

Role of IgG antibodies in association with placental function and immunologic diseases in human pregnancy

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During human pregnancy, the maternal immune system develops and changes, providing protection for the growing placenta and fetus. These protective changes provide mechanisms allowing two genetically different individuals to interact with each other without allograft rejection. In addition to normal pregnancy, some pregnancies may develop under immunologic diseases, during which specific monitoring and medical treatments are essential. The aim of this current review is to provide information regarding the development of human placental function during pregnancy, the immunology of human pregnancy and the role of the placenta in providing the fetal tissue with antibodies (IgG and its subclasses 1–4), which are required for the passive immunization of the newborn. In addition, the available methods for the determination of placental function will be explored. Furthermore, immunologic diseases observed during pregnancy and the possible therapies for these diseases will be assessed.

KEYWORDS: *ex vivo* perfusion model • human pregnancy • IgG antibodies • immunologic diseases and therapy • placental function • placental transfer

The immunology of human pregnancy is very different from nonhuman pregnancy. The implantation and the invasion of the hemochorial placentation implicate direct contact between maternal uterine cells and fetal tissues. These two processes, with the development of the human placenta, are dependent on the existence of two immunological interfaces between the mother and the fetus. The first interface allows interaction between maternal cells and placental trophoblast cells. The second interface occurs toward the end of the first trimester, with the onset of maternal blood flow into the placental intervillous space including direct contact with the syncytiotrophoblast. With the development of the placental tissue (consisting of the decidua cell layer, which is of maternal origin and the remaining placental tissue of fetal origin) throughout gestation, the syncytiotrophoblast produces and releases specifically placental factors such as hormones, cytokines, angiogenic parameters and trophoblast microparticles into maternal blood circulation.

During human pregnancy, the maternal immune system develops protection against

allograft rejection; therefore, the fetus, as a semiallogenic body possessing half of the paternal antigens, is not rejected. In addition to this basic change of the immune system, materno-placental tolerance is developed to protect the pregnancy. This special phenomenon of human pregnancy – maternal–fetal tissue tolerance – is effected via several mechanisms performed by the maternal immune system, placental decidua and trophoblast cells. During pregnancy, the development of antibodies, specifically IgG including classes 1–4, are essential for transplacental transfer to the fetus providing the neonate with passive immunity for the first few months of life. There are many immunological diseases related to antibody functions and production of autoimmune antibodies and it is essential that their treatments be continued during pregnancy.

The aim of the present review is to provide information on the development of human placental function during pregnancy, the immunology of human pregnancy and the role of the placenta in providing the fetal tissue with antibodies (IgG and its subclasses 1–4), which are required for the passive immunization of the

newborn. In addition, the available methods for the determination of placental function will be explored. Furthermore, the immunologic diseases observed during pregnancy and the possible therapies will be assessed.

Development of human placenta

The processes involved in placental development are highly regulated to ensure normal growth of the developing fetal tissues and the maintenance of a healthy pregnancy. A unique function of the placenta as an interface link between maternal and fetal tissues is to fulfill critical roles such as preventing allograft rejection of the fetus, ensuring fetal nutrient supply along with respiratory gas exchange and enabling the transfer of fetal toxic metabolic waste into the maternal blood circulation for elimination. The placenta also functions as an endocrine organ producing steroid and protein hormones for the protection of pregnancy.

After fertilization, the development of the placental and fetal tissues begins and continues for approximately 9 months. After 4 days of this development in the fallopian tube, the formed cellular mass of the morula migrates to enter the uterus with formation the blastocyst (polarized cells with accumulating fluid) at the fifth day. The outer membrane (chorion) and trophoblast layer of the placenta are derived from the trophoctoderm of the blastocyst cells. Other extra-embryonic tissues, including the amnion (inner membrane), develop from the inner cell mass of the blastocyst. The vessels of the placenta are derived from the mesoderm. The implantation process occurs within 5–8 days of fertilization by attaching the blastocyst to an optimal location of the uterus. The implanted blastocyst in the uterine tissue will be supplied from the maternal circulation with oxygen and metabolic substrates, which are required for embryonic developing growth. After this adhesion, the invasion process starts, during which the cytotrophoblast cells from the trophoblast layer migrate deeper into the decidua layer. The formed vacuoles of the embryo become confluent to the uterus with the formation of the lacunar space 13 days after fertilization. The lacunar space will develop into the intervillous space for blood circulation a few weeks later. The placental progenitor stem cell is the cytotrophoblast cell. These cells are responsible for the cellular growth of the placenta, and they have the ability to proliferate and differentiate throughout gestation. The proliferating cytotrophoblast cells provide, after differentiation, the villous syncytiotrophoblasts as the outer cellular layer of the placenta. The other pathway that these cells can take when differentiating is one that forms the extravillous cytotrophoblasts. The specialized epithelium of the syncytiotrophoblast has a number of functions, including transport of gases, nutrient supply, waste elimination and hormone synthesis of either peptides or steroids that regulate placental and fetal growth, and maternal systems with the protection of pregnancy. The extravillous trophoblast (EVT) includes both proliferative and invasive cell layers. The third part of the EVT is a migratory EVT but is neither invasive nor proliferative. This trophoblast cell population forms the cell islands, septum, chorionic plate and chorion laeve. After 4–5 weeks of gestation (WG), both trophoblasts of EVT erupt in columns with the proliferative trophoblast

layer growing at the base, while the invasive trophoblast grows in the distal portion of the column. The invasive component of EVT invades the decidua to form the interstitial EVT including endovascular EVT where the invasive cytotrophoblast cells remodel the spiral arteries. Endovascular invasion, which is also known as the intramural or intra-arterial invasion process, occurs when the cytotrophoblast cells from this layer migrate, with the replacement or displacement of vascular smooth muscle and endothelial cells to remodel the narrow spiral arteries into wide dilated arteries. The formation of anastomoses between the dilated spiral arteries and endometrial maternal veins allows maternal blood circulation in low resistance vessels within the placental lacunar system. Beside the maternal uteroplacental circulation, the fetal blood circulates in the small blood vessels, including fetal capillaries, through the placenta, where the maternal and fetal blood circulation is separated. Maternal blood supplies the placenta and fetus with oxygen and nutrients, and allows the transfer of the metabolic waste from placental and fetal tissue through the umbilical cord to the maternal kidney for elimination.

In current clinical practice, the placental tissues are discarded after delivery, therefore these tissues are easy to obtain for clinical research with few ethical problems. Nevertheless, the ethics of medical research is nowadays, as always, extremely important and the ethical implications of this should always be considered. These cells represent well a promising source for allogenic research due to the low immunogenic properties. The placental mesenchymal stem cells (MSCs) demonstrate an important cell type for clinical research and therapy not only because of their strong ability to proliferate (expand) with remarkable immunosuppressive properties, but also their ability to differentiate into many cellular lineages (plasticity) *in vitro* such as mesoderm, osteocyte, chondrocyte, cardiac muscle and endothelial cells [1]. A recent study comparing stem cells obtained from the placenta versus bone marrow demonstrated that placental cells have stronger immunosuppressive effects than those observed with bone marrow [2]. The determination of the immunologic properties of placental cells has shown their suitable application for the treatment of the critical ischemic limb tissue [3]. Furthermore, these placental cells have demonstrated the ability for multilineage differentiation with high capacity of expansion [4–7]. Stem cells isolated from different tissue layers of the human placenta have been studied with tissue obtained after different periods of gestation using either a mechanical separation method or enzymatic digestion [4–8].

