<th>Groups of infants</th>
<th>Without antigenic effects</th>
<th>With antigenic effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mild</td>
<td>Severe</td>
</tr>
<tr>
<td></td>
<td>Age of infants (days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-7</td>
<td>8 and more</td>
<td>1-7</td>
</tr>
<tr>
<td>Spleen</td>
<td>42.9:1</td>
<td>3.1:1 a</td>
<td>3.0:1 a</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>40.8:1</td>
<td>4.4:1 a</td>
<td>4.8:1 a</td>
</tr>
</tbody>
</table>

a Significantly different from unaffected group (p<0.05-0.01)
b Significantly different from values of the spleen (p<0.05-0.01)
Dystrophic changes in macrophages were seen only in very destructive processes. In such cases, associated with devastation of the lymphoid organs, phagocytosis ceased and the number of macrophages was significantly decreased. In some severely affected cases there was an increase (2-4%) in the number of eosinophils in the lymphoid organs. The immune reaction of LBW fetuses was also characterized by a weak reaction of the thymus.

The immature lymphoid system in LBW fetuses is characterized partly by the absence of plasmocytes and reactive centers in follicles of the spleen and partly by the weak reaction of the thymus and the absence of follicles in the lymph nodes. These last two features are highly characteristic of LBW fetuses (115,116). Moreover, immunoincompetence in these fetuses is manifested by the rapid exhaustion of the lymphoid organs. Various morphological and morphometric signs of such devastation were seen among most severely affected fetuses, all of whom exhibited distinct evidence of decompensation in the development of the immune system. We therefore suggest that antigenic effects do not accelerate the normal development of the immune system in fetuses as proposed elsewhere (119), but interfere with it and may even destroy the processes of normal development, which may be manifested later in childhood. It is suggested that even moderate disease-related antigenic effects can exert a marked influence on the normal development of the immune system. The resulting changes in this system may be regarded as one of the reasons for the LBW of such fetuses.

The immune response to antigenic effects in LBW infants has many features that are typical to fetuses. It is spread throughout the many lymphoid organs and manifested in the thymus, lymph nodes and mainly in the spleen. In LBW infants, this type of immune reaction is maintained until up to 10 months or more, while in full-term infants it is retained for no longer than 3 months after birth.(120). It is manifested as an increase in the number of macrophages and in the transformation of lymphocytes into lymphoblasts. In LBW infants the mature plasmocytes and reactive centers in the follicles of the spleen are absent. This has been explained by the peculiarities of the B-lymphocytes in fetuses and infants (121). Components of the immune system are related to this pathology. For example, the very low levels of IgG serum in LBW infants are connected with a high risk of severe infection and sepsis (122). Septic complications in infants with very low birthweight are accompanied by a significant decreased in the monocyte's phagocytic capacity, and in HLA-DR expression on the monocytes (123).

The morphological changes in the immune organs described herein were seen in all infants studied, who died in the first week and after 4 to 5 months (116). Some peculiarities were found connected with the age of infants and the nature of the effects. In the infants older than 2 weeks, the lymphocytes/lymphoblasts relationship increased under all antigenic effects. Under sepsis, the reaction of neutrophils and eosinophils was found to increase significantly in LBW infants while in similar fetuses it was very weak.

The immune reaction in fetuses and infants can be caused not only by infectious (inflammatory) processes within their own bodies, but also by antigenic influences from the mother. The severe morphological changes in lymphoid organs similar to septical have been seen in fetuses with swelling-type rhesus-HDN and in infants who
were born from mothers with acute appendicitis and periappendicitis or pre-eclampsia and juvenile diabetes (124).

FGR syndrome has many causes. Severe FGR, for example, may be associated with placental damage leading to increased feto-maternal cell traffic which results in a significant increase in the proportion of fetal erythroblasts in the maternal blood (125,126). Artificial FGR in guinea pig fetuses induced by uterine artery ligation causing the hypoxic stress, leads to an increase in medullary erythropoiesis (127). This was manifested in an increase in total erythrocyte precursors and a decrease in total granulocyte precursors.

The reasons for insufficiency of the lymphoid system in LBW infants which develop under disease-related antigenic effects are different. This insufficiency has been found in the weak reaction of the thymus and its reticular epithelium (128), as well as of macrophages (129), of neutrophils (130), of T lymphocytes (131) and of B lymphocytes (122). Similar observations was made in a morphological study of the lymphoid organs (115,116). It appears that owing to the close relationship between different parts of the immune system, the underdevelopment of one part causes disturbances in the others and as a result in the entire system. The low mass of the lymphoid tissue, particularly the low number of lymphocytes, can cause rapid devastation of the immune system under antigenic effects.

A high rate of relationships between different morphological features was found in unaffected LBW infants which reflected good coordination in all the processes of normal ontogenesis of the lymphoid system. The significant changes in these relationships in mild and especially in severely affected neonates can be considered a result of deep disturbances in the normal development of lymphoid organs and their insufficiency under the immune reaction.
References

(To 4.1)


(To 4.2)


82

(To 4.3)


79. Mirkes, P.E., 2002, 2001 Warkany lecture: to die or not to die, the role of apoptosis in normal and abnormal mammalian development, Teratology, 65, 228.


93. Levy, R., and Nelson, D.M., 2000, To be, or not to be, that is the question. Apoptosis in human trophoblast, Placenta, 21, 1.
system from dams administered with ethylnitrosourea (ENU), Histol. Histopathol., 16, 79.

(To 4.4)


immune system in the pathogenesis of RhD-hemolytic disease of newborns, Human Antibodies, 8, 76.
Chapter 5.
Mother-Embryo Immune Conflict as a Reason for Recurrent Spontaneous Early Abortions

Spontaneous early abortions, i.e., the interruption of a pregnancy without outside intervention before 20 weeks' gestation, refers to clinical conditions describing loss of the intrauterine developing product prior to its being considered a viability fetus, the latter conventionally accepted as 500 grams of fetal body weight. Three or more serially miscarriages is the arbitrarily set point at which a patient is labeled a habitual aborter and her miscarriages as recurrent (1). The exact frequency of spontaneous abortion in the general population is unknown, and data in the literature are controversial. Although pregnancy loss is common, affecting 10% to as many as 20% of all pregnancies (1), recurrent miscarriage, the occurrence of at least three consecutive first-trimester pregnancy losses, is seen in only 1% of pregnancies (2). With the availability of sensitive beta-human chorionic gonadotropin serum assays, early pregnancies are now being detected that were formerly written off as simple abnormal prolongations of the menstrual cycle. Delays of 5 to 10 days in the onset of menstrual bleeding are very frequently diagnosed through the beta subunit as spontaneous early abortions. It is therefore estimated that more pregnancies are lost spontaneously than are actually carried to term.

5.1) Possible reasons for the immune conflict

The causes of recurrent abortions are classified as anatomical, genetic, endocrinological, immunological, microbiological, and environmental (1). Most recent studies have focused on potential autoimmune and alloimmune causes, and reports have investigated the potential associations between these autoimmune (antithyroid antibodies and antiphospholipid antibodies) and alloimmune (NK cells, cytotoxic T cells, and embryotoxic) factors and recurrent pregnancy loss.

In general, anatomical abnormalities account for less than 1% of the total number of habitual abortion cases (1). Uterine anomalies cause spontaneous abortions (3,4). Uterine and cervical factors can lead to habitual abortion due to malformation of the Mullerian duct system. A wide variety of congenital defects exist in which there is abnormal fusion of the two ducts. Cases ranging from simple arcuation of the uterine body to complete duplication of the entire uterus and cervix have been seen. In these cases, the spontaneous abortion usually takes place during the second trimester, when the intrauterine cavity becomes inadequate to support a growing fetus.

Morphological changes in the placenta are common reasons for spontaneous recurrent miscarriages. Miscarriage, missed miscarriage, and early- and late-onset pre-eclampsia represent a spectrum of disorders secondary to deficient trophoblast invasion (5). If trophoblast invasion is severely impaired, plugging of the spiral arteries is incomplete, and onset of the maternal intervillous circulation is premature and widespread throughout the placenta. Syncytiotrophoblastic oxidative damage is extensive and likely a major contributing factor to miscarriage.

Placental vasculogenesis is a basic feature in all types of pregnancy and a relationship exists between trophoblast cells and vessels in the chorionic villi with the potential to


influence each other's functions. Defective chorionic villus vascularization is associated with embryonic death and is considered to induce miscarriages (6). Morphological and immunohistochemical markers of cellular stress and damage, such as expression of heat-shock protein 70, formation of N-Tyr residues, and lipid peroxidation, were seen to have increased in tissues obtained from missed miscarriages relative to controls (7). The effect was greatest in those pregnancies of shorter than 77 days' duration and was associated with increased apoptosis and decreased numbers of mitotic cells, indicating that oxidative stress overwhelms cellular antioxidant defense systems. Placental oxidative stress with resultant damage to the syncytiotrophoblast, secondary to early onset of the maternal circulation, may provide an ultimate common mechanism of embryonic death in spontaneous abortions.

Genetic or chromosomal abnormalities are still believed to be the most common etiological factor underlying spontaneous abortions. Most spontaneous abortions occur before 12 weeks' gestation, and most of these are due to chromosomal errors in the conceptus (8). Indeed, up to 50% of the examined first-trimester losses show some kind of chromosomal abnormality (1). Chromosome abnormality is one of the major causes of early spontaneous abortion after in-vitro fertilization (9). It has been speculated that spontaneous, random errors in meiosis or mitosis occur in sperms or in oocytes or during early embryogenesis, that lead to chromosomal damage. Another possibility relies heavily on defects in parental genes that create chromosomal breaks in the embryo. The dramatic rise in the number of Down syndrome cases associated with advanced parental age suggests that spontaneous chromosomal damage is more common with advancing age.

Medical diseases, such as systemic lupus erythematosus, congenital cardiac disease and renal disease, are associated with spontaneous abortions (1). The severity of the underlying disease condition determines the pregnancy outcome. It has been suggested that the high rate of fetal wastage among patients with systemic lupus erythematosus is due to circulating immune complexes. In pregnant patients with congenital cardiovascular diseases, the spontaneous fetal wastage is in excess of 50%. With renal disease, especially with coexistent hypertension, the incidence of fetal loss can be extremely high. Individual, uncontrolled studies suggest that diabetes mellitus, especially when there is poor control of the blood glucose level, can lead to increased fetal wastage.

Syphilis can seriously complicate pregnancy and result in spontaneous abortion, stillbirth, non-immune hydrops, intrauterine growth restriction, and perinatal death, as well as serious sequelae in live-born infected children (10). A literature review for the period 1983-1996 identified 31 prospective studies with appropriate control groups, in which there appeared to be an association, albeit a weak one (odds ratios 4.05), between maternal HIV infection and an adverse perinatal outcome (11). There does, however, appear to be a real and large increase in the risk of infant death associated with maternal HIV infection, especially when an attempt is made to control for confounding. The presence of the virus in the second trimester of pregnancy is not significantly associated with elevated IL-6 levels or with early postamniocentesis pregnancy loss (12).
**Endocrinological abnormalities** are present in about a quarter of the women with unexplained recurrent miscarriage (13). Luteal-phase deficiency was found as a reason in approximately 20% to 35% of aborts (1). Thyroid peroxidase antibodies are present in 10% of women at 14 weeks' gestation, and are associated with an increased pregnancy failure (14).

**Biochemical abnormalities** in the mother's serum have also been found to be a reason for spontaneous abortions. Low plasma folate levels are associated with an increased risk of early spontaneous abortion. Both folate deficiency and folic acid supplements have been reported to increase the risk of spontaneous abortion (15).

**Immunological Factors**

The immunological relationship between the mother and the fetus is a bi-directional communication that is determined, on the one hand, by fetal antigen presentation and, on the other, by recognition of and reaction to these antigens by the maternal immune system. There is evidence that the immunological recognition of pregnancy is important for the maintenance of gestation, and that inadequate recognition of fetal antigens may result in a failed pregnancy. For example, non-polymorphic class I molecules, particularly HLA-G class Ib, are expressed in the extravillous cytotrophoblast, in the endothelial cells of the fetal vessels in the chorionic villi, and in amnion cells and the amniotic fluid (16). These molecules present antigens for gamma/delta T cells and at the same time defends the trophoblast from cytotoxic effector mechanisms. Following recognition of fetally derived antigens, the immune system reacts with a wide range of protective mechanisms.

The maternal immune response is biased toward humoral immunity and away from cell-mediated immunity that could be harmful to the fetus. Cytokines of maternal origin act on placental development. On the other hand, antigen expression on the placenta determines maternal cytokine pattern (16). Normal human pregnancy is characterized by low peripheral NK activity, and increased NK activity seems to play a role in spontaneous abortions of unknown etiology. In early human pregnancy, most uterine lymphocytes are CD56 granulated NK cells, which do not express CD16 or CD3. In early pregnancy, they are enriched at sites where the fetal trophoblast infiltrates the decidua. The dynamics of the appearance of uterine NK cells suggests that one of the functions of these cells is control of placentation.

Endometrial immunological conditions are intrinsically altered in recurrent aborts, and in such cases, endometrial lymphocytes harbor a distinct immunophenotypic profile that precedes implantation (17,18). The prognostic impact of CD8 and CD20 expression supports their predominant role in the development of fetal tolerance, whereas a role for NK cells in the abortion process is suggested by their altered subsets in all repetitive aborts. A higher mean number of CD56+ cells was documented in the endometrium of women with recurrent early miscarriage (19). On the other hand, CD56+3+ T cells were found to play a role in the maintenance of pregnancy (20). The phenomenon of a decrease in the proportion of CD56+3+ T cells in decidual lymphocytes, may be due to an immunological event leading to missed abortion. Despite of these data, it has been noted in recent publications, that
immunophenotypic analysis of the endometrium cannot predict pregnancy outcome in women with recurrent abortions (21).

The placenta is the tissue most involved in immune regulation at the maternal-fetal interface. It is comprised of cells of maternal as well as fetal origin, both of which express molecules (HLA-G by the trophoblast and FasL by the maternal decidual cells) that play a role in maternal-fetal tolerance (22). Mechanisms which protect the fetus from the maternal immune system include the expression of non-classical MHC molecules by trophoblast cells (23,24), T-cell apoptosis (25), and complement regulatory proteins expressed on the trophoblast (26).

Most of the polymorphic MHC class Ia and class II antigens are lacking on the surface of human trophoblastic cells, and this is thought to be critical in preventing deleterious maternal immune responses against the fetus (27). However, transgenic expression of paternal class I MHC molecules does not affect pregnancy rates in animals (28,29), indicating that lack of MHC is not critical in maintaining maternal-fetal tolerance.

During pregnancy, there is a general downregulation of most of the MHC class Ia and class II molecules just before implantation occurs (22). However, certain class Ib molecules and minor paternal MHC antigens are expressed. Typically, absence of MHC should lead to the trophoblast's escape from recognition by cytolytic T lymphocytes (CTL) while rendering them susceptible to NK cells. However, such cytolysis does not take place, and NK cells found in the placenta are of a distinct type and are called uNK cells. These cells are present in the decidua during the first and second trimester, and they modify the uterine arteries to increase blood supply to the fetoplacental unit (30,31). Owing to their increased presence in the decidua and their direct contact with the trophoblast, uNK cells are thought to play a critical role in acceptance/rejection of the fetus.

The extravillous cytotrophoblast expresses the non-classical HLA Ib genes (HLA-E, HLA-F, and HLA-G, HLA-G), possesses a number of immunomodulatory functions, and is connected with immune tolerance in pregnancy (32), inhibits both CTL responses and NK cell functions (33,34), and can induce CD8+ T-cell apoptosis through the Fas/FasL pathway (34,35). In humans, HLA-G is thought to facilitate the expression of HLA-E, by forming a complex with it on the trophoblast cell surface and binding to CD94-NKG2. This trimeric complex then binds to NK cells and leads to inhibition of NK cell activity (22).

The establishment of immune privilege at the implantation site is a result, at least in part, of clonal deletion of immune cells that recognize paternal antigens present in the embryo. This is mediated by the expression of FasL on fetal trophoblast or maternal decidual cells (36,37), where FasL has been shown to promote allograft rejection rather than tolerance (38,39). Fetus-derived FasL has also been shown to be essential for deletion of allospecific maternal T cells during pregnancy (40). FasL formed in the microvesicles of the trophoblast can compete with the classical surface FasL on these cells, thereby promoting fetal rejection (41,42).

Successful pregnancy is maintained by the expression of complement regulatory proteins expressed on the trophoblast, which prevent damage inflicted by complement
activation. Decay-accelerating factor (CD55) and membrane cofactor protein (CD46) are examples of such complement regulatory proteins expressed on the human trophoblast and are crucial for sustaining pregnancy (43).

A clinical association has been established between a history of pregnancy loss in patients with the diagnosis of primary or secondary antiphospholipid syndrome (APS) and the presence of different antiprothrombin antibody subtypes (IgG, IgM and IgA) in patients with APS (44). Women with antiphospholipid antibodies and a history of pregnancy loss are at high risk during pregnancy for another fetal death (45). In patients with recurrent pregnancy loss, anti-phospholipid, anti-Saccharomyces cerevisiae, and anti-prothrombin antibodies were more prevalent than in controls. Anti-prothrombin and anti-phospholipid antibodies were more significantly associated with late vs early pregnancy losses (46).

5.2) Humoral and cellular mechanisms of the immune conflict

Protection of the embryo from the adverse maternal environment during early pregnancy is considered to be achieved by the establishment of a transitory permeability barrier created by decidual cells immediately surrounding the implanting embryo (47). The success of normal pregnancy depends upon the protection and growth of the semi-allogenic embryo within the maternal uterine microenvironment. However, a detailed account of the mechanisms by which the genetically incompatible embryo escapes maternal immunological responses during early pregnancy remains unknown (48). Furthermore, the loss of the zona pellucida from the blastocyst prior to implantation, and the loss of the uterine luminal epithelium at the site of the implanting blastocyst make the embryo more vulnerable to maternal insults. Thus, it is speculated that a special barrier mechanism operates at the maternal-conceptus interface to prevent the passage of harmful stimuli to the embryo.

The formation of an anatomical barrier between mother and fetus, the lack of maternal immune responsiveness, and a lack of expression of allogeneic molecules by the fetus have been proposed as mechanisms accounting for the absence of fetal rejection during pregnancy (49). These mechanisms have helped us begin to understand how rejection of the fetus is avoided; however, they do not completely explain how the fetus evades the maternal immune system. Site-specific suppression, in which maternal immune responses are controlled locally at the mother-fetus interface, plays a fundamental role in controlling maternal allogeneic immune responses.

As already noted, the immunological relationship between the mother and the fetus consists of bidirectional communication which relies on fetal antigen presentation and recognition of, and reaction to these antigens by the maternal immune system (16). An interaction is established during pregnancy between the maternal immune system and fetal cells to enable the survival and the normal growth of the fetus. Fetal cells expressing paternal alloantigens are not recognized as foreign by the mother because of an efficient anatomical barrier and local immunosuppression determined by the interplay of locally produced cytokines, biologically active molecules and hormones (50). A special balance between T helper lymphocytes types Th1 and Th2 has also
been observed at the feto-maternal barrier that contributes to controlling the immune response at this level (51).

The maternal and fetal immune systems temporarily coexist; both are precisely tuned to detect and reject foreign invasion and yet somehow achieve a symbiotic relationship. This mutual state of tolerance is obviously critical for carrying a pregnancy to term. Two active parts of the immune system maintain protection of the mother: (i) a humoral immune system in which foreign tissue invokes an antibody response via B-cell recognition of antigenic surfaces, and (ii) cell-mediated immunity in which T-cells and NK cells seek out and destroy foreign tissue (52). Several mechanisms are thought to invoke immune tolerance of the fetus. These include: absence of MHC-I antigens, presence of unique HLA surface molecules, nonspecific reduction of systemic immunoreactivity, a possible role for blocking antibodies, expression of complement regulatory proteins, and factors of locally reduced immunoreactivity.

It has been well documented that the potential immunological mechanism involved in the maintenance of pregnancy contains several components: (i) the embryo does not engender an immune response, (ii) the maternal immune response is suppressed, (iii) the uterus is an immunologically privileged site, and (iv) the placenta constitutes a barrier between the mother and the fetus. The most important factors for the maintenance of pregnancy appear to lie at the uterus–placenta interface. In particular, expression of FasL and complement regulatory proteins, and failure to express MHC class I and II molecules in the placenta are thought to be crucial factors for maintaining a pregnancy (25,53,54).

Trophoblast cells fail to express MHC class I or class II molecules, except HLA-C and HLA-G (55,56). In addition, the trophoblast also protects itself by expressing FasL (25,54), thereby conferring immune privilege. Fas is expressed on many cells, whereas FasL expression is restricted to sites of immune privilege and activated CTL and CD4+ Th1 cells. FasL expression has been reported in first-trimester and term human placental villi (57), and expression sites of FasL are obviously positioned to induce apoptosis in maternal Fas-positive immune cells, such as NK and T cells (25). Fetal responses are clearly sensitive to the ambient cytokine environment of pregnancy (58), and the capacity of the fetus to produce IL-13 and IL-10 is directly related to the level of these cytokines produced by the mother in response to fetal alloantigens (59).

Responsiveness to paternal HLA antigens is a key factor controlling the activity of the maternal immune system in pregnancy. HLA-G, selectively expressed on the cytotrophoblast, plays the role of protector as opposed to the lysis carried out by the decidual uterine NK cells (60). HLA mismatching between maternal and paternal (fetal) antigens may be a source of the immune stimulation during pregnancy, altering the cytokine balance in the placenta (61). Placental HLA-G proteins facilitate semi-allogeneic pregnancy by inhibiting maternal immune responses to foreign (paternal) antigens which action on the immune cells and may serve as powerful tools in the prevention of immune rejection of the embryo (32). While profound cytokine shifts threaten pregnancy, it has been speculated that mild reactivity between maternal and
paternal (fetal) antigens may activate antigen-presenting cells to provide an important stimulus for fetal immune maturation (particularly Th1 responses) (59).

Several mechanisms have been reported to participate in the maternal-fetal interface. These mechanisms include fetal factors such as trophoblast cell properties and altered MHC class I expression as well as local maternal factors such as specialized uterine NK cells and a shift in the T-helper cell cytokine profile from a type 1 to a type II array (62). Novel immunomodulators are found to be expressed in the local uterine environment to aid in fetal survival. Furthermore, the fetal cells persist in the maternal circulation long after pregnancy is over and may have implications for autoimmune diseases. CD95-L (Fas-L) presenting on trophoblastic cells plays a part in establishing foeto-placental tolerance by inducing apoptosis of immune-defense cells (63). Expression of FasL by the human trophoblast has been accepted as a mechanism providing protection against the lytic action of activated decidual immune cells expressing Fas receptor (64).

Immunologic investigations proved the presence of specific systems which block the function of antipaternal maternal antibodies, as well as the formation of cytotoxic maternal T cells to paternal antigens (65). The system preventing rejection of an embryo as a graft during pregnancy functions at the level of the maternal and fetal tissues and is coded by HLA-G, HLA-E and HLA-C molecules (66). A high level of complement-regulatory proteins (CD46, CD55 and CD59), in response to the synthesis of complement-fixing maternal antibodies to paternal antigens and regulation of the placental HLA expression as a preventive reaction of the fetoplacental unit to the influence of maternal CTL, are the most important protective mechanisms of the placenta (67).

The following protective mechanisms are common for both the placenta and uterus: expression of FasL, prevention of infiltration of activated immune cells, and regulation of immunosuppression, which prevents proliferation of immune cells and high natural immunity (NK cells and macrophages) of the decidua (67). The maternal-fetal interface represents an immunologically unique site that must promote tolerance of the semi-allogenic fetus, whilst maintaining host defense against a diverse array of possible pathogens. Pregnancy is therefore an immunological balancing act. Trophoblasts do not express MHC class I or II, except HLA-C and G, but express FasL, which confers immune privilege (68). Expression of receptor-binding cancer antigen and FasL in the cytotrophoblast may play a role in the downregulation of the maternal immune response, thereby maintaining pregnancy in its early stage.

There appears to be variability in the capacity of women to develop tolerance to paternal antigens with successive pregnancies. Pregnancy may in turn modify maternal immune responses, reducing, for example, maternal allergy (69). In some situations, successive pregnancies have 'more successful Th2 skewing' and lower incidence of Th1-mediated complications (70). However, in other situations, where Th1 responses are adaptive (i.e., in the protection from placent al malaria), higher Th1 responses are seen with successive pregnancies, and these protect the fetus (71). Thus, it appears that while all pregnancies have to cope with a degree of maternal/fetal incompatibility, immune responses in pregnancy vary as a result of a
complex interplay between maternal immune programming and adaptation to environmental factors.

Maternal patterns of immune response can directly influence immune development in offspring. For example, women prone to allergic immune responses to allergens may also have altered immune responses to other antigens including fetal antigens (59). Altered cytokine responses at birth have significant implications for subsequent immunological development and allergic disease (72,73). There is evidence indicating a direct influence of maternal atopy on Th1 dysfunction at birth (74). The recognized predisposition for allergic Th2 responses in atopic women may modify immune responses in pregnancy and directly alter fetal immune responses. There is also growing evidence that altered T-cell cytokine responses in fetal and early postnatal life are associated with allergic disease in pre-schoolchildren (72).

There are no apparent relationships between maternal allergy and cytokine responses to fetal alloantigens (59). In contrast, neonates born to allergic mothers show stronger lymphoproliferative responses to maternal alloantigens. While genetic factors also have a strong influence on fetal immune responsiveness, it has been suggested that the placental microenvironment could be an equally (if not more) important determinant of immune reactivity in the early postnatal period (59). The development of allergic disease at 6 years was significantly associated with stronger maternal responses to fetal alloantigens. These data may explain the suggestion that maternal influences during gestation have a stronger influence than those of the father on the development of allergic disease in offspring (75). Specifically, maternal lymph proliferation, IL-13 and IFNγ responses were higher in response to fetal alloantigens if children subsequently developed allergic disease. Thus, allergic outcomes appear to be more strongly associated with direct maternal–fetal immune interactions than 'genetic risk'.

The newborn's immune system grows rapidly from its small size at birth primarily by exposure to the intestinal microflora normally obtained from the mother at and after birth. While building up its immune system, the infant is supported by the transplacental IgG antibodies, which also contain anti-idiotypic antibodies, possibly also actively priming the offspring (76). The immune system develops in fetal life and is qualitatively quite complete at delivery, although certain cytokines are produced only at low levels. Also, many cells such as phagocytes and dendritic cells are not yet adequate in number and function (77). The lymphocyte population is very limited, and the immune system of new-born mice, for example, is reported to be only a few percent of that of an adult (78). The major impetus for the expansion of the lymphoid population is exposure to the microbial flora colonizing the gut from birth on. The neonate clearly needs help from the mother for immediate protection, for colonization with the mother's gut flora, and for the long-term buildup of its own immune system. This immunological support arrives via the placenta and the milk.
5.3) Pathological changes in the placental barrier as a reason for spontaneous early abortions

The mother establishes a special interaction with the fetus in pregnancy, allowing its normal survival despite the different antigens. The main factors contributing to these favorable conditions for the fetus are efficient local immunosuppression and the formation of a protective anatomic barrier between the mother and the fetus (79,80). The placental barrier is responsible for the normal functioning and development of these two immunologically different organisms (81,82), as it allows them to tolerate one another and escape from the immune allogeneic mother-fetus conflict (64,83).

An example of such a conflict can be seen in the hemolytic disease of fetuses and newborns caused by maternal anti-rhesus antibodies to erythrocyte antigens inherited from the father (84). Early pregnancy loss, the reasons for which in 50% of the cases remain unknown, has also been proposed to be due to maternal-embryonic conflict (85). Different maternal cells cross the maternal-fetal barrier and participate in spontaneous abortions (CD45RO/UCHL1+ cells) or cause growth delay and recurrent reproductive failure (CD5+ cells) (86). A high rate of apoptosis in cells of the chorionic villi, especially of the syncytiotrophoblast, has been described in spontaneous early aborts and was explained as an increase in the activity of immune processes in the placenta (68,87).

We have described orphological changes in the placental barrier in spontaneous early abortions under the maternal-embryonic immune conflict, and the role of maternal IgG, IgA and IgM as well as of some immunocompetent cells and apoptosis-related components in these changes (88). We expected this approach to provide a better understanding of the etiology and pathogenesis of early allogeneic maternal-fetal immune conflict as a possible reason for spontaneous early abortions. Using immunohistochemical methods, we examined the chorionic villi and other tissues obtained from 54 aborts between weeks 3.5 and 8 of pregnancy (89). The material was divided into two groups (Table VII). Group I (control) contained 15 medically recommended and spontaneous early aborts with no signs of inflammations or pathological immune processes. Group II contained 39 spontaneous early aborts with acute chorionic villitis. Table VIII shows the relationship between the characteristics of the mothers with fetal disorders and the distribution of cases with recurrent early pregnancy loss among the studied population of patients.
Table VII. The number and pregnancy age of aborts

<table>
<thead>
<tr>
<th>Groups of patients</th>
<th>Age (weeks)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.5-4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>I</td>
<td>3 (20.0)</td>
<td>5 (33.3)</td>
<td>1 (6.7)</td>
<td>2 (13.3)</td>
<td>4 (26.7)</td>
</tr>
<tr>
<td>II</td>
<td>10 (25.6)</td>
<td>10 (25.6)</td>
<td>11 (28.2)</td>
<td>6 (15.4)</td>
<td>2 (5.2)</td>
</tr>
</tbody>
</table>

In parenthesis, number of aborts in %.
Group I (control), medical and early spontaneous aborts without signs of inflammations or pathological immune processes. Group II, early spontaneous aborts with acute villitis. Note high similarity in the age of aborts in both studied groups. After week 8, cases of the group II have not been found.

Table VIII. Relative distribution of cases with recurrent early pregnancy loss among the studying population of patients (% to total number of patients).

<table>
<thead>
<tr>
<th>Groups of patients</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, control without infectious and immune conflicts</td>
<td>16</td>
</tr>
<tr>
<td>2, cases with intrauterine growth restriction without infections</td>
<td>5.6</td>
</tr>
<tr>
<td>3A, cases with ascending infection of the birth canal, chorioamnionitis and infection of embryos</td>
<td>15.2</td>
</tr>
<tr>
<td>3B, cases with ascending infection of the birth canal and chorionic intervillous spaces with deleterious of villi</td>
<td>13.6</td>
</tr>
<tr>
<td>4, cases with immune mother-fetus conflict</td>
<td>49.6</td>
</tr>
</tbody>
</table>

Age of patients varied between weeks 3.5 and 8.
In the chorionic villi from group II, changes were related to all structures and were manifested as acute villitis. Disorders of the villous capillaries (thrombovasculitis) were manifested in apoptosis, disruption of the endothelium and of erythroblasts, in mucous swelling of the capillary basal membrane and in coagulation of blood proteins. Igs were found in some of the endothelial cells and in erythroblasts. Apoptotic cells were TUNEL-positive. p53 was present in the damaged capillaries but was not seen in the destroyed capillaries.

A basic feature of pregnancy is placental vasculogenesis, and a relationship exists between trophoblast cells and vessels in the chorionic villi. Defective chorionic-villus vascularization is associated with embryonic death and is considered to induce miscarriages (6). The number of villi with vessel destruction in aborts with acute villitis (group II) varied from 3% to 33%, averaging 12.5±1.3% (89). As a final result of capillary destruction and their disappearance, the average number of normal capillaries per villus was twofold lower in group II than in group I (2.04±0.6 and 4.53±0.3, respectively, p<0.001), whereas the number of avascular villi was threefold higher in cases from group II compared to those from group I (Fig. 5).

Considerable changes were seen in the monocytes (Kaschenko-Hofbauer cells) and promonocytes in the chorionic villi (89). Many of them were in different phases of apoptosis, and they contained p53, but not Fas or FasL. The number of promonocytes increased sharply in group II relative to group I (Table IX). In some cases, the number of promonocytes reached 92% and even 100% of all mononuclear phagocytes. A high number of phagolysosomes per cell section was seen in monocytes and promonocytes in group II (Table IX). Many of phagolysosomes contained IgG and IgA (up to 50 and even 100 per cell section).
Fig.5. The number of morphologically damaged chorionic villi (% to the total number of villi). 1, Villi with normal capillaries; 2, Villi with spasmodic capillaries; 3, Villi with villitis and thrombovasculitis; 4, Avascular villi; 5, Edemic villi; 6, Intravillous hemorrhages. Values in the groups II were significantly different from the group I (p<0.05-0.001).
Table IX.
The number of monocytes and promonocytes in the chorionic villi and of macrophages in the maternal decidua (After ref. 89)

<table>
<thead>
<tr>
<th>Groups a</th>
<th>Mononuclear phagocytes in chorionic villi</th>
<th>Number of phagolysosomes with Igs in mononuclear phagocytes in chorionic villi</th>
<th>Macrophages in decidua</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of monocytes and promonocytes /50,000 µm² (%)</td>
<td>Number of lysosomes/cell section</td>
<td>IgG</td>
</tr>
<tr>
<td>I</td>
<td>7.8±0.7</td>
<td>2.0±0.3</td>
<td>19.1±1.8</td>
</tr>
<tr>
<td>II</td>
<td>6.5±0.6</td>
<td>54.1±4.0 c</td>
<td>40.1±1.4 c</td>
</tr>
</tbody>
</table>

a See footnotes to Table VII.
b Significantly different from the number of mononuclear phagocytes in chorionic villi, p<0.001.
c Significantly different from the values in the group I, p<0.01-0.001.
Subsequent phases of apoptosis and even complete disruption could be seen in the syncytiotrophoblast, sometimes in the cytotrophoblast, and in some of the capillaries and mononuclear phagocytes. In such areas of the trophoblast, the positive reaction to bcl-2, an antagonist of apoptosis, disappeared, but FasL, a promoter of apoptosis, was seen. IgG and IgA were often seen in the syncytiotrophoblast and very seldom in the cytotrophoblast. IgM was found only in the apical microvilli of the syncytiotrophoblast.

