Immunological challenges during pregnancy : preeclampsia and egg donation

Hoorn, M.L. van der

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Immunological paradox

Human pregnancy is an interesting immunological paradox. The fetus is a semi-allograft, carrying paternal and maternal genes but is not rejected by the maternal immune system. The placenta is a key player in maintaining the pregnancy, since this fetus-derived organ is in direct contact with the mother. At this fetal-maternal interface, cells of the mother come in direct contact with cells of the fetus. This thesis describes the results of investigations on the immune regulation at the fetal-maternal interface with emphasis on two immunological challenges during pregnancy. First, preeclampsia, which might be immunologically related to host versus graft disease as seen in solid organ transplantation and second, egg donation (ED) pregnancies, which show that even complete allogeneic fetal allografts can be tolerated by the mother. The immunological mechanisms involved in acceptance of the totally allogeneic fetus in ED pregnancies are not well understood yet. It is possible that it leads to differential immunological regulation. This hypothesis is tested in this thesis. This general introduction will give an overview of placenta development, general immunology, immunology at the fetal-maternal interface, preeclampsia and ED pregnancies.

1. Placenta development

1.1 Placenta

The development of the placenta is essential for fetal growth, development and maintenance of (un)complicated pregnancy. The in growth of the placenta in to the maternal endometrium promotes acceptance of the fetal allograft, and the placenta serves metabolic and endocrine functions. Already at the time of fertilization placental development starts. The placenta develops from fetal derived cells. Around four days after fertilization the blastocyst consists of two cell types: the inner cell mass, which will form the embryo and the trophoblast, which will form the placenta and fetal membranes. During implantation the blastocyst will invade the uterine decidualized epithelium. The stem cells of the placenta are progenitor villous trophoblast cells. They can develop into invasive extravillous trophoblast or into syncytiotrophoblast (Figure 1). The core of the highly branched villi is surrounded by two types of non-invasive trophoblast; the mononuclear cytotrophoblast and, when fused, it forms the multinuclear syncytiotrophoblast which overlies the villi. The syncytiotrophoblast has direct contact with the surrounding floating maternal blood. The syncytiotrophoblast layer does not divide but is able to shed syncytiotrophoblast microparticles, which will enter the maternal blood via the intervillous space [1]. Nutrients in the maternal blood will transport across the two layers of trophoblast in to fetal blood vessels. These fetal blood vessels originate from the umbilical cord arteries, to supply each villus. Waste products and deoxygenated blood are transported in fetal arteries to chorionic villi. The fetal vein carries oxygenated blood and nutrients from the placenta to the fetus. Floating villi are not in contact with the decidua and are surrounded by the maternal blood which is present in the intervillous space. Other villi are attached to the decidua basalis and are called anchoring villi.

Extravillous trophoblast invades the maternal decidua and is thereby responsible for anchoring the placenta to the maternal myometrium. Invasive extravillous cytotrophoblast become either interstitial trophoblast cells or multinucleated placental bed giant cells [2]. These cells interact with decidual cells in the decidua basalis. Furthermore, extravillous cytotrophoblast cells invade the uterine spiral arteries, becoming endovascular trophoblast and partly replacing endothelial cells. This gives the fetus access to the maternal vascular system to assure the supply of oxygen and nutrients. A balance of this invasion is very important; the cells need to invade enough for the anchoring and to receive nutrients, on the other hand over-invasion of trophoblast cells