Exploring methods of human placental function

The comparison of maternal and fetal (umbilical cord) blood levels of any substance or medicine provides information on their concentrations at a particular time point of gestation after delivery. The obtained values do not indicate the mechanism by which the transfer rate and direction occurred between maternal and fetal compartments. Intermammalian species show that the structure [9,10], transport [11] and endocrine functions [12] of the placenta are strongly varied compared with other organs. In addition, differences in fetal susceptibility to teratogens also exist between species [13–15] as do rates and extent of fetal development during

pregnancy. The animal species that most closely mimic human placental characteristics have a hemochorial histological organization, such as rodents and, primarily, primates. Clearly, primates would be the species of choice, but they are expensive and can be difficult to work with, so rats and mice are more commonly used. While the human placenta is hemomonochorial in classification, composed of a single layer of trophoblast (syncytium) in direct contact with the maternal blood, rats and mice have three trophoblast layers (hemotrichorial) in a labyrinthine placenta [16,17]. This anatomical difference is reflected by different diffusion patterns between maternal and fetal circulations, with concomitant differences in permeability to various substances. A review by Moffett and Loke demonstrated that differences in the immunological reaction exist between the two species [18]. The placental endocrine functions differ very strongly between mice and humans [12]. In addition, in humans, the yolk sac is present only very early in pregnancy, whereas in rodents it persists throughout gestation, encloses the fetus and performs important transport functions [19]. For example, the transfer of IgG and its subclasses from the maternal to the fetal circulations in humans occurs via Fc receptors (FcRs) in the placenta, which can be readily demonstrated using *ex vivo* perfusion [20], while in mice the yolk sac is the organ responsible for IgG materno–fetal transport [21,22]. The weight ratio of the fetus to the placenta in the mouse reaches 30:1 at birth, which is significantly higher than that of man, which reaches 6:1 at delivery [23]. The higher ratio rate shown for the smaller placenta of the mouse demonstrates that the smaller placenta in rodents can more efficiently nourish the fetus than the large placenta in humans.

The placental perfusion technique began in the 1960s allowing the study of tissue functions under *ex vivo* conditions [24–26]. The first described perfusion method of the isolated human placental cotyledon was reported in 1967 by Panigel *et al.* [27], and in 1970, Nesbitt *et al.* introduced the dual perfusion model including both maternal and fetal circuits in an apparatus [28].

The easy availability of the human placental tissue with its known biological resistance to hypoxia and ischemia allows the tissue to be suitable for clinical research *in vitro* [29,30]. However, these investigations with placental tissue at delivery (with mature organs) have certain limitations, because the provided information may not reflect the structure and biological function of the placenta earlier in gestation. Therefore multiple models, such as cells and tissue explants cultured from various placental layers and gestational age, and *ex vivo* perfusion of human placental tissues at term, have been used to explore a wide variety of functions such as cellular proliferation and differentiation, endocrine function as well as permeability with hormone production, drug transport and mechanism with determination of the influx and efflux transfer direction, and metabolism [31,32]. The *ex vivo* perfusion model allows the study of these parameters in the presence of different substances and medicines, as well as of the drug kinetic profile and the chemical action on the placental tissue, which are difficult to obtain under *in vivo* conditions [31,32]. This method has been used in the last 40 years to study the transfer of many substances such as nutrients, hormones, proteins, therapeutic agents and drugs of

abuse, and offers an extremely useful tool for therapeutic drug development [31,32]. The increasing demand of the clinicians to reach better decisions about which medicine should be used with the optimal dosage regimens in pregnancy to minimize the fetal exposure and toxicity could be drawn from experimental data on placental drugs investigated using the *ex vivo* placental perfusion model [31,32].

Placental transport mechanisms

The syncytiotrophoblast, the only layer of the human placenta that is directly in contact with maternal blood, represents the main site of nutrient and drug uptake, transfer and metabolism essential for the placental and fetal growth as well as the waste clearance into the maternal circulation [33]. For the efficiency of nutrient uptake and transfer into the fetal circulation, there are several mechanisms developed in placental tissue to control molecule direction and metabolism in the fetal tissue [34,35]. The formation of the syncytiotrophoblast occurs from the differentiation of the cytotrophoblast cells, which are in a layer below the syncytiotrophoblast. The polarized plasma membranes of the syncytiotrophoblast provide the brush-border membranes with the creation of a microvillous structure to increase the area facing into the maternal blood. The plasma membrane without the brush-border structure is at the lower end of the trophoblast facing the stomal area and the fetal blood vessels. The placental transport mechanisms and associated transport proteins were recently reviewed by Malek and Mattison [32].

Transport of IgG & its subclasses across the human placenta

Maternal and fetal infections are important causes of mortality and morbidity during pregnancy. Due to the undeveloped immune system in the fetus and newborn, immune protection is provided during pregnancy and is dependent on the placental maternal-to-fetal supply of maternal antibodies, which are taken up from maternal blood to be released into fetal blood circulation. This antibody transmission to the fetal tissue is apparently limited to IgG and its subclasses (1–4) with exponential profiles during the second half of gestation. The fetal transmission with all IgG subclasses is an important feature of pregnancy to provide passive immunity for the extra-uterine and neonatal survival against infections [21,36,37].

Immunoglobulins are Y-shaped molecules containing two antigen-binding fragments (Fab) and the stem of the Y-shape (Fc fragment) (FIGURE 1). Specific binding of antigens at the Fab fragments results in the formation of immune complexes, whereas the Fc fragment interacts with effector systems of the immune response, such as complement receptors or FcRs on the surface of certain subpopulations of white blood cells [38]. This starts a reaction cascade ultimately leading to the elimination of the antigen. In the human, class G immunoglobulins are the predominant antibodies, which in the serum are present in four subclasses differing in their Fc regions leading to different affinities to FcRs. FcRs, apart from eliciting an immune response against the invasion of the body by antigens, are also involved in the transport of free