The group of changes described for damaged tissues of the placental barrier was characteristic of the cases with acute villitis and was absent in the cases of non-antigenic spontaneous abortions (89). Some fragments of this damage, such as destruction of the syncytiotrophoblast and apoptosis of villous cells, have been described previously as manifestations of spontaneous abortions or mother-fetus conflict (90,91).

Because the apoptotic destruction of cells occurs over a few hours (in rats, between 2 and 6 h; our unpublished observations), pathologists should pay attention not only to the phenomenon of apoptosis but also to its consequences, such as an increase in the number of avascular edemic villi and a decrease in the average number of capillaries in tertiary villi.

Changes found in components of the placental barrier such as the trophoblast, capillaries, stroma and phagocytes of the chorionic villi, can be indicative of acute villitis. Two trends can be recognized in this process: destructive and proliferative (compensatory). Destructive processes were found in all mentioned tissues. Acute thrombovasculitis developed in blood vessels of the tertiary villi. This was manifested in apoptosis of the endothelial cells and erythroblasts, mucous swelling of the basal membrane and coagulation of blood proteins. As a result, the capillaries were completely destroyed and disappeared, without hemorrhaging or fibrosis (Fig. 6).

Phagocytes in the injured villi were also destroyed by apoptosis. Trophoblasts, especially the syncytiotrophoblast (91,92), were destroyed by apoptosis (93) without phagocytosis of the destroyed particles. Phagocyte destruction involves the participation of p53 whereas destruction of the trophoblast occurs in the presence of Fas and FasL, with neutralization of bcl-2. Similar data have been published regarding the human placental villi obtained from pregnancies complicated by intrauterine growth restriction (94,95).

Proliferative changes take place mainly in monocytes and promonocytes of the chorionic villi. Intensive destruction of monocytes is compensated for by the formation of an extremely high number of promonocytes: the amount of the latter cells in the group II increased 20-fold relative to the group I (89). The average number of phagolysosomes in sections of group II monocytes and promonocytes was significantly higher than their number in the group I and in decidual (maternal) macrophages (Table IX). This indicates a sharp increase in the phagocytic activity of monocytes and promonocytes at the very beginning of embryogenesis, i.e., at 3.5 to 4 weeks of pregnancy. Massive phagocytosis of maternal IgG, IgA and IgM reflects the
protective reaction of embryonic monocytes and promonocytes to intensive attacks by these Igs.

In conclusion, we suggest that destruction of the chorionic villi causes a decrease in the average number of capillaries and an increase in the number of avascular villi. Massive proliferation of promonocytes as well as the phagocytic activity of the promonocytes and monocytes can serve as additional proof of the assumption that the immune response of the embryo is already present at the very beginning of its development, during weeks 3.5 to 4 of pregnancy. The presence of maternal IgG, IgA and IgM in the high amount in the destroyed villous cells and especially in the phagolysosomes of the monocytes and promonocytes, and the absence of maternal immunocompetent cells in the placental barrier, suggest maternal Igs as a possible reason for the observed destructive changes in the chorionic villi. This fact is particularly important for a better understanding of the etiology and pathogenesis of allogenic maternal-fetal immune conflict and as a consequence spontaneous early pregnancy loss.
(Color Fig.)

Fig. 6. The chorionic villi under allogenic mother–embryo conflict.

A. 3.5- to 4-week pregnancy. Acute thrombovasculitis: apoptosis of the endothelial cells and of erythroblasts, blood clots in the capillaries, apoptosis of a phagocyte (head of an arrow). H&E. x400.

B. The same case as in A. Note apoptotic destruction of the capillary walls in the chorionic villus, of mononuclear phagocytes (heads of arrows), and of the trophoblast. TUNEL. x400.

C. 6- to 7-week pregnancy. Note avascular oedemic tertiary villi as a result of acute villitis. In the stem villus (the upper right part of the Fig.), blood vessels present and content erythroblasts. CD34. x100.

D. 5-week pregnancy. Destruction of the trophoblast. Large number of CD45LCA-positive different types of maternal leukocytes (macrophages, lymphocytes, NK – red color) are seen in fibrin clots and not penetrated in the villous stroma. Embryonic mononuclear phagocytes are CD45LCA-negative (heads of arrows). x200.
References

(To 5.1)

37. Qiu, Q., Yang, M., Tsang, B.K., and Gruslin, A., 2005, Fas ligand expression by ma-ternal decidual cells is negatively correlated with the abundance of leukocytes present at the maternal-fetal interface, J. Reprod. Immunol., 65, 121.
40. Vacchio, M.S., and Hodes, R.J., 2005, Fetal expression of Fas ligand is necessary and sufficient for induction of CD8 T cell tolerance to the fetal antigen H-Y during pregnancy, J. Immunol., 174, 4657.
105

(To 5.2)


74. Prescott, S., Jenmalm, M., Bjorksten, B., and Holt, P., 2000, Effects of maternal allergen-specific IgG in cordblood on early postnatal development of allergen-specific T-cell immunity. Allergy, 55, 470.

(To 5.3)


Chapter 6.
The Fetal Immune System in Normal Development and Under Pathological Effects

6.1) The common immune system in human fetuses

In mammals, the immune system begins its maturation early in fetal life. In humans, for example, B lymphocytes have developed in the liver by 9 weeks of gestation and are present in the blood and spleen by 12 weeks (1). T lymphocytes start to leave the thymus from about 14 weeks of gestation and subsequently, cells with helper and suppressor phenotypes appear in the spleen. The relative lack of development of secondary lymphoid tissues in healthy fetuses most probably reflects a lack of antigenic stimulus.

Although there is considerable exchange of materials, the fetus remains largely separated from the mother's tissues. The fetus is inside the mother's uterus but is never in direct physical contact with the uterine walls. Two membranes—the trophoblast (chorion) and the amnion—surround the human fetus, and the inner space is filled with the amniotic fluid. Only a thin tube—the umbilical cord—penetrates these membranes to connect the fetus and the mother. Physiological exchange between mother and fetus occurs only at the interface where the umbilical cord fuses with the uterine walls. This interface, where fetal tissues and maternal tissues interact, is called the placenta.

Different genes are expressed in the trophoblast (fetal part of the placenta) and amniotic membrane. For example, HLA-A and B class I genes are downregulated in human trophoblast cells, whereas non-polymorphic class I molecules, e.g., HLA-G class Ib, are expressed in the extravillous cytotrophoblast and also in the endothelial cells of fetal vessels in the chorionic villi as well as in the amnion cells and amniotic fluid (2). HLA-G presents antigens for γ/δ T cells and at the same time defends the trophoblast from cytotoxic effector mechanisms. Since polymorphic major histocompatibility (MHC) is absent from the trophoblast, presentation of fetally derived antigens is unlikely to be MHC-restricted. Most γ/δ T cells recognize unprocessed foreign antigens without MHC.

In the decidua (maternal part of the placenta), γ/δ TCR-positive cells significantly increase in number, and most of these cells are in an activated form due to recognition of conserved mammalian molecules on the trophoblast (2). Following recognition of fetally derived antigens, the maternal immune system reacts with the setting in of a wide range of protective mechanisms.

Many observations suggest that a successful pregnancy is associated with an altered TH1/TH2 balance, an important prerequisite to the maternal immune system not rejecting the fetus (3-5). Maternal immune response is biased toward humoral immunity and away from the cell-mediated immunity which could be harmful to the fetus. Cytokines of maternal origin act on placental development. On the other hand, antigen expression on the placenta determines maternal cytokine pattern. Increased concentrations of proinflammatory cytokines in the amniotic fluid indicate the
presence of intra-amniotic or placental inflammation and increase the risk of preterm birth, cerebral palsy, and bronchopulmonary dysplasia (6,7)

Normal human pregnancy is characterized by low peripheral natural killer (NK) activity, and increased NK activity seems to play a role in spontaneous abortions of unknown etiology (2). In early human pregnancy, most uterine lymphocytes are CD56 granulated NK cells, which do not express CD16 or CD3 (8). In early pregnancy, uterine NK cells become enriched at sites where the fetal trophoblast infiltrates the decidua (9). The dynamics of the appearance of uterine NK cells suggests that one of the functions of these cells is control of placentation (10,11).

Another protective mechanism operating in favor of pregnancy is progesterone-dependent immunomodulation (12). The biological effect of progesterone is mediated by a 34-kDa protein termed progesterone-induced blocking factor (PIBF). PIBF, synthesized by lymphocytes of healthy pregnant women in the presence of progesterone, inhibits the release of arachidonic acid and NK activity, and modifies the cytokine balance. PIBF supports TH2 cytokines, inhibits NK cells, and induces an increased production of non-cytotoxic blocking antibodies (13,14). The production of pro-inflammatory, cytotoxic cytokines, such as INFγ, TNFα, and IL-2, is reduced.

In the fully developed placenta, the fetal-derived trophoblast forms fingerlike villi that penetrate and intermingle with the surface layer (endometrium) of the uterus (15). The fetal circulation extends down the umbilical cord and branches into the capillaries inside these villi. The villi are surrounded by a network of intervillous spaces, and the mother's endometrial arteries fill these spaces with blood. Endometrial veins remove this blood. As a result, maternal blood flows continuously around the villi, and they are the sites for the exchange of materials between the fetal and maternal circulatory systems. The mother's circulatory system is not continuous with that of the fetus. Blood does not normally flow from the mother to the fetus and back; only materials carried in the blood are exchanged. Therefore, maternal blood cells such as B lymphocytes are not normally transferred to the fetus, although the antibodies produced by B lymphocytes do cross the placenta (16-19). This separation of circulatory systems is very important for immunological reasons. It is known that half the fetus's genes come from the mother and half from the father. The father's genes are "foreign" to the mother, and this difference is potentially sufficient to trigger an immune response. The separation of the mother's and fetus's tissues and blood reduces the likelihood that the maternal immune cells will encounter fetal cells and launch an attack against the fetus.

As for the placenta itself, although white blood cells such as T lymphocytes and NK cells are plentiful in the endometrium, they do not react against the villi of the fetal chorion (20-22). The reason for this is not completely known, but it appears that proteins on the surface of the villi keep them safe.

It is known that fetal cells occasionally enter the maternal circulatory system, and the result is documented medical tragedy (23,24). The transfer of blood cells in the other direction, from mother to fetus, does not have the same effect, because the fetal immune system is not fully developed and cannot respond to any foreign antigens carried by maternal cells. In addition, the few maternal blood cells that may leak through the placenta to the fetus are not enough to launch an immune response against
fetal antigens (25). Recent studies have shown that certain ante-partum conditions, such as placental insufficiency or chorioamnionitis, significantly increase the chances of maternal cells entering the fetus (26,27).

Studies on the role of the maternal immune system during pregnancy have focused mainly on the aspect of immune tolerance to the invading trophoblast and, therefore, embryo. While this is a critical aspect of reproductive immunology, it is also important to consider the function of the fetal immune system in the promotion of implantation and maintenance of pregnancy (28). The process of embryonic development is accompanied by the phenomenon of programmed cell death or apoptosis (29,30). Apoptosis is not the final stage in tissue development. The quick and effective removal of apoptotic cells by tissue macrophages is vital preventing "leakage" of self-antigens and promoting the production of proliferative/survival factors. One of the key requirements of apoptotic cell clearance is the resolution of inflammatory conditions, which, as in the case of pregnancy, may have lethal consequences (31).

**6.1.1) Immune components in experimental fetal pathology**

The role of the immune system in fetal pathology has been studied in many experiments with laboratory animals. In nude mice, for example, it has been demonstrated that the bone-marrow-derived non-lymphoid thymus cells, most likely the Ia-antigen-positive thymic macrophages of dendritic cells, are responsible for the induction of tolerance to MHC antigens in developing T lymphocytes (32). The administration of drugs during pregnancy may result in potential long-term effects on the developing immune system. Offspring of such pregnancies may suffer an increased incidence or severity of autoimmune diseases as a result of placental transfer of deleterious agents (33).

Ovine abortions caused by *Chlamydia aborts* resulted in the appearance, in the placental tissue, of a high number of cells expressing the macrophage-associated molecule CD14 and cells expressing MHC class II molecules (34). Many cells expressing mRNA encoding for tumor necrosis factor-alpha (TNFα) were observed. The fetal immune response included small numbers of CD4+ and CD8+ cells, γδ T cells and B cells. Production of TNFα by fetal macrophages expressing MHC II molecules may be of considerable significance in the pathogenesis of abortion.

*Helicobacter pylori* infection can induce activation of resident uterine immune cells and/or recruitment of cells at the endometrial level. *H. pylori* infection of pregnant mice was accompanied by macrophage activation in the endometrium, and by an increase in the number of CD4+ and CD8+ lymphocytes and of INFγ and MHC II expression (35). During pregnancy, preferential induction of Th2-type cytokines down regulates Th1-type responses, allowing fetal survival.

Microbial infections of pregnant Sprague-Dawley rats with *Mycoplasma pulmonis* chorioamnionitis were accompanied by accumulation of neutrophils in the capsular decidua, elevated mRNA levels of TNFα and IL-6 in the placental tissues, and the secretion of TNFα by the placenta during late gestation (36,37). Experimental inoculation of cattle with the parasite *Neospora caninum* in early gestation caused an immune response in the placenta (38). Pathological changes in the placenta consisted
mainly of CD3+ lymphocytes, dominated by CD4+ and γ/δ TCR+ cells, with CD8+ cells present to a lesser extent. It is possible that a pro-inflammatory Th1 response early in gestation leads to destruction of the placental tissues themselves and is thus incompatible with fetal survival.

A low number of T cells (at most, 10% of those seen in the adult airways) was found in different parts of the respiratory tract in horse fetuses (39). The low level of MHC class II expression in the fetus, together with the reduced number of T cells, was consistent with the suggestion that the fetal immune system requires exposure to airborne stimuli for the full development. The low level of MHC II expression in the mare may have been reflecting the immunosuppression that accompanies pregnancy.

6.1.2) The immune components in human fetal pathology

Characteristics of the fetal immune system under pathogenic effects are very similar between experimentally caused pathology and that which develops as a result of maternal or fetal illness. Immunosuppressive and other drugs administered to mothers during pregnancy and lactation might affect the development of the fetal and neonatal immune system (40,41). Chorioamnionitis or intrauterine fetal pneumonia caused by *Chlamydia trachomatis* (42) or *Candida colonies* (43) or other infections can be considered examples of fetal disorders in humans. Fetuses younger than 13 weeks showed no inflammatory response or cells positive for IgGs and proliferating *Candida colonies* which were evident in the lungs. The 16- and 22-week-old cases revealed a unique giant cell response in the terminal airways and increasing numbers of Ig-positive cells, with an increased proportion of IgA-positive cells in the older cases.

Severe chorioamnionitis is associated with a nonspecific inflammatory response comparable to that of neonatal sepsis (44) characterized by shrinkage of the thymus and spleen depletion, involving both B and T lymphocytes (45). Chorioamnionitis accompanied by an intrauterine inflammatory response of the fetal lungs is characterized by a severe infiltration of macrophages, neutrophils, and lymphocytes, and by sharply increased expression of IL-8 mRNA (46). Chorioamnionitis increases fetal intrahepatic myelopoiesis as one defense mechanism and induces a fetal extramedullary hematopoietic response in the second trimester of gestation (47). Fetal myelopoiesis significantly increases with leukocyte clustering.

One of potentially life-threatening disease for the both mother and fetus is pre-eclampsia, during which increased production of chemokines and leukocyte activation have been described in the fetal circulation (48). The activation of neutrophils and monocytes in the fetus involves enhanced chemokine activation, possibly contributing to the fetal morbidity in this disorder. The activation of neutrophils and monocytes is accompanied by raised plasma levels of the chemokines IL-8 and growth-related oncogene-α. The NK cell counts of umbilical blood in preeclamptic fetuses, as well as the proliferative and killing abilities of these cells, are significantly increased (49).

In fetuses with Down's syndrome, a statistically significant depletion in the total number of CD3+ T cells and a significant increase in the CD8/CD4 ratio during the second trimester of gestation has been found (50). The increased number of B cells along with primary follicles in fetuses with Down's syndrome, implies that at least part of the thymic medulla works and behaves like a peripheral lymphoid organ,
receiving mature lymphocytes and turning them from inactive to immunoefficient cells (51). The inflamed thyroid gland was shown to be capable of accumulating fetal cells, including T cells and dendritic cells (52). Such active immune cells may have a profound regulatory influence on autoimmune thyroiditis in pregnancy and the postpartum period.

An important cause of respiratory distress in newborn infants is meconium aspiration syndrome (MAS) (53). Approximately 12% to 15% of human infants are born through meconium-contaminated amniotic fluid (54), and these infants are much more likely to develop respiratory distress and require respiratory support (55). When meconium is present in the amniotic fluid, about 5% of neonates will develop MAS, and about 5% or more of these infants will die (54). The pathophysiology of MAS involves airway obstruction, surfactant dysfunction, and pulmonary inflammation (56). Meconium may interfere with the function of alveolar macrophages by decreasing their phagocytic activity (57) and inducing oxidative stress and apoptosis (58).

Rheumatic autoimmune diseases are high prevalent in women, particularly during their childbearing years. If the maternal disease is characterized by the presence of IgG-isotype autoantibodies, they can cross the placenta and potentially cause antibody-mediated damage to the fetus (59). This is typically the case in the so-called neonatal lupus erythematosus. A similar mechanism has been shown in infants of patients with immune thrombocytopenic purpura and, less frequently, in those from mothers with anti-phospholipid syndrome.

6.2. The secretory immune system in human fetuses

6.2.1) Lymphoid-epithelial components of the secretory immune system in self-protection of human fetuses

Some researchers are of the opinion that the SIS evolves after birth as a reaction to massive microbial invasion and the introduction of large amounts of different foreign antigens through the mucous membranes of the upper respiratory, digestive and urogenital tracts (60,61). However, as has been shown by the other authors and stated in previous chapters of this book, components of the SIS, such as SC, J chain, IgM, IgA and lymphocytes, are already present not only in human fetuses in the third trimester of gestation (62,63), but also in embryos of the first trimester (64,65).

Maturation of the immune system is well known to start early in human fetal life (65). As noted at the beginning of this chapter, B lymphocytes develop in the liver by 9 weeks of gestation and are present in the blood and spleen by 12 weeks. From 14 weeks, T lymphocytes leave the thymus, and subsequently cells with helper and suppressor phenotypes appear in the spleen. The lack of secondary lymphoid tissues in healthy fetuses most probably reflects a lack of antigen stimulus. On the other hand, newborn plasma contains adult levels of IgG which are acquired across the placenta from the mother.

In human fetuses, components of the SIS have been found in the epithelium of the salivary glands and mouth (66,67), trachea and lungs (68,69), and digestive tract
Small amounts of SC appear in the intestinal mucosa before week 29 of gestation, and its quantity increases rapidly thereafter (72). Secretory IgA-containing epithelial cells have been found in the respiratory tract and intrahepatic bile ducts of fetuses at 20 to 21 weeks of gestation (73). Immunocompetent mucosa-associated cells (dendritic cells, T lymphocytes, B lymphocytes, and macrophages) have been found in the human fetal larynx after week 14 of gestation (74). SC of fetal urogenital origin has been found in the amniotic fluid (75). Lymphoid cells expressing IgA and IgM, as well as other immunocompetent cells, have been described in fetuses of the second trimester of gestation (76), particularly, in the gut and amniotic fluid (77). From 11 to 14 weeks of gestation, CD68+ and CD40+ cells are present throughout the lamina propria. With the emergence of lymphoid aggregates (14-16 weeks), dendritic cells and B lymphocytes are detected in the fetal gut; however, their expression is restricted to the lymphoid aggregates. Lymphoid follicles forming after 16 weeks of gestation contain MHC II-positive cells of different subtypes of T lymphocytes.

Components of the SIS during the second trimester of gestation (weeks 13-25) were studied in 36 human fetuses obtained as a result of medically or socially recommended abortions (group I) or which had died of different causes -- abruptio placentae, placenta previa, and chorioamnionitis (group II) (78). The first group included 21 cases with no signs of foreign antigenic influences. The second group included 15 cases of acute chorioamnionitis with sepsis, aspiration syndrome or meningitis. In both groups, fetuses were of similar gestational ages.

**Fetuses without antigenic effects (group I)**

Elements of the SIS were widespread in the different organs of these fetuses. SC, J chain, IgA, IgM and IgG were found in the epithelium and glands of the digestive organs such as the mouth cavity, pharynx, esophagus, stomach and intestine, throughout the respiratory tract (larynx, trachea, lungs) and urinary tracts (kidneys, ureters, bladder), in hepatocytes and the epithelium of the bile duct, and in acini and ducts of the pancreas, in the follicular epithelium of the thyroid and the ovaries, in the epithelium of the Fallopian tubes and uterus, in the epididymis and rete testes, in the epithelium of the choroid plexuses of the cerebral ventricles, in the mesothelium of the pleura, epicardium and peritoneum, and in the epithelium of the skin, sweat and sebaceous glands. A few macrophages, B cells and different subsets of T lymphocytes were observed in these structures. All of these SIS elements were already observed in 13-week-old fetuses and were maintained during the whole second trimester of gestation.

The immunoreactivity of the different SIS components varied in different organs. The SC and J chain usually reacted intensively, except for cells in the distal part of the hypophysis and in pancreatic islands, where the SC was weakly reactive. In Leydig’s cells of the testes and some other organs, where only the J chain was found, the SC was absent. Expression of IgA, IgG and especially of IgM, was very low in the large intestine and hepatocytes in the center of liver lobules. IgM was sometimes absent in the choroid plexuses of the brain. SIS components were not seen in the gray substance of the brain, myocardium, intestinal goblet cells, skeletal muscles,
Fibroblasts, chondrocytes or osteoblasts. A small number of B lymphocytes expressing IgA and IgM was seen in different organs (Table X).

**Fetuses with antigenic effects (group II)**

In fetuses which had been subjected to massive antigenic effects at chorioamnionitis, expression of the SC and J chain was no different from that observed in group I (Fig. 7). Immunoreactivity of IgA, IgG and especially IgM was weak or even absent in the epithelium of the skin, respiratory, digestive and urinary tracts, hepatocytes, tubules of the kidneys, and choroid plexuses of the brain. The number of IgA- and IgM-positive lymphocytes increased in the spleen and lymph nodes, lungs, and in the mucous membranes of the stomach and intestine (Table X).

It has been shown that the whole SIS-protein-complex is already present in many tissues of the human fetus in the second trimester of gestation (78). Such tissues include mainly the widely spread border tissues covered with the epithelium or its analog, such as the mesothelium of serous cavities. Our findings of the SIS protein components in the fetal respiratory, digestive and urogenital tracts are very similar to those observed in children and adults (63,79).

In fetuses, there are cellular components of the SIS, such as lymphocytes of different subsets, including IgA- and IgM-containing B cells, and macrophages. However, their amounts are small (Table XI), and they are diffusely located in subepithelial tissues and intraepithelial spaces. Special lymphoid-epithelial structures, such as tonsils, solitary follicles (nodules) and aggregated follicles (Peyer's patches), which are typical of the adult SIS (62), were not found in second-trimester fetuses. Such peculiarities in the cellular structure of the fetal SIS as part of the common lymphoid system of fetuses can be explained by its "immaturity".

The total mass of lymphoid tissue in second-trimester fetuses is minute. The total weight of the main lymphoid organs such as the spleen and thymus is 0.092±0.02% of mean body weight in 13- to 15-week-old fetuses, 0.36±0.07% in 23- to 25-week-old fetuses, and 0.73±0.09% in 38- to 40-week-old fetuses. Lymph nodules are few and are of microscopic size. Some lymphoid tissue structures are absent. Reactive centers of lymph nodules are not seen, even in the infected fetuses. The process of transformation of Ig-synthesized B lymphocyte stops at the immunoblast stage, and these never become mature plasma cells in cases with acute infections (80).

The massive antigenic effects under chorioamnionitis (group II fetuses) caused a two- to fourfold increase in the number of IgA- and IgM-synthesizing lymphocytes in the spleen, lymph nodes, and respiratory and digestive tract organs where SIS is localized and where the antigenic effect is manifested at its highest level (Table X). As a result, Ig synthesis was activated in the common immune system and in the SIS. The immunoreactivity of IgA, IgM and IgG in the epithelial cells sharply decreased or disappeared altogether. Sometimes, IgA and IgG immunoreactivity was seen in the fibrin in the bronchial cavity or in the stomach mucus as a manifestation of the exocrine secretion of IgA by the epithelium. The described changes could be
considered a morphological manifestation of SIS functional activity in human fetuses in the second trimester of gestation.

The components of the fetal SIS, such as IgA and IgM, are of fetal origin (60). Maternal IgG has been found in the fetal epithelium, and this is considered an additional proof of insufficient functional activity of both the common and secretory fetal immune systems (78). Under massive antigenic attack, immunoreactivity of Igs in the fetal epithelial cells decreases to complete disappearance, whereas reactivity of the SC and J chain does not change (Fig. 7). It can be supposed that SC and J chain do not leave the cells during SIS function, and their discovery in the cavities of some organs (such as the amnion, 75) may result from cell shedding and destruction. Cells without exocrine secretion in some organs, such as the distal lobe of the hypophysis, pancreatic islets, etc., contain only the J chain without SC. Our study demonstrated that the SIS is widely present and functionally active in fetuses in the second trimester of gestation. It is not restricted to mucosal membranes, it is present in the fetal organs (Fig. 8) and it plays an important role in the immune defense of the entire fetuses and its strategically important organs against foreign antigenic influences.
Fig. 7.

C. A 23-old fetus. Chorioamnionitis. SC in the epithelium of the bronchus (b), esophagus (e), thymus and thyroid (t). ×100.

Fig. 8.

A. A 17-week-old fetus. The kidneys. SC in the epithelium covering of glomerulus (heads of arrows), loop of Henle (small arrows), collecting tubules (ct). x100.
B. The same case. IgA in the epithelium of convoluted (small arrows) and collecting (a large arrow) tubules. x200.
C. A 20-week-old fetus. The bladder. SC in the superficial epithelium. x400.
Table X.
The number of IgA- and IgM-positive lymphocytes in different fetal organs (per 50,000 µm², mean ± SE) (After ref. 65)

<table>
<thead>
<tr>
<th>Organs studied</th>
<th>Group I fetuses</th>
<th>Group II fetuses</th>
<th>Group I fetuses</th>
<th>Group II fetuses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>0.8±0.3</td>
<td>2.3±0.5 a</td>
<td>3.0±0.6</td>
<td>5.4±0.9 a</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>1.1±0.4</td>
<td>2.6±0.6 a</td>
<td>2.3±0.5</td>
<td>5.2±1.2 a</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.2±0.1</td>
<td>0.9±0.3 a</td>
<td>0.3±0.1</td>
<td>1.6±0.5 a</td>
</tr>
<tr>
<td>Stomach, Small intestine</td>
<td>0.4±0.2</td>
<td>1.8±0.5 a</td>
<td>1.9±0.7</td>
<td>6.9±1.8 a</td>
</tr>
<tr>
<td>Liver</td>
<td>1.2±0.4</td>
<td>2.4±0.7</td>
<td>1.9±0.4</td>
<td>4.8±1.2 a</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.2±0.1</td>
<td>0.8±0.4</td>
<td>0.6±0.4</td>
<td>2.5±0.8 a</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.2±0.2</td>
<td>0.6±0.3</td>
<td>1.3±0.7</td>
<td>1.9±0.8</td>
</tr>
<tr>
<td>Choroid plexus</td>
<td>0.3±0.2</td>
<td>0.6±0.3</td>
<td>1.3±0.7</td>
<td>1.9±0.8</td>
</tr>
</tbody>
</table>

* Significantly different from Group I, p < 0.05.
Groups of fetuses: I, without antigenic attacks; II, with antigenic attacks.

Table XI. The number of immunocompetent cells in the liver of embryos and fetuses (mean±SE in 50,000 µm²) (After ref. 65)

<table>
<thead>
<tr>
<th>Groups of patients a</th>
<th>Age of pregnancy (weeks)</th>
<th>Macrophages</th>
<th>CD3+ T cells</th>
<th>CD20+ B cells</th>
<th>IgA+ B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3.5-4 to 6</td>
<td>11.7±1.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>7 to 8</td>
<td>12.7±1.7</td>
<td>single</td>
<td>single</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>9 to 12</td>
<td>19.3±2.4 b</td>
<td>4.6±0.8 b</td>
<td>3.9±0.7 b</td>
<td>0.8±0.4</td>
</tr>
</tbody>
</table>
6.2.2) Components of the secretory immune system in fetal membranes and decidua

Researches performed to date have been restricted to the study of single components of the SIS in human fetal membranes or the placenta. We are not aware of any report on the role of SIS in the whole complex of the maternal-fetal barrier in the first trimester of pregnancy. Because of its ontogenic importance, we investigated the distribution of SIS components in fetal membranes (amnion, yolk sac, chorion) and the decidua throughout the first trimester of pregnancy (64). Specimens from 17 embryos (3.5-4 to 8 weeks of pregnancy) and 9 fetuses (9 to 12 weeks) were divided into those that had not been exposed to massive foreign antigenic effects (group I) and those that had suffered acute chorioamnionitis (group II).

Embryos and early fetuses without massive antigenic effects (group I)

The presence of SIS protein components (SC, J chain and Igs) was found in tissues of the gestational sac of both fetal and maternal origin as early as the fourth to fifth week of development, and throughout the rest of the first trimester of pregnancy. In 3.5-4- to 6-week-old embryos not exposed to massive foreign antigenic influences, SC, J chain and IgG were observed in the epithelium of the amnion, in the syncytiotrophoblast of the chorion and in the yolk sac endoderm (Fig. 9). CD68+ macrophages were found to contain J chain, IgA and IgG (Tables XII, XIII), but not SC or IgM. Lymphocytes were not found. Decidual cells were reactive to SC, J chain and IgA. Single lymphocytes and macrophages were seen in the stroma of the decidua, and only a few of them were positive to IgG, IgA and IgM.

In 7- and 8-week-old embryos, immunoreactivity of SC, J chain and IgG was not changed in the amniotic epithelium, endoderm of the yolk sac, trophoblastic and decidual cells. A slight increase in the reactivity of IgA was seen in the latter cells. Groups of CD3+ T lymphocytes and CD20+ B lymphocytes were seen in angioblasts of the yolk sac and the aorta at week 8, while the other subsets of T cells and Igs-containing cells were not found (Table XII).

In fetuses at 9 to 12 weeks of pregnancy, immunoreactivity of SC, J chain and IgG was not changed in the above-mentioned tissues, whereas reactivity to IgA and IgM increased. The number of macrophages increased in the stroma of trophoblastic villi (Table XIII). At week 9, single T and B lymphocytes were observed, and at weeks 10 to 11, IgA+ and IgM+ lymphocytes were found. The number of different subsets of lymphocytes increased in the decidual tissue (Table XII). Fibrotic avascular villi were...
seen in a case with acardius amorphus. SC, J chain, IgA and IgG were found in the amniotic epithelium and in the trophoblastic and decidual cells.

In fetuses during the last trimester of pregnancy, the amniotic fluid contains, in addition to the predominant maternal IgG, different molecular forms of fetal Igs (81). IgG is found to be the major isotype and to contain mother-derived tetanus antitoxins. IgA is much less abundant, whereas no IgM can be detected. Amniotic fluid of human fetuses at a gestational age of 26 to 40 weeks contains low levels of IgA of fetal origin and SC in its free form (75, 82). IgG, IgA, and SC are detected in the fetal urine and, therefore, can reach the amniotic fluid via this route. Their function as an immune barrier against infection and against mother-derived autoantibodies has been suggested.

**Embryos and early fetuses with massive antigenic effects (group II)**

Massive antigenic exposure due to chorioamnionitis during the first trimester caused little change in the distribution and immunoreactivity of SC and J chain in the amniotic epithelium, trophoblastic and decidual cells. SIS protein components were even found in embryos with heavy abnormalities such as acardius amorphus. Reactivity to IgG and IgA decreased to its disappearance, while reactivity to Igs increased significantly in the perivillous fibrin. Lymphocytes were seen only after week 9 whereas macrophages were observed in high numbers as early as the fourth week of pregnancy (Table XIII). In the decidual tissue, the number of all types of immunocompetent cells, including Igs-synthesizing lymphocytes and plasma cells, increased sharply (Table XII).

The cellular components of the SIS in all fetal membranes of 3.5-4- to 8-week-old embryos were represented only by macrophages. CD3+ and CD20+ lymphocytes were seen to the end of week 8, and IgA+ and IgM+ lymphocytes were found only at weeks 10 to 11. This is in accordance with other authors' observations regarding the development of B cells and Ig-synthesizing lymphocytes (18).

The Igs seen in the embryonic tissue appeared to be of maternal rather than embryonic origin. The placental barrier has been shown to be permeable for maternal IgG (17, 19, 83). Specific IgG and IgA were found in the coelomic fluid of 6- to 12-week-old embryos and fetuses suffering from rubella, cytomegalovirus and *Toxoplasma gondii* infections in amounts similar to their concentrations in the maternal blood (16). These results suggested that maternal IgG and IgA are potentially available to the embryo as early as week 6 into development. This is in accordance with our findings that IgG and IgA but not IgM are present in macrophages of the chorionic villi.

Macrophages from different embryonic and fetal organs of the first trimester of pregnancy were found to be immunoreactive to IgG and IgA but not to IgM (65). We therefore suggested the presence of transport and defensive functions among macrophages. Thus, during the embryonic period, when the common (systemic) immune system is still only beginning to develop, Igs of maternal origin appear to be functioning in the embryonic SIS.