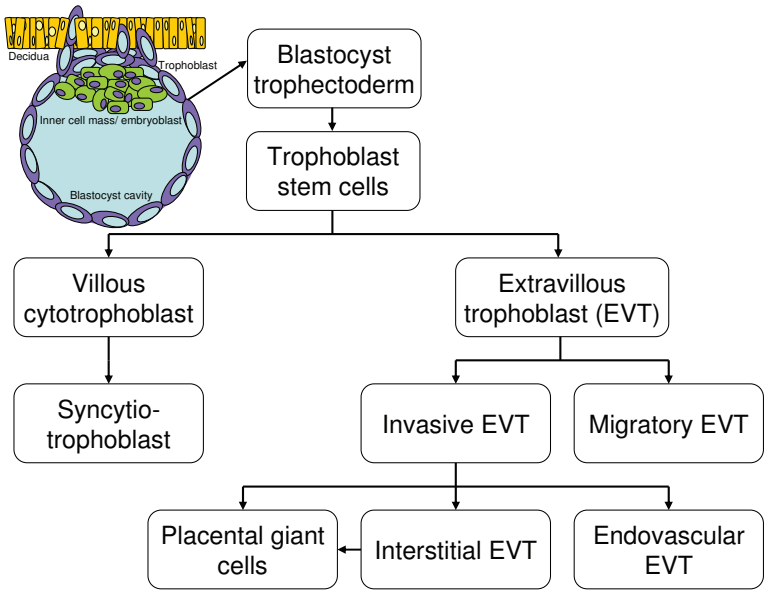

Figure 1 Flowchart of trophoblast development. The trophoblast stem cells differentiate in different trophoblast cells. Migratory EVTs are found in the chorionic plate and cell islands. The syncytiotrophoblast forms a superficial layer facing the intervillous space. EVTs are the basic material for all the non-villous parts of the placenta. In figure 4 the different types of trophoblast are shown in its environment.

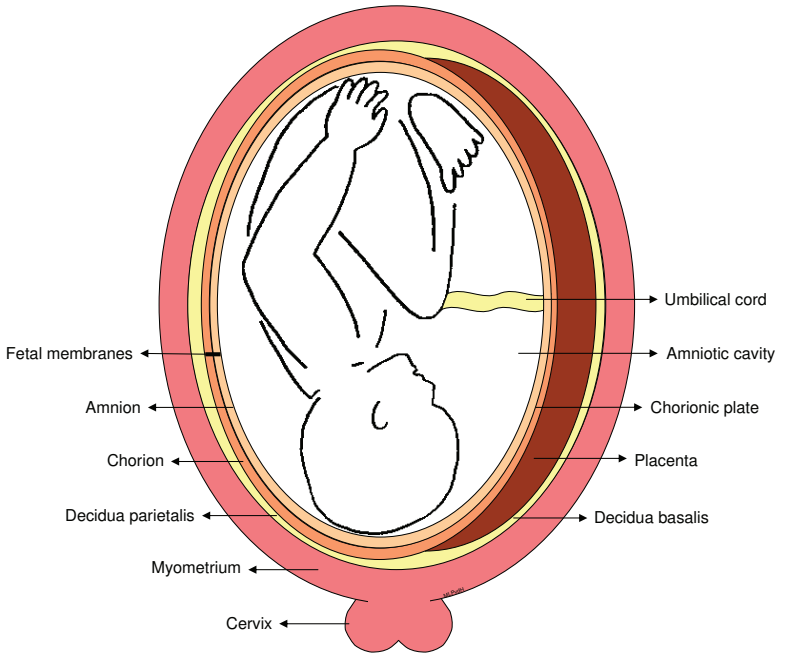

Figure 2 Uterus, placenta and fetal membranes. The fetal membranes consist of the amnion, chorion and the decidua parietalis. This latter layer is adjacent to the maternal myometrium. The placenta consists of the chonionic plate, villi and the decidua basalis which is adjacent to the maternal myometrium. The fetus is connected to the placenta via the umbilical cord.

has to be limited to protect the mother from hazardous complications like placenta accreta. In healthy pregnancies the extravillous cytotrophoblast cells invades as far as the inner third of the myometrium. Failure of this regulation, like inadequate placental invasion, might play a role in preeclampsia and fetal growth restriction. On the other hand, excessive invasion might lead to placenta accreta, a condition in which the placenta is abnormally deep attached in the endometrium and the myometrium. A schematic overview of the placenta and fetal membranes in relation to the fetus is depicted in Figure 2.

1.2 Fetal membranes

The fetal membranes surround and protect the fetus throughout gestation. Their function includes turnover of amniotic fluid and enzymatic activity during the initiation of labor. They are composed of four layers, from fetal to maternal side: amnion, chorion, trophoblast and decidua. The amnion consists of the amniotic epithelium and the amniotic mesoderm. The latter is divided in to the basal membrane, a compact stromal layer and a fibroblast layer. Amnion is adjacent to the chorion which facilitates sliding of the amnion across the chorion. The chorion is composed of the chorionic mesoderm, which includes blood vessels and a basal membrane. The chorion is adjoining the trophoblast layer. These trophoblast cells constitute a population of extravillous trophoblast. The decidual layer forms the maternal component of the membranes. In Figure 3 the layers are schematically shown.