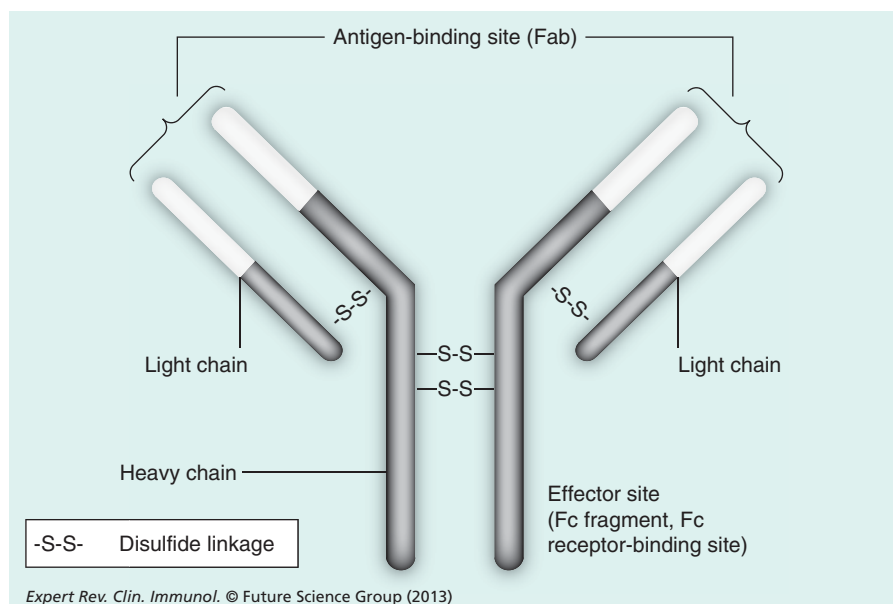


Figure 1. Structure of a typical immunoglobulin (antibody) protein. Two identical heavy chains are connected by disulfide linkages. The antigen-binding site is composed of the variable regions (white) of the heavy and light chains, whereas the effector site of the antibody (which controls whether it agglutinates antigens, binds to macrophages, enters into mucous secretions or binds to placental Fc receptor) is determined by the amino acid sequence of the heavy chain constant region.

antibodies across various tissue barriers [39]. A full complement of maternal antibodies crosses the intestine or placenta providing the fetus and the neonate with a protective shield against infections during the first months of newborn life.

***In vivo* observations of IgG transport during pregnancy**

The immune protection provided by maternal antibodies is essential for newborns during the first few months of life after delivery and the transfer of these antibodies in different species reach the fetus or newborn via different routes [40]. In the human, the placenta is the predominant route of IgG transfer in the maternal-to-fetal direction [41,42]. Immunoglobulins in the fetal circulation almost exclusively consist of maternal IgG, and a wide spectrum of different antibodies such as specific IgG antitetanus toxoid, antigroup-A streptococcal carbohydrate and antiherpes simplex virus have been described [43,44]. Throughout evolution, the placenta developed a complex transport system to allow a large variety of highly specific antibodies to cross the different tissue layers without interfering with the protective function of the placental barrier between the circulating blood in maternal and fetal tissue. In a recent study, suggestive evidence is provided that maternal antibodies, reaching the fetus via the placenta or the infant via breast milk, do not only provide passive immunity against postnatal infection but may act as immunomodulatory agents helping to develop specific and long-lasting immune responses in the infant [45].

The observations described below are derived from various studies reflecting the *in vivo* conditions [36,46]. In these studies, paired samples representing maternal and fetal blood were collected between 17 and 41 WG, samples of fetal vein blood were collected

by cordocentesis by (17–36 WG, $n = 91$) and from the umbilical vein directly at delivery (37–41 WG, $n = 16$), while the corresponding maternal blood samples were obtained from a peripheral maternal vein. Additional samples (from the group of 37–41 WG) were collected from the same pregnant individual at 10, 20, 30 WG and at term ($n = 16$). The mean concentrations of IgG and IgA in the maternal sera at 9–16 WG were 13.72 ± 2.53 g/l IgG and 3.95 ± 1.23 g/l IgA. Both IgG and IgA levels throughout gestation reached, at delivery, a concentration of 60–70% when compared with the initial concentration at 10 WG. The concentration ratio of IgG1:IgG2 in the maternal blood was 2:3 and remained unchanged throughout gestation (17–41 WG). The lower levels of IgG3 and IgG4, which together were less than 10% of total IgG, remained unchanged throughout gestation.

The initial observed level of fetal IgG (17–22 WG) was below 10% of the corresponding maternal concentration and at

term the value of fetal IgG (11.98 ± 2.18 g/l) represented 130% of the maternal concentration. In addition to the continuous increasing of the fetal IgG during pregnancy, the positive correlation observed between fetal and maternal IgG levels supports the mechanism by which the fetal IgG is of maternal origin. In contrast, IgA levels in the same fetal sera remained at a concentration 1000-times below the maternal levels. The initial level of IgG subclasses measured for IgG1 (0.93 ± 0.42 g/l) was 300% higher than that of the IgG2 concentration. Both IgG1 and IgG2 showed different concentration profiles during pregnancy. An exponential rise of IgG1 with a slightly linear rise of IgG2 was observed where the concentration IgG2 was seven-times lower than IgG1. While the highest transfer rate to the fetus was found for IgG1, IgG2 showed not only the lowest transfer rate but also its concentration remained below the maternal level. The other two subclasses IgG3 and IgG4 reached similar concentrations to the corresponding levels in the maternal sera at term, but their concentration profiles showed an exponential rise throughout gestation. However, the ratio of fetal to maternal concentration was slightly higher for IgG3 than for IgG4 [36,37]. At all stages of pregnancy, the concentrations of IgG2, IgG3 and IgG4 representing fetal blood showed that these subclasses remained significantly below the levels of IgG1 [37,46–48]. The placental transfer affinity of immunoglobulin antibodies, which represents the differences in the transfer rates of the immunoglobulins in the maternal-to-fetal direction, was concluded with the following sequence: IgG1 > IgG4 > IgG3 > IgG2, with no transfer activity for IgA.

Interestingly, small newborns demonstrated whole IgG levels that were lower than those of normal-weighted newborn peers [49]. This observation suggested that there is an impact on placental

IgG transport providing a lower level of IgG1 in small newborns [48,50]. Additional observations supported the specificity of the human placental IgG transport with much lower affinity to IgA. The fetal-to-maternal ratios of specific IgG antibody levels against tetanus toxoid (TT; anti-TT-IgG) versus IgA at term showed that the ratio for anti-TT-IgG was 1.4, which was 1000-times lower for IgA. In addition, the ratio of antibody (anti-TT-IgG) versus antigen (TT-Ag) was similar in both fetal and maternal sera with comparable correlation. This observation supports the phenomenon that fetal antigen could cross the placenta in the form of an antigen-antibody complex.

The fetal transmission of antigen by placental tissue was supported by animal experiments, after which the theory of transplacental immunization in pregnant rats was transferred to fetuses, due to the detection of fetal IgM of anti-TT in newborns [51]. Further observations in humans were also supportive for this phenomenon. The tetanus vaccination in the last few months of human pregnancy showed the ability of fetal tissue to be immunized by the detection of IgM anti-tetanus in the sera of the cord blood after delivery [52]. The induction of such antibody in fetal blood could possibly be due to the transferred TT, or through reactions with antibodies of the anti-idiotypic species [52]. Because placental transport of antibodies is limited to the species of IgG, the existing fetal IgM in the blood of either the umbilical cord or the newborn has to be of fetal origin. Other studies of maternal immunization demonstrated the detection of higher levels of fetal vaccine IgG antibodies, without the transfer of the specific T lymphocytes responsible for the reaction with such vaccine-antigens or the production of neonatal antibody [53].