In the decidua, cellular components of the SIS exhibit a full immune response during the entire first trimester of pregnancy (84). The small amount of each type of these cells (Table XII) suggests that this response is manifested very weakly, reflecting
mainly the reaction of a pregnant mother (her decidua) to embryonic antigens. In the decidua, the number of lymphocytes, including those synthesizing IgG, IgA and IgM, plasma cells and macrophages increases significantly, even during the earliest embryonic period. Such an increase was found even relative to their amount in adult ruptured uterine tubes (Table XII).

Chorioamnionitis with accompanying massive antigenic effect is characterized by changes in protein and cellular components of the SIS. Igs disappear from the fetal membranes and decidua. With chorioamnionitis, the cellular composition is different in the embryonic and maternal parts of the gestational sac. In fetal membranes, only macrophages were found to react to the antigenic effect. The absence of a response by lymphocytes of the studied subsets showed that in 3.5-4- to 8-week-old embryos, even a massive antigenic effect does not accelerate lymphocyte maturation. A weak lymphocytes reaction was seen after only 9 to 10 weeks of pregnancy. In the maternal decidua with chorioamnionitis, immune-response development was analogous to an intensive immune reaction in adults (84).

Data from the literature (85) and our observations (64,65) show a different origin and composition of immunocompetent cells and a different course of immune reactions in embryonic and maternal parts of the gestational sac. We conclude that two SIS are present at the border between the maternal and embryonic tissues. These systems are already in place at the beginning of the embryonic period, weeks 4 to 5, function during the entire first trimester of pregnancy and are the main immune mechanism underlying the barrier between these two organisms. We suggest that the SIS plays an important role in the regulation of pregnancy and in the development of fetal tolerance.
(Color Fig.)

Fig. 9.
A 4-week-old normal human embryo. Microphotographs of the gestational sac.
A. SC (brown staining) in the decidual cells (d) and invasive trophoblast (it). ×100.
B. J chain in both the cytotrophoblast and syncytiotrophoblast and in the macrophages (heads of arrows). x200.
C. IgG in the syncytiotrophoblast and macrophages (heads of arrows). x200.
D. IgA in the syncytiotrophoblast and cytotrophoblast and in the macrophages (heads of arrows). x400.
E. Gestational sac of a 8-week-old embryo. SC in the amniotic epithelium (a) and yolk sac endoderm (heads of arrows). A blood island (a white arrow). x200.
Table XII.  
The number of lymphocytes and macrophages in the decidua (in 50,000 µm², mean±SD) (After ref. 64)

<table>
<thead>
<tr>
<th>Groups of patients</th>
<th>Week of pregnancy</th>
<th>T lymphocytes</th>
<th>CD4+</th>
<th>CD20+ B cells</th>
<th>B lymphocytes and plasma cells producing IgG+ IgM+</th>
<th>IgA+</th>
<th>CD68 + Macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CD3+ CD8+</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>4 to 6</td>
<td>0.8±0.6</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
<td>0.5±0.5</td>
<td>0.6±0.3</td>
<td>0.3±0.2</td>
</tr>
<tr>
<td></td>
<td>7 to 8</td>
<td>0.9±0.4</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
<td>0.7±0.4</td>
<td>0.3±0.2</td>
<td>0.2±0.2</td>
</tr>
<tr>
<td></td>
<td>9 to 12</td>
<td>1.5±0.7</td>
<td>0.5±0.3</td>
<td>0.9±0.5</td>
<td>0.9±0.6</td>
<td>0.8±0.4</td>
<td>0.2±0.2</td>
</tr>
<tr>
<td>II</td>
<td>4 to 6</td>
<td>6.7±1.4 a</td>
<td>1.8±0.8</td>
<td>4.4±1.1 a</td>
<td>14.8±2.2 a</td>
<td>4.3±1.3 a</td>
<td>8.1±1.9 a</td>
</tr>
<tr>
<td></td>
<td>7 to 8</td>
<td>6.9±1.3 a</td>
<td>2.1±0.8 a</td>
<td>3.7±0.8 a</td>
<td>11.3±2.1 a</td>
<td>1.8±0.9</td>
<td>1.0±0.4</td>
</tr>
<tr>
<td></td>
<td>9 to 12</td>
<td>8.2±1.9 a</td>
<td>2.8±0.7 a</td>
<td>4.5±1.2 a</td>
<td>16.2±3.3 a</td>
<td>6.9±1.9 a</td>
<td>9.3±2.6 a</td>
</tr>
<tr>
<td>Uterine tube</td>
<td>4 to 6</td>
<td>4.4±0.8</td>
<td>1.3±0.6</td>
<td>3.2±0.9</td>
<td>2.9±0.8</td>
<td>2.2±0.9</td>
<td>0.8±0.3</td>
</tr>
</tbody>
</table>

\(^a\) Significant difference compared to similar parameter in the group I, p < 0.05-0.01.
Table XIII.
The number of lymphocytes and macrophages in the chorionic villi (in 50,000 µm², mean±SD) (After ref. 64)

<table>
<thead>
<tr>
<th>Groups of patients</th>
<th>Week of pregnancy</th>
<th>T lymphocytes</th>
<th>CD20+ B cells</th>
<th>Igs-producing B lymphocytes</th>
<th>CD68+ Macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CD3+ CD8+</td>
<td>CD4+</td>
<td>IgG+ IgM+</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>4 to 6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10.7±1.2</td>
</tr>
<tr>
<td></td>
<td>7 to 8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9.2±1.1</td>
</tr>
<tr>
<td></td>
<td>9 to 12</td>
<td>0.18±0.09</td>
<td>0.06±0.04</td>
<td>0.09±0.07</td>
<td>19.8±2.3</td>
</tr>
<tr>
<td>II</td>
<td>4 to 6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>17.4±1.9</td>
</tr>
<tr>
<td></td>
<td>7 to 8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>33.6±3.9</td>
</tr>
<tr>
<td></td>
<td>9 to 12</td>
<td>0.72±0.15</td>
<td>0.16±0.19</td>
<td>0.18±0.11</td>
<td>33.4±4.9</td>
</tr>
</tbody>
</table>
a Significant difference compared to similar parameter in the group I, p < 0.05-0.001.
b Significant difference compared to similar parameter of the previous age embryos in the same group, p < 0.05-0.001.
References

(To 6.1)


Chapter 7.
The Secretory Immune System and the Placental Barrier

7.1) The placental barrier: its morphology, function and pathology

The placental barrier between the mother and her fetus is the main structure providing normal functioning and development of these two immunologically different organisms (1). The placenta contains fetal and maternal parts. The fetal part (villous and membranous) is composed of fetal tissues such as the trophoblast, amnion, mesenchyme and blood vessels carrying fetal blood. Aside from the lymphocytes, the fetal part of the placenta contains many macrophages which are responsible for immune and non-immune phagocytosis, fixation of maternal antibodies and production of cytokines (2).

The maternal part includes decidual tissues and intervillous spaces filled with maternal blood. The border between the maternal and fetal tissues passes not only between the trophoblast and the maternal blood but also at the contact surfaces of the trophoblast and decidual tissue (3). In the decidual tissues, about 10% of the cells are lymphocytes and about 20% are macrophages. These cells of maternal origin are immunocompetent and participate together with the decidual cells in various immune reactions.

The concept of placental barrier was evaluated about 20 years ago using advances in ultrastructural analysis and in transport physiology (4). Structurally, the barrier effect is grounded by the syncytiotrophoblast's continuity, basal and plasma membrane electrical charges and by basement membrane porosity. The continuity of the aqueous phase for diffusion operates through intercellular gaps, fenestrations (rat, rabbit) or transcellular channels (guinea pig). However, these connections are not apparent in the human syncytiotrophoblast. In terms of molecular size selectivity, the human hemochorial placenta with a pore radius of 10 nm appears much less selective than the epitheliochorial one in animals. The main structural and functional units of the placenta are the chorionic (trophoblastic) villi within which the fetal blood is separated by only three or four cell layers (placental membrane or barrier) from the maternal blood in the surrounding intervillous spaces (5). The metabolic capacity of the placental cells (trophoblast, macrophages) participates in the barrier effect by metabolizing or by converting some substrates.

Trophoblast specificity in the location of enzymes, carriers and receptors on the outer (maternal side) and on the basal (fetal side) plasma membranes, and in the release of secretory products, contributes to maintaining separate fetal and maternal compartments. Trophoblastic cells, macrophages, and perhaps also fetal blood cells can form a sequential barrier blocking maternally activated defense cells bearing CD95 molecules and thereby blocking anti-fetus CD95+ T lymphocytes from entering apoptosis (6).

Trophoblastic cells participate in the establishment of specific interactions between the maternal and fetal circulation in the placenta. Mammalian embryos have an intimate relationship with the placental vasculature from which they obtain essential
nutrients for growth. The vascular bed exhibits interesting specificity because number and diameter of maternal vessels change dramatically during gestation and, in rodents and primates, the terminal blood space becomes lined with placental trophoblast cells rather than endothelial ones (7). The transition from endothelium-lined artery to trophoblast-lined (hemochorial) blood space is associated with giant trophoblast cells. The shaping of the maternal blood spaces within the labyrinth is dependent on chorioallantoic morphogenesis.

The placenta is the highly specialized organ of pregnancy that supports the normal growth and development of the fetus. Growth and function of the placenta are precisely regulated and coordinated to ensure that the exchange of oxygen and nutrients between the maternal and fetal circulatory systems as well as removal carbon dioxide and other waste products operate at maximal efficiency (5,8,9). Oxygen transport is limited by placental blood flow, but the transport of glucose and amino acids is determined by the abundance and activity of specific transport proteins. Glucose in the maternal blood passes freely through pores of the cytotrophoblast, transported into the cytoplasm of syncytiotrophoblast I via GLUT1, an isoform of facilitated-diffusion glucose transporters, then enters syncytiotrophoblast II through the gap junctions, and finally leaves syncytiotrophoblast II via GLUT1 and enters the fetal blood through pores of the endothelial cells (10).

Placental metabolism of glucose has major effects on both the quantity of carbon and nitrogen delivered to the fetus, and on the composition of the involved substrates. The placenta's capacity for glucose transport under moderate glucose deprivation is up regulated in part by increased expression of the GLUT3 transport protein (8). During severe glucose deprivation however, placental transfer and fetal uptake of glucose are constrained in proportion with the maternal supply, leading to fetal growth retardation.

The placenta metabolizes a number of substances and can release metabolic products into the maternal and/or fetal circulation (11). The placenta can help to protect the fetus against certain xenobiotic molecules, infections and maternal diseases. In addition, it releases hormones into both the maternal and fetal circulations to affect pregnancy, metabolism, fetal growth, parturition and other functions. Many changes in placental function occur to accommodate the increasing metabolic demands of the developing fetus during gestation. Some drugs are pumped across the placenta by various active transporters located on both the fetal and maternal sides of the trophoblast, and pinocytosis and phagocytosis are considered too slow to have any significant effect on fetal drug concentrations (11).

Placental inflammatory disorders represent a diverse and important category of pathological processes leading to fetal and neonatal morbidity and mortality. These processes can be divided into two broad subcategories, those caused by microorganisms and those caused by host immune responses to non-replicating antigens. The mechanisms by which these inflammatory processes cause death and disability are diverse and can be separated into four distinct classes: i) placental damage with loss of function, ii) induction of premature labor and subsequent preterm birth, iii) release of inflammatory mediators leading to fetal organ damage, and iv)
transplacental infection of the fetus (12). Each specific inflammatory process can be modulated by properties of the specific organism, the route and timing of the infection and variations in the host’s genetic background and immune responsiveness.

The main pathology of the placenta is the inflammation of the chorionic (trophoblastic) villi termed villitis. The inflammatory response in placental villitis of unknown etiology is characterized by the invasion of fetal villi by maternal T cells and associated with focal destruction of the syncytiotrophoblast (13). Placentas with villitis exhibit significantly higher syncytiotrophoblast intercellular adhesion molecule-1 expression than placentas without villitis (14).

Villitis caused by *Toxoplasma gondii*, *Trypanosoma cruzi*, or *Paracoccidioides brasiliensis* is characterized by rupture of the trophoblastic barrier and influx of immune cells into the villi (15). In some cases, placental toxoplasmosis is accompanied by granulomatous villitis (16). The immune response in villitis consists of maternal lymphocytes as the predominant intravillous population: mainly T cells (CD3, CD8 and CD4) and only rarely B lymphocytes (17). CD68+ macrophages and CD8+ T lymphocytes make up the major portion of the cell population in villitis caused by *T. cruzi* (18).

Placental mesenchymal dysplasia is found in association with intrauterine fetal death. In severe cases, the placenta is markedly enlarged (up to 1,050 g), and approximately 80% of it is occupied by extraordinarily enlarged villous structures with a myxoid appearance (19). Histologically, the dysplastic villi have myxoid stroma and a decreased number of fetal vessels, which are occasionally seen to be obliterated. There is no abnormal trophoblastic proliferation. Large-sized fetal vessels in the chorionic plate frequently contain organized thrombi.

*Placental hemorrhagic endovasculitis is found in association with stillbirth and with abnormal growth and development in live births* (20). *Lesions occurring with significant frequency in such placentas include villitis of unknown etiology, chorionic thrombi, villous fibrosis, erythroblastosis, and meconium staining. The segmental pattern of villous fibrosis and the high incidence of growth restriction, erythroblastosis, and meconium suggest a chronicity of adverse intrauterine events that may precede fetal loss.*

There is a direct link between parasitic malarial infection of the mother and syncytiotrophoblast damage (21). Placentas with active malaria infection showed erythrocyte adhesion of infected cells to the syncytiotrophoblast, syncytiotrophoblast damage, increased syncytiotrophoblast and localized destruction of the villi. Past malarial infection is characterized by syncytiotrophoblast disruption and fibrinoid-type fibrinoid (FTF) deposition. Perivillous FTF deposition is consistent with increased syncytiotrophoblast and both increased lesions and syncytiotrophoblast knots have been associated with reductions in birth weight. Syncytiotrophoblast destruction could have serious implications, impairing fetal growth and, in some cases, providing a previously unrecognized pathway for congenital infection.
Massive chronic intervillositis is a placental lesion associated with malarial infection. It is characterized by a prominent inflammatory infiltrate in the intervillous space, composed mainly of monocytes and macrophages which can simulate a maternal malignant disorder involving the placenta (22).

7.2) The role of SC and J chain in maternal immunoglobulin transport through the placental barrier

Transfer of Igs through the placenta is considered here as an example of the presence of the barrier portion of the SIS. Maternal Igs are important in the immune protection of the embryo/fetus, especially in the first trimester, when the embryo’s immune system has not yet developed. Ig transport is already seen in early embryos, at 3.5-4 to 5 weeks (23). SC, J chain and Igs are located in both layers of the trophoblast in a very similar manner, which may help them unite into one complex, that is unique to the trophoblast (Fig. 10).

Although it is accepted that maternal Igs are able to pass through the decidua (maternal part of the placenta) to the amnion and can reach the fetus' lungs and intestines via the amniotic fluid, it has been claimed that only IgG passes through the placenta and that all other types of Igs (IgA, IgM, IgD) do not (24,25). However in some pathological cases, such as cytomegalovirus, Toxoplasma gondii and rubella, transport of IgA from mother to fetus has been reported (25,26). Prematurity and LBW are associated with impaired placental transfer of IgG subclasses 1 and 2 (27). Maternal IgE has also been found in the umbilical blood of newborns whose mothers had allergies (28). Fetuses begin synthesizing their own Igs after the ninth week of intrauterine development (29,30).

Igs have to pass three different parts of the fetal portion of the human placental barrier during their transport to embryos/fetuses (31-34). The first part, the trophoblast (the fetal part of the placenta) with its two layers, the cyto- and syncytiotrophoblast, contains SC. In embryos without the effect of foreign antigens, SC was found either in both layers of the trophoblast or accumulated in one of them (Fig. 10). J chain, IgG and IgA were found in both layers of the trophoblast, corresponding with changes in SC accumulation. IgM was sometimes found in the apical microvilli of the syncytiotrophoblast immersed in the maternal blood lacunae. The second part of the placental barrier is the stroma of the trophoblastic villi. In non-infectious aborts, about 60% of the villi contained IgG, 25% contained IgA, and about 12% contained IgM. Fcγ receptors were not seen in half of the embryos but were found in the fetuses. Capillaries, the third part of the placental barrier, were already detected in embryos in each of the trophoblastic tertiary villi, 3 to 22 µm from the basal membrane of the trophoblast. In second-trimester fetuses, they often contacted the basal membrane. In third-trimester fetuses, such contact was rare but the number of capillaries rose. At all stages studied, a weak reaction to SC receptors was sometimes noted in the endothelium of the capillaries.

Igs can be transported from the stroma of the trophoblastic villi through the endothelium of the blood capillaries, which contains the receptors FcγRII (35) and
FcRn (neonatal Fc receptors) (24). The close contact between the basal membrane of the cytotrophoblast and the capillaries (35,36) aids in transporting Igs into the fetal blood. Embryonic erythroblasts, along with the other nucleus-containing blood cells (monocytes, lymphocytes, leucocytes, etc.), are able to catch Fc receptors (37,38), and a loss of this ability parallels the loss of nuclei in the erythrocytes after week 9 of intrauterine development. The presence of the Ig-containing erythroblasts allows us to consider these cells as part of the cellular Ig-transport system in embryos. The finding of Igs in the blood plasma in the fetal capillaries (39) indicates the manner in which Igs are transported in the bloodstream.

Our observations have shown that in normal pregnancy, mainly IgG passes through the placental barrier (40). Transport of IgA was seen in 78% of the samples, whereas no transport of IgM was detected. In cases with moderate inflammation of the birth canal, transport of IgG and IgA, and to a lesser extent IgM, increased. In cases with severe inflammation, transport of all types of Igs increased, with IgG showing the highest level.

In all embryos and fetuses with moderate inflammation of the birth canal, the permeability of the placental barrier was seen to increase (41). More than 82% of the villi contained IgG, 34% of them contained IgA, and 18% contained IgM (Fig. 11). The number of CD68+ monocytes increased sharply (Fig. 12, 13). The concentration of Fcγ receptors increased from 5% to 25% in embryonic monocytes to 90% in fetal cells after week 13. J chain was seen in 63% of all monocytes; some of them contained IgG. In embryos with acute infections and severe inflammation of the birth canal, distinct reactions to SC, J chain, IgG and IgA, and a weaker one to IgM were seen in all cases in the cytotrophoblast and in the apical microvilli of the syncytiotrophoblast. In erythroblasts, SC was not seen but J chain, IgG, IgA and, rarely, IgM were found.

Transport of Igs from the placenta into the fetal blood is also performed by monocytes till week 9. Monocytes are located close to the basal membrane of the cytotrophoblast, in the Ig-impregnated stroma, and near capillaries (42,43). This location allows them to catch Igs from the basal membrane of the trophoblast or from the tissue fluid in the stroma, and to transfer them to the capillaries. Monocytes contain a complex of Fcγ receptors (35,39), Fcδ receptors (35,43) and, possibly, the Fc receptor for IgM, because this last receptor has been found in the cytoplasm of monocytes. Fc receptors located on monocytes catch Igs with their Fc fragments and build the immune complexes (39). The SC and FcRn are absent in monocytes (23,35).
Fig. 10.
A. The placenta of a 13-week-old fetus with low antigenic effect. Note the fetal SIS contents of SC (brown staining) in syncytio- and cytotrophoblast, and in arterial myocytes (an arrow). Macrophages (heads of arrows) do not contain SC. x400.
B. The placenta of a 23-week-old fetus without an antigenic effect. Note IgA in the cyto- and syncytiotrophoblast, in the endothelium of blood capillaries, in the stroma of chorionic villi and monocytes. This indicate on transport of IgA throughout the placental barrier. x200.
C. The placenta of a 21-week-old fetus with moderate antigenic effect. Note IgM in the chorionic villi (v), in the invasive trophoblast (it), and in the deciduas (d). In the villous stroma IgM is absent. x200.
D. The placenta of a 20-week-old fetus. Chorioamnionitis. Fetal part of the SIS: note a large amount of CD68+ macrophages (brown staining) in chorionic villi. x200.
Fig. 11. The number of tertiary chorionic villi which stroma contains Igs (% of the total number of tertiary villi). Material contained embryos of 3.5 to 8 weeks of gestation and fetuses of 9 to 12 weeks. Groups of patients studied: I, without inflammatory; II, with moderate inflammatory; III, with severe infection of the birth canal.
Fig. 12. The number of macrophages containing Igs (% of the average number of CD68+ monocytes). Groups of material studied, see footnotes to Fig. 11.
Fig. 13. The average number of monocytes in villi per 50,000 nm$^2$ in embryos (E, 3.5 to 8 weeks of gestation) and fetuses (F, 9 to 12 weeks). Groups of material studied, see footnotes to Fig. 11.
In early human embryogenesis, at 3.5-4 to 5 weeks, Fcγ receptors were seen in 57% of the monocytes (23). The role of monocytes in the transport of Igs appears to be quite important. The number of monocytes in the trophoblastic villi, even in normal embryos, is significantly higher than the number of macrophages in the decidua. At term, the number of monocytes reaches 81% of the total number of CD68+ cells, and they show a strong reaction to FcγRIII receptors and Igs. In cases with infections, the number of monocytes increases sharply. Monocytes, together with Igs, pass between the endothelial cells into the capillaries and via the bloodstream, reaching all of the organs and tissues of the embryo/fetuses.

Although many reviews have been devoted, in recent years, to the study of transcellular Igs transport (44-46), opinions differ with respect to the role of J chain in this process. Some think it plays a key role in involving polymeric (p)Igs in transepithelial transport and exocrine secretion (45). Others have written about the non-essential role of J chain in Ig transport (47), and still others do not even mention J chain in their description of this process (48). The mechanism of interaction between J chain and pIgR/SC, and the biological function of the former remain unknown.

J-chain participation in transcellular transport of Igs should be considered in terms of the three phases of this process: endocytosis, transcytosis and exocytosis (23). The heart, most of the endocrine glands, gonads, brain, the epithelium of the renal canals and capillary endothelium, etc., were found to be among the organs in human embryos and fetuses whose parenchyma cells contain J chain and Igs and are free of SC. The presence of Igs in the cytoplasm of these cells, which are not able to synthesize Igs themselves, indicates the presence of endocytosis, i.e. Ig internalization, without the participation of SC.

Fc receptors can carry out the SC function in endocytosis by joining to the Fc fragment of Igs. Fc-αR, Fc-γR, and others have been described in this respect (49-51). FcRn participates in the transport of IgG through the cyto- and syncytiotrophoblast of the placenta (52) and through some types of the epithelium (53-55). The role of J chain in endocytosis with the participation of Fc receptors has not been described, and the mechanism underlying their interaction as well as the relationship between J chain and SC remain elusive (45).

Our morphological observations indicate that in cells containing J chain and free of SC the process of exocytosis does not occur. Igs exocytosis in SIS organs containing SC and J chain, such as the epithelium of the stomach and intestine, acinis and pancreatic ducts, is accompanied by a sharp decrease in the immune reactivity of both Igs and SC (30). In contrast, the immune reactivity of J chain does not decrease.

7.3) The secretory immune system as part of the placental barrier

The placenta contains the SC, IgA and IgM (56,57), components of the SIS which are characteristic for adults and protects the organism against invading microorganisms and foreign antigens. In human fetuses, the SIS has been found in the third trimester of gestation (58,59) and involved in immune disturbances in fetuses of ill mothers (56,60). The role of the SIS in the placental barrier has been described elsewhere (2).
In our studies of the placentas of 32 human fetuses who died during the second trimester of pregnancy (weeks 13 to 25) of different causes, such as abruptio placenta, placenta praevia, chorioamnionitis, or abortion for medical or social reasons, the material was divided into three groups (2). Group I consisted of seven placentas with little lymphoid-macrophageal infiltration into the tissues. Group II consisted of 12 placentas with moderate infiltration that reflected weak or moderate immune processes in the placenta. Group III included 13 placentas with acute chorioamnionitis and deciduitis, complicated by severe foreign antigenic effects such as fetal sepsis or pneumonia (61).

In the fetal part of the placentas with low and moderate lymphoid-macrophageal infiltration (groups I and II), SC and J chain were consistently found in the cytoplasm of the cyto- and syncytiotrophoblast of the chorionic villi, in the epithelium of the amnion, and in the arterial myocytes of the umbilical cord and stem villi. IgA and IgM were also detected in the cytoplasm of trophoblast but their immunoreactivity in the myocytes was weaker than in the other structures. IgG was found in the cyto- and syncytiotrophoblast. There were few lymphocytes, and about half of the B lymphocytes were positive to IgA or IgM (Table XIV). Macrophages were abundant in placentas with low infiltration, and even more so in those with moderate infiltration (Table XIV). They contained J chain but not SC. From 50% to 55% of macrophages contained IgG, but no IgA or IgM were found.

In the placentas with acute chorioamnionitis (group III), the rate of immunoreactivity and the distribution of SC and J chain were similar to those found in the other two groups. However, the reactivity of IgA, IgM and IgG in the chorionic and amniotic epithelia was low. Whereas the number of lymphocytes was similar to that in the other groups, the number of macrophages increased and the amount of IgG-positive macrophages decreased sharply (Table XIV). Inflammation was seen in the fetal membranes.
Table XIV. Cellular composition of infiltrates in the fetal part of the placenta
(No. of cells/50,000 µm²) (mean ± SE). (After ref. 2)

<table>
<thead>
<tr>
<th>Groups of patients</th>
<th>T cells</th>
<th>T helpers</th>
<th>T killers/ suppressors</th>
<th>B cells</th>
<th>IgA+ B cells</th>
<th>IgM+ B cells</th>
<th>Macrophages</th>
<th>IgG+ macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td>I- Weak infiltration</td>
<td>0.26±0.14</td>
<td>0.09±0.14</td>
<td>0.09±0.04</td>
<td>0.64±0.14</td>
<td>0.22±0.12</td>
<td>0.06±0.06</td>
<td>20.0±2.7</td>
<td>11.5±2.8</td>
</tr>
<tr>
<td>II- Moderate infiltration</td>
<td>0.20±0.12</td>
<td>0.11±0.12</td>
<td>0.08±0.03</td>
<td>0.60±0.10</td>
<td>0.28±0.14</td>
<td>0.10±0.10</td>
<td>27.5±1.9</td>
<td>14.0±3.2</td>
</tr>
<tr>
<td>III- Acute inflammation</td>
<td>0.63±0.18</td>
<td>0.17±0.04</td>
<td>0.23±0.07</td>
<td>0.31±0.11</td>
<td>0.19±0.08</td>
<td>0.25±0.13</td>
<td>37.7±2.5</td>
<td>3.1±0.9</td>
</tr>
</tbody>
</table>

*a* Significantly different from group I, p<0.01  
*b* Significantly different from group II, p<0.01

Table XV. Cellular composition of infiltrates in the maternal part of the placenta
(No. of cells/50,000 µm²) (mean ± SE). (After ref. 2)

<table>
<thead>
<tr>
<th>Groups of patients</th>
<th>T cells</th>
<th>T helpers</th>
<th>T killers/ suppressors</th>
<th>B cells</th>
<th>IgA+ B cells</th>
<th>IgM+ B cells</th>
<th>Macrophages</th>
<th>IgG+ macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td>I- Weak infiltration</td>
<td>0.43±0.10</td>
<td>0.10±0.20</td>
<td>0.47±0.17</td>
<td>0.45±0.07</td>
<td>0.24±0.09</td>
<td>0.30±0.12</td>
<td>2.86±0.34</td>
<td>1.55±0.81</td>
</tr>
<tr>
<td>II- Moderate infiltration</td>
<td>0.69±0.27</td>
<td>0.18±0.08</td>
<td>0.47±0.19</td>
<td>0.68±0.24</td>
<td>0.22±0.11</td>
<td>0.16±0.08</td>
<td>4.32±0.27</td>
<td>4.4±1.01</td>
</tr>
<tr>
<td>III- Acute inflammation</td>
<td>8.4±1.65</td>
<td>3.5±0.65</td>
<td>1.89±0.28</td>
<td>2.15±0.4</td>
<td>35±0.4</td>
<td>3.1±1.02</td>
<td>16.3±1.62</td>
<td>7.2±1.82</td>
</tr>
</tbody>
</table>

*a* Significantly different from group I, p<0.01  
*b* Significantly different from group II, p<0.01
In the maternal part of the placentas with low and moderate lymphoid-macrophageal infiltration (groups I and II), SC and J chain were found in the decidual cells. The immunoreactivity of IgA, IgM and IgG was low in approximately half of the decidual cells, and a few lymphocytes were present (Table XV). The number of macrophages in the maternal part of the placentas was significantly lower than in the fetal part in the same groups of patients (Fig. 14).

In the maternal part of the placentas with acute chorioamnionitis (group III), SC and J chain were present in 50 to 70% of the decidual cells; immunoreactivity of IgA, IgM and IgG was very low in 50 to 60% and absent in 23% of the cases. In these placentas, the density of all subsets of lymphocytes studied as well as macrophages increased substantially (Table XV).

The data presented herein demonstrate that in the second trimester of gestation both fetal and maternal parts of the human placenta contain all the typical components of the SIS. In the fetal part of the placenta, SC, J chain, IgA, IgM and IgG are found mainly in the cyto- and syncytiotrophoblast of the chorionic villi and in the epithelium of the amnion. Different subsets of lymphocytes are present in the corresponding stroma. Macrophages are abundant and contain J chain and IgG. All of these components, except IgG, are of fetal origin and are hallmarks of the SIS (58,59,62). In the maternal part of the placenta, the proteins of the SIS are found in the decidual cells, whereas macrophages and different subsets of lymphocytes are seen in the decidual stroma.

Moderate activation of immune processes was not accompanied by significant changes in SIS composition. Large antigenic attacks in acute chorioamnionitis appear to have been the result of a reduction in the immunoreactivity of Igs in both parts of the placenta, and their appearance in the fibrin of the intervillous spaces. These changes are, perhaps, a consequence of Ig excretion.

A change in the content of immunocompetent cells, as a result of increased antigenic attack, make up the second characteristic of the functional SIS activity. In the fetal part of the placenta, a moderate increase in antigenic attack (group II) and the more severe cases of chorioamnionitis (group III) were mostly accompanied by a significant increase in the number of macrophages. Under chorioamnionitis, the number of T-cell killers and suppressors increased non-significantly, while the number of T helpers, B lymphocytes, and IgA- and IgM-positive cells remained unchanged or had a tendency to decrease. In the maternal part of the placenta, a moderate intensification of the immune processes could also be inferred from the moderate reaction of macrophages, without changes in the number of lymphocytes. Chorioamnionitis caused a sharp reaction of the immune system that was manifested in the significant, 5- to 20-fold increase in the number of all types of immunocompetent cells, including Ig-producing B cells.

Thus, a significant difference exists in the reactivities of the fetal and maternal parts of the placenta. The reaction of the fetal part is mainly characterized by the large participation of macrophages, while the reaction of lymphocytes is very weak. Such a reaction is highly typical of the fetal immune response, when insufficiency of the fetus's common immune system and its SIS develops (61).
Fig. 14. The number of macrophages in fetal (F) and maternal (M) parts of the placenta (in 50,000 nm²) in different groups of patients. Groups of patients: I, with little lymphoid-macrophage infiltration; II, with moderate infiltration; III, with acute chorioamnionitis and deciduitis.
At the same time, the reaction of the immunocompetent cells of the maternal part of the placenta is more intense and highly true-to-type. All subsets of lymphocytes studied participated in this reaction, corresponding to the adult immune response (63). Thus, immune reactions of the maternal part of the placenta are similar to the respective reactions of adults whereas the immune reactions of the fetal part of the placenta are similar to those of fetuses.

The SIS has different functions in the fetal and maternal parts of the placenta. Whereas the maternal part protects the mother against paternal antigens from the fetus, the fetal part protects the fetus against macromolecules and microorganisms that can enter from the mother. These data suggest that the placenta has two SISs that differ in their origin, structure, function and especially in their immune reactions (40). All of the components of both parts of the SIS are already present in 13-week-old fetuses and remain during the entire second trimester of pregnancy.

The placental barrier is composed of villous structures, such as the syncytio- and cytotrophoblast, their basal membrane, the stroma, the endothelium and basal membrane of the villous capillaries (2). Nevertheless, the fetus remains vulnerable to foreign antigens that can penetrate through the fetal membranes, the amniotic fluid, and the contacts between the villi and the decidua. This explains the presence of SIS proteins and immunocompetent cells not only in the chorionic villi but also throughout the chorion, amnion and decidua, and these structures should therefore be included in a definition of the placental barrier.

The placenta is a large organ: it weighs about one-third of the fetus, and is almost 300 times heavier than the fetal spleen (2). The active area of the chorion of 36- to 40-week-old fetuses can amount to 11.0±1.3 m² (64). These characteristics reflect the importance of the fetal and maternal parts of the placenta, including their separate SIS, as a major component of the extra-corporal system regulating the mother-fetus relationships.
References
(To 7.1)


(To 7.2)


(To 7.3)


Chapter 8.
Immune Systems in the Pathogenesis of RhD-Hemolytic Disease of Fetuses and Newborns

In previous chapters, we showed that fetal cells occasionally enter the maternal circulatory system, as reflected by ensuring documentation of medical tragedy. When the fetus has the red blood cell D antigen of the Rh group (the fetus is Rh-positive) and the mother does not (she is Rh-negative), the entry of fetal blood cells into the mother's blood will cause an immune response against the antigen. The mother produces antibodies against the D antigen, and these may cross the placenta and start destroying the fetal red blood cells: a condition called hemolytic disease of the newborn (HDN).