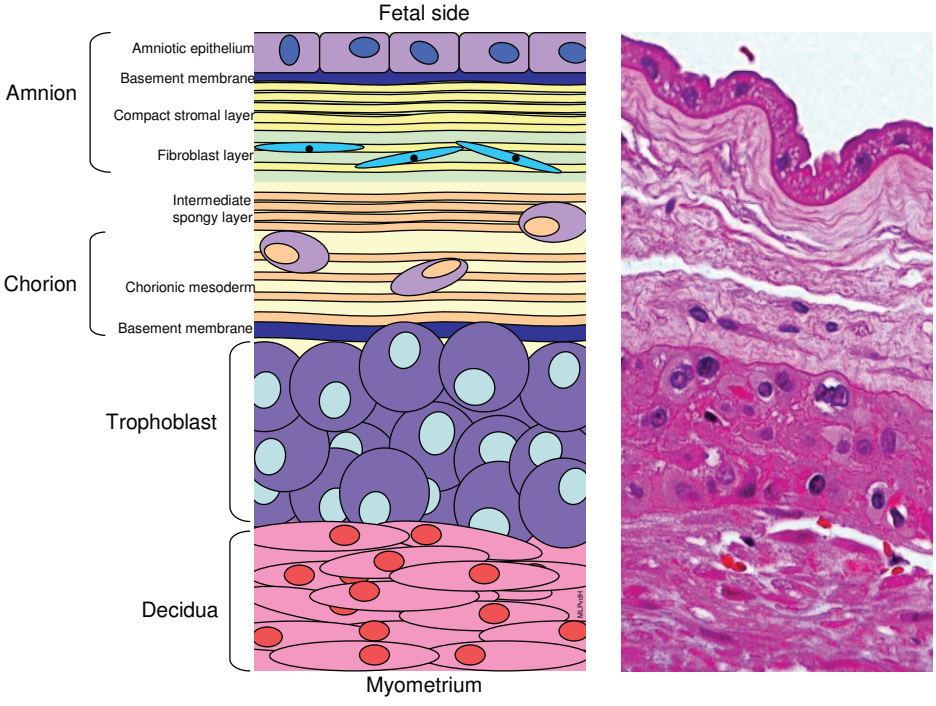

Figure 3 Fetal membranes. The layers of the fetal membranes schematically illustrated at the left panel and a histological picture at the right panel (H&E staining). From the fetal to the maternal side the fetal membranes consists of the amnion, chorion, trophoblast layer and the decidua parietalis.

1.3 Decidua

At term the decidua can be divided in to two parts. The maternal side of the placenta is the decidua basalis (Figure 4). This is the site where implantation has taken place and where the placenta has been developed. Furthermore, upon implantation, this is the first location where fetal-maternal contact takes place. The second part of the decidua is the decidua parietalis. This is the maternal side of the fetal membranes (Figure 3).

The fetus is never in direct contact with maternal tissues. In the decidua fetal and maternal cells come in contact, also referred as the fetal-maternal interface. There are three contact locations. First, the decidua parietalis, the maternal part of the membranes contacts the non-invading trophoblast of the chorion. Second, the decidua basalis (Figure 4), the maternal part of the placenta interacts with invading villous trophoblast and third maternal peripheral blood contacts the syncytiotrophoblast layer during utero-placental circulation.

The investigation of immunological mechanisms at the fetal-maternal interface gives insight in the processes leading to the acceptance of the fetal allograft.

2. Immunology

2.1 Immune system

The immune system protects the human body against diseases by identifying and killing pathogens and tumor cells. In order to function properly the cells of the immune system must distinguish between the own healthy cells and pathogens like virus, bacteria and parasites. The innate immune system attacks pathogens in a non-specific manner. The human immune system is able to adapt over time to recognize pathogens more efficiently and creates immunological memory. This part of the immune system is referred to adaptive or acquired immunity.