Ex vivo investigations of IgG transport & associated specificity

Using the *ex vivo* model for perfusion of the human placenta [20,54], the transplacental transfer of human serum proteins such as IgG and its subclasses, IgA, TT-AG and anti-TT-IgG, was studied. To determine the transfer specificity of the human placenta, IgG was labeled with biotin (IgG-BT) and ^{14}C -labeled bovine serum albumin (^{14}C -BSA) was used as a permeability marker for the transfer of macromolecules. Both perfusates of the maternal and fetal side were recirculated (closed system). After an initial stabilization phase of 2 h (control phase), media were changed on both sides and the experimental phase of the perfusion was continued for 4–6 h. Two experimental groups were compared: in group A ($n = 3$) no test protein was used, while in group B, the perfusion medium contained IgG (Sandoglobuline®, CSL Behring, CA, USA, 6–10 g/l), anti-TT-IgG (21–25 mg/l), TT-Ag (0.19–0.24 mg/l) and IgA (0.13–0.19 g/l), only on the maternal side. In the same medium of the maternal side labeled, IgG-BT (2 g/l) and ^{14}C -BSA (30–40 nCi/ml) were added. In both groups, the metabolic activity of the placental tissues showed stable conditions with comparable constant rates of glucose consumption and lactate production with similar accumulation rate of both hormones hCG and human placental lactogen. In group A, the protein washout levels of the endogenous IgG and IgA were observed, at the end of the control phase, the fetal level

was 5- and 1000-times lower for IgG and IgA, respectively, than the maternal level. These levels remained constant during the experimental phase, showing 50–80% of the levels detected in the control phase with a stable level during the last 4 h of perfusion. In group B, various proteins were tested, the IgG-BT, IgA and ^{14}C -BSA on the fetal side were barely detectable within 1 h of the perfusion, with no further change in the level of these proteins in the remaining 4 h of the experiments. In contrast to these proteins, the detection of IgG and its subclasses in the fetal perfusate was barely detectable within the first experimental 2 h, but in the remaining 4 h of the perfusion a linear increasing profile of all IgG subclasses was observed. The calculated ratio of IgG1 to IgG2 at the end of the experiments was significantly higher in the fetal (3.8:1) than in the maternal perfusate (1.8:1), indicating that placental tissue has a preferential mechanism to the transfer of IgG1. The concentration of TT-Ag in the fetal perfusate was detectable after sample concentration by ultrafiltration at the end of the perfusion. The concentration ratio of the fetal perfusate (F) and maternal perfusate (M) multiplied by 100 ($F:M \times 100$) for the samples obtained after 6 h of perfusion was used to estimate the differences of placental permeability between these proteins. The labeling of IgG with biotin (IgG-BT) has reduced its transfer ($\text{IgG-BT} = 0.04 \pm 0.01$) by factor 10 when compared with the transfer of the normal IgG (0.49 ± 0.08) or the specific anti-TT-IgG (0.46 ± 0.11) antibodies, indicating that these last two antibodies have the same placental transfer activity. The relative F:M ratio created for TT-Ag (0.48 ± 0.12) and anti-TT-IgG (0.46 ± 0.11) was 4–50-times (significantly) higher than for ^{14}C -BSA (0.12 ± 0.03) and IgA (0.01 ± 0.01), respectively. Regarding the larger molecular weight of the antibodies (IgG and anti-IgG), and the antigen (TT-Ag) when compared with BSA, or to the same structure of IgA, it can be suggested that the first three proteins are transferred across the placenta by a specific transport mechanism.

The placental transmission of maternal antibodies (IgGs) into the fetal blood circulation required the transfer across various cell layers including the villous syncytiotrophoblast, stroma and the endothelial cells of the fetal capillaries inside the villous tissue. A number of different *ex vivo* models were developed with human placenta to study the antibody transport mechanisms. In addition to the fast development of knowledge in molecular biology, more recent technological advances in the field of histochemistry allowed for a more precise allocation of different antibodies and their receptors to defined subcellular structures [38,55]. The various subtypes of FcRs and their respective isoforms are differently expressed in the various tissue components of the human placenta and play different roles in the maternal-to-fetal transfer of IgG [56]. The neonatal Fc receptor (FcRn) had originally been described in the intestinal brush-border of the neonatal rat [57–60] and was found in the murine fetal yolk sac [61]. A human ortholog of the FcRn was identified as mRNA and protein in the human placental syncytiotrophoblast [62–64], it is generally accepted that FcRn mediates the uptake of IgG at the surface the human placental syncytiotrophoblast. The formation of an endosome after the binding of IgG to its FcRn protects the transfer of IgG without

degradation across the placenta in the maternal-to-fetal direction (FIGURE 2) [39]. In some animals such as rats, mice and ruminants, the expression of the FcRn is presented in the gut of the infant with the transfer of IgG into the colostrum within the first 24 h of life. The current knowledge of the interaction between the FcRn receptor and IgG or its Fc fragment and its relevance for different functions has previously been reviewed [65]. The mutated IgG Fc fragments were used to study the role of the receptor for the maternal-to-fetal transfer of IgG and the associated half-life of IgG in the blood circulation. Ultimate proof of the essential role of FcRn in providing an intact IgG from the maternal to the fetal side came from the experiments with mutated IgG in the *ex vivo* perfusion model of the human placenta. The maternofetal transfer of IgG1 as a wild-type antibody was compared with a recombinant, humanized (IgG1) antibody [66]. Understanding the specifics of IgG transfer and the importance of the FcRn was further advanced by testing, in the perfused human placenta, the transit of a chimeric mouse/human IgG Fab fragment used for anticoagulant therapy (abciximab). No intact transmission of Fab fragments without the associated Fc portion could be demonstrated but, much like albumin, the Fab fragment was catabolized by the human placenta [67]. In contrast to abciximab, rituximab, another chimeric mouse/human intact IgG and monoclonal anti-CD20 antibody, was documented to cross into the human fetus and reduce B-lymphocyte activity, both in the mother and in the newborn [68]. Thus, the importance of the Fc

end of the intact IgG is critical for the transfer of intact IgG, be it human or mouse/human chimeric. The specific binding of the Fc γ -portion of IgG to the receptor on the surface of the placental syncytiotrophoblast is considered to be the first important step of the mechanism of maternal-to-fetal transport of IgG [69]. This selective binding of the maternal IgG is followed by the coating of IgG in vesicles for its transmission across the three layers of the placenta [70]. The coated vesicles are apparently to protect IgG against lysosomal protein degradation, providing an intact IgG to the fetal blood. Many studies were performed to detect IgG in the placental tissue layers, applying various approaches with different immunohistochemistry methods [71–73]. The immunohistochemistry methods have clearly shown that all three subtypes of the Fc γ -receptors (I, II and III) detected the human placenta [74,75]. These studies have also shown that the expression of the three Fc γ receptors were detectable in the placental Hofbauer cells and the stromal macrophages. The role of these two kinds of cell in the passage IgG is unclear. It appears that isoforms of the three Fc γ Rs may bind IgG providing immune complexes as a protective barrier across the placenta supplying the fetus with intact IgGs [76–78]. The main route of IgG transfer is principally mediated through the FcRn receptor, with differences between species [79]. While IgG is extensively transferred during the last part of gestation in primates (including humans) via the chorioallanotic placenta, with similarity found in rabbits and guinea pigs via inverted yolk sac splanchnopleure [79], the other rodent newborns of rats and mice receive their passive immunity predominantly after birth [79].