This disease was first described by a French midwife in 1609, in a set of twins: the first twin was hydropic and stillborn, and the second was deeply jaundiced and subsequently died of what we now call kernicterus (cit. after 1). The two conditions were not associated again until 1932, when Diamond et al. (2) demonstrated that hydrops and kernicterus were two aspects of the same disease in which hemolysis of the red cells of fetuses and neonates results in extramedullary erythropoiesis, causing hepatosplenomegaly and an outpouring of erythroblasts into the circulation, a condition they termed erythroblastosis fetalis.

The identification of the cause of the hemolysis had to await the discovery of the Rh system in 1940 and the determination, shortly thereafter, that hemolytic disease of the fetus had occurred in an RhD-positive fetus carried by an RhD-negative woman who had been immunized by transplacental passage of RhD-positive red cells during a prior pregnancy (3). Maternal IgG antibodies to RhD traverse the placenta, coating and destroying the RhD-positive fetal red cells and initiating the chain of events that leads to death from fetalis hydrops in 25% of affected fetuses and death from kernicterus in 25% of affected neonates.

Despite the fact that great strides have been made in the ability to determine fetal RhD status, and in preventing and managing of RhD isoimmunization (4-7), HDN remains a great problem in obstetrics and pediatrics (8). The maternal reaction to the RhD antigen is one of strongest and most common maternal alloimmune reactions (1). Although the use of RhD-immunoprevention over the last three decades has made a substantial contribution to the decline of RhD-HDN, RhD sensitization continues to occur in RhD-negative women at a rate 30% to 40% when they are bearing an RhD-positive child (9,10). Despite the availability of an effective preventive measure (11,12), RhD-HDN continues to contribute significantly to infant morbidity and mortality (13). Until recently, ultrasound examination, amniocentesis, and fetal blood sampling are, in many cases, the only tools available to assess the status of fetuses and the risk for hemolytic disease in RhD-sensitive women (14,15). The application of genetic determination is only now taking its first steps towards clinical use (5,16-18).

Modern immunology added new approaches for a better understanding of the pathogenesis of this disease. HDN is no longer considered to result erythrocyte lysis with its attendant consequences, such as hyperbilirubinemia, anemia and compensatory erythroblastosis (1,19). When an RhD-negative mother is exposed to the RhD-positive red cells (usually by transplacental haemorrhage), she develops allo-
anti-D which crosses the placenta and then results in the destruction of fetal red cells. Clinical manifestations of HDN range from asymptomatic mild anemia to hydrops fetalis or stillbirth associated with severe anemia, hyperbilirubinemia and jaundice (20).

Unfortunately, little attention has been paid to the role of the immune system in fetuses and newborns, which are capable of producing an immune response during the second half of the gestational period (21). The fact that Igs are themselves antigens needs to be taken into account. As Rh IgGs, they can induce an immune reaction via the formation of anti-Ig antibodies (22-24). The possibility of immune complex (IC) formation during HDN, such formation having been seen in different infectious diseases of fetuses and newborns (25,26), and a role for these complexes in the pathogenesis of this disease remain unclear.

The mechanism of erythrocyte destruction has been studied in the icteric form of HDN but it has not been proven in the hydropic form of the disease. There is no theory to explain the appearance of different forms of HDN which agrees with the modern conception of pathology and immunology of fetuses, newborns and the placenta. Our knowledge regarding the hydropic form of HDN is scant, and this is one of the reasons for the negative results obtained during the course of its prevention and treatment (10-12). These problems are discussed in more detail in this chapter.

### 8.1) The immune response of fetuses and newborns in RhD conflict

The immunocompetence of fetuses during the second half of gestation and of newborns has been described in many publications (27-30). The immune response of fetuses and newborns is different from that of children and adults. Fetuses synthesize a large amount of IgM together with IgA (31-33) and IgE (34-36) as a manifestation of their immune response to antigenic attack. The synthesis of IgA, IgG and IgM by thymic B cells has been shown in pig fetuses (37).

The fetal immune response to antigenic attacks has its own unique morphological features (38,39). Many T and B lymphocytes are transformed into lymphoblasts, and there is an increase in the number of IgM-synthesizing cells and macrophages (40-42). The reactive centers in the spleen and lymph-node follicles and the mature plasma cells are not formed in fetuses and newborns (43,44), as they are in children and adults (45,46).

The blood level of IgG in infants with any form of icteric HDN (with and without anemia) has been found to vary between 1241±92 and 1395±115 mg/100 ml compared to 12.2±3.3 mg/100 ml in healthy newborns (47). Blood levels of the other types of Igs (IgM and IgA) were also significantly higher in infants with icteric HDN. In infants with the hydropic form of HDN, the blood level of IgG was also higher compared to healthy newborns, but the blood levels of IgA and IgM were significantly lower (Fig. 15).

Morphologically, icterus without anemia is characterized by a significant increase in the number of lymphoblasts (B and T lymphocytes) in the spleen and lymph nodes:
from 2.0±0.8 and 2.4±0.2 in controls to 10.6±3.4 and 10.8±1.6 in the ill infants (47). The number of IgM-positive cells, macrophages and siderophages also increases significantly. In the thymus, features of accidental involution in the weight and area of the cortex are seen. Extramedullar erythroblastosis is low or nonexistent in the spleen, liver and other organs.

In cases of icterus with anemia, the morphological changes of the spleen, lymph nodes and thymus are more distinct (47). The number of IgM-positive cells increases significantly whereas there are only a few solitary IgA-positive cells. B and T lymphocytes and IgM-positive cells are accumulated not only in the spleen and lymph nodes, but also in the liver and lungs. The number of follicles in the spleen per 10,000 μm² of slide section decreases, as does their area. The area of the lymph-node parenchyma also decreases as a result of the lower total number of lymphoid cells. Erythroblastosis is high in the liver and spleen. In the thymus, phase III of accidental involution (AI) is found.

The hydropic form of HDN, seen in 60% to 80% of the cases in immature 20- to 32-week-old fetuses (47), is characterized by a significant decrease in the total number of all types of lymphocytes, IgM-positive cells and macrophages. The follicles in the spleen disappear or are present as solitary groups of lymphocytes. In the red pulp of the spleen and in the liver, the number of nuclear erythrocytes, especially erythroblasts, increases significantly. Lymph nodes exhibit signs of devastation: sinuses are wide, containing single macrophages without phagocytic features. The parenchyma contains only stromal cells and a small amount of lymphocytes. The thymus shows phases III-IV of AI with an increased number and area of thymic corpuscles, which sometimes form cysts.

Although both variations of the icteric form of HDN (with and without anemia) exhibit great similarity in their immune-response characteristics, the icteric form with anemia exhibits those characteristics to a more salient degree. However, this form of HDN exhibits signs of immune-insufficiency. There is a decrease in the total number of T- and B-lymphocytes and lymphoblasts per 10,000 μm² of spleen area, in the number of follicles and their total area, as well as in the areas of the lymph-node parenchyma and of the thymus cortex decreased (47). Concomitant to these features, the number of cells decreases to 30% to 50% of their number in healthy newborns.
Fig. 15. The serum levels of Igs in newborns with different forms of RhD disease (% of healthy newborns)

Groups of infants: A, icteric form without anemia; B, icteric form with anemia; C, hydropic form.
In the hydropic form of HDN, the immune system manifests signs of strong inhibition. The serum levels of IgM and IgA, and the number of IgM⁺ cells decreased significantly (Fig. 15). The number of lymphocytes decreases by up to 10% relatively to healthy newborns. The number of macrophages decreases sharply, and their cytoplasm exhibits dystrophic changes.

An increase in the weight of the spleen and liver is related to erythroblasts and does not compensate for the decreased number of lymphocytes participating in the immune response. These observations confirm the decompensation of the immune system found in fetuses which have died of the hydropic form of HDN (47), or of leukemia with hepatosplenomegaly and fetalis hydrops (48). The development of graft-vs-host disease (GVHD), which sometimes accompanies massive blood transfusions in severe cases of the hydropic form of HDN can also be considered a consequence of this decompensation (49).

The morphological features of the immune system's decompensation during HDN are similar to those described in sepsis of newborns (50,51), except that in the former, these features are more salient. No neutrophilic infiltration of the spleen or lymph nodes has been found in HDN (47). It seems that the syndrome of immune system decompensation is similar to the immune response to infectious and other antigenic effects and depends on the duration and intensity of these effects. The low resources of the immune system in fetuses with HDN may also contribute to this decompensation.

The morphological manifestations of the immune response are different in the different forms of HDN. They are mild in the icteric form without anemia and exhibit characteristics of severe decompensation in the hydropic form. Similar differences have been found with other features of HDN which were directly connected to the effect of RhD antibodies. Anemia and erythroblastosis, AI of the thymus and changes in organ weight, are not significant in the icteric form of HDN, but reach their highest levels in the hydropic form. For example, spleen weight in healthy fetuses increased from 1 g in 24-week-old fetuses to 15 g at week 40 of gestation (52). In the hydropic fetuses, spleen weight increased to the end of gestation up to 476%, and in some cases even up to 1300% (47). Because the rate of cell division is limited, such growth must be the consequence of prolonged processes and should be explained as being the result of the different intrauterine durations of the RhD-antibody effect.

Icterus without anemia should be considered an acute form of the disease, with the effects of RhD antibodies being manifested shortly before or even during delivery. In hydropic fetuses, the effects of RhD antibodies continue for weeks, and sometimes months (47). Icterus with anemia occupies an intermediate position between these two forms of HDN.

Which antigenic effects induce an immune response in infants with HDN? It cannot be microbial complications, such as pneumonia, or an accidental antigenic influence, because the immune reaction in HDN is always manifested at full strength even in cases without complications. We suggest that in HDN the immune response develops as a result of the effects of maternal antigens crossing the placenta. The maternal RhD IgG is one such antigen. Igs contain a large number of antigenic determinants,
idiotypes, including variable domains. Anti-idiotypic antibodies can be formed against every idiotype and against different parts of the IgG molecule (53,54), including antibodies against RhD antibodies.

IgG is connected to the FcγRI cell receptors (55), fixated with complement (56), and then passed through the placenta (57,58). The intensity of this passage during the last weeks of gestation is in agreement with our data relating to the differences in the intrauterine duration of the various forms of HDN. Subclasses IgG1 and IgG3 bind with high affinity to macrophage FcγRI receptors (59). As a result of their synergistic action, the attraction to erythrocytes and their phagocytosis by macrophages increase (60,61), resulting in the increased destruction of erythrocytes during HDN (62). This may be the reason why the titer of IgG subclasses is higher in severe cases of HDN (63,64).

One of the reasons for HDN could be the effect of maternal lymphocytes which pass into the fetal organism as in GVHD. The ability of maternal T lymphocytes to cross the placenta has been described in many publications (65,66), but the fetus has several ways of inhibiting such allogenic lymphocytes (67,68). Nevertheless, this mechanism can be destroyed by congenital immunodeficiency (69-71) or other events, such as a large blood transfusion in LBW infants (72,73).

The pathomorphological and clinical features of GVHD differ from those of HDN (74,75). In an in vitro-developed mixed culture of lymphocytes from RhD− mothers and their RhD+ newborns, a high rate of cellular lysis (58-100%) was seen either via the activation of the maternal lymphocytes and inhibition of newborn lymphocytes, or via the activation of the newborn lymphocytes and inhibition of maternal lymphocytes (76). These data showed that a newborn has his own lymphocytes which react against the maternal ones. Moreover, in male infants with HDN, the karyotype of all lymphocytes was male (46XY), and not female (46XX) as would be expected in lymphocytes of maternal origin (77). All of these studies show that maternal T lymphocytes are not present in the fetus during HDN.

Thus, the icteric form of HDN exhibits distinct features of fetuses' or newborns' immune reaction to external antigenic effects (47,78,79). Similar changes have also been observed in infants with infectious diseases (80-82), maternal pre-eclampsia (83), chorioamnionitis (84), and of other diseases having allogenic effects on fetuses and newborns (43,44).

8.2) The immune antigen-antibody complexes in RhD HDN

Antigen–antibody reactions may occur at the cellular and molecular levels. The first type of reaction develops between cell antigens and antibodies, as in, for example, the relationship between RhD+ erythrocytes and RhD antibodies. The second type of reaction occurs between molecular antigens, such as microbial toxins or separate protein molecules, and their corresponding antibodies. This reaction may be accompanied by the formation of ICs (85,86), namely, soluble ICs (87). A complement may constitute the third component of such a reaction. It participates, for example, in modulating the production of reactive oxygen species by polymorphonuclear leukocytes stimulated by IgG ICs (88).
ICs can circulate in the blood (circulating ICs) and may be eliminated by monocytes and macrophages (89), or removed with the urine (90). IC can be retained in tissues (deposited IC), particularly in the walls of small arteries of different organs, in the glomeruli and epithelium of renal tubules (when removed in the urine) and in the choroid plexus of the brain (91,92). IC deposition may have pathogenic effects on virus infections in fetuses (93).

In the blood of healthy newborns, IC are absent or present in low amounts (47), but they are present in high concentrations in neonates with various diseases (94,95). C1q-bearing ICs, for example, exert complex effects on mature T cells that include both pro- and anti-inflammatory responses. Immuneologic maturation is required for these effects, as cord blood T cells are relatively hyporesponsive to C1q-bearing ICs relatively to adult T cells (96). ICs appear as a manifestation of both infectious and inflammatory immune processes (97), as a reaction to fetal and neonatal cytomegalovirus infection (98), rubella (99), early Lyme disease (100), tropical spastic paraparesis (101), syphilis (102), herpes virus infection (103), and in preeclamptic gestations (104). The ICs themselves do not cross the placenta, but are trapped in the placental stroma (105,106). Transfer across the syncytiotrophoblast of the chorionic villi is mediated by the neonatal Fc receptor. The receptors responsible for trapping ICs appear to be FcγRIa, FcγRIIa, and FcγRIIIa on stromal macrophages, and FcγRII on fetal capillary endothelial cells.

The fixation of exogenous complement occurs in HDN as a result of the activation of erythrocyte receptors to complements CR1 and CR3 (47). No circulating ICs were found in the blood of healthy newborns, but they were found at low concentrations in newborns with signs of intrauterine infections such as maternal chorioamnionitis and neonatal bronchopneumonia. Deposited ICs were absent in the tissues of newborns who died accidental deaths. Igs and exogenous complement were located on the surface of erythrocytes in only 8% of the infants. Fixation of endogenous complement was not detected.

In newborns with the icteric form of HDN without anemia, no circulating ICs were found at delivery. These ICs appeared on days 2 and 3 post-delivery in moderate amounts (Table XVI). Igs were revealed on the surface of erythrocytes in 90% of the infants. Exogenous complement was found in 100% of the infants, whereas endogenous complement was found in only 20% of the cases studied (47). It seems that this form of HDN is an acute process which develops shortly before or even during delivery, and the fetus has no time to develop its immune reaction to its full extent. In dead newborns, deposited IC are not detected in the tissues. A small amount of circulating ICs and a lack of deposited ICs were found in newborns with congenital bronchopneumonia (47).

In icterus with anemia, 90% of the newborns had a high amount of circulating ICs, which did not change on days 2 and 3 after birth (Table XVI). Igs were found on the surface of erythrocytes in 78% of the infants, exogenous complement - in 100% of them, and endogenous complement - in 20% of the cases (47). Deposited ICs were found in 62% of the dead infants, in the walls of the small arteries of the spleen, lungs, skin, in the choroid plexus, glomeruli and renal tubules, but morphological features in these cases were scarce. ICs contained exogenous complement, but IgM,
IgA and IgG were not found. In parallel, small pathological features were revealed in the arteriolar wall of these organs: pyknosis of endothelial nuclei, fibrinoid necrosis of subendothelial tissue and the adjacent muscular cells, lateral thrombus with IgM and complement.

In the hydropic form of HDN, circulating ICs were absent (Table XVI). Igs on the surface of erythrocytes and exogenic complement were found in 100% of the cases studied (47). Endogenous complement was revealed in only 30% of infants. In the epithelium of the convoluted renal tubules, there was a weak reaction between Igs and endogenous complement, and a high level of exogenous complement. It seems that the absence of circulating ICs in newborns with the hydropic form of HDN is a result of decompensation of their immune system.

We suggest that in newborns with HDN there are two types of the immune reaction. One is the known interaction between fetal RhD+ erythrocytes and maternal anti-RhD IgG (64,107). RhD antigen was found in many organs and in the in vitro developed cells (108). However, there are only a few publications describing morphological changes which could be related to the direct effects of RhD IgG on organs and tissues other than erythrocytes (47,109). The second reaction is the immune response of the fetus or newborn to molecular maternal antigens with the formation of antibodies and circulating ICs. Fetuses should be considered at risk for HDN due to maternal red cell alloantibodies (110,111). Antibodies in the IC are represented by IgM and are, therefore, of fetal origin. The most likely suggestion is that the ICs arise from maternal molecular antigens, including RhD IgG, that cross the placental barrier, and fetal anti-idiotypic IgM.

These data reflect the relationship between IC formation and the time needed to form an immune response in the fetus. The formation of antibodies as a primary immune reaction was revealed on days 3 to 5 and peaked on days 14 to 25 after the start of the antigenic effect (112). This is in agreement with the supposed antenatal duration of both variants of the icteric form of HDN. The age of gestation or degree of immaturity are less significant, because after the 34th week of gestation, when the icteric form of HDN develops, the fetus possesses immunocompetence. However, in the hydropic form of HDN, which begins after 16 to 20 weeks of gestation, decompensation of the immune system can be related to a weak reserve of this system in LBW fetuses (1).
Table XVI.
Concentration of circulation immune complex (IC) (IU=\text{E}_{450} \times 10^3) in the serum of newborns (mean ± SD) (After ref. 47)

<table>
<thead>
<tr>
<th>Groups of infants</th>
<th>n</th>
<th>IC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control\textsuperscript{a}</td>
<td>24</td>
<td>3.9±1.4</td>
</tr>
<tr>
<td>Icterus without anemia at birth</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>The same on days 2-3 of disease</td>
<td>9</td>
<td>17.1±4.4\textsuperscript{b}</td>
</tr>
<tr>
<td>Icterus with anemia at birth</td>
<td>10</td>
<td>22.3±6.1\textsuperscript{b}</td>
</tr>
<tr>
<td>The same on days 2-3 of disease</td>
<td>10</td>
<td>24.5±6.8\textsuperscript{b}</td>
</tr>
<tr>
<td>Hydropic form of HDN</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Control includes healthy newborns and those who suffered of pneumonia.

\textsuperscript{b} Significantly different from controls, p<0.05-0.01.
In some cases of the icteric form of HDN, the amount of circulating ICs can be significantly higher than that in congenital bronchopneumonia (47) and is similar to that in congenital cytomegalovirus infection or rubella (98,99), or other prolonged fetal infections. This reflects the significance of such immune processes during HDN.

The formation of large amounts of circulating ICs may have some pathological consequences. As with any acute process, ICs are deposited in the walls of small arteries with the subsequent development of microangiopathy (47). This process is accompanied by purpura until the appearance of generalized disseminated intravascular coagulation of the blood, as occurs with fulminate meningococcemia (113). In HDN, these processes are rarely seen, and only weakly manifested. This means that the pathogenic effects of ICs during HDN are not highly significant.

IC formation may be one of the mechanisms underlaying the binding and elimination of strange antigens from the organism. In the blood, ICs are fixed on the receptors of erythrocytes (114) and passed to the liver or spleen where they are destroyed by phagocytosis. In this case, the erythrocytes play the role of a transport system, and are not destroyed. ICs are removed from the organism through the kidneys, sometimes with no damage (90). The morphological features of this process, including IgM deposition and strong fixation of exogenous and endogenous complements, were found in the renal glomeruli and epithelium of convoluted tubules during icterus with anemia and the hydropic form of HDN.

Significant negative relationships between the blood level of ICs and illness severity, the rate of Ig fixation on erythrocytes and the amount of indirect bilirubin were found in cases with icteric HDN ($r = -0.55$, $p<0.05$) (47). The immune destruction of erythrocytes in the icteric form of HDN is weaker at high blood concentrations of IC. This indicates the significant role of IgM and IC in the elimination of maternal RhD IgG. The higher the number of RhD IgG bound to ICs, the lower their amount fixed to erythrocytes, and the lower the amount of the destroyed erythrocytes as well as of toxic indirect bilirubin. It seems that the absence of jaundice in the anemic form of HDN and the subclinical characteristic of this disease (1) can be explained not only by sufficient function of the liver but also by the fact that the elimination of RhD IgG proceeds mainly as part of the IC.

During HDN, the infant's endogenous complement does not participate in the reaction between RhD$^+$ erythrocytes and anti-RhD IgG. But in HDN, receptors for complements CR1 and CR3 are activated on blood cells (110,111), whereas the blood level of complements decreases as a result of their consumption. At the same time a small amount of endogenous complement on the surface of erythrocytes was found in 24.5% of the cases studied (47). The participation of the endogenous complement in the formation of ICs during HDN was revealed in about 60% of the cases.
Mechanism of immune destruction of erythrocytes in RhD-HDN

Erythrocytes are destroyed in two main manners: i) intravascular lysis, and ii) extravascular erythroclasia via phagocytosis by macrophages (115-117). The first process is manifested by an increase in free hemoglobin (Hb) in the blood plasma. Hb is connected with haptoglobin in a complex which is captured by hepatocytes and converted in their cytoplasm. As a result, the amount of iron from the destroyed heme accumulates in hepatocytes in the form of hemosiderin. Hb has almost the same molecular mass as albumin (60 kD) and without haptoglobin easily crosses the renal filter. When the resources of haptoglobin in the blood are exhausted, Hb appears in the primary urine. About 10% of it is reabsorbed by the epithelium of the renal tubules (118), when it causes iron accumulation in the epithelium. Necrosis of the epithelial cells is accompanied by the appearance of hemosiderin in the urine. In healthy adults, plasma levels of Hb are 0.6 to 1.0 mg/dl, and in newborns, the number varies between 1.0 and 20.0 mg/dl (116).

Extravascular destruction of erythrocytes is performed mainly by macrophages of the spleen (119) and, to a lesser extent, by those of the bone marrow, liver, lymph nodes and blood monocytes (120-122). During the enzymatic disintegration of erythrocytes, indirect bilirubin is produced from the heme, and its level in the blood increases. Iron from heme in the form of hemosiderin accumulates in the cytoplasm of macrophages. This last process occurs only in the organs in which erythrocytes are being destroyed. Later, iron in the form of ferritin is transported by blood in the liver and bone marrow (123). There is an introductory stage to the destruction of erythrocytes. First, they divide in the blood vessels into small (0.5-3.0 μm) rounded erythrocyte fragments or micro spherocytes containing Hb (124). At this stage, the plasma level of Hb does not increase. Then, during the next hour, 50% to 80% of these erythrocyte fragments undergo phagocytosis by macrophages (125).

The destruction of erythrocytes during RhD HDN is considered to be extravascular erythroclasia (126). The mechanism of hemolysis in fetuses and newborns with HDN, especially in its hydropic form, was detailed in one of our publications (47). Based upon the results of plasma-Hb determinations, patients were divided into three groups. The first group included healthy newborns or infants who had died of nonimmune and noninfectious diseases and served as a control. The second group included newborns with the icteric form of HDN and a level of Hb similar to controls. The third group consisted of patients with a high plasma-content of Hb who showed the hydropic form and sometimes the severe icteric form of HDN with anemia.

In control newborns, plasma Hb level was 15 ± 8.7 mg/dl with variations between 2.8 and 25.0 mg/dl (Fig. 16). Single erythrocytic fragments (0-1 per 10,000 μm²) were seen in the blood smears and in vessels of the spleen, liver, lungs and kidneys. The number of siderophages and the level of phagocytosis of erythrocytes by macrophages in the spleen and bone marrow were low (0-5 per 10,000 μm²). Ferritin was present in the veins of the spleen and liver in the form of small separate crystals.

In newborns with the icteric form of HDN without anemia, the plasma level of Hb
was 11.5±8.3 mg/dl, with variations between 0.6 and 24.1 (Fig. 16). The number of erythrocytic fragments was 5 to 10 per 10,000 μm² in blood smears and 10 to 20 per 10,000 μm² in the spleen. Morphological features were similar in all infants who had died of the icteric form of HDN. The number of macrophages with 'swallowed-up' erythrocytes and of siderophages in the red pulp of the spleen increased to 20 to 30 per 10,000 μm². Hemosiderin and lipofuscin were found, but free Hb in the spleen was absent. In 14% of the infants who died at 3 to 4 days of age, a high number of large ferritin crystals was found in the veins of the spleen and liver. Phagocytosis of erythrocytes was moderate in the bone marrow and weak in the liver and lymph nodes. A small amount of hemosiderin was discovered in the Kupffer cells and hepatocytes. Traces of hemosiderin were found in the epithelium of the renal convoluted tubules in 2.6% of the cases. The concentration of iron in the spleen increased to 0.73±0.13 mg/g, compared to 0.12±0.01 in controls (Table XVII).

In infants with the hydropic form of HDN, plasma Hb increased to up 145.0±42.3 mg/dl, with variations from 65 to 250 mg/dl and more (Fig. 16). The morphological features were similar for all dead patients of this group. The number of erythrocytic fragments in blood smears and in the red pulp of the spleen was 5 to 10 per 10,000 μm². In the other organs, only solitary erythrocytic fragments were observed. Only 27% of the infants studied showed weak phagocytosis of the erythrocytes by macrophages in the spleen. The amount of siderophages was small, and free Hb was found in the red pulp of the spleen. Iron was deposited in the fibers of the trabecular capsule and in the stroma of the spleen. Hemosiderin was found to a large extent in the hepatocytes, in 61% of the renal-tubule epithelium, and rarely in the epithelium of the thyroid, pancreas and thymus. The concentration of iron in the liver and kidneys increased significantly, as compared with the other forms of HDN (Table XVII).

Summarizing these data, it can be concluded that although HDN exhibits a common mechanism for the immune destruction of erythrocytes, this process is manifested in different ways in the disease's various forms. In the icteric form, erythrocytes are destroyed mainly through extravascular erythroclasia by phagocytosis and enzymatic disintegration in the cytoplasm of macrophages. Prior to this process, a large proportion of the erythrocytes are fragmented into microspherocytes. The largest amount of these fragments appears in the red pulp of the spleen. The blood levels of Hb during massive fragmentation do not increase, i.e. this destruction is not accompanied by the discharge of Hb (127,128).
Fig. 16. Blood levels of Hb in newborns (mg/dL).

Groups of infants: A, healthy control; B, with icteric form of HDN without anemia; C, with hydropic form of HDN and icteric form with anemia.
Table XVII.
Iron concentration in different organs of infants died of HDN (mean±SD)
(After ref. 47)

<table>
<thead>
<tr>
<th>Organs</th>
<th>Control</th>
<th>Icterus without anemia</th>
<th>Icterus with anemia</th>
<th>Hydrops</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>0.12±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.73±0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.84±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.24±0.01&lt;sup&gt;c,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver</td>
<td>0.21±0.02</td>
<td>0.33±0.05</td>
<td>0.56±0.07&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>0.68±0.07&lt;sup&gt;c,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.07±0.01</td>
<td>0.07±0.01</td>
<td>0.17±0.04&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>0.36±0.01&lt;sup&gt;c,d,e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Control group includes infants died of nonimmune and noninfectious diseases.
<sup>b</sup> mg/g of a raw organ.
<sup>c</sup> Significantly different from controls, p<0.05-0.01.
<sup>d</sup> Significantly different from icterus without anemia, p<0.05-0.01.
<sup>e</sup> Significantly different from icterus with anemia, p<0.05-0.01.
Fragmentation is considered a physiological process designed to eliminate old and defective erythrocytes, a process that accompanies disorders characterized by hemolytic anemia (129,130). Our data are in agreement with this suggestion. We found a large number of erythrocytic fragments in the lung alveoli during hemorrhaging and in the vessels of macerated stillborns (47). Fragmentation in the biting off of part of an erythrocyte by a macrophage was not observed in HDN. In fact, in HDN, fragmentation cannot develop due to mechanical trauma to the erythrocytes, because of the paretic condition of the spleen sinuses. The most likely mechanism of erythrocyte fragmentation in HDN, as well as in some other diseases (131,132), consists of changes in erythrocyte structure as a result of biochemical and biophysical processes.

In HDN, as in other alloimmune processes (133,134), the fragmentation begins with maternal IgG. Fifty or more molecules of IgG are bound to the surface of an erythrocyte in healthy people, and this number increases significantly in patients with hemolytic anemia (135). Cellular death of erythrocytes during HDN manifests itself in their fragmentation into membrane-bound bodies. This process initiates as an antibody-dependent mechanism and finishes with macrophage phagocytosis. Thus, this process can be considered apoptosis which in HDN develops into non-nucleate erythrocytes, similarly to that which has been described in other disorders (136-138).

Phagocytosis of fragments and whole erythrocytes in HDN proceeds mainly via macrophages of the red pulp of the spleen. Follicular macrophages do not participate in this process. Phagocytosis by macrophages of the liver, bone marrow and lymph nodes is weak. The utilization of nuclear erythrocytes proceeds mainly in these organs. Each macrophage 'swallows' 5 to 30 erythrocytes and their fragments. The specific histochemical reactions exhibit disintegration of the erythrocytes in the macrophages. Hemosiderin is formed as a result of hem destruction. Due to erythroclasia, the amount of iron in the spleen increases six- or sevenfold relative to controls (Table XVII). From here iron is transported to the liver, where it is deposited. This transport is manifested by an increase in the amount of ferritin and by a positive Perl's reaction of the blood plasma from trabecular veins of the spleen and intrahepatic branches of the portal vein on days 3 to 4 of the disease.

In icterus without anemia, there are no signs of intravascular lysis of the erythrocytes. In icterus with anemia, the main mechanism of erythrocyte destruction is their fragmentation and phagocytosis by macrophages. High intravascular lysis of erythrocytes was found in 6% of the cases, and in 27%, weak intravascular lysis of erythrocytes was exhibited (47). In the hydropic form of HDN, intravascular lysis of erythrocytes becomes the main route of erythrocyte destruction. The clinical and morphological features of this process are similar to those described as hemolysis in other diseases (139,140). In the hydropic form of HDN, the blood-plasma levels of Hb ranged from 65 to 250 mg/dl and more, reflecting intensive hemolysis (116). A large amount of iron is accumulated in the hepatocytes: three- to fivefolds more than in controls. Hb passes through the renal filter (115) and is found as Hb cylinders in the renal tubules. The reabsorption of Hb and its utilization by the epithelium of convoluted tubules (118) causes a three- to fivefold increase in the iron concentration in the kidneys. Because free Hb was found in the red pulp of the spleen, we suggest that intensive lysis of erythrocytes occurs there. In the spleen, the free Hb impregnates the collagen fibers of the capsule, trabeculae and stroma and is transformed into ferro-
The hydropic form of HDN is accompanied by weak fragmentation of the erythrocytes. Their phagocytosis is insignificant or even absent. Lone siderophages were found in 27.5% of the cases studied (47). The absence of phagocytosis may be regarded as reflecting decompensation of macrophage function and of the immune system as a whole. It is not related to the high percentage (76%) of prematurely born infants with the hydropic form of HDN because in infants born at term with this form of the disease, erythrocyte destruction also proceeds via intravascular lysis.

The above described observations have enabled us to add to the body of knowledge related to HDN pathogenesis. The development of the fetal/newborn immune response is shown to be an important process which develops as a result of the effects of maternal antigens, including RhD IgG, crossing the placental barrier (141). This means that the infant is not a defenseless victim in the RhD conflict. The immune response exhibits distinct proliferative characteristics in the icteric forms of HDN. In the hydropic form, severe decompensation of the immune system develops. A direct effect of maternal T lymphocytes (as in GVHD) is not found with HDN.

The various forms of HDN are characterized by the different durations of its intrauterine course. This is proven by the clinical and morphological features connected with the direct effects of RhD IgG: degree of anemia, erythroblastosis, changes in organ weight and morphology. Icterus without anemia runs an acute course and begins not long before delivery. The hydropic form of HDN continues for a long time, perhaps a month or longer. Icterus with anemia holds an intermediate position.

The immune destruction of erythrocytes is effected by different mechanisms in the various forms of HDN. In the icteric form, extravascular erythroclasia is the main mechanism responsible for this process. Phagocytosis of erythrocytes and their fragments occurs mainly in the spleen. Great similarities with apoptosis can be seen in this process. Intravascular lysis is absent in icterus without anemia, but was found to a low degree in 27% of the infants which died of icterus with anemia. In the hydropic form of HDN, intravascular lysis is the main mechanism of erythrocyte destruction. Insignificant erythroclasia was seen in only 27.5% of the cases studied.

The post-natal duration of the effect of RhD IgG is short. Although there are possible long-term complications (anemia, disturbances in the immune response, etc.), direct effects of RhD antibodies last for less than 5 to 7 days after birth. This can be explained by the effective destruction and elimination of these antibodies from the infant's body, a process that takes place via two different mechanisms. The first is the fixation of RhD IgG on the erythrocytes of the fetus or newborn, and their subsequent phagocytosis and destruction together with the erythrocytes. The process is accompanied by severe complications such as massive erythroclasia, anemia and formation of a large amount of toxic indirect bilirubin. The second mechanism manifests itself in the formation of ICs consisting of maternal RhD IgG and antiidiotypic fetal IgM, and their consequent removal through the kidneys or utilization by macrophages. This mechanism is not as severe for an infant as the first, because it does not entail destruction of the erythrocytes and development of hyperbilirubinemia. The active immune reaction of the fetus, the formation of ICs and the
prevalence of the second mechanism for removing maternal antibodies lessen the severity of the HDN.

References


(To 8.1)

61. Shepard, S.L., and Hadley, A.G., 1997, Monocyte-bound monoclonal antibodies inhibit the Fc gamma RI-mediated phagocytosis of sensitized red cells: the efficiency and mechanism of inhibition are determined by the nature of the antigen, Immunology, 90, 314.


(To 8.2)


glomerulonephritis with monoclonal IgG deposits: a distinct entity mimicking immune-complex glomerulonephritis, Kidney Int., 65, 85.


108. Westhoff, C.M., 2007, The structure and function of the Rh antigen complex,
Semin. Hematol., 44, 42.
111. Denomme, G.A., and Fernandes, B.J., 2007, Fetal blood group genotyping, Transfusion, 47(1 Suppl), 64S.

(To 8.3)


Chapter 9.
Pathology of the Immune System in Human Fetuses and Newborns Affected by Infectious Diseases

Infectious and inflammatory diseases are the most widespread reasons for pregnancy loss, stillbirth and neonatal death. Susceptibility of low birth weight (LBW) newborns to infections derives from deficiencies in their cellular and humoral immunological mechanisms. Studies of morphological and morphometric features of the immune reaction of fetuses and newborns have revealed fetal immune incompetence as one of the major reasons for their death or retarded postnatal development (1,2).

To better understand the role of the fetal immune system in the reaction of fetuses and newborns to infectious diseases, we studied the changes in the number of transcriptionally active nucleolar organizer regions (NORs) in splenic lymphoid cells, lymphocytes (resting cells) and lymphoblasts (activated cells) of LBW and full-term fetuses and newborns with microbial infections such as bronchopneumonia and sepsis (3).

9.1) Nucleolar organizer regions in the lymphoid cells of fetuses and newborns with bronchopneumonia and sepsis

NORs are the defined sites in the cell nucleus for the RNA synthesis on chromosomal matrices of DNA (4), and NOR-associated proteins reflect cell-cycle activity during cell differentiation and proliferation (5). These proteins are demonstrated in interphase nuclei by the argyrophilic (silver-staining) nucleolar organizer regions (AgNOR). NORs are important for regulating protein synthesis, and their increase in number from weeks 19 to the 35 of gestation, for example, is indicative of immune system maturation in human fetuses (6,7). NORs are characterized by high polymorphism, as it has been found in 9- to 12-week-old human fetuses (8), and also by high variability in their numbers during human development. In newborns and infants, a relatively high modal number of NORs is found, whereas in older individuals this number is significantly reduced. It has been suggested that at a young age due to the obvious enhanced growth and differentiation, more gene sites may be transcriptionally active and show a higher number of AgNORs, but with advancing age and development, many of these may be gradually repressed or inactivated.

AgNOR evaluations are performed for processes in which cell proliferation plays an important role, such as embryogenesis. It has been shown, for example, that gut-associated lymphoid cell proliferation and maturation in 10- to 35-week-old fetuses can be assessed by determination the number of AgNOR dots (7). NORs have been used to study fetal thymuses (9). It has been shown that type I epitheliocytes (subcapsular-perivascular) of the cortex presents a higher number of AgNORs relative to other cell types between developmental weeks 10 and 15. This reflects their intense protein synthesis, a fact that explains the increased secretion of β2-microglobulin, which releases immature lymphocytes from the yolk sac and liver. A gradual increase in the average number of AgNORs was observed in all thymic epitheliocytes between weeks 10 and 35. This increase might be due to the intense
functional activity of all of the epitheliocytes participating in the proliferation, differentiation and issue in the circulation of mature T lymphocytes, which takes place after week 17 of development. The 17-week-old thymus appears fully differentiated, and begins producing the main type of thymocytes from this stage on throughout life.

NORs were used to study of the cellular activity of the embryonal mesenchyme in order to determine the origin of the primitive lymph vessels (9). A statistically significant difference in an average AgNOR numbers in lymph vessels vs. blood vessels was found in the endothelial and mesenchymal cells during weeks 10 to 15 of gestation. After week 20, no statistically significant difference was found in this parameter. It has been suggested that development of the lymph vessels follows that of the blood vessels. Furthermore, the intense protein synthesis between weeks 10 and 15 of development is an additional proof for the view that the primitive lymph vessels derive from clefts into the embryonal mesenchyma and not from capillary offshoots of the blood vessels endothelium.

AgNOR technique was used in experimental teratology to evaluate the causes of fetal limb deformity under maternal administration of retinoic acid (10). It was found that in 15-day-old rat fetuses, hypoplasia and disorientation of hindlimbs are present in 90% of the cases. The histological study showed a reduction in mitotic and NOR activities of mesenchymal cells, an increase in volume of the vascular lumen, a reduction in the volume of nerve structures, and a reduction in the percentage of pre-rhabdomyoblastic cells. Somitic NOR activity decreased relative to the control group. This finding suggests that a particular pathology of the somites might be involved in clubfoot pathogenesis, and that this pathology is related to a decrease in NOR activity.

We studied of the role of NORs in blast-transformation of lymphocytes in human fetuses and newborns under local (bronchopneumonia) and generalized (sepsis) microbial infections (3). The material for comparison included three groups of 22- to 42-week-old fetuses and newborns. The first group (without infections or other antigenic effects) contained 25 fetuses and newborns who died as a result of intranatal asphyxia, respiratory distress syndrome (RDS), hyaline membrane disease of the lungs, or brain hemorrhage. The second group included 20 fetuses and newborns who died of bronchopneumonia. The third group consisted 27 newborns who died of sepsis. Spleen growth and differentiation in the first group were accompanied by a decrease in the number of follicles per 10,000 μm² and an increase in their area with gestation (Table XVIII). The number of small and medium-sized lymphocytes/10,000 μm² of follicular area increased whereas the number of lymphoblasts decreased. This resulted in a decrease in the mean nuclear diameter of the lymphoid cells. The mean area of the lymphoid cells and the number of AgNOR/nucleus also decreased. A high negative correlation was found between these parameters and fetal age ($r = -0.68, p < 0.01$). The number of mitotic cells was very low, $0.36\pm0.06/10,000 \mu m^2$, representing 0.14% of total cells. In newborns during the first week of life, the number of AgNORs/nucleus decreased from $1.64\pm0.12$ to $1.28\pm0.04 (p < 0.01)$ and then increased within 2 to 4 weeks to $1.5\pm0.04 (p < 0.01)$. These data exhibit good relationships between some cell parameters and AgNOR features (Table XIX).
Table XVIII. Relationship to gestational age in some morphometric and image analysis parameters of lymphoid cells of the splenic follicles in fetuses and infants died without infectious effects (After ref. 3)

<table>
<thead>
<tr>
<th>Parameters studied</th>
<th>22 to 30</th>
<th>32 to 42</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphometric analysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of follicles/10,000 μm$^2$</td>
<td>5.58±0.6$^a$</td>
<td>3.7±0.6$^b$</td>
</tr>
<tr>
<td>Average area of a follicle/10,000 μm$^2$</td>
<td>2.7±0.6</td>
<td>4.21±0.3$^b$</td>
</tr>
<tr>
<td>Total number of lymphoid cells$^c$</td>
<td>127.2±14.9</td>
<td>129.6±9.8</td>
</tr>
<tr>
<td>Number of small and medium lymphocytes$^c$</td>
<td>73.2±3.6</td>
<td>86.6±5.3$^b$</td>
</tr>
<tr>
<td>Number of lymphoblasts$^c$</td>
<td>6.98±0.6</td>
<td>4.24±0.6$^b$</td>
</tr>
<tr>
<td><strong>Image analysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuclear area, μm$^2$</td>
<td>22.3±1.3</td>
<td>15.65±1.3$^b$</td>
</tr>
<tr>
<td>Area of AgNOR, μm$^2$</td>
<td>2.2±0.14</td>
<td>1.55±0.2$^b$</td>
</tr>
<tr>
<td>Number of AgNOR/nucleus</td>
<td>1.5±0.08</td>
<td>1.12±0.07$^b$</td>
</tr>
</tbody>
</table>

$^a$ Mean ± SD.

$^b$ Significantly different from values in young fetuses, $p<0.05$-0.01.

$^c$ Per 10,000 μm$^2$. 
Table XIX.  
Coefficients of correlation between AgNOR features and some cell parameters 
(After ref. 3)

<table>
<thead>
<tr>
<th>AgNOR features</th>
<th>No of small and medium lymphocytes</th>
<th>Number of lymphoblasts</th>
<th>Number of mitoses</th>
<th>Nuclear area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear area</td>
<td>- 0.67</td>
<td>0.61</td>
<td>0.49</td>
<td>-</td>
</tr>
<tr>
<td>AgNOR area</td>
<td>- 0.77</td>
<td>0.85</td>
<td>0.58</td>
<td>0.81</td>
</tr>
<tr>
<td>No AgNOR/ nucleus</td>
<td>- 0.69</td>
<td>0.67</td>
<td>0.47</td>
<td>0.98</td>
</tr>
</tbody>
</table>
Table XX.
Effects of infectious antigens on some morphometric and image analysis parameters of lymphoid cells of the splenic follicles in fetuses and infants (After ref. 3)

<table>
<thead>
<tr>
<th>Parameters studied</th>
<th>Without infection</th>
<th>With pneumonia</th>
<th>With sepsis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphometric analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of follicles/10,000 μm²</td>
<td>5.1±0.5⁵</td>
<td>4.9±0.6</td>
<td>1.32±0.4³</td>
</tr>
<tr>
<td>Average area of a follicle/10,000 μm²</td>
<td>4.5±0.5</td>
<td>3.4±0.7</td>
<td>1.63±0.2³</td>
</tr>
<tr>
<td>Total number of lymphoid cells c</td>
<td>129.3±6.2</td>
<td>112.7±3.6³</td>
<td>92.3±5.2³</td>
</tr>
<tr>
<td>Number of small and medium lymphocytes c</td>
<td>83.6±5.6</td>
<td>56.3±3.4³</td>
<td>63.1±2.2</td>
</tr>
<tr>
<td>Number of lymphoblasts c</td>
<td>5.7±0.5</td>
<td>32.8±3.1³</td>
<td>24.5±1.7³</td>
</tr>
<tr>
<td>Number of mitoses c</td>
<td>0.36±0.06</td>
<td>1.28±0.24³</td>
<td>0.43±0.06</td>
</tr>
<tr>
<td><strong>Image analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuclear area, μm²</td>
<td>20.5±1.14</td>
<td>23.2±1.02</td>
<td>20.4±0.77</td>
</tr>
<tr>
<td>Area of AgNOR, μm²</td>
<td>1.9±0.2</td>
<td>2.7±0.2³</td>
<td>2.04±0.2</td>
</tr>
<tr>
<td>Number of AgNOR/nucleus</td>
<td>1.4±0.1</td>
<td>1.7±0.06³</td>
<td>1.4±0.06</td>
</tr>
</tbody>
</table>

⁵ Mean ± SD.
³ Significantly different from values in the first group, p<0.05-0.01.
⁶ Per 10,000 μm².
The development of an infectious disease such as bronchopneumonia in the perinatal period is characterized by very active transformation of lymphocytes into lymphoblasts, resulting in a significant increase, compared to patients without infections, in the number of lymphoblasts and a significant decrease in the number of lymphocytes (Table XX). The main types of cells were CD20+ cells (B lymphocytes) and CD3+ cells (T lymphocytes). CD45RO+ cells were seen in lower amounts (3). The number of dividing lymphoid cells remained small (1.28±0.24/10,000 μm², or 1.58% of all cells), whereas the number of AgNORs and their area increased significantly. The area of the lymphoid follicles and the number of cells/10,000 μm² decreased, reflecting a decrease in the total number of lymphoid cells in the spleen: from 2936±139/10,000 μm² of follicular area in infants without infection to 1894±54/10,000 μm² in infected infants.

Sepsis causes severe morphological changes in the lymphoid organs (Table XX). The number of the splenic follicles and their area as well as the number of lymphoid cells in the follicles decreased relative to the group with bronchopneumonia. These changes were reflected in the presence of so-called "bare" central arteries in the spleen. Transformation of lymphocytes into lymphoblasts decreased, and this was manifested by a 25% decrease in the number of lymphoblasts as compared to the number in bronchopneumonia. The number of mitotic cells decreased from 1.28±0.24 in cases with bronchopneumonia to 0.43±0.06/10,000 μm² with sepsis. The number of AgNORs/nucleus decreased and their area tended toward a decrease. Some dystrophic and necrotic changes were found in lymphocytes. A good correlation was found between the number of AgNORs and the number of lymphoid cells ($r = 0.67$ and 0.85), as well as between the former and the rate of mitosis ($r = 0.97$).

The development of lymphoid organs (growth, differentiation and maturation) in 22- to 42-week-old fetuses is accompanied by an increase in the relative number of small and medium lymphocytes and by a decrease in the relative number of lymphoblasts (1,2). The percentage of lymphocytes in fetal white blood cells is 79% in early second- and third-trimester fetuses, but decreases to 40% at birth (11). Age-related changes have been found in cytokine production, immunoproliferative T-lymphocyte response and NK cell activity in newborns, children and adults (12). After birth, alterations in some of the morphological and morphometric parameters of the splenic cells and their AgNORs were seen even in uninfected newborns (2,3). The changes were usually weak and are overshadowed by the high antigenic effects that accompanying physiological contamination of the infant after birth, especially as related to infections.

The development of infection causes considerable changes in the infant's lymphoid system, and the main manifestation of this process is the transformation of lymphocytes into lymphoblasts (1,2). The number of lymphoblasts in bronchopneumonia increased to up 32.85±3.11/10,000 μm², as compared to 5.69±0.48/10,000 μm² in infants who died of noninfectious diseases. The number of mitoses per 10,000 μm² in the lymphoid cells of infants who died of bronchopneumonia increased to 1.28±0.24, as compared to 0.36±0.06 in children who died of non-infectious diseases. This may explain the absence of germinative centers in the splenic lymphatic follicles. An
increase in AgNOR numbers also accompanies the process of lymphocyte transformation to lymphoblasts in newborns subjected to infectious influences (3). Both fetuses infected with cytomegalovirus, rubella and toxoplasmosis and their mothers have significant identifiable changes in white cell counts and T-lymphocyte subpopulations compared to controls (13). The percentage of CD3+ and CD8+ lymphocytes was significantly higher in infected mothers compared to controls, while the percentage of CD19+ lymphocytes and the CD4+/CD8+ ratio were lower. Infected mothers carrying infected fetuses had significantly lower white blood cell counts compared to those infected mothers without fetal infection. The percentage of CD3+ T lymphocytes was significantly higher and the CD4+/CD8+ ratio lower in infected fetuses compared to controls and noninfected fetuses in infected mothers. This is in line with observations relating to an extreme increase in RNA and protein synthesis, which was found to parallel the activation of the lymph system during immune reaction (14,15).

In the presence of severe infections such as sepsis, the size and number of AgNORs per nucleus decrease significantly, reflecting inhibition of the infant's immune system. This process is thought to be a manifestation of the general decompensation of the lymphoid system which develops as a result of severe antigenic effects. In newborns who died of sepsis, there was an almost 15-fold decrease in splenic lymphatic cells as compared to unaffected infants. It has been suggested that in newborns suffering from bronchopneumonia there was already a significant decrease in the number of these cells, including lymphoblasts, and that this process just became more severe in sepsis (3).

The spleen is the main organ of the immune reaction in fetuses and newborns, and its follicles are the main site of lymphoid cell concentration. The disastrous decrease in the number of these cells reflects an inadequacy of the newborn's immune response to the severe infections. A possible explanation for this is that an increases in the number of lymphoblasts in fetuses and newborns occurs not only by mitotic division, as in children and adults, but also by transformation of lymphocytes to lymphoblasts (16). This results in the rapid depletion of the total amount of lymphatic cells in the organism.

The generalized devastation of lymphoid organs is the major pathological process reflecting the exorbitant antigenic effects on a developing organism under sepsis. In septicemia, for example, the more common type of sepsis in the perinatal period, this devastation is the basis for pathomorphological diagnosis of the disease. A similar picture characterizes the Rh-HDN or severe inflammatory processes in pregnant mothers (17). A high correlation between the changes in NORs in lymphocytes and lymphoblasts and the state of the lymphoid organs in sick infants suggests that these changes occur not only in the lymphocytes of the splenic follicles but also in those of the peripheral blood.

Changes in NORs and nuclei of the splenic lymphoid cells may reflect their role in the immune reaction of fetuses and newborns to infections such as bronchopneumonia and sepsis. These data agree with the pathomorphological picture and reflect a close relationship between NORs and the synthesis of DNA, RNA and proteins in cells (5). The changes in lymphoid cell NORs are connected with these cells' reaction to
infectious antigenic effects. NORs changes can be used as a reliable parameter in diagnostic practice for evaluating the immune reactive process in newborns.

9.2) Intraorganic immunity of fetuses and newborns with infectious diseases

At least two types of IgA circulating in the blood and lymph are recognized: serum IgA and the so-called secretory IgA (sIgA). The latter is secreted by cells within mucosal membranes, and is more important than sIgG or sIgM (18). sIgA is considered to be an important part of an organism's protective mechanism against penetration of infection through the mucosal membranes of the respiratory and gastrointestinal tracts, and urogenital organs.

In fetuses, IgG and IgA responses are expressed by B cells (19,20). T helper cells are present and functional, but their capacity to drive IgG and IgA responses is impaired. Development of clonal diversity for both T and B cells begins during the first trimester of human gestation and is far advanced by mid-gestation (21). It has been well documented that in normal human intrauterine development, IgA increases on the syncytiotrophoblast at 8 to 10 weeks of gestation; IgE is present on the surface of the trophoblast only during spontaneous labor (22); and IgG is observed on the syncytiotrophoblastic cell membrane and basement membrane and in the cytoplasm and nuclei at all stages of gestation (23,24). The detection of the sIgA in the amniotic fluid in the early periods of pregnancy leads to the assumption of IgA in the amniotic membranes (25). The presence of IgA in the chorion and decidua suggests that the placenta is a first barrier against infection of the amniotic cavity (26,27).

In addition to the predominant maternal IgG, the amniotic fluid contains different molecular forms of fetal Igs. These function as an immune barrier against infection and against mother-derived autoantibodies. In studying the molecular status of antibodies in the human amniotic fluid, IgG is found to be the major isotype, IgA is much less abundant, and IgM is not detected at all (21). IgA is monomeric, with a low level of sIgA and with various amounts of free SC. The presence of a low level of SC-containing Igs of small size is confirmed during the last trimester of pregnancy.

Abnormal fetal development caused by infections or inflammation is characterized by a high blood concentration of different types of Igs (28,29), but this is not the case with antigenic effects (30). Several types of autoantibodies can be found in some sera of newborns, and increased IgM concentration may reflect a polyclonal antibody response (31). Cytomegalovirus infection or Toxoplasma gondii cause the appearance of IgM antibodies in the fetal blood (32,33). Neospora caninum-specific fetal IgG and IgM antibodies were detected in cattle inoculated with N. caninum at mid-gestation (34). Elevated IgA and IgM levels have been found in abnormalities of the central nervous system and in congenital malformations (35,36).

Amniotic fluid levels of sIgA increase significantly during normal pregnancy (21,37). sIgA has been estimated in the amniotic fluid of the third trimester of pregnancy and in mucus samples of pharyngeal cavities and urine of newborns to test the hypothesis that there is a connection between sIgA content in the amniotic fluid and fetal pulmonary maturity (38). Small amounts of SC and only a few IgM-, IgD- and IgG-producing cells were present in the tracheal surface and gland epithelium during the fetal period and increased towards term, but no IgA- or IgE-producing cells were
found (39). These features probably reflect local activation of the immune system in response to environmental factors.

In fetuses with prematurely ruptured membranes, an increase in the concentration of IgA has been found in the chorioamniotic membrane (40). Preterm premature rupture of membranes and microbial invasion of the amniotic cavity are associated with a robust host inflammatory response in the fetal, amniotic, and maternal compartments (41). IL18, a proinflammatory pleiotropic cytokine that has been implicated in the host defense against infection, increased in cases of the microbial invasion of the amniotic cavity (42).

In the premature infants who were either stillborn or died shortly after delivery (gestational age 24-32 weeks), in full-term infants who died during the first 3 weeks after birth, and in infants who died in the postneonatal period, only a few IgM- and IgG-producing cells were present in the duodenal mucosa throughout the period studied, while no IgA immunocytes were seen before the first week after birth (39). The appearance of IgA immunocytes suggests that the intestinal immune system is modulated in response to environmental factors shortly after birth.

Among fetal infections, candidal chorioamnionitis is an uncommon and apparently rather indolent intrauterine infection in which the fetus is able to marshal some of the immunological forces at its disposal against an easily visualized antigen impinging on lung mucosal surfaces (43). Chorioamnionitis was associated with an intrauterine inflammatory response of the fetal lung characterized by a severe infiltration of macrophages, neutrophils, and lymphocytes as well as by increased expression of IL8 mRNA (44). Apoptosis and proliferation are important features of chorioamnionitis-associated lung injury: chorioamnionitis induces apoptosis of distal airway epithelial cells via the caspase-8 pathway and interferes with the normal proliferative activity of epithelial, endothelial, and smooth muscle cells in fetal lungs (45).

The inflammatory response in candidal chorioamnionitis is manifested in the Ig-containing lesions, which probably originated in dense-staining plasmacytoid and immunoblastic cells in the inflammatory infiltrates (46,47). The finding of giant cell pneumonitis suggests that the fetus can mount a brisk inflammatory and immune response at as early as 18 weeks of gestation and that mucosal exposure to this antigen can result in IgA production by the lungs. A similarly high amount of specific T. gondii IgA antibody has been found in cord blood and in neonatal blood, 64% and 66%, respectively (48).

The presence of granular deposits of Igs within the vessel walls with acute atherosis may be related to an immunological disorder, probably mediated by immune complexes. Acute atherosis associated with human fetal growth restriction (FGR) is manifested in massive intramural deposits of IgM, and slight deposits of IgA and IgG (49,50). No intramural deposition of Igs or complement has been observed in vessels with or without physiological changes. In severe pregnancy-induced hypertension, there are depositions of immunocomplex and complement on the vessel walls of the chorionic villi and decidua (51,52). Immunological factors play an important role in the development of this disorder: the positive expression rates of the IgA, IgG, IgM and C3 in the vascular wall were significantly higher than those in normal-term pregnancies. Patients with severe vessel lesions had a significantly greater incidence of fetal loss than those with only mild to moderate lesions.
Evaluation of the maternal-fetal interface reveals an increased deposition of Igs that may be associated with a common antigen as an immunological etiology for preeclampsia (53). Antiphospholipid antibodies, for example, may play a pathogenic role in some cases of preeclampsia: elevated levels of IgG or IgM to cardiolipin and phosphatidylserine were detected in 11% of women with preeclampsia in the third trimester, compared to only 3% in controls (54).

Chorioamnionitis is considered an important risk factor for early-onset infection in premature newborns. Septicemia, pneumonia or omphalitis were documented in 20% of infected premature newborns, and inflammatory lesions in the placenta were observed in all of them (55). The probability of neonatal infection in premature newborns was 62.5% when polymorphonuclear neutrophils were present in the chorion and amniotic membrane, as compared to 0.5% when these tissues were normal.

The frequency of clinical chorioamnionitis in preterm premature rupture of the fetal membranes increases with the duration of the interval between membrane rupture and delivery (56). The prevalence and severity of pathological evidence of intrauterine infection is also correlated with the interval between membrane rupture and delivery. The amount of IgA in the chorioamniotic membrane was 24.58 mg/dl in patients whose membranes had been ruptured for longer than 10 h, as compared to 2.52 mg/dl in membranes which had been ruptured for less than 10 h (40). These data indicate that the increasing IgA in patients after 10 h of latency probably represents the beginning of an ascending colonization of bacteria which could be a source of the impending infection.

A characterization of the fetal-derived inflammatory cell reaction may be important in understanding of both the intrauterine and the antenatal immunological response of the neonates to viral infection. The marked hyperplasia of fetal-derived placental macrophages (Hofbauer cells) is considered an example of the immunological features of the fetal inflammatory response to placental cytomegalovirus infection (57,58). Lymphocytic villitis is characterized by the presence of T-cell and not B-cell antibodies. The plasmacellular villitis contains both IgG- and IgM-secreting cells at as early as the second trimester of gestation. No IgA-positive plasma cells are observed. CD3+ lymphocytes predominated in syphilitic villitis, with slightly more CD8+ cells than CD4+ cells (59). CD68 and HLA-DR-positive cells are as frequent as CD3+ cells, but B-lymphocytes are rare.

Neither serum IgM nor IgA or sIgA cross the placenta (60). In fetuses, the serum IgA is formed in the presence of perinatal infections (61) and other antigenic effects such as HDN (17). A newborn receives sIgA with the colostrum and maternal milk (62). The sIgA plays an important role in the development of local immune reactions in the gastrointestinal tract normalizing microbiocenosis (63). This is expressed by an increased amount of IgA-secreting cells and SC synthesis (39). The high importance of sIgA in children is reflected in the fact that the synthesis of the child form of sIgA and its movement through the mucosal membranes increase sharply during the first three months after birth and it reaches adult levels in 2-year-olds (64).

Neither serum IgM nor IgA or sIgA cross the placenta (60). In fetuses, the serum IgA is formed in the presence of perinatal infections (61) and other antigenic effects such as HDN (17). A newborn receives sIgA with the colostrum and maternal milk (62). The sIgA plays an important role in the development of local immune reactions in the gastrointestinal tract normalizing microbiocenosis (63). This is expressed by an increased amount of IgA-secreting cells and SC synthesis (39). The high importance of sIgA in children is reflected in the fact that the synthesis of the child form of sIgA and its movement through the mucosal membranes increase sharply during the first three months after birth and it reaches adult levels in 2-year-olds (64).

The distribution and functional activity of sIgA in mucosal membranes and lymphoid organs were studied in full-term and LBW fetuses and newborns (65). Thirty-eight
fetuses and newborns were divided into three groups. The first group (without infections) contained 15 fetuses and newborns who had died as a result of non-antigen-induced diseases, such as intranatal asphyxia, RDS, or brain hemorrhage. The second group included 10 fetuses and newborns who had died of bronchopneumonia. In the third group, 13 fetuses and newborns were included who had died of sepsis.

In fetuses from the first group (20 to 21 weeks of gestation), IgA was found in two types of cells: one type presented B lymphocytes of the spleen and lymph nodes; the other presented the epithelium of the trachea and bronchi, their submucosal glands, and the epithelium of hepatic bile ducts. This IgA is excreted into the lumina of the bronchi and bile ducts, and is, therefore, considered to be sIgA. The number of IgA-secreting epithelial cells varied in the organs studied from 2 to 8 cells/10,000 μm², i.e., from 11% to 26% of the total number of epithelial cells on a slide.

The number of IgA-secreting cells increased with the time of gestation. sIgA was not found in the epithelium or lumina of bronchioles, or in the epithelium of the pancreas or renal pelvis. Secretory IgM and IgG were also not found in any of these organs during gestation. However, synthesis of IgM in the spleen proceeded at a higher rate than that of IgA, especially in the presence of antigenic effects. Maternal sIgG enters the fetus with the amniotic fluid from the 18th week of gestation (66). The absence of sIgM and sIgG shows that in newborns sIgA plays a more pronounced role than other types of Igs. Infection caused a significant decrease in the number of sIgA-containing epithelial cells (Table XXI). In the bronchi, for example, their number decreased from 30% in controls to 18% in newborns who had died of broncopneumonia and to 14% in infants who had died of sepsis. In the intrahepatic bile ducts, these cells numbered 48%, 36%, and 14%, respectively. At the same time, the number of IgA-containing granules in the lumina of these organs increased significantly, showing that infection intensifies sIgA secretion.
Table XXI.
The number of IgM- and IgA-containing lymphocytes and epithelial cells in fetuses and newborns with different antigenic and non-antigenic effects (After ref. 65)

<table>
<thead>
<tr>
<th>Type of Ig and organs studied</th>
<th>Groups of patients (^ a )</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM&lt;sup&gt;+&lt;/sup&gt; lymphocytes:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In the follicles of the spleen</td>
<td></td>
<td>1.95±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.61±0.68&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.94±0.78&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>In the red pulp of the spleen</td>
<td></td>
<td>0.83±0.11</td>
<td>1.64±0.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.18±0.42&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>In lymph nodes</td>
<td></td>
<td>1.21±0.11</td>
<td>2.81±0.54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.14±0.53&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>IgA&lt;sup&gt;+&lt;/sup&gt; lymphocytes in the spleen</td>
<td></td>
<td>0.07±0.01</td>
<td>0.18±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.28±0.03&lt;sup&gt;c,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>IgA&lt;sup&gt;+&lt;/sup&gt; epithelial cells in trachea</td>
<td></td>
<td>4.48±0.92</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IgA&lt;sup&gt;+&lt;/sup&gt; epithelial cells in bronchi</td>
<td></td>
<td>5.32±0.77</td>
<td>2.88±0.82</td>
<td>2.12±0.62&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>IgA&lt;sup&gt;+&lt;/sup&gt; epithelial cells in bile duct</td>
<td></td>
<td>5.03±0.88</td>
<td>4.87±0.98</td>
<td>2.48±0.6&lt;sup&gt;c,d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

\(^ a \) Groups of patients: I, died without antigenic effects; II, died of bronchopneumonia; III, died of sepsis.

\(^ b \) Mean ± SE.

\(^ c \) Significance different from group 1, \( p<0.05-0.01 \).

\(^ d \) Significance different from group 2, \( p<0.05-0.01 \).
sIgA participates in different immune processes, such as inhibition of microbial adherence, antigen exclusion, virus and toxin neutralization, and modulation of enzyme activity (67). Fixing on receptors for Fc, fragments of macrophages (18) and neutrophils (68), sIgA may participate in phagocytosis, cause the degranulation of eosinophils (69), provide an alternative way of fixing complement (70), and may participate in antibody-dependent cytotoxicity.

In the presence of infection, the number of sIgA-containing epithelial cells decreased, while the number of IgA-positive lymphocytes in the spleen increased, reflecting the increased synthesis of IgA in this organ (65). The low and inverse correlation between the number of IgA-synthesized lymphocytes and sIgA-containing cells in the epithelium of bronchi \( r = -0.34 \) and bile ducts \( r = -0.31 \) can be explained in several different ways. First, the synthesis of serum IgA and sIgA originates from separate pools of B lymphocytes, and therefore, their contents do not overlap (64). Secondly, although the spleen is the main organ of the lymph system in fetuses and newborns, IgA synthesis occurs in other organs as well, such as the liver, lymph nodes and lymph nodules of the gastrointestinal tract. Finally, a decrease in the number of IgA-positive epithelial cells may be caused by the rapid exhaustion of their SC, the cellular amount of which decreases as a result of IgA secretion into the organ lumina. This last phenomenon can be considered a manifestation of the 'immaturity' of the immune system and its consequent rapid exhaustion under even weak antigenic effects (1,2).

The presence of sIgA in the mucosal membranes of the trachea, bronchi and intrahepatic bile ducts is related to their importance in protecting against physiological contamination by microbes after birth and in preventing inflammatory processes. The presence of a large amount of sIgA in mid-gestation fetuses (20-21 weeks), is considered to be evidence of early maturation of the immune system, or at least this component of it.

### 9.3) Insufficiency of the immune system in fetuses and infants with pneumonia and sepsis

In higher vertebrates, the ability to respond to antigen develops in a slow, controlled, stepwise fashion as a function of ontogeny. The process takes months in humans and lambs, and days to weeks in mice. In humans, the ability to mount an effective humoral response to antigens, including pathogenic bacteria and vaccines, develops in a sequential fashion and is not fully mature until well after infancy (71). The delay in the ability to respond to specific antigens increases young infant's susceptibility to infection, particularly those that are born prematurely. The capacity of lymphocytes to generate a heterogeneous repertoire of antigen-binding receptors lies at the heart of their ability to mount a specific humoral response to diverse antigens. Antibody repertoire development appears to be endogenously controlled and adheres to an individualized developmental progression that probably contributes to the relative immaturity of the neonatal immune response (72). The reasons underlying constraint of the antibody repertoire in the first and second trimester, and its slow developmental progression during the third trimester and early infancy, remain a mystery.
An increase in the synthesis of IgG, IgA and IgM immunoglobulins is one of the characteristics of defense mechanisms which protect fetuses against infection. Infants with congenital toxoplasmosis, for example, show evidence of increased intrauterine IgM and IgA synthesis (73,74). In the serum of newborns and infants born to mothers suffering from different viral and parasitic disease (rubella virus, cytomegalus virus, Listeria monocytogenes, Chlamydia trachomatis and T. gondii), IgA and IgM were found at very high levels, significantly higher than in controls samples (75-77).

Inflammatory diseases and, especially, sepsis can cause infant mortality, premature birth, and LBW infants. The peculiarities of the immune-response mechanism and its relation to pathogenesis in neonates is generally described only as "immature and naive" (78). Thus, the well-known susceptibility of LBW infants to infections is blamed on deficiencies in cellular and humoral immunological mechanisms. Fetal immune incompetence is considered to be a major reason for the high death rate of infants or for retardation of their postnatal growth and development (79). Different parts of the immune system in such infants show signs of underdevelopment, yet little information exists on the possible contribution of the pathology of immune organs to the birth and death of LBW neonates.

Lymphoid organs develop in a controlled, stepwise fashion during ontogeny (80). Organization of the primary structures is not complete until the end of the second trimester. Expression of an expanded repertoire could be deleterious to an infant with a disorganized lymphoid system; expression of the conserved fetal repertoire may play an important protective role, or expression of a "mature" repertoire could be deleterious to the developing infant or to the fetal-maternal balance. Clearly, however, processes critical to the establishment of a mature repertoire are active and changing during the third trimester of gestation. The appearance of secondary structures that represent a reaction to antigen in the primary lymphoid nodules, i.e., follicles, is first observed at 30 weeks of gestation (2). Antigenic effects, such as neonatal sepsis and chorioamnionitis, induce morphological modifications and shrinkage of the lymphoid organs, particularly the thymus (81). Fetuses with chorioamnionitis or neonatal sepsis show spleen-cell depletion, involving both B and T lymphocytes.