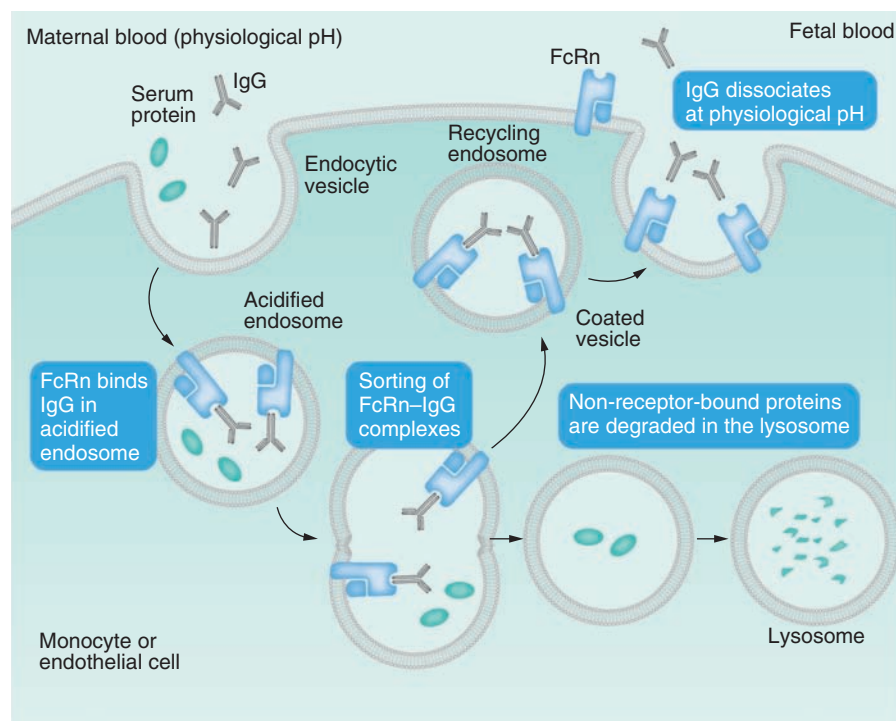


Figure 2. IgG active transport mechanism in the maternal-to-fetal direction.

FcRn at the maternal–placental interface (syncytiotrophoblast) binds IgG from maternal blood circulation in a pH-dependent way, transports it over placental tissue cell layers via transcytosis, and releases the bound IgG in the fetal blood circulation.

FcRn: Neonatal Fc receptor.

Adapted with permission from [38].

Immunologic diseases of human pregnancy & suggested therapies

Allergic disease in pregnancy

Most allergens are normally harmless substances that produce allergic disease through their impact on the immune system. The resulting allergic reaction caused by insect stings, food and reaction to medicines could induce a life-threatening reaction known as anaphylactic shock. Recent studies have clearly shown that children with the susceptibility to develop allergic diseases including asthma in later life could be associated with the exposure of environmental factors during pregnancy [80,81]. Additional studies observing the stress of the maternal prenatal period have suggested that maternal prenatal stress is a potent factor in fetal programming to develop asthma in children [82,83]. Prevention and treatment of allergic asthma was recently reviewed by Cadavid *et al.* [84]. The authors suggest that asthma is the most common respiratory disorder in the reproductive age of women. This respiratory disorder affects 5% of pregnancies in industrialized countries [85].

Impacts of severe and uncontrolled asthma on pregnancy have been reported to associate with additional high risk of complications such as pre-eclampsia, preterm birth, carrying infants with low birthweight or infants with congenital malformations, intrauterine growth restriction, or perinatal death. Conversely, women with well-controlled asthma and appropriate treatment have little or no increased risk of adverse maternal or fetal outcomes [86,87]. Asthma therapy using classical drugs are divided into two treatments, the first one, called long-term control therapy, to prevent the manifestations of asthma (inhaled corticosteroids, long-acting β -agonists, leukotriene modifiers, cromolyn and theophylline), while the second therapy, called the rescue therapy, aims to provide quick relief of symptoms (mainly short-acting inhaled β -agonists). Oral corticosteroids can either be used to treat an asthma exacerbation as a form of rescue therapy or to treat as long-term control therapy for patients with severe, persistent asthma [86,88]. Substances like bradykinins, cytokines, prostaglandins, leukotrienes and thromboxane, which are known as inflammatory mediators, initiate the cascade of the anaphylactic reaction.

In contrast to nonpregnancy, these mediators in pregnancy may induce cardiovascular collapse following an edema or respiratory compromise. Other disorders such as hypoxia inducing poor neurologic outcome or fetal death were observed in association with these inflammatory mediators [89]. The treatment of the anaphylactic reaction in pregnancy is similar to nonpregnancy, using epinephrine, H1 and H2 histamine antagonists and corticosteroids [90].

Inflammatory bowel disease in pregnancy

The majority of patients with inflammatory bowel disease (IBD), who are affected by Crohn's disease with or without ulcerative colitis, are in their reproductive years, so there are concerns about pregnancy and bearing children. Therefore, the placental transfer function of the immunosuppressants and monoclonal antibodies used for the treatment of IBD as well as the safety of these therapies during pregnancy was recently reviewed [39,91,92].

The use of prescription drugs in pregnancy always raises the question of the impact on pregnancy including the fetal risk of teratogenicity and malformation of the newborn. Therefore, drug therapy during pregnancy has to ensure the optimal health of the pregnancy and the growing fetus without any impact on placental function. In most cases of IBD in pregnancy, a normal outcome was observed with inactive disease, and a small risk of early termination of pregnancy and premature baby with low birthweight [92]. The following immunosuppressants and monoclonal antibodies (biologics) used for the therapy of IBD are derived from Chaparro and Gisbert [92]. Methotrexate for human pregnancy is contraindicated because of the associated risk for malformation, while thiopurines are safe and well tolerated throughout gestation. An additional immunosuppressant drug, cyclosporine, used for the treatment of ulcerative colitis with steroid refractory during pregnancy can be considered effective and safe. Anti-TNF- α therapies are apparently safe for use in pregnancy, as the malformation risk was similar to those seen in nonpregnancy. Transport mechanisms across the human placenta

are known to include simple (passive transfer) and facilitated diffusion, and active transport. Thiopurines are transported by passive transfer. The transport across the placenta of corticosteroids, methotrexate and cyclosporine is modulated by protein 'pumps'. Infliximab and adalimumab bind to the FcRn-receptor, as do other immunoglobulin proteins. The mechanisms of the diffusion of certolizumab across the placental barrier, however, remain unknown.