In diseases based on bacterial or viral infections, B and T lymphocytes play an essential role, which is reflected by their percentages in the blood. In infants with acute upper respiratory tract infections, for example, percentages of B lymphocytes in the peripheral blood are markedly increased (82,83). Early intrauterine rubella infection has a profound effect on the developing immune system in fetuses that is manifested in complete immune paralysis, Ig abnormalities, and loss of antibodies to rubella (84). These defects are transient, but the absence of IgA may be permanent. No such defects have been observed in other congenital viral infections, but precocious development of Igs and germinal follicles does occur.

Neonatal bacterial sepsis is often characterized by a fulminate clinical course and highly elevated plasma levels of proinflammatory cytokines (85). Activation of cord blood cells by infectious stimuli, such as Streptococcus agalactiae, is comparable to the adult immune response in terms of expression of proinflammatory cytokines. The malaria parasite Plasmodium falciparum caused an increase in the synthesis of Igs, especially of IgG, the concentration of which in the cord blood was 69%, as compared to 6% and 4.4% IgM and IgE, respectively (86). Neonates born to malaria-
positive mothers mounted predominantly Th2-type immune responses. It appears that neonates born to malaria-infected mothers may relatively be high susceptible to malaria attack during the first years of life.

The immune response to microbial effects in fetuses and newborns with pneumonia is manifested in specific differences in the morphological features compared to those seen in children and adults. Such an immune response has been termed "immune insufficiency" (1,2). The role of the lymphoid system in fetuses and neonates who succumb to severe antigenic effects, such as pneumonia and sepsis, has been studied using morphological, morphometric and immunohistochemical analyses (87).

In the aforementioned study of three groups of fetuses and newborns - those who died of non-antigen-induced diseases (intranatal asphyxia, RDS, or brain hemorrhage), those who died mainly of bronchopneumonia and alveolitis-pneumonia accompanied by RDS, hyaline membrane disease and inborn heart disease, and those who died with sepsis - it was found that in the absence of antigenic effects, the lymphoid organs (thymus, spleen and lymph nodes) are morphologically formed by 22 to 24 weeks of gestation. The thymus consists of a cortex, medulla, and thymic corpuscles (Table XXII). The spleen consists of white and red pulps with differentiation of follicles and periarterial lymphoid sheaths (PALS) in the white pulp (Table XXIII). Follicles are quite pronounced in the lymph nodes.

When the microbial antigenic effects are mild (e.g., in alveolitis-pneumonia), the immune reaction is generalized and spreads to all of the lymphoid organs. It is manifested in an increase in the number of CD20+ B lymphoblasts and fewer small lymphocytes, as a result of their activation (blast transformation) (Table XXIII). There are high amounts of T lymphocytes in the PALS, IgM and IgA-synthesized cells and a high number of macrophages and dendritic cells (65). The accidental involution (AI) of the thymus reaches the third phase, as reflected by a significant increase in the number of thymic corpuscles (Table XXIV). These changes are different from those described for children and adults. The germinal centers of the follicles, massive apoptosis of lymphocytes, multiplication of lymphoblasts and the formation of mature plasmocytes, which are characteristic in children and adults, are absent in infants (1,2).
Table XXI.
Morphometric parameters of the thymus in different groups of fetuses
(mean±SE) (After ref. 87)

<table>
<thead>
<tr>
<th>Parameters studied</th>
<th>Without antigenic effects</th>
<th>With bronchopneumonia</th>
<th>With sepsis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (weeks)</td>
<td>25.6±1.3</td>
<td>30.3±1.3</td>
<td>32.3±1.4</td>
</tr>
<tr>
<td>Area of cortex</td>
<td>59.7±4.5</td>
<td>55.4±4.6</td>
<td>32.6±3.3 b,c</td>
</tr>
<tr>
<td>Area of medulla</td>
<td>24.8±2.7</td>
<td>31.6±1.3 b</td>
<td>50.8±3.9 b,c</td>
</tr>
<tr>
<td>Thymic corpuscules</td>
<td>2.5±0.6</td>
<td>3.3±0.3</td>
<td>5.9±0.5 b,c</td>
</tr>
<tr>
<td>Trabeculae</td>
<td>15.5±1.2</td>
<td>13.8±1.2</td>
<td>17.2±0.8 c</td>
</tr>
</tbody>
</table>

a As a percentage of the total area of the whole slide.
b Significantly different from group 1, p<0.02-0.01.
c Significantly different from group 2, p<0.05-0.01.
Table XXII.
Morphometric parameters of the spleen in different groups of fetuses (mean±SE) (After ref. 87)

<table>
<thead>
<tr>
<th>Parameters studied</th>
<th>Without antigenic effects</th>
<th>With pneumonia</th>
<th>With sepsis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (weeks)</td>
<td>25.6±1.4</td>
<td>29.6±1.3</td>
<td>31.7±1.2</td>
</tr>
<tr>
<td>White pulp $^a$</td>
<td>23.0±0.9</td>
<td>24.1±1.4</td>
<td>12.6±1.3 $^{b,c}$</td>
</tr>
<tr>
<td>Red pulp $^a$</td>
<td>67.2±1.0</td>
<td>67.1±3.1</td>
<td>80.2±1.3 $^{b,c}$</td>
</tr>
<tr>
<td>Number of follicles/1 mm$^2$</td>
<td>6.4±0.3</td>
<td>4.5±0.4 $^b$</td>
<td>2.0±0.1 $^{b,c}$</td>
</tr>
<tr>
<td>Number of cells in follicles/10,000 μm$^2$</td>
<td>152.5±3.0</td>
<td>132.6±8.0 $^b$</td>
<td>116.3±4.0 $^{b,c}$</td>
</tr>
<tr>
<td>Percentage of lymphocytes</td>
<td>74.8±3.0</td>
<td>52.5±4.7 $^b$</td>
<td>52.5±2.6 $^b$</td>
</tr>
<tr>
<td>Percentage of lymphoblasts</td>
<td>2.7±2.3</td>
<td>23.1±0.4 $^b$</td>
<td>21.3±1.8 $^b$</td>
</tr>
<tr>
<td>Percentage of dendritic cells</td>
<td>2.3±0.6</td>
<td>4.4±0.1 $^b$</td>
<td>4.7±0.6 $^b$</td>
</tr>
<tr>
<td>Number of cells in red pulp/10,000 μm$^2$</td>
<td>88.7±2.2</td>
<td>81.1±5.5</td>
<td>66.5±2.5 $^{b,c}$</td>
</tr>
<tr>
<td>Percentage of lymphocytes</td>
<td>27.4±1.9</td>
<td>27.4±3.2</td>
<td>31.1±1.8</td>
</tr>
<tr>
<td>Percentage of lymphoblasts</td>
<td>2.6±1.3</td>
<td>10.7±0.8 $^b$</td>
<td>10.9±1.2 $^b$</td>
</tr>
<tr>
<td>Percentage of macrophages</td>
<td>2.1±0.4</td>
<td>3.9±0.2 $^b$</td>
<td>5.4±0.5 $^{b,c}$</td>
</tr>
<tr>
<td>Percentage of neutrophils</td>
<td>0.3±0.1</td>
<td>0.4±0.1</td>
<td>4.2±0.6 $^{b,c}$</td>
</tr>
</tbody>
</table>

$^{a,c}$ See footnotes to Table XXII.
Table XXIV.
Area of thymic corpuscles in fetuses and infants (as a percentage of the total area of the whole slide) (After ref. 87)

<table>
<thead>
<tr>
<th>Groups of patients</th>
<th>Stillborns</th>
<th>0-2</th>
<th>3-6</th>
<th>7-13</th>
<th>14-28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without antigenic effects</td>
<td>2.01±0.4</td>
<td>2.9±0.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>With pneumonia</td>
<td>1.96±0.4</td>
<td>2.6±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>7.9±0.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>With sepsis</td>
<td>3.4±0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.1±0.2&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>7.4±0.5&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>8.5±0.5&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>7.4±0.3&lt;sup&gt;a,c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significantly different from group 1, <i>p</i><0.01.
<sup>b</sup> Significantly different from other infants in the same group, <i>p</i><0.01.
<sup>c</sup> Significantly different from infants in other groups, <i>p</i><0.01.
The morphological features of immune insufficiency in infants can be displayed even under mild antigenic effects: the number of cells in the splenic follicles decreases by 30%, and the area of the parenchyma of lymph nodes decreases by 21%. In LBW neonates, the thymus is characterized only by the first phase of AI and by weak proliferation of the reticular epithelium. All of these data characterize the immune response of fetuses and newborns to antigenic effects as a special form of the fetal type (1,2).

Among fetuses and newborns with bronchopneumonia, the lymphoid organs showed changes reflecting a marked generalized immune response (87). The number of CD20⁺ lymphoblasts in the spleen and lymph nodes is four to eight times higher and the number of lymphocytes, especially B cells, in the splenic follicles is significantly lower compared to counterparts without antigenic effects. The germinal centers in the follicles are not formed, the number of mitotic and apoptotic cells is low, and the mature plasma cells are absent. There is an increase in the number of CD3⁺ T lymphocytes in the PALS, dendritic cells in the follicles of the white pulp, and of macrophages in the red pulp of the spleen (Table XXIII). There is a significant decrease in the number of follicles in the spleen (per mm²), and in the number of cells and the parenchyma of the lymph nodes. In the thymus, both AI and development of the thymic corpuscles are correlated with age: AI in the second and third phases is seen only in full-term neonates or in LBW infants who died after the second week of life (1,2).

In the group with sepsis, there are fewer proliferative and many more destructive changes in the lymphoid organs (87). The spleen and lymph nodes demonstrate blast-transformation of CD20⁺ B lymphocytes and an increase in the number of macrophages and dendritic cells. There is a sharp decrease in the number of lymphoid cells, which is especially noticeable in T and B lymphocytes and IgM-containing cells. The number of follicles is one-third that in fetuses without antigenic effects, and their cellular area is reduced by half. In the spleen, which is the main immune organ in fetuses and newborns, the number of lymphoid cells decreases by 60% to 75% compared with their number in neonates without antigenic effects. A particularly sharp decrease in the total number of lymphoid cells is seen in LBW infants in whom the entire mass of the lymphoid organs and, particularly, the spleen is five to seven times smaller than in full-term infants (1). Devastation of the cortex of the thymus and of other lymphoid organs is found in the most severely affected cases. The third phase of AI is found in the thymus. The area of the thymic corpuscles increases significantly (Table XXIV) and persists of "pearls" from the horn-like epithelium. The marked proliferation of thymic corpuscles in these cases can be considered an adaptive reaction of infants to an abnormal situation.

The morphological signs of decompensation of the lymphoid system afflicted by severe antigenic effects have been observed in cases in which the lymph system was incapable of providing an adequate immune response. This inability can develop under mild antigenic effects in parallel with the so-called immune incompetence or immunodeficiency, seen in LBW infants with immaturity or genetic immunodeficient syndrome (88). Unfortunately, the terms of immune incompetence or immunodeficiency describe only insufficiency of the immune system and cannot be considered a phenomenon of decompensation. The latter can develop under extremely severe antigenic microbial exposure and manifests itself in intoxication and
dysfunction of different organs and systems. Decompensation has also been observed in fetuses under massive non-microbial antigenic effects, consequences of the edemic form of HDN or preeclampsia (2). However, the morphological features of sepsis described herein, and especially the increase in the number of neutrophils, did not develop under these conditions.

It can be suggested that sepsis in infants is a result of generalized decompensation of the lymphoid system, especially of its B and T parts. Participation of neutrophils and eosinophils in the response to sepsis can be considered a manifestation of an organism's compensatory reaction, an additional mechanism of an antimicrobial defense. This reaction increases in parallel to the advance of gestation and postnatal age (1).

There are three possible causes of decompensation of the lymphoid systems in sepsis: i) invasion by even a small amount of highly pathogenic microbes, ii) massive infection by moderately pathogenic microbes, and iii) inadequacy of the immune system, or immune incompetence. This last parameter is a common reason for the development of sepsis in infants. The process begins locally, and eventually the entire immune system exhibits features of decompensation, such as underdevelopment of the thymus, of T and B lymphocytes, neutrophils, and macrophages (89). A similar picture is observed in pre-term LBW infants (90,91). The low weight of the lymphoid organs is also relevant to the development of immune incompetence, because such organs become exhausted under even weak antigenic affects.
References

(To 9.1)


(To 9.2)


75. Lazzarotto, T., Varani, S., Spezzacatena, P., Gabrielli, L., Pradelli, P., Guerra, B., and Landini, M.P., 2000, Maternal IgG avidity and IgM detected by blot as diagnostic tools to identify pregnant women at risk of transmitting cytomegalovirus, Viral Immunol., 13, 137.
numbers of IL-4 and IFN-gamma secreting cells in paired maternal cord blood from South West Cameroon, Int. J. Infect. Dis., 9, 159.


Intrauterine or fetal growth restriction (FGR) is associated with a heterogeneous group of conditions ranging from fetal chromosomal aberrations to maternal malnutrition. FGR is the second most important cause of perinatal morbidity and mortality (1). Adverse effects, however, are not limited to the perinatal period because FGR is associated with sequelae, such as a permanent neurological damage (2). Furthermore, a pathophysiological concept has emerged linking FGR to increased susceptibility in adulthood to diseases, such as hypertension, type-2 diabetes, and atherosclerosis (3), as well as with increased risk of neonatal morbidity and mortality and alterations in physical and mental development during early childhood (4).

10.1) Geographic peculiarities of immunoglobulins in FGR

The blood IgG level is directly related to the neonate's birth weight being higher in infants with normal birth weight than in those with low birth weight (5). The lower level of IgG in low-birth-weight (LBW) infants can probably be attributed to blockage of the IgG-specific Fc-receptor sites in the placenta due to acute atherosis and reduced uteroplacental perfusion. Cord blood levels of IgG, IgA and IgM in newborn FGR infants were found to be significantly lower than those in infants with growth that was adequate for their gestational age (6). Lower levels of cord IgG in FGR may be due to a defect in the active transport of IgG across the placenta. Lower levels of cord IgM and IgA suggest an impairment in Ig synthesis in FGR infants. No differences were observed in maternal Ig concentrations among the study groups.

In the adult human thymus, cytoplasmic IgG is detected most frequently with lesser amounts of IgA and IgM (7,8). In the fetal thymus, the significant number of Ig-containing cells present a distribution similar to that seen in the adult, with IgG predominating. IgM is demonstrated more frequently than IgA. There is a tendency for the number of Ig-containing cells to rise after birth and to continue into early adult life, sometimes continuing past the age of 20. Although Ig-containing cells represent only a minor population, their presence must be taken into account when considering the function of the thymus and the mechanism of involution.

The humoral and cellular immune status of preterm and small-for-dates babies was evaluated in Indian newborns (9). Neonates with severe FGR and preterm babies had significantly lower levels of IgG, but not of IgM or IgA. The preterm babies had a significantly higher percentage of B lymphocytes, although the absolute count was not significantly different from normal newborns. The babies with severe FGR had a significantly lower absolute count of B cells, as well as lower absolute and percentage counts of E-rosette-forming cells relative to normal newborns. These findings suggested that LBW babies with severe FGR are at greater risk of developing...
bacterial infection due to deficiencies both humoral and cellular immune host defenses. In contrast, preterm babies are immunologically competent, even though passively transferred maternal IgG levels are low.

Sizeable concentrations of IgG were present at birth in Nigerian LBW babies (2,500 g or less) (10). The mean IgG level in such babies (1360 mg/100 ml) was significantly lower than in babies with average and above average weight. The mean maternal and cord IgG levels were approximately equal in all except in LBW babies. Mothers with higher IgG levels than in their corresponding babies were most prominent in the LBW group. There was no significant difference between the maternal or cord serum's respective IgA and IgM levels. The mean cord serum IgA and IgM levels were about 4% and 10% respectively, of those in the mothers' serum. Compared with Caucasians, relatively higher quantities of IgD were found in the Nigerian mothers.

Serum levels of IgG, IgM and IgA were different between appropriate-for-date (AFD) and small-for-date (SFD) infants in Japan (11). The serum IgG level was higher in the AFD infants' blood than in their mothers'. The fetus-to-mother ratio was 1.25±0.22. The fetus-to-mother ratios of IgG in term SFD infants and premature infants were lower than in term AFD infants. The placental transmission of IgG increased with time of gestation weeks went by, and the fetus-to-mother ratio reaching 1.0 by the 38th week of pregnancy. The serum IgM level was lower in SFD infants vs. AFD infants, whereas no differences were found in the serum IgA levels between the two groups of infants.

In a similar study performed in Tanzania, the mean serum levels of total protein, albumin, and IgG in mothers who delivered AFD infants were 6.8 g/100 ml, 2.9 g/100 ml, and 1,840 mg/100 ml, respectively, whereas those from their infants were 6.9 g/100 ml, 4.1 g/100 ml, and 1,471 mg/100 ml (12). The synthesis of IgM and IgA during fetal life appeared to be activated at an earlier gestational age than in infants in Western countries. This fact subsequently resulted in a higher detectable amount of IgM and IgA in the cord blood of the AFD infants. IgG in Tanzanian mothers was generally higher than in the corresponding cord blood sera, which is contrary to the finding in Europe.

**10.2) Changes in the placenta as a reason for FGR**

**10.2.1) Morphological aspects**

The placenta plays a key role in fetal nutrition. It mediates the active transport of nutrients and metabolic wastes across the barrier separating maternal and fetal compartments, and modifies the composition of some nutrients through its own metabolic activity (13). The function of the placenta is essential to the growth of a healthy fetus; it is becoming apparent that the activities of the placenta are in turn modulated by signals originating from the fetus. Communication between the placenta and fetus is especially critical in intrauterine growth retardation.
Normal fetal growth depends on the genetically predetermined growth potential and is modulated by fetal, placental, maternal, and external factors (14). Fetuses with growth restriction (FGR) are at high risk for poor short- and long-term outcomes. Although there are many underlying etiologies, FGR resulting from placental insufficiency is most relevant clinically because the outcome can be altered by appropriate diagnosis and timely delivery. A diagnostic approach that aims to separate FGR resulting from placental disease in the constitutionally small fetuses from that with other underlying causes (e.g., aneuploidy, viral infection, non-aneuploid syndromes) needs to integrate multiple imaging modalities. In placental-based FGR, cardiovascular and behavioral responses are interrelated with disease severity.

From the beginning of the pregnancy, the placenta exerts its effects on fetal growth via metabolic and endocrine mechanisms (15). To achieve this, the placenta exchanges a wide array of nutrients, endocrine signals, cytokines and growth factors between the mother and the fetus. These exchanges modulate or programmed fetal growth and development. The mother's nutritional and hormonal state from as early as the first few days after fertilization, can influence the growth rate of the placenta and the fetus as well as the length of gestation. Thus influences on placental development and their consequences will clearly have an impact on the placental control of fetal growth. Variations in the maternal environment and consequent perturbation of the metabolic and endocrine environment of the placenta and fetus are responsible for the associations between prenatal growth of the placenta and its fetus and the subsequent risk of adult disease.

Changes in the placenta are reflected in fetal weight (16). With large fetuses, there is a relatively small volume of the fibrinoid substance, moderate synthesis by the syncytiotrophoblastic epithelium of glucosaminoproteoglycans (GAPG), slight lymphoplasma cell infiltrates with a marked suppressor influence, a lack of human lymphocyte antigens (HLA) on the trophoblast and the formation of adequate interrelations between the immune-dependent placental structure and the thymus. It may be that all of these features allow the large fetuses to be retained as fetuses for up to 38-40 weeks or even more. In cases of FGR, there is a sharp increase in the fibrinoid substance, focal enhancement of GAPG production by the syncytiotrophoblastic cells, a decrease in the suppressor activity and the appearance of HLA on the trophoblast. This is probably a manifestation of a premature exhaustion of compensatory-adaptive reactions in the placenta.

A placental lesion, characterized by fibrinoid and trophoblastic necrosis with massive infiltration of the intervillous space by mononuclear cells (massive chronic intervillositis, MCI), was observed in cases of FGR and of sudden intrauterine fetal death (17). The lesions are characterized as chronic villitis of unknown etiology or as anchoring villitis. A high number of acute atherosis-like lesions in the spiral arteries of parietal and/or basal decidua were observed. Patients with MCI showed a higher incidence of FGR with lower than control values of infant weight, length, and pondered index. Massive chronic intervillositis may represent an extreme variant of villitis of unknown etiology.

A prominent feature of the placentation process is an extensive transformation of the 120 spiral arteries that supply the intervillous space of the placenta with maternal
blood (18). The transformation implies a destruction of the muscular wall of the spiral arteries resulting in wide vessels with low resistance and high flow rates (19). In pregnancies with placental insufficiency, this adaptive remodeling of the spiral arteries is incomplete (20). In addition, a prominent feature of the insufficient placenta is thrombotic infarction (21).

Fetal growth is largely determined by the availability of nutrients to the fetus and regulated by the hormones of the fetal somatotrophic axis, and in particular insulin-like growth factor (IGF)-1 (22). Placental function in turn, is heavily influenced by the maternal and fetal growth hormones (GH). The placenta itself is also an active endocrine organ and it produces a large number of hormones including GH and IGF-1 as well their corresponding receptors. Thus the placenta can no longer be considered merely a passive conduit for fetal nutrition. Rather, it is actively involved in the integration of nutritional and endocrine signals from the maternal and fetal somatotrophic axes. Inadequate growth in utero is associated not only with adverse fetal, perinatal, and neonatal outcomes, but also with an altered propensity for disease later in life (23). Conversely, fetal overgrowth is also associated with increased medical risks for both the mother and fetus. The interaction between the fetal genome and the intrauterine environment determines in large part how fetal growth will progress. The placental, maternal, and fetal somatotrophic axes (in particular, GH and IGF-1) play key roles in modulating this interaction.

FGR is a significant cause of infant mortality and morbidity. It is now clear that FGR infants exhibit higher rates of coronary heart disease, type-2 diabetes, hypertension and stroke as adults. Therefore, fetal growth not only impacts the outcome of the perinatal period, but also impacts adult well-being (24). The etiologies of FGR are numerous, but are often associated with abnormalities in placental structure and function. The processes of implantation and placentation require the production of a plethora of growth factors, cell-adhesion molecules, extracellular matrix proteins, hormones and transcription factors. Many of these exhibit altered expression within the placenta in FGR pregnancies, particularly, with respect to placental vascular structure and function.

A morphological electron microscopy study showed that in cases of FGR, villi of human placentas appear longer, thinner, and less vascularized, compared to the normal condition (25). Fibrinoid, an extracellular material of hematic origin, frequently fills the villous stroma. The density of apical microvilli appears considerably reduced and occasional microvillus-free areas are observed. Moreover, the underlying basal membrane appears significantly thicker than that of the normal syncytiotrophoblast. A study of apoptosis as a possible cell-deletion mechanism in growth restriction showed that most of the typical apoptotic features appear indifferently in both FGR and normal pregnancy. It has been suggested that growth retardation might be correlated with a complex of structural changes, suggestive of materno-fetal traffic downregulation mechanisms.

The placenta is an important functional unit for gas transfer between mother and fetus. The placental membrane, consisting of the trophoblast layer interposed between the maternal and fetal blood, is known to play an active role in respiration intensity. It is accepted that FGR and increased rate of fetal mortality can usually be seen at high
altitude. It has been shown that trophoblast cells may play an important role in the gas transfer mechanism in a hypoxic state at high altitude, such as in Nepal (26). The gross characteristics of placental pathology in Nepalese placentas obtained from cases with FGR were represented by marked subchorionic fibrin deposits and increased chorionic cysts in contrast to low incidence of intervillous thrombosis compared with their Japanese counterparts. The incidence of chorangiosis and chorangioma of the placental villi in the Nepalese group was significantly higher than in the Japanese group. Accompanying an increase in the vasculosyncytial membrane in the villi, thickness and separation of the syncytiotrophoblast basement membrane and increased apoptosis of syncytial cell nuclei were recognized. Characteristic ultrastructural features of the chorionic villi in the Himalayan placentas included an increase of mitochondria and cystic formation of rough endoplasmic reticulum, in addition to the appearance of lamellar bodies similar to alveolar epithelial type-II cells in organelles of the syncytium. These ultrastructural changes of the placental villous capillaries may be described as hypervascularization caused by the chronic hypoxic state.

Defects in all the trophoblast-differentiation pathways -- endovascular, interstitial and chorionic villous -- play a role in the pathogenesis of early-onset FGR (27). There are two types of extravillous trophoblast: endovascular trophoblast, which forms the definitive placenta by occlusion of the spiral arteriole at the implantation site, and interstitial extravillous trophoblast, responsible for the anatomical erosion of the distal spiral arteriole and the secretion of angiogenic and vasodilator signals to improve uterine blood flow. Defective endovascular erosion may render the basal plate inadequate to meet the demands of the fetus. Failed interstitial invasion of the spiral arterioles may lead to failure of local angiogenic and systemic cardiovascular adaptation signals which in turn could be the underlying basis for early-onset FGR and pre-eclampsia.

10.2.2) Biochemical aspects

FGR is considered an abnormal pregnancy. It is not a specific disease entity per se, but rather a complex of several fetal and/or maternal disorders. Analysis of the modalities of the uteroplacental circulation and maternal blood entrance has demonstrates physiological hypoxia ending with the first trimester of pregnancy. Moreover, in vitro culture of the first-trimester villous explants has shown the role of oxygen in extravillous cytotrophoblast proliferation, decidual invasion and spiral artery remodeling (28). Oxygen appears to be a key factor controlling the mechanism of placentation by regulating the transcription of several genes, such as the vascular endothelial growth factor and leptin, among others. These genes are turned on or off as a function of the oxygen partial pressure via an oxygen sensor. Oxygen is also implicated in the development of several pathologies of pregnancy. It is involved, for example, at different steps in the cascade of events leading to preeclampsia. Decreased oxygen partial pressure is considered a possible reason for abnormal development of the trophoblast villous tree in FGR, in maternal anemia or in pregnancies in regions at high altitude.

Under hypoxic conditions, litter size in rats is reduced and insulin-like growth factor binding protein (IGFBP-1), a secreted protein that binds IGFs in extracellular
environments, is up-regulated in the maternal serum and in the fetal liver and heart (29). Tissue-specific induction of calcium homeostasis-related genes and suppression of growth-related genes are observed, suggesting mechanisms underlying hypoxia-related FGR. Furthermore, induction of inflammation-related genes in placentas exposed to long-term hypoxia (11 days) suggests a mechanism for placental dysfunction and impaired pregnancy outcome that accompany intrauterine hypoxia. Hypoxia treatment not only resulted in FGR, it also caused a significant delay in the developmental rate and timing of morphogenesis in vital organs (30). Elevated IGFBP-1 mediates hypoxia-induced FGR and developmental delay by binding to and inhibiting the activities of IGFs. Knockdown of IGFBP-1 significantly alleviated the hypoxia-induced growth retardation and developmental delay. Over expression of IGFBP-1 caused growth and developmental retardation under normoxia. Furthermore, reintroduction of IGFBP-1 to the IGFBP-1 knocked-down embryos restored the hypoxic effects on fetal growth and development. The induction of IGFBP-1 expression may be a conserved physiological mechanism to restrict IGF-stimulated growth and developmental processes under hypoxic stress.

IGF-I is known to play a role in placental and fetal growth. Immunoreactive IGF-I was detected in the cyto- and syncytiotrophoblast, amnion, endothelial cells of the fetal capillaries and in the decidua in both normal and FGR placental tissue (31). Stronger immunostaining and an increased number of positively stained cells were found in the decidua of the FGR placenta. Intense immunostaining was also found in endothelial cells, smooth muscle cells and fibroblasts of the umbilical vein. IGF-I immunoreactivity was also present in the stroma (Hofbauer cells and/or fibroblasts) of FGR chorionic villi. Expression of IGF-I is normally high at specific sites in the placenta and umbilical cords, indicating a paracrine and/or endocrine function. The increased expression of IGF-I in the placenta of FGR fetuses indicates its involvement in restoring normal growth by means of a positive feedback mechanism.

Gestational and fetal size effects were found on the receptor density of endothelins (ET), potent vasoconstrictor peptides, within the trophoblastic stem villi (32). Smooth muscle cells expressed ET receptors predominantly in the proximal regions of the chorionic villous tree and in the deciduas, and at a lower density on paravascular stromal cells in the stem villi. The localization of ET-1 immunoreactivity differs in human placental tissues from third-trimester normal vs. FGR pregnancies (33). The localization of ET-1 immunoreactivity was significantly higher in the capillary endothelial cells of the trophoblastic villi as well as in the endothelial, decidual, and trophoblastic cells of the basal plate in placentas from normal pregnancies than in FGR pregnancies. It has been suggested that the lower expression of ET-1 in placental tissues from FGR pregnancies might be secondary to an adaptive mechanism designed to reduce the vasoconstrictor effect of ET-1.

Human angiogenin, a potent inducer of neovascularization, is involved in the morphological and angiogenic changes in the placenta necessary for a successful fetal outcome during pregnancy. It has been shown that placental explants from patients with FGR secrete in vitro angiogenin at 1.3- to 1.6-fold higher than normal term levels at 24 and 72 hr of culture, respectively. (34). Expression profiles of angiogenin from term and FGR cases are in agreement with its mRNA levels and immunoblot...
resultss. The significantly elevated levels of angiogenin in the FGR placenta may provide a molecular mechanism for the abnormal placental development.

Some hormones play an important role in regulating fetal growth. The role in this process of 11\(\gamma\)-HSD2, a known factor in determining fetal morbidity and in the subsequent development of cardiovascular disease in adulthood, has been studied as an example (35). In the placenta, 11\(\gamma\)-HSD2 activity is thought to protect the fetus from the deleterious effects of maternal glucocorticoids. Patients with apparent mineralocorticoid excess owing to mutations in the 11\(\gamma\)-HSD2 gene invariably exhibited reduced babies' birth weight, accompanied by reduced placental 11\(\gamma\)-HSD2 activity in pregnancies complicated by FGR. This is reflected by evidence of hypercortisolemia in the fetal circulation in small babies. Placental 11\(\gamma\)-HSD2 mRNA levels were significantly lower in FGR pregnancies than in gestationally matched, appropriately growing placentas.

10.2.3) Transport across the trophoblast in FGR

The causes of FGR are multifactorial and largely unknown; however, altered placental transport has been implicated (36). The placental transporting epithelium -- the syncytiotrophoblast -- is polarized with the apical microvillous membrane and basal plasma membrane. These two plasma membranes constitute the major barrier between the mother and her fetus, and most nutrients and metabolites are actively or passively transferred across them to and/or from the fetus. Some of the evidence for a link between disturbed placental transport across these membranes and FGR is considered below.

Development of the fetal immune system begins at the early stages of gestation (37). It is competent to respond to intrauterine infections from as early as 12 weeks and has full functional potential at birth. Maternally acquired IgG is available for up to 9 months of life until the infant's own immune system has been adequately primed and activated following first exposure to specific antigens. The normal fetomaternal immune relationship represents a remarkably harmonious association between two genetically disparate individuals. Maternal IgG antibodies are transmitted across the trophoblast by receptor-dependent mechanisms to provide immediate protection for the neonate against environmental pathogens. Leakage of fetal erythrocytes, leukocytes and platelets into the maternal circulation can elicit IgG isoantibodies that take advantage of the same mechanisms to gain access to the fetus, with pathological consequences (38). Autoantibodies in women with various disease states may also pass into the fetus, but these normally produce only mild and transient effects. The fetal trophoblast is able to act as a protective barrier by virtue of its unique properties, including a lack of conventional class I and class II human lymphocyte antigen (HLA) molecules, which render it nonsusceptible to immune attack (38). Maternal immune cell traffic across the placenta occurs only at a very low level, if at all, in normal pregnancy. This may take place to a greater degree in some of the rare instances of fetal graft-versus-host disease, but this is complicated by the associated fetal immunodeficiency.
FGR can be characterized as a condition in which the fetus has failed to achieve its genotypical growth potential. FGR is associated with increased perinatal morbidity, higher incidence of neurodevelopmental impairment, and increased risk for a number of diseases in adulthood, such as cardiovascular disease and diabetes (39,40). The altered growth pattern seen in many cases of FGR is believed to be caused by impaired placental transport functions and blood flow (36). FGR is associated with changes in fetal and/or maternal levels or placental secretion of a number of hormones and growth factors, providing the underlying rationale for most of the effectors tested. Fetal levels of IGF-I (41), leptin (42), and epidermal growth factor (43) as well as maternal levels of growth hormones (44) are reduced in FGR. FGR placentas show higher than normal placental amounts of IGF-II transcripts (45). There are contradictory results concerning maternal and fetal cytokine levels in FGR: some studies show elevated levels of cytokines, others show reduced levels, and still others show no change in cytokine production in association with FGR (46,47).

Vascular placental insufficiency is considered a common pathogenic factor in human FGR. In experiments with ischemic conditions in rabbits, it has been shown that the change in activity of the brain-type isozyme of creatine kinase (CK), involved in energy generation and regulation, can be used as a marker for responses to ischemia in rabbit tissues, including placenta (48). A significant transient increase in CK-specific activity was found in the kidney and the cerebellum but not in the other organs tested, at 24 and 48 h after ligation. This increase was not seen with adenylate kinase, another enzyme involved in energy generation and regulation. It has been proposed that an increase in CK-specific activity could serve as a metabolic marker of vascular insufficiency in rapidly developing tissues, representing part of a compensatory mechanism to overcome an energetic gap induced by ischemia and, as a result, manifested in retarded fetuses.