Hemolytic disease of the fetus & the newborn

The pathology of Rhesus isoimmunisation disease can occur when the blood of the mother and the fetus is not compatible, that is, when a mother is carrying Rh-negative blood, while placenta and fetus have Rh-positive blood. Following this condition, during the first pregnancy and during delivery, contamination can occur between maternal and fetal or placental blood as well as through amniocentesis, abortion or miscarriage; maternal blood becomes sensitized with the production of antibody against the Rh-positive fetal blood, affecting subsequent pregnancies, during which maternal antibodies can attack fetal red blood cells (RBCs), inducing hemolytic disease in the fetus. In future pregnancies, the mother's antibodies can attack the RBCs of the unborn baby, resulting in hemolytic disease. The severity of the hemolytic disease varies from mild to severe anemia, which can even lead to fetal death.

If fetal and maternal blood that are Rh-positive carry the same D genotype, maternal antibodies have no impact on fetal blood. Pregnant women with Rh-negative blood have no D genotype (Rh-positive) DNA. Therefore, the detection of D genotype DNA in the maternal blood indicates that the fetus is Rh-positive [93,94]. Approaches for the protection of the fetus and newborn against hemolytic disease with exploring action mechanisms of anti-D as a prophylactic agent were recently reviewed [95]. The highly immunogenic antigen of the D polypeptide is responsible for the clinic feature of anti-D responses in D-negative pregnancies [96]. Two different observations have provided the decision to prevent D alloimmunization through treatment with anti-D [97,98]. The first observation was the lower impact on the RBCs (hemolytic disease) in the fetus and newborn under conditions of ABO incompatibility between maternal and fetal blood. This incidence was thought to occur due to the destructive effect of antibodies (anti-A or anti-B of IgM) on the fetal blood cells before recognition by the immune system [97]. The second observation was obtained from animal models showing that RBC immunization could be inhibited by antibodies [99] and the mechanism of action was termed as antibody-mediated immune suppression [100]. Based on these observations, the neutralization effect of anti-D on D antigen of the RBCs has allowed therapeutic approaches to prevent D immunization of the fetal blood [97,98]. Although the therapy with anti-D has been applied for over 30 years, anti-D therapy has been associated with many problems. The preparations of anti-D are currently obtained from human plasma donors, who are immunized with D antigen, and the supply of such preparation is limited. While the initial thought of the transmission risk of serious infectious diseases with anti-D preparations

was apparently minimal, reports of hepatitis C virus transmission have been demonstrated [96]. In addition, many theoretical concerns could be associated with such preparations including Creutzfeldt–Jakob disease [96], or distribution of unknown new viruses or pathogens into defenseless inhabitants. The development of several anti-D monoclonal or recombinant antibodies has been initiated [101,102], as new approaches to increase the specificity of anti-D replacing the polyclonal antibodies obtained via human plasma preparations. However, the anti-D monoclonal or recombinant antibodies demonstrated inconsistent effects; few of them inversely enhanced the response of anti-D [102]. The ability to block the activation of an immune response using anti-D in the presence to D-positive RBCs has been observed with various experiments performed in animal models, during which the antigen-specific IgG with the corresponding antigen was administered [89,103]. The mechanism of action studied in animal models with antibody-mediated immune suppression has provided relevant information for the design and selection of the monoclonal or recombinant anti-D antibodies, with the optimized ability to prevent an immune response in the presence of a D-antigen. The immune response activation induced through the ligation of the antibody on a specific antigen receptor on the surface of the B cell, which in turn enhances the interaction between B cells and T cells in the blood [104]. The interaction between an antigen and a naive B cell has been studied in various models [105]. The antigen can be directly recognized by a B cell or its existence in the surface of either follicular and migrating dendritic cells or macrophages of subcapsular sinus type [105]. The detailed mechanism regarding the recognition of D-positive cells by B cells in blood needs further investigation.

Rh immunoglobulin

The Rh immunoglobulin (RhIg) is a purified blood product containing antibodies to Rh factors. Rh factors are inherited proteins found on the surface of the RBCs. Rh hemolytic disease can be treated by the administration of RhIG to an Rh-negative woman who was exposed to Rh-positive blood during a previous pregnancy. The prophylactic treatment with RhIG administration is provided during the pregnancy and after the delivery of the newborn with Rh-positive blood (within 72 h of delivery). The standard treatment is the use of 300 µg of RhIG administered to unsensitized woman with Rh-negative blood at 28 WG and after delivery within 72 h, or either after an abortion or the rupture of a tubal pregnancy [106]. The calculated dose of RhIG is based on the count of fetal blood detected in the maternal blood, provided by maternal screening using a blood test called Kleihauer–Betke.

Intravenous immunoglobulin (IVIg)

Intravenous IgG (IVIg) is a purified IgG solution obtained from plasma, which was separated from population-pooled donor blood. Patients with the indication of either primary or secondary immunodeficiency disorders, or cancer patients that are characterized by absent or deficient antibody production, require IVIg as replacement therapy due to their inability to produce antibodies [107]. This therapy is also useful for the treatment of newborns

with indications of severe isoimmune hemolytic jaundice or isoimmune thrombocytopenia [108,109]. A dose range between 500 and 1000 mg/kg of IVIg is usually infused during 2–6 h; depending on the course of the therapy, a similar dose can be administered every day. The therapeutic application with IVIg has been comprehensively investigated in newborns with fulminant sepsis, provided there is no health impact and it is acknowledged as safe treatment [109–112].

Intrauterine transfusion

The standard therapy of intrauterine transfusion (IUT) used for the treatment of an anemic fetus, which is particularly caused by the cell alloimmunization of the fetal RBCs, has a substantial risk for the development of the fetus. The estimated rates of complications and fetal mortality were up to 9 and 5%, respectively [113,114]. The highest rates of fetal morbidity observed during the second trimester of gestation in association with IUT are demanding further investigation [114,115]. This observation is apparently not only explained by the difficulties associated with IUT, but also due to the anemic condition developed by the premature anemic or hydropic fetus or both together [116].