Amino acids have multiple functions in fetoplacental development. The supply of amino acids to the fetus involves active transport across the trophoblast and metabolism within it. Transport occurs through various amino acid-transport systems located on both the maternal- and fetal-facing membranes. The capacity of the placenta to supply amino acids to the fetus develops during pregnancy through alterations in such factors as the surface area and expression of the specific time-dependent transport system. In FGR, both placental surface area and amino acid uptake decrease in human and experimental animal models (49). In an ovine model of FGR produced by hyperthermia-induced placental insufficiency, umbilical oxygen and the rate of uptake of essential amino acids were significantly reduced in the most severe cases, in concert with decreased fetal growth. These changes indicate that severe FGR is likely associated with a shift in amino-acid transport capacity and metabolic pathways within the fetoplacental unit. Under normal conditions, after transport across the trophoblast amino acids are actively incorporated into tissue proteins or oxidized. In a sheep growth-retarded fetus, which was hypoxic, hypoglycemic and hypoinsulinemic, there appeared to be net effluxes of amino acids from the liver and skeletal muscle, suggesting changes in amino acid metabolism. Potential changes may be occurring in the insulin/IGF-I signaling pathway, including decreased production and/or activation of specific signaling proteins leading to a reduced protein synthesis in fetal tissues. Such observations in the placental
insufficiency model of FGR indicate that this combination decreases fetoplacental amino acid uptake and disrupts insulin/IGF signaling in the liver and muscles in FGR.

FGR is characterized by a reduction in fetal plasma concentrations of a number of essential amino acids. The activity of placental transporters for cationic and neutral amino acids is reduced in FGR: mediated uptake of lysine was reduced by 44% in the basal membrane and uptake of leucine was lower in the both microvillous and basal membranes relative to control vesicles (50). Intravesicular glycine (2 mM) increased the uptake of leucine by 98% in the microvillous membrane. These data suggest that a reduced glycine gradient in the FGR placenta, due to reduction in amino-acid transporter system A activity, impairs leucine transport to the fetus, providing an additional mechanism for the reduced placental transport of leucine in FGR.

The activity of system A has been shown to be reduced in the syncytiotrophoblast microvillous membrane in FGR. However, the impact of these changes on transplacental transport is difficult to assess without information on system A activity in the basal plasma membrane (51). In term of FGR neonates, microvillous membrane system A activity was unaltered compared to controls (52). In preterm FGR neonates (gestational age 28-36 weeks), the system A activity in the microvillous membrane was reduced by 50% as compared to controls (gestational age 28-35 weeks). In all preterm FGR pregnancies, signs of severe fetal compromise were present, whereas term FGR neonates were less affected. These data support the view that the activity of microvillous membrane system A is related to the severity of the compromise in FGR. The markedly reduced system A activity in the microvillous membrane in preterm FGR together with the unaltered activity in the basal membrane is consistent with decreased transplacental transport of neutral amino acids in this pregnancy complication.

Taurine is an essential amino acid during fetal life and appears to be vital for the growth of the fetus and for the development of the fetal central nervous system. In FGR, fetal plasma concentrations of taurine are reduced, due to its altered placental transport (53). Transplacental transfer is the fetus's primary source of taurine. In FGR, the placental transport capacity of taurine is reduced and fetal taurine levels are decreased (54). The taurine transporter (TAUT), detected as a single 70-kDa band, is primarily localized in the syncytiotrophoblast microvillous plasma membrane, and its expression is unaltered in FGR. None of the tested hormones, e.g., leptin and growth hormone, altered TAUT activity significantly. The syncytiotrophoblast TAUT is strongly polarized to the maternal-facing plasma membrane. Microvillous membrane TAUT expression is unaltered in FGR, suggesting that the reduced microvillous membrane taurine transport in this condition is due to changes in the transporter's activity. Nitric oxide plays an important role in downregulating microvillous membrane TAUT activity in FGR.

The placenta plays a critical role in providing an environment that supports optimal fetal growth, by being the site of nutrient transfer from the mother to the fetus and of waste secretion from the fetus to the mother, acting as a barrier against pathogens and the maternal immune system, and serving as an active endocrine organ capable of secreting hormones, growth factors, cytokines, and other bioactive products. Among
the hormones produced by the placenta are members of the growth hormone/prolactin gene family, the placental lactogens and prolactin-related proteins (55). The placental lactogens are secreted into the maternal and fetal circulations and seem to mediate their effects through unique receptors. The placenta is also primarily responsible for elimination of fetal acid equivalents of respiratory and metabolic origin. Intrauterine sampling of the fetal cord blood has shown that growth-restricted fetuses are more prone to develop acidosis in utero than normally grown fetuses (56). This most probably contributes to the adverse outcome associated with FGR.

Several major acid/base-regulating proteins have been demonstrated in the placental syncytiotrophoblast. Two key transporter proteins, the Na⁺/H⁺ exchanger (NHE) (57) and the Cl⁻/HCO₃⁻ exchanger (58) are localized to the microvillous plasma membrane. The main functions of the NHE are maintenance of intracellular pH, vectorial Na⁺ transport, and regulation of cell volume. This family of proteins consists of at least six isoforms, NHE1 to NHE6, and catalyzes the extrusion of one H⁺ per Na⁺ ion entering the cell down its electrochemical gradient. Although NHE-1 is ubiquitously distributed (59), NHE2-6 has a more restricted pattern of expression (60,61). NHE-1 serves housekeeping functions in most cell types, whereas NHE-2 and NHE-3 are epithelial isoforms, implicated in vectorial Na⁺ transport. NHE isoforms are present in the syncytiotrophoblast plasma membranes of the human placenta (61-64). Inhibition of NHE has been shown to severely impair recovery from an intracellular acid load, both in the first trimester of pregnancy and at term (65). The presence of a pH gradient in isolated microvillous membrane vesicles suggests a role for NHE in regulating intracellular pH, which is critical for the proper functioning of enzymatic and transport functions in the placenta (57,66). These observations also raise the possibility that syncytiotrophoblastic NHEs are involved in the removal of acid equivalents from the fetal compartment. The activity of NHE in microvillous membrane from FGR and control placentas has been investigated in term as well as in preterm preparations, and found to be either unaltered (62) or reduced (67).

Regulation of syncytiotrophoblast intracellular pH is critical to optimum enzymatic and transport functions of the placenta (68). The expression of NHE isoforms 1, 2, and 3 was approximately 10-fold greater in the microvillous membrane than in the basal membrane isolated from preterm and term placentas obtained from uncomplicated and FGR pregnancies. NHE-1 and NHE-2 are localized to the microvillous membrane and basal membrane and of NHE-3 to the microvillous membrane, basal membrane, and cytoplasm of the syncytiotrophoblast (63,68). NHE-1 expression in the microvillous membrane from preterm FGR placentas was reduced by 55%, compared to gestational age-matched controls, whereas NHE-1 expression was unaltered in the term FGR placentas relative to their matched controls. The activity of NHE in the microvillous membrane from the FGR preterm placentas was reduced by 48% relative to controls. In contrast, NHE activity in the microvillous membrane of term FGR was unchanged. The reduced activity and expression of NHE in the microvillous membrane of preterm FGR placentas may compromise placental function and may contribute to the development of fetal acidosis in the preterm FGR fetus (68).

NHE-3 also shows intense staining in the cytoplasm. This is in agreement with studies of other epithelia showing storage of this isoform in the plasma-membrane vesicles,
termed recycling endosomes (69,70). These endosomes are incorporated into the apical membranes on demand and represent a dynamic and mobile pool of NHE-3 in the renal epithelium, for example. This also appears to be the case in the human syncytiotrophoblast (68). In other polarized transporting epithelia, such as those in the kidneys and intestine, a consistent pattern is observed with regard to the NHE isofrom distribution. NHE-2 and NHE-3 are inserted into the apical membranes, and NHE-1 is distributed to the basal membrane (71). This asymmetrical distribution dictates the function of NHE isoforms in these epithelia. The basal localization of NHE-1 is thought to mainly regulate intracellular pH, and the apical NHE-2 and 3 aid in vectorial Na\(^+\) transport (72). The placental syncytiotrophoblast displays a different pattern of polarization, with the greatest abundance of all three isoforms localized to the microvillous membrane.

The high abundance of NHE isoforms in the microvillous membrane is consistent with protons being transported from the syncytiotrophoblast into the maternal circulation for subsequent elimination by the maternal kidneys. Besides pH regulation of the syncytiotrophoblast, placental NHE is likely to be involved in vectorial Na\(^+\) transport (73). The anion exchanger is also more abundant in the microvillous membrane than in the basal membrane (74) and possibly acts in conjunction with NHE to perform vectorial NaCl transport. Furthermore, these exchangers could be important for creating an acidic microenvironment in the vicinity of the microvillous membrane. This has been shown in the small intestine, where the increased extracellular proton concentration enhances the uphill transport of oligopeptides (75). The presence of co-transporters coupling the uptake of protons to the import of lactate (76), organic cations (77), and peptides (78) has been described in the chorionic microvillous membrane. The expression of NHE in the basal membrane implies that the syncytiotrophoblast has the ability to transport protons into the fetal compartment. However, NHE activity in the basal membrane, if present, is likely to be low (68). It is possible that NHE localized in the fetal facing plasma membrane is important for optimal function of other transporters by establishing an acidic microenvironment.

A significant reduction in microvillous-membrane NHE activity was found in the FGR preterm-delivery samples in comparison with appropriate-growth-for-gestational age samples (67,68). In the examination of preterm and term FGR, both the microvillous membrane NHE activity and NHE-1 expression were found to be down-regulated in the preterm FGR group, compared to age-matched controls (68). The fact that the preterm FGR microvillous membrane, but not the term FGR microvillous membrane, shows a significant reduction in NHE indicates that both groups are associated with different pathophysiological conditions. The growth retarded fetuses delivered preterm appear to have a decreased capacity to maintain basic homeostatic parameters. The term "growth retarded fetuses" might represent a subgroup of FGR with less severe growth restriction and/or better compensatory mechanisms that allow those pregnancies to progress to term.

The findings in primary villous samples (65), a choriocarcinoma cell line (79), and isolated membrane vesicles (57,66) suggest that NHE represents one of the key mechanisms for intracellular pH regulation in the syncytiotrophoblast. Both NHE activity and NHE-1 expression are reduced in the microvillous membrane from preterm FGR placentas. A decreased capacity to clear the syncytiotrophoblast of
protons might adversely affect placental enzymatic and transport functions. A reduction in NHE activity could lead to lower intracellular pH, which may impair nutrient transport and other placental functions. Growth restricted fetuses are prone to developing chronic acidosis in utero (80). It is likely that several factors contribute to this acidosis such as impaired placental blood flow resulting in hypoxemia (56) and the reduced fetal kidney function (81). It is ultimately the placenta that is responsible for removing acid equivalents from the fetal compartment, either by transporting protons to the mother or bicarbonate to the fetus. It is likely that transcellular transport of protons across the syncytiotrophoblast plays a role in the regulation of fetal pH, and NHE in the microvillous plasma membrane of the syncytiotrophoblast represents a key mechanism in this proton-transport route. The decreased microvillous membrane NHE protein expression and activity in preterm FGR has therefore been suggested to contribute to the development of fetal acidosis (68).

Inadequate placental transport of glucose has been implicated as a pathophysiological mechanism in FGR. Glucose transporter (GLUT) protein is normally abundant in the syncytiotrophoblast (82), with GLUT density being approximately threefold higher in the syncytiotrophoblast microvilli than in the basal membranes. In the latter, the density of GLUT is maintained from 16 weeks of gestation, increased twofold in the second trimester and remains unaltered thereafter to term. GLUT densities in term and preterm FGR placentas were unaltered. These data suggest that despite the fact that GLUT is the main glucose transporter protein isoform in the human syncytiotrophoblast, fetal hypoglycemia in FGR is not due to a decrease in placental glucose transporter density.

FGR increases the risk of developing glucose intolerance and cardiovascular disease in adulthood. Fetal exposure to excess glucocorticoids may contribute to FGR. Two glucose transporters, GLUT1 and GLUT3, are expressed in the placenta. In rodent placenta, for example, GLUT1 is replaced by GLUT3 during late gestation (83). An increased placental GLUT1 protein expression may reflect an attempt to increase placental or fetal glucose supply in order to attenuate the reductive effect of excessive exposure to glucocorticoids on fetal growth, whereas suppression of peroxisome proliferator-activated receptor-gamma protein expression during cardiac development may contribute to the increased risk of developing heart disease found in people who had below average birth weight.

10.3) Maternal environment and FGR

FGR presents complex management problems for clinicians. It is estimated that 13.7 million infants are born annually with FGR, comprising 11% of all births in developing countries (84). Failure of a fetus to achieve its growth potential imparts a significantly increased risk of perinatal morbidity and mortality (85). Consequently, the obstetrician must recognize and accurately diagnose inadequate fetal growth and attempt to determine its cause. Growth aberrations, which are the result of intrinsic fetal factors such as aneuploidy, multifactorial congenital malformations and fetal infection, carry a guarded prognosis. However, when FGR is caused by placental abnormalities or maternal disease (see below), the growth aberration is usually the
consequence of inadequate substrates for fetal metabolism and, to a greater or lesser extent, decreased oxygen availability. Careful monitoring of fetal growth and well-being, combined with appropriate timing and mode of delivery, can best ensure a favorable outcome.

FGR is multifactorial process. Studies in humans and animals have shown that the maternal environment is the most important determinant of the newborn weight, accounting for more of the similarity in sibling birth weights than genetic affinity (86). In addition to a direct relationship with the degree of maternal plasma volume expansion, many clinical factors are associated with FGR. These factors include multiple gestation, fetal genetic, and chromosomal anomalies (Down's syndrome and Turner's syndrome), infections such as TORCH syndrome (acronym for toxoplasmosis, rubella, cytomegalic disease, and herpes), and various maternal disorders including anemia, severe chronic asthma, chronic renal disease, heart disease and hypertension. Maternal stress factors, including narcotic addiction, cigarette smoking and chronic alcoholism, are also associated with FGR, as are placental anomalies including hemangiomas, placental infarcts, a single umbilical artery, and small placental size. Poor nutritional status of the mother at conception and inadequate energy and protein intake during pregnancy may also result in FGR. Because children born with FGR are not a homogeneous group, they have a broad spectrum of growth, health, and developmental outcomes. In general, they have higher rates of subnormal growth, morbidity, and neurodevelopmental problems. The biomedical mechanisms reflected in nutritional, infection-related, hormonal, and metabolic parameters are not likely to be independent causative factors of FGR, but important mediating factors of a pathological process set in motion by other agents and insults.

10.3.1) Effects of mother's diet

Nutrition is the major intrauterine environmental factor that alters expression of the fetal genome and it may have lifelong consequences. This phenomenon, termed "fetal programming", has led to the recent theory of "fetal origins of adult disease" (87). Namely, alterations in fetal nutrition and endocrine status may result in developmental adaptations that permanently change the structure, physiology, and metabolism of the offspring, thereby predisposing individuals to metabolic, endocrine, and cardiovascular diseases in adult life. Animal studies have shown that both maternal under- and overnutrition reduce the placental-fetal blood flow and stunt fetal growth. Impaired placental synthesis of nitric oxide (a major vasodilator and angiogenesis factor) and polyamines (key regulators of DNA and protein synthesis) may provide a unified explanation for FGR in response to these nutritional extremes that have the same pregnancy outcome. There is growing evidence that maternal nutritional status can alter the epigenetic state (stable alterations of gene expression through DNA methylation and histone modifications) of the fetal genome. This may provide a molecular mechanism for the impact of maternal nutrition on both fetal programming and genomic imprinting. Promoting optimal nutrition does not only ensure optimal fetal development, it also reduces the risk of chronic diseases in adults.
FGR is a result of a complex pathology caused by multiple factors of fetal, placental, and maternal origin. Hormones and growth factors released as a result of maternal-fetal physiological interactions play an important role in fetal well-being and fetal outcome. Both maternal malnutrition and anemia are associated with various degrees of FGR. A relationship between decreasing birth weight percentiles and increasing fetal morbidity and mortality has been demonstrated. Maternal anemia and/or malnutrition are recognized to be the most frequent causes of FGR and small-for-gestational-age births in developing countries, such as India (84). The percentage of small-for-gestational-age neonates born to malnourished and anemic mothers was significantly higher than those born to mothers who were either malnourished or anemic. Significantly higher levels of hormones and growth factors, such as GH, prolactin, HPL and IGF-1, were observed in the cord blood of neonates born to malnourished and anemic mothers, indicative of an adaptive response on the part of the fetus designed to overcome an in utero growth disadvantage. Anoxemia-related fetal perturbations may have unique features that make them distinguish them from nutrient-deficiency-related FGR.

Relationships have been found between blood levels of vitamins and FGR. In a poor population of South Asia (Lahore, Pakistan), the occurrence of FGR increased in women with low maternal and cord concentrations of folate (vitamin B12) and high maternal levels of total homocysteine (88). In term, but not preterm, deliveries with FGR, maternal and cord blood folate levels were half those in deliveries of normal-birth-weight infants. The risk of FGR was reduced among women with folate levels in the highest quartile. Total homocysteine levels were higher in women delivering FGR infants. There was an inverse correlation between the cord blood folate levels and total homocysteine levels. Increased risks for hypertensive illness and premature delivery were found in women in the highest quartile of total homocysteine.

In Chinese women, elevated homocysteine and suboptimal vitamin B12 and B6 status may increase the risk of preterm birth (89). An elevated homocysteine level was associated with a nearly fourfold higher risk of preterm birth. The risk of preterm birth was 60% lower among women with a vitamin B12 level of 258 pmol/L or higher than among vitamin B12-deficient women, and was 50% lower among women with 30 nmol/L or higher vitamin B6 than among vitamin B6-deficient women.

Marked differences in vitamin A, folate, and iron concentrations in the cord blood between growth-retarded babies and their normal counterparts were established in Sao Paulo, Brazil (90). The percentages of FGR with the abnormal levels of nutritional indices relative to normal babies were 33.1 vs. 14.6 for vitamin A, 25.7 vs. 19.9 for red blood cell folate, and 37.0 vs. 21.4 for Hb, but similar for ferritin. The percentages of FGR mothers with abnormal levels of nutritional indices compared to normal mothers were 1.1 vs. 1.4 for vitamin A, and 36.8 vs. 32.1 for folate. The FGR mothers were less often anemic (43.2 vs. 50.8%), but tended to have higher levels of ferritin (37.6 vs. 23.9%) compared to normal mothers. These results indicate that micronutrient deficiency is the result of being born small rather than vice versa. The high levels of ferritin in FGR mothers may reflect subclinical maternal infection contributing to FGR. Maternal micronutrient deficiency is unlikely to be a causative factor for FGR in this population.
Mean folate intake was shown to be correlated with circulating concentrations of folate in the serum of 28-week-pregnant women (91). The women with a low mean daily folate intake (less than 240 μg) had an approximately twofold greater risk of preterm delivery and delivery of low birth weight (LBW) infants after maternal characteristics, energy intake, and other correlated nutrients were controlled for.

Vitamin A was found to be significantly correlated with birth weight, head circumference, length, and gestational period (92). There was also a significant positive relationship between zinc and birth weight. In contrast, copper showed a negative correlation with birth weight and head circumference. Vitamin E and magnesium were not associated with any of the anthropometric measurements, although magnesium showed an increasing trend with birth weight. These data suggested that most of the mothers of the subjects studied may have been marginal with respect to vitamins A and E and zinc. In those with LBW babies, a higher intake would have improved their nutritional status and possibly the outcome of their pregnancy.

An antioxidant nutrient balance is important for pregnant women who are exposed to various oxidants through food, drinking water, or inhaled air. Maternal oxidative stress during pregnancy plays an important role in fetal growth. Maternal serum vitamin C level during the second trimester (24-28 gestational weeks) is positively correlated with birth weight and length in full-term babies (93). An increase of 1 μg/ml in the serum vitamin C level increased the birth weight by 27.2 g and the birth length by 0.17 cm. The birth weight and length were highest when the levels of both vitamins C and E were high.

Serum protein levels are not predictive of birth weight or growth retardation at birth, but are significantly correlated with a number of other measures of nutritional status. Serum albumin levels at 18 weeks of pregnancy were inversely correlated with birth weight (94). This negative correlation was explained by an inverse relationship between albumin concentration and maternal body-mass index. There was no significant correlation between albumin levels at 30 weeks and birth weight, or between birth weight and the concentrations of the other two proteins studied at either gestational age. In individual subjects, the concentration of each protein correlated significantly with the concentration of the other proteins, and the levels at 18 weeks correlated with those at 30 weeks.

Maternal blood levels of trace elements were shown to have a great influence on pregnancy and on fetal growth. In studying the associations between blood zinc concentrations and various measures of pregnancy outcome and neonatal conditions at birth, the plasma zinc concentration was shown to decline as gestation progressed from week 6 to week 34 of gestation (95). After plasma zinc concentration was adjusted for gestational age, it was not significantly associated with any measure of pregnancy outcome or neonatal condition.

During pregnancy, trace elements are indispensable for sustaining life not only the mother's but also the fetus's. Fetal growth has been shown to be associated with altered levels of trace elements in the maternal and fetal blood, and the placental tissue (96). Compared with appropriate-for-gestational-age cases, FGR cases show
higher magnesium, copper, and selenium concentrations in the umbilical cord arterial serum, and higher magnesium and selenium concentrations in the placenta tissue, but no significant differences appeared for the elements measured in the maternal and umbilical cord venous serum. The ratio of umbilical cord venous vs. maternal serum concentration was elevated for copper, and the ratios of umbilical cord arterial vs. umbilical cord venous concentration were elevated for copper and zinc, but there were no differences in the placenta tissue vs. maternal serum concentration ratios in FGR. Reduced consumption efficiency of these four essential trace elements may be closely associated with retarded fetal development.

Low levels of zinc in polymorphonuclear white cells were found in women giving birth to small-for-gestational-age babies (97). The maternal plasma zinc and albumin levels 24 to 48 h after delivery were lower than in non-pregnant control women. A combination of smoking and/or low zinc levels was found in 85% of mothers having small-for-gestational-age babies. Despite the commonly accepted facts that zinc and folate are important for the fetal growth, maternal zinc assessed by serum and dietary intake was not associated with birth weight or length of gestation (98). The indirect measures of maternal nutritional status, including maternal pre-pregnancy weight and weight gain during pregnancy, were stronger predictors of adjusted infant birth weight than energy intake or intake of zinc and folate.

10.3.2) Effects of mother's diseases

The most frequent cause of LBW is intrauterine infection associated with intrauterine growth restriction and premature labor. The microorganisms most frequently associated with LBW are *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Escherichia coli*, group B Streptococci and *Chlamydia* spp. (99). Intrauterine infection by these and other microorganisms are associated with a vigorous inflammatory response at the maternal–fetal interface that is characterized by the increased concentrations of T helper-1 cytokines (100-102), nitric oxide production (103,104), prostaglandin production (105,106), and increased numbers of apoptotic cells (107) and neutrophils in the placenta and amniotic fluid. These alterations ultimately result in decreased fetal and placental development as well as premature activation of the fetal hypothalamic–pituitary–adrenal axis to initiate labor (108).

There are different diseases which in pregnant women can have a detrimental effect on the fetuses. Disorders occurring in early life may underlie abnormal functional development in later life, whereas growth is mainly determined during the second half of pregnancy. For example, in the first trimester of type-1 diabetic pregnancy, the embryo and fetus are often smaller than normal (109), but there is no relationship between the degree of early growth delay and birth weight. Mean growth delay per fetus in early diabetic pregnancy was negatively correlated with the occurrence of no-coincidence between behavioral state parameters at 36 weeks of pregnancy.

Maternal cardiovascular adaptation has to provide the uterine perfusion necessary to meet the requirements of the growing fetus by providing transport of nutrients and oxygen to the placenta and fetus (110). Thus, uterine blood flow is inextricably linked
to fetal growth and survival. Reductions in uterine blood flow can occur under acute or chronic conditions or their combination. Chronic reductions in uterine blood flow can be observed in pregnancy-induced hypertension, diabetes mellitus in pregnancy and FGR. Chronic restrictions in uterine blood flow will elicit a placental and fetal response in the form of growth adaptation to the reduced supply of oxygen and nutrients to the conceptus. If compensatory growth restriction reaches its limits, intrauterine fetal distress can ensue.

In mothers with preeclampsia, fetuses often develop the growth retardation. By means of perinatal inquiry, not only was the frequency of preeclamptic toxemia proven, but also the frequency of toxemia in mothers with newborns of different weights (111). In the overweight newborns, toxemia is less often caused by proteinuria than in the underweight babies. These findings can be explained by the hypothesis that preeclamptic toxemia is a compensatory mechanism in FGR. For the fetus, which is insufficiently supplied by the placenta, this regulatory mechanism enhances the placental blood supply. The higher incidence of toxemia in pregnant women with overweight babies is explained by the increased demand on the placenta; developing toxemia entails better placental blood supply to the fetus; in this case, the toxemia is compensated for. In the decompensation stage with FGR, all reserves are mobilized via increased permeability of the vessels, which leads to improved passage through the placenta, but also to proteinuria and increased incidence of edema.

In Norway, among 12,804 deliveries over a 3-year period, 307 live singleton infants were born after preeclamptic pregnancies (112). Preeclampsia was defined as an increase in diastolic blood pressure (of at least 25 mmHg to at least 90 mmHg) and proteinuria after 20 weeks gestation. Preeclampsia was associated with a 5% reduction in birth weight. The risk of having a small-for-gestational-age infant was dramatically higher in women with recurrent preeclampsia. In severe preeclampsia, the reduction was 12%, and in early-onset disease, birth weight was 23% lower than expected. The risk for being small for gestational age was four times higher in infants born after preeclampsia than in control pregnancies. Among nulliparae, preeclampsia was associated with a nearly threefold higher risk of small-for-gestational-age babies, and among paras, this risk was particularly high after recurrent preeclampsia.

Gestational hypertension and/or preeclampsia, the most important gestational diseases that also cause FGR, are characterized biochemically by high blood levels of such glycoproteins as activin A (βA) and inhibin A (αβA) (113). The serum levels of activin A and inhibin A are higher in preeclampsia, and the presence of FGR does not significantly modify these concentrations. Similarly, inhibin-subunit mRNA levels in placentas from preeclampsia are higher than in controls, and FGR does not significantly affect this expression. It has been suggested that the increased placental expression of inhibin-subunit mRNA is part of the mechanism leading to the increased serum levels of activin A and inhibin A. In a comparative study of women in whom gestation was complicated by preeclampsia and women with isolated FGR, neurokinin B was found to potentially be involved in pregnancy hemodynamic adaptation via the nitric oxide production (114). In pregnancies complicated with preeclampsia and FGR, the increased neurokinin B plasma level correlated well with
the increased nitric oxide metabolite level, which may represent a compensatory mechanism for improving blood flow to the uteroplacental unit.

Early fetal growth delay (7-14 weeks of gestation) has been reported in insulin-dependent diabetic pregnancies and in several animal models (115). Macrosomia is a classic feature of infants from such mothers. Early growth delay was greater in the fetuses that subsequently developed macrosomia. Similar results were found for the abdominal circumference measurements. Fetal growth delay occurs in the first half of the insulin-dependent diabetic pregnancy, followed by a phase of increased growth. Although the mechanism governing the early growth delay is unclear, authors speculated that it may be due to a "toxic" effect of glucose or some other metabolite, and that the subsequent increased growth relates to fetal hyperinsulinism which develops from weeks 15 to 20 of gestation.

Recognition of the specific dynamics and characteristics of different morphological patterns can be useful for early detection of the at-risk fetus (116). There is a significantly larger abdominal circumference in the early vs. the late patterns in the fetuses obtained from diabetic women during their third trimester of pregnancy and in gestational diabetes small-for-gestational-age infants. Maternal weight, glycemia after therapy, rates of fetal macrosomia, and a large-for-gestational-age outcome were not significantly different between gestational diabetes mellitus and impaired glucose tolerance (117). In the late second and early third trimester, maternal pregnancy obesity and a previous large-for-gestational-age pregnancy appear to have the strongest influence on the fetus's growth, while later, in the third trimester, during the period of maximum fetal growth, maternal glycemia predominates.

Microalbuminuria and nephropathy in diabetic subjects have been linked to low birth weight or short stature in adulthood. Low birth weight and low pondered index at birth were shown in men and women with microalbuminuria, compared to those who were normal albumin uric (118). The albumin excretion rate of the subjects exposed in utero to maternal starvation was not significantly different from that of subjects exposed in infancy or those who were not exposed. Consistent relationships between short stature and microalbuminuria and nephropathy in non-diabetic and diabetic subjects might suggest that more subtle anthropometric indices relate to the low nephron number at birth, or that postnatal or genetic influences underlie the observed link.

### 10.3.3) Effects of mother's smoking

Among the etiological factors responsible for FGR, one-third of the variations in birth weight are determined by genetic variables and two-thirds by environmental factors (119). Among the preventable, environmental causes of FGR, mother's smoking during pregnancy is by far the most important one, responsible for more than one-third of all FGR newborns (120,121). The effect of maternal smoking on LBW appears to be attributable to FGR rather than preterm delivery. Smoking is associated with at least a doubling of the risk of FGR, regardless of whether LBW or preterm
birth are also present, suggesting that FGR is the key factor mediating the effect of smoking on the birth weight. Social differences in maternal smoking may be responsible for the similarity of the rates between cities with distinct levels of socioeconomic development (122): a wealthier city showed higher rates of maternal smoking, attendance in the private sector, and obstetric intervention than a less developed one.

An association between the intensity and duration of cigarette smoking during pregnancy and the frequencies of LBW, preterm births and FGR was shown in a cohort performed of 5166 live births in Brazil (123). Mean birth weight was 3,169 g. The prevalence of LBW, preterm birth and FGR was 9.1%, 8.0%, and 8.9%, respectively. The prevalence of smoking at conception was 33.2% and 26.2% of the mothers smoked during the entire pregnancy; and 43% of the mothers’ partners smoked. Children whose mothers smoked during pregnancy had a birth weight that was 142 g lower than those of non-smoking mothers. Mothers who smoked for part or all of the pregnancy were 1.59 times more likely to deliver a LBW infant than non-smokers. The risk of FGR was 2.07 times higher in mothers who smoked. Women who stopped smoking during the first trimester had a risk similar to that of nonsmokers. There was a direct dose-response association between the number of cigarettes smoked and the risk of growth retardation. Women whose partners smoked were also at higher risk of having a child with growth retardation.

An association was described between reductions in adjusted birth weight among women who smoked throughout pregnancy and the amount of cigarettes they smoked per day (124). For low/moderate smokers (15 cigarettes per day or less), infant birth weights were reduced by up to 168 g relative to infants of non-smoking women. In heavy smokers (more than 15 cigarettes per day), the reduction in body weight reached 288 g. A decrease in birth weight (179 g) was found among smokers who reported quitting early in pregnancy. Heavy maternal smoking can affect any point in the pregnancy, including solely in the early months, resulting in LBW.

Different statistical methods were used to estimate the effect of smoking on infant birth weight independent of gestational age and maternal weight gain during pregnancy (125). After adjustment for non-modifiable factors, smoking accounted for 1.5 to 3.1% of the variance in gestational age at delivery. It accounted for 5.3 to 7.7% of the variance in net maternal weight gain after adjustment for non-modifiable factors and gestational age. After adjustment for gestational age, net maternal weight gain and the non-modifiable factors, smoking accounted for 2.7 to 5.2% of the variance in infant birth weight. Most of the gain in the infant birth weight upon quitting smoking was due to the independent effect of smoking on FGR, with a much smaller increase related to maternal weight gain and a slightly longer gestational age.

A synergistic effect has been suggested when smoking is combined with preeclampsia, causing a lower birth weight than would be expected from their additive effects (120). In smoking women suffering from preeclampsia, the fetuses often develop growth retardation. Nicotine, a vasoconstrictive substance, increases blood pressure, thereby causing a higher incidence of preeclamptic toxemia in smoking pregnant women (111). By means of perinatal inquiry, not only was the frequency of preeclamptic toxemia proven in smoking women, so was the frequency of toxemia in
mothers with different weights of newborns. However, results from other researchers do not support a synergistic effect of smoking and preeclampsia on fetal growth (126). Birth weight reductions related to maternal smoking appear to be added to those caused by preeclampsia, suggesting that there is no synergism between smoking and preeclampsia effects on growth restriction (112).

Possible interference with the placentation or implantation has been suggested by the observed increased frequency of spontaneous abortions of a chromosomally normal conceptus in smoking women (127). On average, infants born to women who smoke during pregnancy are 200 g lighter than those born to comparable women who do not smoke. The finding of antepartum bleeding of unknown cause is found consistently been found more often in smokers, relative to nonsmokers. Sudden infant death syndrome is closely associated with both the frequency and level of maternal smoking during pregnancy.

10.3.4) Effects of mother's alcohol consumption

In humans, fetal alcohol syndrome (FAS) has been estimated to occur in between 1 in 600 and 1 in 1000 live births in the USA, France, and Sweden (127). The likelihood of miscarriage increases directly with alcohol consumption. The risk of abortion is twice as high in women consuming one ounce of absolute alcohol as infrequently as twice a week. Despite the many facts proving that both cigarette smoking and alcohol consumption during pregnancy have potential detrimental effects on fetal growth, there appears to be a wide spectrum of fetal phenotypic response to the effects of alcohol (127). This phenotypic variability may be partially explained by the dose, timing, and pattern of gestational exposure, the metabolism of the mother or fetus, or other environmental or genetic factors. Infants with the unique combination of anomalies termed FAS are at the most severe end of the spectrum. The abnormalities most typically associated with alcohol teratogenicity can be grouped into four categories: central nervous system dysfunctions, growth deficiencies, a characteristic cluster of facial abnormalities, and variable major and minor malformations.

One of the most common and serious defects associated with ethanol teratogenicity is mental retardation (127). Recent evidence supports the concept of a prenatal origin to the problem. At birth, in infants with FAS, there is a deficiency in both their length and weight, usually at or below the 3rd percentile for both parameters. Growth and mental deficiency are seen in many conditions, but the rather striking facial appearance of children with FAS confirms the diagnosis. The characteristic face in small children includes short palpebral fissures, a short upturned nose, a hypoplastic philtrum and maxilla, and a thinned upper vermilion.

Increasing prenatal alcohol exposure was shown to be associated with LBW and gestational age, higher lead levels, higher maternal age, and lower education level, prenatal exposure to cocaine and smoking, custody changes, lower socioeconomic status, and paternal drinking and drug use at the time of pregnancy (128). Children with any prenatal alcohol exposure were more likely to have higher child behavior
checklist scores on externalizing (aggressive and delinquent) and internalizing (anxious/depressed and withdrawn) syndrome scales and total problem score.

Heavy drinking during pregnancy is an established risk factor for FAS and other adverse perinatal outcomes. In a prospective investigation based on 2,714 singleton live births at Yale-New Haven Hospital in the USA, performed from 1988 to 1992, mild drinking (defined as more than 0.10-0.25 ounce of absolute alcohol per day) during the first month of pregnancy, was found to be associated with an effect on FGR (129). Overall, drinking during the first month of pregnancy suggested a curvilinear effect on growth retardation, with consumption of more than I oz. of absolute alcohol per day showing increased risk. Drinking during the seventh month of pregnancy was associated with a uniform increase in the odds of a preterm delivery. Differences in the risk estimates for FGR and preterm delivery were thought to indicate etiological differences between these outcomes and critical periods of exposure. LBW is not a useful neonatal parameter for this exposure because it is a heterogeneous mix of preterm delivery and FGR. Despite the observed protective effects of mild drinking on FGR, the increased risk of preterm delivery with alcohol use supports a policy of abstinence during pregnancy.

Among women having live births, alcohol consumption during pregnancy was significantly related to having a LBW baby (less than 2500 g), similar to the other factors, such as race, age, mother's education, prenatal care, prematurity, gestational age, and smoking (130). Women who drank more during pregnancy also smoked more, and were younger and less educated than women who drank in lower amounts or not at all. The effect of alcohol is significant for the occurrence of LBW, fetal death and infant death, analyzed by multivariable logistic regressions. Results indicated that alcohol has an important relationship with birth outcome, but that the alcohol effect on mean birth weight is small relative to that of other risk factors, accounting for the non-significant result in the multiple linear regression. First-trimester alcohol consumption (average: four drinks per week) was associated with a 155 g reduction in fetal growth (124). The association, observed with all types of alcohol consumption, was stronger among smokers (-270 g) but was also present in nonsmokers (-115 g). It seems that even moderate alcohol drinking may serve as a cause of LBW.

Although heavy maternal alcohol consumption during pregnancy has been associated with FGR, paternal alcohol consumption was not associated with any of the fetal growth measures after adjustment for other variables (131). The adjusted odds ratio for moderate consumption of three or more drinks per week was 2.6 for LBW, and 2.3 for FGR. Examining the combined effect of smoking and alcohol consumption revealed a synergistic effect was found for LBW but not for FGR. Moderate alcohol consumers had an average birth weight decrement of 143 g, which varied with smoking. There was little association of alcohol consumption with preterm delivery (less than 37 weeks).

Studies with animal models of alcohol-related birth defects suggest that reductions in circulating thyroid hormones, including thyroxine, may be a persistent postnatal effect of fetal alcohol exposure. The few clinical reports of children with FAS describe thyroid hormone levels that are within normal limits. It was shown that alcohol intake
and smoking each had a substantial negative impact on birth weight, gestational age at birth, and fetal growth, assessed as birth weight corrected for gestational age (132). Infant thyroxine levels were positively related to the birth weight and gestational age and were more strongly related to the fetal growth. Infant thyroxine levels were not significantly influenced by either maternal smoking or alcohol consumption. Smoking- and alcohol-related reductions in birth weight, gestational age, or fetal growth were not significantly associated with variations in infant thyroxine levels. Interesting questions remain regarding species differences and the influences of maternal alcohol consumption on thyroxine metabolism as a mechanism for alcohol-related birth defects. However, the current data do not support the hypothesis that maternal alcohol consumption, or smoking, during pregnancy leads to compromised thyroid system function in human newborns.

10.3.5) Effects of drinking water

There are many uncertainties regarding the risk of adverse pregnancy outcomes associated with exposure to by-products of drinking water disinfection. In Montreal, Canada, 493 hospital-based cases of FGR were assumed to be a result of drinking water containing trihalomethanes (133). Mothers and newborns were characterized for two genetic polymorphisms, one in the CYP2E1 gene (G1259C), and the other in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene (C677T). Exposure to specific and total trihalomethanes from drinking water, determined for 458 cases and 426 controls, did not result in an increased risk of FGR. However, a significant modifying effect was observed in comparison of newborns with and without the CYP2E1 variant: among newborns with the variant, the adjusted odds ratio for FGR associated with exposure to average total trihalomethanes above the 90th percentile (corresponding to 29.4 μg/L) was 13.20. The findings suggested that exposure to trihalomethanes at the highest levels can affect fetal growth but only in genetically susceptible newborns.

A great risk for FGR was found in communities whose drinking water contains elevated levels of herbicides. Communities in southern Iowa, US, for example, whose drinking-water supply was contaminated with herbicides, had elevated rates of FGR compared to neighboring communities with different water supplies (134). Multiple linear regression analyses revealed that levels of the herbicides atrazine, metolachlor, and cyanazine were each significant predictors of community FGR rates after controlling for several potentially confounding factors including maternal smoking and socioeconomic variables. The association with FGR was strongest for atrazine, but all three herbicides were inter-correlated.

10.3.6) Effects of socioeconomic factors
Embryonic and fetal growth depends on genetic and environmental factors, and the process is a result of the interaction between these factors. About 7% to 9% of live-born infants have below-normal birth weight (below the 10 percentiles). The rate and extent of FGR varies by ethnicity and socioeconomic status (135). Maternal factors such as inadequate or severe malnutrition, chronic maternal diseases, birth order, multiple births, and parental genetic factors are suspected causes of FGR. Chronic maternal malnutrition and other poverty-related factors are likely to be important determinants of FGR in developing countries (136). A study of relationships between birth weight, sociodemographic variables and maternal anthropometry showed that in an inner urban area of Dhaka, Bangladesh, about 21% of the babies were of LBW, less than 2,500 g of the mean birth weight (137). LBW was more common in younger (younger than 20 years) and older (older than 30 years) mothers, in the low-income group and in women with little or no education. The mean birth weights among a higher-educated, higher-income group and of male children were on average 290, 260 and 120 g, respectively, higher than in uneducated, lower-income groups and female children. The sensitivity (69%) and specificity (68%) were best for maternal weight. A regression analysis found that mother's weight at term was the best predictor of LBW, while maternal weight combined with age, educational level, and income group correctly predicted slightly more than 35% of LBW.

LBW for gestational age is associated with increased risk of developing cardiovascular and diabetic diseases later in life (138). These associations are the main basis for the 'Fetal origins hypothesis' (the 'Barker hypothesis') that malnutrition-related FGR has long-lasting physiological and structural effects that predispose the individual to diseases later in life (139). Fetal malnutrition has two main causes, in particular, poor maternal nutrition and placental insufficiency.

Some authors are of the opinion that maternal malnutrition was not prevalent in the majority of the populations in which the fetal origins hypothesis was tested (140). These authors suggested that there is no reason to believe that poor maternal nutrition was present in the populations used to study associations between birth weight and hypertension in children (141). Socioeconomic conditions are claimed not to confound the association between fetal weight and life-long health risks. This implies that significant maternal undernutrition was present in the upper social classes, for example in Sweden, in the 1920s, which seems unlikely (142). The cases with monozygotic twins, in which the LBW twin had the highest risk of diabetes later in life, cannot be explained by differences in maternal nutrition (143). There are marginal effects of nutritional intervention to prevent FGR in populations with a low prevalence of nutritional deficiency (144). Finally, only extreme maternal undernutrition, such as the Dutch famine, reduces the birth weight to the extent that an increase in the risk of adult disease would be expected (145).

Higher rates of FGR are not necessarily be observed in poorer areas relative to wealthier ones (122). In Ribeirao Preto, for example, a city located in the most developed area in Brazil, a FGR rate was 18%, and a similar rate (18.5%) was found in Sao Luis, located in one of the poorest areas in the country. It would appear that the early detection of FGR followed by cesarean section in the wealthier city is what is actually associated with increased LBW and FGR rates, but reduced stillbirth and infant-mortality rates.
Maternal hypertension is a risk factor of placental insufficiency measured as prevalence of small-for-gestational-age infants (146). Coagulation disorders and dyslipidemia are more often found in preeclamptic women, among whom placental insufficiency and FGR are more prevalent (147,148). Thus, established risk factors for cardiovascular diseases and diabetes are present with a higher prevalence in pregnant women with placental insufficiency. Associations between maternal blood pressure, LBW and hypertension in the offspring have been described (149,150). The association between LBW and the risk of adult cardiovascular diseases may therefore indicate that placentation in women with cardiovascular risk factors is impaired, resulting in placental insufficiency and small infants (140). Pathogenically, it is conceivable that 'hypertensive' spiral arteries are more resistant to transformation, that dyslipidemia may induce acute atherosis and that coagulation disorders result in local thrombosis and placental infarctions.

It was suggested that in adequately nourished populations, maternal cardiovascular risk factors may increase the risk of adult diseases in the offspring via two mechanisms (140): inheritance (151), and impairment of the normal placentation process resulting in placental insufficiency (147,149). The latter may result in FGR which itself can cause adult disease. Alternatively, the association between LBW and adult disease may also be an epiphenomenon, leaving inheritance as the main explanation for the fetal origin hypothesis, in well-nourished populations.

A significant association between low serum folate levels and FGR was found in pregnant women at 30 weeks gestational age, each of whom had been provided with folate supplementation at enrollment in prenatal care (152). Because high folate levels are most likely explained by recent folic acid intake, it has been speculated that the decreased fetal growth associated with a low folate level may be related to a combination of psychological and behavioral characteristics for which the low serum folate level is only a surrogate measure. Poor psychological scores, including measures of depression, anxiety, self-esteem, mastery, stress, and social support, were significantly related to the lower serum folate levels. In women with both good and poor psychosocial scores, the high folate level was significantly associated with increased birth weight, a relationship that persisted even after adjusting for maternal race, body mass index, smoking, history of a LBW infant, and infant gender. It has been suggested that women with good psychosocial scores are more likely to take folate, but that the use of folate itself is related to a lower risk of FGR and increased birth weight.

10.3.7) Genetic predisposition to FGR

FGR is an important cause of small stature in children presenting to pediatric endocrinologists (153). FGR has to be differentiated from familial ('constitutional') short stature, where the growth deficit is genetically determined and/or induced by smallness of the mother (maternal constraint). Intrinsic fetal anomalies such as chromosomal abnormalities, primary growth failure syndromes, congenital infections and congenital anomalies are of equal importance with maternal disorders, in particular chronic use of alcohol, tobacco and narcotics, and pregnancy complications.
such as hypertension and preeclampsia, in causing FGR. The relative importance of placental abnormalities and environmental factors (with the exception of malnutrition) appears to be small. Genetic predisposition and abnormal trophoblastic function are thought to contribute to the development of preeclampsia. Multiparous women developed severe preeclampsia and subsequently delivered a live growth-retarded infant with trisomy 13 (154).

LBW in healthy term neonates is associated with fetal inherited prothrombotic risk factors (155). The proportion of children in the LBW quartile increased from 23.7% to 30.5% to 48.0% for children with no defect, only a single heterozygous defect and multiple or homozygous defects, respectively. The respective adjusted odds ratios (95% confidence intervals) of thrombophilia for birth weight in the lowest quartile (lowest deciles) were 1.53 (0.76-3.08) in carriers of one prothrombotic risk factor and 4.01 (1.48-10.84) in subjects carrying multiple or homozygous defects.

Genetic factors may interact with altered intrauterine growth causing the risk of cardiovascular and renal diseases (156). Subjects homozygous for the D allele of the ACE gene are predisposed to both cardiovascular complications and nephrosclerosis. Altered intrauterine growth is associated with a reduced number of nephrons at birth, has a negative influence on the development of the cardiovascular system and favors the occurrence of hypertension, insulin resistance, hypercholesterolemia and hyperuricemia in adult life.

Frequencies of ACE, Apo-E gene polymorphisms, apolipoprotein-B (Apo-B) mutation and lipid compositions were determined in full-term newborn infants with FGR (157). An insertion/deletion polymorphism with a significantly increased frequency was observed in the FGR group (65%) as compared to the control group (33%). When the distribution of the Apo-E gene polymorphism (E2, E3 and E4) was studied, no difference was found between the FGR and control groups with respect to frequency. No Apo-B gene mutation was identified in the study groups. An insertion/deletion polymorphism is responsible, at least in part, for the etiology of intrauterine growth restriction (IUGR). Levels of total cholesterol and Apo-B are elevated in FGR infants, suggesting a linkage between LBW and atherosclerosis.

A number of genetic and environmental factors are taken into account in relation to fetal pathology and as responsible for FGR. It is likely that a gene polymorphism or mutation that is susceptible to reproductive failure has a beneficial effect on the process of human reproduction with or without the environmental interaction. Pregnancies with a mosaic trisomy for chromosomes 7, 2 and 14 resulted in pre- and postnatal growth restriction (158). These chromosomes are known or suspected to harbor imprinted genes, so that an unbalanced gene dosage in a subset of cells during embryonic development could lead to early impairment of placental function. Inherited thrombophilia, such as factor V Leiden, prothrombin, and methylenetetrahydrofolate reductase mutations, gene polymorphisms of detoxification enzyme (CYP1A1), growth factors (insulin-like growth factor-I), and hormones such as angiotensinogen and CYP17 are involved in the pathogenesis of FGR (159). The inherited thrombophilia, gene polymorphisms of coagulation and anticoagulation factor such as thrombomodulin, endothelial protein C receptor, plasminogen activator inhibitor 1, and factor XIII, human lymphocyte antigen (HLA-G), detoxification
enzymes (glutathione-S-transferase M1), cytokines such as IL-1 and IL-6, hormones (CYP17), vasodilators (nitric oxide synthase 3), and vitamins (transcobalamin) are involved in the pathogenesis of sporadic and recurrent miscarriage. The factor V Leiden mutation has genetic advantages that are believed to confer improved implantation rate in *in vitro* fertilization and a reduction in maternal intrapartum blood loss. The CYP17 A2 allele has bidirectional effects on human reproduction, including increases in susceptibility to recurrent miscarriage and fetal growth enhancement.

**10.3.8) Racial effects on FGR**

Marked racial variation in birth-weight percentiles by gestational age has been described (160). Compared with extremely-low-risk white mothers, the risk of a small-for-gestational-age infant was 2.64 times greater for extremely low-risk African American mothers, and the risk of infant mortality was 1.61 times greater for the latter. For the extremely low-risk group, the infant mortality rates of African American and white infants at or below the 10th percentile of birth weight for gestational age of their respective maternal race group were essentially identical after controlling for gestational age. In the other words, race differences in fetal growth patterns remained after controlling for risk status. Efforts to remove racial disparities in infant mortality need the development of etiological pathways that can explain why African Americans have relatively higher rates of preterm births and higher infant mortality rates among term and non-small-for-gestational-age infants.

Maternal serum α₂-macroglobulin was elevated at as early as 18 weeks of gestation in women destined to have a growth-retarded infant, and this elevation persisted through 30 weeks of gestation (161). Furthermore, levels were higher in white vs. black women, in smokers vs. non-smokers, and in thin vs. heavier women. When the effect of α₂-macroglobulin on birth weight was evaluated in a multiple regression analysis adjusting for gestational age, race, body size, smoking, fetal sex, and a history of a LBW infant, high α₂-macroglobulin levels were associated with a statistically significant decrease in birth weight. The effect was greater in smoking women. This relationship did not appear to be associated with differences in serum zinc or hematocrit levels.

Low nephron number has been related to LBW and hypertension. In the southeastern United States, the estimated prevalence of chronic kidney disease due to hypertension is five times greater for African Americans than for white Americans. The relationships between the total glomerular number (Nglom), blood pressure, and birth weight were found to be different between southeastern African Americans and white Americans (162). For African Americans, the relationships between the mean arterial blood pressure (MAP), Nglom, and the birth weight were not significant. For white Americans, they were as follows: MAP and Nglom (r=-0.45), Nglom and birth weight (r=0.57), MAP and birth weight (r=-0.42). For African Americans, the average glomerular numbers for normotensive and hypertensive patients were not significantly different. For white Americans, the average glomerular numbers for normotensive and for hypertensive patients were significantly different. It can be concluded that the low
nephron number and, possibly, LBW play a role in the development of hypertension in white Americans but not African Americans.

Fundamental differences in the etiology of IUGR have been found between European and Asian women (163). Fetoplacental function tests discriminated well between poorly grown and normally grown fetuses in European mothers. In contrast, apart from human placental lactogen, the individual values of poorly grown Asian fetuses fell within the normally grown range. Hb values rose in the second trimester in European pregnancies associated with poor growth, whereas the values fell in the Asian pregnancies for both normally grown and poorly grown fetuses.

Fetal growth during pregnancy was shown to be related to ethnic factors, such as African and European origin (164). Bi-parietal diameter was significantly smaller among African fetuses mainly during late pregnancy, and that of the femur was greater during the entire pregnancy, whereas the transverse diameter of the fetal abdomen did not differ between the two groups. Ethnic factor remained significant when taking into account confounding factors. Observed differences appeared to be more likely related to a racial factor than to hypotrophy.

Racial differences in hematocrit levels and the relationship between low and high hematocrit were found to be connected with FGR and preterm delivery (165). In black women, hematocrits of 27% to 30% were associated with lower reductions in the rates of FGR and preterm delivery. At 31 to 34 weeks, 40% or more of the hematocrits were associated with significantly higher odds ratios for FGR in both blacks and whites.

Research based on hospital records demonstrated that many births classified as normal according to conventional demographic measurements are FGR when evaluated clinically. Moreover, in analyses of birth outcomes, one must focus on a third dimension, maturity, in addition to birth weight and gestational age. Although clinical studies allow more precise classification, the small number of cases tends to result in unreliable estimates of rates and in loss of generalizability. The fetal growth ratio, a measure recently shown to be a valid proxy for maturity, was used to develop a classification system based on combinations of weight, gestational age, and maturity, which were applied in a comparative analysis of a large data set. The results evaluated large differences in the distribution of compromised births across racial and ethnic groups, as well as significant race/ethnic differentials in the risk of infant mortality associated with adverse outcomes (166). Considerable racial/ethnic variations were found across birth outcome categories; differences persisted in the adjusted parameter estimates, and the effects of other risk factors on birth outcomes were similar in direction, but varied somewhat in magnitude (167). The odds of compromised birth outcomes were much higher among African Americans than among Mexican Americans or non-Hispanic whites.

Ethnicity and birth place affect prenatal care and birth outcomes but are probably not as significant as racial differences. Poor outcomes without elevated newborn costs may indicate less access to high-quality neonatal care among some ethnic groups. With the influx of Latin American immigrants to the United States and the relatively high fertility of Hispanic women, the importance of understanding patterns of birth
outcomes within the heterogeneous Hispanic community is growing (168). White women of Puerto Rican descent have a significantly higher risk than both non-Hispanic whites and other Hispanic whites of having LBW babies. However, their infants do not have an increased risk of mortality. Mexican-born white women begin prenatal care later than their US-born counterparts, but do not have worse birth outcomes. The sharpest contrasts are between non-Hispanic black and non-Hispanic white women born in the same place. The findings indicate that disparities by race may be at least as important as variations in birth place and ethnicity. Puerto Rican white women who gave birth in New Jersey were twice as likely, relative to their US-born non-Hispanic white counterparts, to have a LBW infant and to have an infant who died in the first year of life. Women of Puerto Rican descent, regardless of whether they were born in the US, initiated prenatal care later than all other whites, except the infants born in Mexico, and their infants had the highest rates of LBW and mortality among all whites. Although the multivariate results indicated that ethnic Puerto Rican Black women begin prenatal care earlier and have better birth outcomes than non-Hispanic Blacks, the descriptive statistics showed that Puerto Rican blacks and whites have similar levels of prenatal care use and birth outcomes. Poor outcomes without concomitant increases in hospitalization costs may be a sign of low access to high-quality neonatal care.

In studying the perinatal outcomes in Hispanic, black, and white non-Hispanic women in the USA, it was demonstrated that although foreign-born Mexican American women have many demographic and socioeconomic risk factors, their rates of LBW infants and infant mortality are similar to those of white women. This phenomenon has been termed an epidemiological paradox. The sociodemographic risk profile and perinatal outcomes were studied in women of Asian Indian birth and compared to those of foreign-born Mexican American and US-born black and white women (169). When compared with whites, US-born Blacks and foreign-born Mexican mothers were at increased risk for adverse perinatal outcomes on the basis of higher levels of inadequate prenatal care, teen births, medical paid delivery, and lower levels of maternal and paternal education. Foreign-born Asian Indian mothers had good prenatal care, were rarely teenagers, had dramatically higher levels of both maternal and paternal education, and had the lowest percentage of medical paid deliveries. Black infants had the highest rates of prematurity, FGR, LBW, and fetal, neonatal, and postneonatal mortality. Paradoxically, despite their high-risk profile, Mexicans did not have elevated levels of LBW or neonatal mortality. Conversely, Asian Indian infants, although seemingly at low sociodemographic risk, had high levels of LBW, growth retardation, and fetal mortality. Logistic regression analysis of independent risk factors for giving birth to an LBW infant showed higher maternal education, early access to prenatal care, and having private insurance to be protective in white non-Hispanic and black but not in Asian Indian or Mexican-born women. It can be concluded that despite their high socioeconomic status and early entry into care, foreign-born Asian Indian women have a paradoxically higher incidence of LBW infants and fetal deaths relative to US-born whites. Factors that protect from giving birth to a LBW infant in white women were not protective among Asian Indian women.
Fetal growth patterns among different US racial/ethnic groups varied markedly and, across the gestational age range, there was considerable oscillation in the relative ranking of any one group's birth-weight-percentile value in comparison to the others (170). Males had relatively higher birth-weight-percentile values than females. The proportion of infants with a birth weight value less than the US population's 1994-1996 10th percentile value for their corresponding gestational age was 7.87 for non-Hispanic whites, 15.43 for non-Hispanic African Americans, 9.30 for Hispanics, and 8.81 for Native Americans. While the factors underlying trends and population subgroup differences in fetal growth are unclear, nutrition, smoking habits, health status, and maternal morbidity are possible precursors for some of the variations in fetal growth patterns.

Maternal nativity status, along with ethnicity, may serve as an important axis of differentiation in birth-outcome studies. This conclusion was derived from the finding of substantial maternal nativity differences in risks of infant mortality and LBW between US- and foreign-born women, with the magnitude of the nativity effect varying significantly across racial/ethnic groups (171). Overall, foreign-born status was associated with 7% and 20% lower risks of LBW and infant mortality, respectively. However, the reduced risk of adverse pregnancy outcome associated with immigrant status tended to be substantially higher for blacks, Cubans, Mexicans, and Chinese than for other ethnic groups.

Maternal characteristics and birth outcomes of Mexican-born vs. native-born mothers in the US and North African mothers living in France and Belgium vs. French and Belgian nationals were compared (172). The adjusted odds for LBW were lower for immigrants than natives/nationals by 32% in the US, 32% in Belgium, and 30% in France. The adjusted odds for preterm births were lower for immigrants compared with natives/nationals by 11% in the US and by 23% in Belgium. In France, the odds for preterm births were comparable for immigrants and naturalized mothers. Infants of immigrant mothers also had higher mean birth weights in all three countries.

Associations were examined between obesity, diabetes, and three adverse pregnancy outcomes, such as primary cesarean delivery, preterm birth, and LBW, in different racial/ethnic groups, and it was shown that obesity and diabetes are independently associated with adverse pregnancy outcomes (173). Chronic and gestational diabetes were significant risks for a primary cesarean and for preterm birth in all women. Diabetes as a risk for LBW varied by group. For example, whereas chronic diabetes increased the risk for LBW among Asians, Hispanics, and whites (adjusted odds ratios were 2.28, 1.69, and 1.59, respectively), it was not a significant predictor of LBW among blacks.

Although a decline in neonatal mortality has been widely publicized in the United States, research suggests that clinicians may still be underestimating the chances of survival of an infant who is born too early or too small and may overestimating the eventuality of serious disability. Clinicians may have the current and needed ethnic- and race-specific estimates of the "chances" of early survival for newborn infants. With this in mind, birth weight/gestational age-specific neonatal mortality rates for the three largest ethnic/racial groups in the United States: non-Hispanic whites, Hispanics, and non-Hispanic blacks were examined (174). Marked racial variation
in birth weight and gestational age-specific mortality has long been recognized, and growing concerns are being raised about ongoing and increasing racial disparities in pregnancy outcomes. The overall neonatal mortality rates for whites, Hispanics, and blacks were 3.24, 3.45, and 8.16 neonatal deaths per 1,000 live births, and the proportion of births at less than 28 weeks gestation was 0.35%, 0.45%, and 1.39%, respectively. Newborns who weighed under 1,500 g comprised less than 2.5% of all births in each racial/ethnic group but accounted for more than 50% of neonatal deaths. For whites, Hispanics, and Blacks, more than 50% of the newborns that were 24 to 25 weeks of gestational age survived. For most combinations of birth weights less than 3,500 g and gestational ages less than 37 weeks, the neonatal mortality rate was lowest among blacks, compared with whites or Hispanics. At these same gestational age/birth weight combinations, Hispanics have slightly lower mortality rates than whites. For combinations of birth weights below 3,500 g and gestational ages of 37 to 41 weeks, Hispanics had slightly lower neonatal mortality rate. At these birth weight/gestational age combinations, which describe approximately two-thirds of all births, blacks had the highest neonatal mortality rate. Compared with earlier reports, these data suggest that a substantial improvement in birth weight/gestational age-specific neonatal mortality has occurred in the United States. Regardless of ethnicity/race, the risk of a neonatal death does not exceed 50% (the suggested definition for the limit of viability), except for birth weights under 500 g and gestational ages less than 24 weeks. Notwithstanding, ethnic/racial variations in neonatal mortality rates continue to persist, both in overall rates and within birth weight/gestational age categories. Blacks continue to have higher proportions of preterm and LBW births, compared with either whites or Hispanics. At the same time, blacks experience lower risks of neonatal mortality for preterm and LBW infants, while having higher risks of mortality among term, post-term, normal birth weight, and macrosomic births.

Race differences in the proportion of LBW attributable to maternal cigarette smoking in a low-income population were described (175). Non-Hispanic whites had a much higher prevalence of smoking and were heavier smokers than African Americans. For both moderately LBW and very LBW, the population attributable risk percentages for smoking were twice as high for non-Hispanic whites as for African Americans. Overall, after adjustment, 30.7% of low birth weight births among non-Hispanic whites and 14.4% of low birth weight births among African-Americans were attributable to smoking.

The relationship between maternal birth weight, infant IUGR, and prematurity was determined (176). Race-specific rates of small-for-gestational-age (weight for gestational age in the lower 10th percentile) and preterm (less than 37 weeks) infants rose as maternal birth weight declined. The adjusted (controlling for maternal age, education, marital status, parity, prenatal care utilization, and cigarette smoking) odds ratio (95% confidence interval) of small-for-gestational-age for maternal LBW (less than 2,500 g) among African Americans and whites was 1.7 and 1.8, respectively. The adjusted odds ratio (95% confidence interval) of prematurity for maternal LBW (less than 2,500 g) among African Americans and whites was 1.6 and 1.3, respectively. The racial disparity in the rates of small-for-gestational-age and prematurity persisted independent of maternal birth weight: adjusted odds ratio
equaled 2.2 and 1.5, respectively. Maternal LBW is a risk factor for infant IUGR and prematurity among African Americans independent of maternal risk status during pregnancy; it is a risk factor for infant IUG among whites. Maternal LBW fails to explain the racial disparity in the rates of small-for-gestational-age and premature infants.

10.4) Effects of FGR on postnatal growth and development

FGR is a frequent cause of perinatal morbidity as well as of impaired growth during childhood, and is associated with significant perinatal and childhood morbidity (85). Epidemiological studies suggest that FGR is a significant risk factor for the subsequent development of chronic hypertension, ischemic heart disease, diabetes, and obstructive lung disease in adult life. FGR contributes disproportionately to neonatal mortality and morbidity in both preterm and term babies, and is a predisposing factor to major psychiatric sequelae, such as depression, suicide and suicide attempts (177). FGR is usually due to maternal effects, fetal factors, or placental insufficiency (178). Newborns with FGR are at increased risk of developing a metabolic syndrome later in life, namely obesity, arterial hypertension, hypercholesterolemia, cardiovascular disease, impaired glucose tolerance, or diabetes mellitus type 2. This association is the result of the adaptational changes in the fetal endocrine-metabolic mechanisms to the impaired intrauterine milieu that occur to ensure survival in the short term. The persistence of these changes after birth can be detrimental in adult life. Furthermore, premature adrenarche, as well as ovarian hyperandrogenism, seem to be associated with FGR in girls, demonstrating that FGR may have long-lasting effects on both somatic health and reproductive functions. Finally, intrauterine exposure of the fetus to stressors may affect the individual's ability to face stress in postnatal life. Therefore, if optimization of the individual somatic and psychosocial well-being is the golden goal of medicine, special attention should be paid to maintaining an optimal intrauterine milieu - devoid of any stressors and with adequate nutrient supply, and to reserve ideal psychosocial support for the pregnant woman. Some catch-up growth has been observed in about 70% of children with FGR during the first year of life (153). Many FGR children show major or minor birth defects which may be predisposing factors or may also coexist because of common underlying factors producing both small stature and structural anomalies.

IUGR fetuses are at risk for the development of adult hypertension and related cardiovascular diseases. IUGR may be linked to congenital oligonephropathy and potentially to hypertension in later life (179). IUGR appears to be associated with a decrease in fetal renal volume: renal volume in the IUGR fetuses was 31% (95% CI, 20%-40%), which was less than that in the group of fetuses that were not IUGR after an adjustment was made for gestational age The ratio of renal volume to estimated fetal weight was 15%, which was less than the same ratio in the fetuses that were not IUGR.

IUGR has an effect on the clinical course and prognosis of IgA glomerulonephritis (IgA GN) in children (180). A significantly higher mean percentage of sclerotic
glomeruli was found in children with IUGR than in those without IUGR (33 vs. 13%). At the end of the follow-up period, a significantly higher incidence of arterial hypertension was observed in children with IUGR than in those without IUGR (50 vs. 11%). An increased risk of developing arterial hypertension and glomerulosclerosis was demonstrated in children with IgA GN who had suffered from IUGR with a birth weight below the 10th percentile for gestational age. IUGR may therefore help in identifying early in the course of IgA GN those children who are at higher risk of an unfavorable course.

References


(To 10.1)

(To 10.2)


43. Shigeta, K., Hiramatsu, Y., Eguchi, K., and Sekiba, K., 1992, Urinary and plasma epidermal growth factor levels are decreased in neonates with intrauterine growth retardation and in their mothers, Biol. Neonate, 62, 76.


distribution and functional analysis of the human Na\(^+\)/H\(^+\) exchanger isoform
62. Hughes, J.L., Doughty, I.M., Glazier, J.D., Powell, T.L., Jansson, T., D'Souza,
S.W., and Sibley, C.P., 2000, Activity and expression of the Na\(^+\)/H\(^+\) exchanger
in the microvillous plasma membrane of the syncytiotrophoblast in relation to
Expression of the mRNAs and proteins for the Na(+)/H(+) exchangers and their
regulatory factors in baboon and human placental syncytiotrophoblast,
Endocrinol., 142, 3685.
64. Fliegel, L., Haworth, R.S., and Dyck, J.R., 1993, Characterization of the
placental brush border membrane Na+H+ exchanger: identification of thiol-
function in human placenta: gestational changes in intracellular pH regulation,
Placenta, 17, 661.
and Ganapathy, V., 1986, Na\(^+\)/H\(^+\) exchanger of human placental brush-border
67. Glazier, J.D., Cetin, I., Perugino, G., Ronzoni, S., Grey, A.M., Mahendran, D.,
activity of the system A amino acid transporter in the microvillous plasma
membrane of the human placenta and severity of fetal compromise in
Activity and protein expression of the Na\(^+\)/H\(^+\) exchanger is reduced in
syncytiotrophoblast microvillous plasma membranes isolated from preterm
intrauterine growth restriction pregnancies, J. Clin. Endocrinol. Metabolism,
87, 5686.
and Aronson, P.S., 1997, Monoclonal antibodies for high-resolution localization
70. Cavet, M.E., Akhter, S., deMedina, F.S., Donowitz, M., and Tse, C.M., 1999,
Na\(^+\)/H\(^+\) exchangers (NHE1–3) have similar turnover numbers but different
percentages on the cell surface, Am. J. Physiol., 277, C1111.
71. Wakabayashi, S., Shigekawa, M., and Pouyssegur, J., 1997, Molecular
physiology of vertebrate Na\(^+\)/H\(^+\) exchangers, Physiol. Rev., 77, 51.
72. Chow, C.W., 1999, Regulation and intracellular localization of the epithelial
isoforms of the Na\(^+\)/H\(^+\) exchangers NHE2 and NHE3, Clin. Invest. Med., 22,
195.
maternofetal clearance of sodium across the near-term rat placenta, Q. J. Exp.
Physiol., 74, 557.
74. Powell, T., Lundquist, C., Doughty, I.M., Glazier, J.D., and Jansson, T., 1998,
Mechanisms of chloride transport across the syncytiotrophoblast basal
membrane in the human placenta, Placenta, 19, 315.


(To 10.3)


110. Warkentin, B., 1994, Fetal development in late gestosis and nicotine consumption, Geburtshilfe Frauenheilkd., 54, 262.


(To 10.4)