Antiphospholipid syndrome & thrombophilia disorders in pregnancy

The pathogenesis of the antiphospholipid syndrome and thrombophilia disorder in pregnancy were recently reviewed by Pierangeli *et al.* [117] and Willis *et al.* [118]. The antiphospholipid syndrome (APS) is a disease induced by the pathogenic antiphospholipid antibodies (aPL) including anticardiolipin antibodies. The aPL belong to the large antibody family that bind the negative charge of phospholipids such as cardiolipin and many phosphatid-molecules including phosphatidylcholine, phosphatidylinositol, phosphatidylglycerol, phosphatidylserine and phosphatidic acid. This disorder is considered a 'rare disease' as reported by the Office of Rare Diseases at the NIH USA [201]. APS affects approximately 200,000 persons in the USA, where women indicate the largest fraction of the patients (80%). There are many people that show no problems of APS although they carry the aPL. The risks of this syndrome are dependant on the blood concentration of the aPL; a higher risk of complications with the event of thrombosis is associated with the higher levels of aPL. The daily health risks are headaches and migraines with giddiness, image disturbance, dermatologic disorders and vein thrombosis inducing either heart attack and/or stroke. In addition, the APS is a disease induced by the pathogenic aPL, and mediating the recurrent pregnancy loss and thrombosis, through action on various cellular antigens. Other complications of the APS and lupus anticoagulant in terms of pregnancy complications are recurrent pregnancy loss, fetal loss and fetal growth restriction, autoimmune thrombocytopenia and pre-eclampsia [119]. These complications are induced by an autoimmune process, separating this syndrome into those women with connective tissue disorders and others having systemic lupus erythematosus diseases. Systemic lupus erythematosus is a long-term autoimmune disorder that may affect the skin, joints, kidneys, brain and other organs. Despite the available knowledge

regarding the aPL mechanisms of action, which induce a procoagulant phenotype in the vasculature, a cellular proliferation abnormality and differentiation in placental tissues to cause the typical clinical features, these processes still remain incompletely understood. It is also known that various inflammatory processes provide the essential link between the observed phenotype of procoagulant and the actual development of thrombus, and is an important mediator of the placental injury in patients with APS. Even less well-understood are the processes underlying the ontogeny of these pathogenic antibodies. The hypothesis, with multiple mechanisms that are associated with thrombosis and pregnancy morbidity, is recently reviewed by a few publications [120,121].

The level of medical care with APS in pregnant women is considered in obstetrics as a high-risk condition, and the institutional medical care with consideration associated with these high-risk patients is further discussed according to [202]. Patients with symptoms of thrombosis and thromboembolism should be counseled and educated regarding frequent examination, if the signs or symptoms occurring include thrombosis or thrombus formation, decreased movement of the fetus or pre-eclamptic indications. By evidence of pre-eclampsia or of fetal growth restriction, ultrasonography examination is recommended beginning at 18–20 WG with further examination every 3–4 weeks until the end of gestation.

The placental hormone human chorionic gonadotropin (hCG) is an important parameter to monitor the viability of the developing pregnancy. If the monitored level of the hCG showed a normal increasing profile with doubling of concentration every 2 days during the first month of pregnancy, the predicted success rate of pregnancy is approximately 85%. When in the same period, the level of hCG shows an abnormal (slower) profile, 70–80% of the monitored cases provide poor outcome. The risk of bleeding should be considered before initiation of heparin therapy in a pregnancy with an expected poor outcome under the condition of uncomplicated APS pregnancy. Therefore, ultrasonography should be used to determine the fetal growth viability at 30–32 WG, which could also show the insufficiency of the utero–placental blood circulation in the APS patient. Under these circumstances, low-molecular-weight heparin is recommended. In addition, it is important to counsel the patient with the adverse effect of heparin in producing osteoporosis, which occurs in approximately 1–2% of the cases. Medicines like chloroquine and other cytotoxic agents should not be recommended during pregnancy and therefore it is also recommended that patients stop therapies with these agents a few months before pregnancy. In contrast, warfarin is a good substitution for heparin use during the postpartum period to limit the risk of induced osteoporosis. Patients using anticoagulation therapy with either heparin or low-molecular-weight heparin should be examined for bone density to limit the risk of osteopenia. IVIg therapy is thought to have three mechanisms to modulate the action of anticardiolipin [202]:

- The possible existence of anti-idiotypic antibodies in the IVIg preparation; these antibodies could bind the

autoantibody-producing complexes of idiotype–anti-idiotype dimers, and allow neutralization of the impact of the autoantibody;

- The binding and downregulation of receptors on the surface of the B cell by anti-idiotype antibodies, decreasing the production of the autoantibodies;
- The anti-idiotype antibodies could possibly bind receptors on the surface of the regulatory T cells, leading to the suppression of lymphocyte production and reduction of the activation of B cells to produce autoantibodies.

Thyroid dysfunction & autoimmune disease in human pregnancy

The many important physiological and hormonal changes described for normal pregnancy have been previously described [32]. There are several changes that are associated with the function of the maternal thyroid gland including metabolism, hemodynamic circulation and immunologic activity during pregnancy and after delivery. In the first trimester, the fetus is completely dependent on maternal production of thyroid hormones. From the second trimester the fetus produces its own thyroid hormones. However, the supply of iodine to the fetus and newborn is still provided by the mother, this supply is essential for the production of the thyroid hormones. Different from nonpregnancy physiology, during pregnancy the thyroid function is influenced by two hormones: hCG and estrogen. These hormones induce the increase of the thyroxin-binding globulin in maternal blood, which is the mechanism responsible for increasing the synthesis rate by up to 50% of the thyroid hormones [122,123].

In addition, the observed higher rate of glomerular filtration explains the increased iodine levels [122,124]. In the first half of gestation, the placental transfer of maternal iodide and iodothyronines is essential for the development of the fetal tissues [122]. Thereafter, the fetal production of thyroid hormones initiates and continues after delivery, while iodine continues to be supplied by the mother, and after delivery is supplied via breast milk [122,124].

The deficiency of iodine causes not only metabolic changes but is also responsible for goiter (enlargement of the thyroid gland in the neck region) in the mother and fetus [122]. The lower levels of free thyroxin and triiodothyronine in the presence of higher levels of thyroid-stimulating hormone, thyroxin-binding globulin and thymoglobulin, which is due to a mild deficiency of iodine, were frequently observed in the second half of gestation. While thyroid autoimmunity ameliorates during pregnancy, after delivery the autoimmune impact on the thyroid gland dysfunction is aggravated. The autoimmune thyroid disorders can be divided into two diseases; Graves' disease is associated with hyperthyroidism, while hypothyroidism is known as Hashimoto's disease (thyroiditis [125]). Both diseases are rarely observed in human pregnancy. Both types of thyroid dysfunction can possibly lead to harmful complications in maternal organs and the development of fetal organs; early recognition and treatment is essential to prevent the intensifying

of the autoimmune disorder after delivery. Inadequate treatment in women with hyperthyroidism disorder can possibly lead to exacerbation of the thyroid dysfunction (thyroid storm), which induces premature labor, bleeding delivery with small babies, fetal tachycardia (fast heart rate), stillbirths and pre-eclampsia, and possibly congenital malformations including neurological problems [203]. Similar disorders were also observed in untreated pregnant women with hypothyroidism [203].

Methimazole (Tapazole) or propylthiouracil (PTU) are anti-thyroid drug therapies and both are registered in the USA for the therapy of hyperthyroidism disorder [203]. Both of these drugs are known to transfer across the human placenta, inducing a potential impact on the fetal thyroid function and both can cause newborn goiter. The maternal treatment of hyperthyroidism with PTU in pregnancy was historically preferred because PTU is apparently transferred less across the placenta than Tapazole. However, recent studies comparing both drugs demonstrated that both are safe for administration in pregnancy. Although both drugs did not show any risk regarding the malformation of the newborns, the lowest possible drug dose is recommended to prevent any possible impact on fetal thyroid development.

The therapy of hypothyroidism in pregnancy is the same as the one used for nonpregnancy, using an adequate thyroid replacement therapy with a synthetic thyroid hormone called levothyroxine [203]. Untreated newborns with hypothyroidism using the same drug levothyroxine can cause a few disorders in children such as cognitive, brain and neurological development problems associated with malformations. Therefore, in the USA, all delivered newborns are screened for the hypothyroidism syndrome.

The potential of using stem cells derived from human placenta for immunotherapy

The placenta represents a valuable, promising and significant source of stem cells [126,127]. Stem cells derived from placental layers and membranes (amnion, chorion, decidua, and so on) have shown their ability in proliferation with high capacity of renewal expansion capacity and differentiation into multilineage cells, demonstrating even stronger capacity than those shown for bone marrow-derived stem cells [2,4–7]. The first therapeutic applications for extraembryonic MSCs have been reported within the last decade [128,129]. Infusion of human term placenta-derived MSCs into a patient with acute myeloid leukemia has been performed. The patient developed clinically negative symptoms and died at 68 years of age from respiratory failure. The authors concluded that his death was not a consequence of the infusion of placental MSCs [129]. Moreover, MSCs isolated from human term placental tissue have been used for the treatment of 12 patients with Crohn's disease [204]. Briefly, the study was performed using placental stem cell cultures derived from normal human full-term placental tissue (PDA). The patients, with active moderate-to-severe Crohn's, were given two infusions of PDA, 1 week apart. Six patients received a lower dose of the cells and six received a higher concentration, or high dose, of PDA. Unpublished results of the study indicated a clinical remission among four patients in the low-dose group. At this stage, the authors of the study declined

to speculate on why the more distinctive results were seen in the patients who received the lower, and not the higher dose. These preliminary approaches could be very important in the future for the treatment of inflammatory diseases during pregnancy.

Conclusion

In human pregnancy, the transport of antibodies to the fetus is limited to IgG. IgG is actively transported across the placenta to the fetus to provide immunological protection to the newborn via passive immunization for the first weeks of life. The transfer of maternal IgG to the fetal blood circulation is an active Fc-receptor-mediated transport mechanism. The transfer of IgG to the fetus in the first trimester is minimal with an exponentially increasing profile in the third trimester. Although all IgG subclasses (1–4) are found in the fetal serum, transport of IgG1 is predominant. The transport capacity of IgG1–4 is in the following sequence: IgG1 > IgG4 > IgG3 > IgG2.

The *ex vivo* model of the human placental tissue regarding the transport and the specificity of IgG and its subclasses yielded similar results to those obtained from *in vivo* studies, indicating the importance of the *ex vivo* model for the investigation of placental function. In addition, this method has provided details on the IgG transport mechanism across the human placenta.

The most common respiratory problem in women of reproductive age is allergic asthma, which affects 5% of pregnancies in industrialized countries. Immunotherapy in order to avoid asthma-related problems during pregnancy reduces the risk of fetal programming for allergies in later life of the newborn.

Although the exposure *in utero* to immunosuppressants is associated with a low risk for neonatal adverse outcome or derangement of normal immunologic function, most medications used for the therapy of IBD are compatible with pregnancy, with the exception of methotrexate, which is contraindicated during pregnancy. Corticosteroids, thiopurines, methotrexate and cyclosporine cross the placenta by passive diffusion. Other medications, like adalimumab and infliximab, which are monoclonal IgG antibodies, cross the placenta by binding to FcRn. The safety of these drugs needs further investigation regarding their effects on placental function and fetal exposure. Knowledge concerning the pharmacokinetics, transfer and metabolism of a drug can help distinguish between drugs that readily cross the placenta and expose the fetus with a harmful concentration.

The treatment of hemolytic disease has changed from fetal blood transfusion to maternal therapy with anti-D IgG. The therapy with anti-D IgG has brought major successes associated with a significant improvement in the outcome of affected pregnancies. It is likely that the invasive treatment with anti-D IgG will be a better choice than fetal blood transfusion in the future.

The APS is induced by the presence of pathogenic aPL, mediating the reproductive infertility as well as the recurrent pregnancy loss and thrombosis typical of the disease. Despite knowledge that such antibodies are acting on various cellular antigens and inducing a procoagulant phenotype in the vasculature and placental tissue providing the typical clinical features, the fully detailed processes need further investigation.

Thyroid autoimmunity disease ameliorates during pregnancy and aggravates after delivery. Graves' disease is rare during pregnancy and provides the most frequent cause of hyperthyroidism, while Hashimoto's thyroiditis is the most frequent cause for hypothyroidism. Both types of thyroid dysfunction may lead to detrimental complications in mother and baby and therefore timely recognition and treatment is essential. Postpartum autoimmunity most frequently exacerbates in the form of postpartum thyroiditis, which presents with diverse clinical presentations and may lead to permanent hypothyroidism.

Expert commentary & five-year view

Existing antibodies for the treatment of autoimmune diseases during pregnancy need further research establishing new strategies to extend the plasma half-life of these antibodies similar to those explored with nonpregnant blood in order to produce better efficacies with less frequent administration of these medicines during pregnancy [130]. These new strategies will assist reducing not only the frequency of the drug administration but also the cost of these antibodies, which will allow better application of these medicines in developing countries, which have a substantial fraction of immune disease.

The development of medicines for better therapeutic options in pregnancy represents the most challenging area, with important opportunities in clinical medicine and developmental biology, with a substantial impact on millions of pregnant women and newborns in western and developing countries.

All the cited studies mentioned in the current review are of clinical academic interest. The authors believe that the drug development of human pregnancy will remain neglected by

pharmaceutical industries and research for pregnant patients will remain supported by academic healthcare providers [32]. Additional surprises will remain because drug migration from nonpregnancy to use in pregnancy will always associate with concerns regarding maternal toxicity, inducing abortion, miscarriage and malformation. These problems occur because drug development for nonpregnancy does not consider the three compartments (maternal, placental and fetal) that have different biology in pregnancy [32]. There are clearly strong inherent difficulties associated with research on human pregnancy, including the associated ethical problems providing complications in creating terms and conditions for future therapy and treatment with drugs/medicines in clinical trials. Hopefully, the current advance in technology will aid the scientist interaction in the multidimensional field either to improve or create better/new therapeutics and management of pregnancy.

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Key issues

- There are strong inherent difficulties associated with research on human pregnancy, including the associated ethical problems providing complications in creating terms and conditions for future therapy and treatment with drugs/medicines in clinical trials.
- In the human, the placenta is the predominant route of IgG transfer in the maternal-to-fetal direction; the immune protection provided by maternal antibodies is essential for newborns during the first few months of life after delivery.
- The ex vivo perfusion model of human placenta provides an important tool in exploring medicines for therapies of immunologic diseases.
- Existing antibodies for the treatment of autoimmune diseases during pregnancy need further research in order to produce better efficacies with less frequent administration of these medicines during pregnancy.

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