2.2 Innate immunity

The innate immune response provides immediate, but non-specific first line of defence against pathogens. The main function is recruitment of immune cells to the sites of infection, through the production of cytokines. Furthermore, it activates the complement cascade, kills pathogens by white blood cells and leads to activation of the acquired immune system by antigen presentation. Upon an infection inflammation is one of the first responses of the immune system. The individual recognizes infection by pain, swelling, redness, heat and a possible dysfunction of the targeted tissue. This occurs because chemokines are produced and attracts neutrophils and macrophages, which then releases cytokines and thereby triggering other parts of the immune system. The complement system refers to a cascade of reactions which eventually helps the immune system to recognize and kill pathogens. Natural killer (NK) cells, mast cells, basophils, eosinophils, macrophages, neutrophils and dendritic cells belong to the innate immune system. Phagocytes (macrophages, neutrophils and dendritic cells) are able to engulf pathogens, which results in the release of cytokines and products that kills the engulfed pathogen. The cells of the innate immune system are able to activate the acquired immune system.

2.3 Acquired immunity

The acquired immune response is highly specific for a particular pathogen improving with successive encounters via memory. T and B cells are involved in the acquired immunity. B cells are involved in the humoral immune response and T cells are involved in the cell mediated immune response. T cells recognize antigens in the complex of the major histocompatibility complex (MHC), presented on the cell surface. When T cells are activated they replicate and these cells can develop in to memory cells. Memory T cells have developed the skills to recognize antigens since they have previously encountered and responded to an antigen in a prior infection. If the pathogen is recognized again throughout life time, this will elicit a faster and a stronger immune response.

The differences between the two immune responses are obvious. The innate immune response is initiated almost immediately after infection, whereas adaptive immunity takes longer to develop. Innate immunity uses generalized and invariant mechanisms to recognize pathogens. Innate immunity is often unable to eradicate the pathogens completely, and it does not provide a stronger immunity to re-infection. In contrast, the adaptive immune response involves specific recognition by highly specific receptors on lymphocytes. This response is powerful enough to eradicate the infection and provides immunological memory. However, both immune responses work together and are able to protect an individual from harmful pathogenic infections. If an individual's immune response does not work properly, this may lead to serious complications. For example immunodeficient patients, who are not able to eradicate an infection are at a higher risk to die upon an infection. On the other hand autoimmune diseases like, diabetes or rheumatoid arthritis, are the result of an immune system which does not work appropriately.

2.4 Human leukocyte antigens

Pathogen recognition requires the ability to distinguish self from non-self. The MHC plays a pivotal role in this process. The MHC is a region of highly polymorphic genes, located in humans on the short arm of chromosome six. The human MHC system is called human leukocyte antigens (HLA). The protein products of the HLA genes are divided in to two major groups: class I and class II. The structure of these proteins is comparable. HLA class I molecules include HLA-A, -B, and -C, which are expressed on all nucleated cells and platelets. HLA class I molecules do not bind to peptides derived from pathogen-derived proteins until the peptides have been transported into the endoplasmatic reticulum. Transport to the endoplasmatic reticulum does not occur until after proteolytic cleavage of the pathogen proteins has occurred in the cytoplasm [3]. Once the peptide has bound a HLA class I molecule, this complex will be transported to the cell surface for the presentation to CD8 T cells (Figure 5) [4]. The HLA class I molecules inspects the intracellular environment. HLA class II molecules includes HLA-DR, -DQ and -DP, they are found an a few specialized cell types; macrophages, dendritic cells and B cells. HLA class II molecules bind pathogen derived peptides in a location inside endocytic vesicles, where the pathogens proteins are present (Figure 5). A peptide will bind to HLA class II molecule and this complex will be transported to the cells surface for the presentation to CD4 T cells [5]. The HLA class II molecules presents peptides derived from proteins from the extracellular environment.

The T cells recognize peptides bound to HLA molecules. To bind specifically the T cell receptor must recognize both the peptide and the part of the HLA molecule surrounding the peptide. This leads to antigen recognition and hence T cell activation. CD4 T cells, also known as T helper cells or regulatory cells, function by secreting cytokines that instruct other cells to acquire effector function. They only recognize antigens presented by HLA class II molecules. CD8 T cells differentiate into cytotoxic effector cells and kill the target cells that they recognize. These cells only recognize antigens presented by HLA class I molecules.

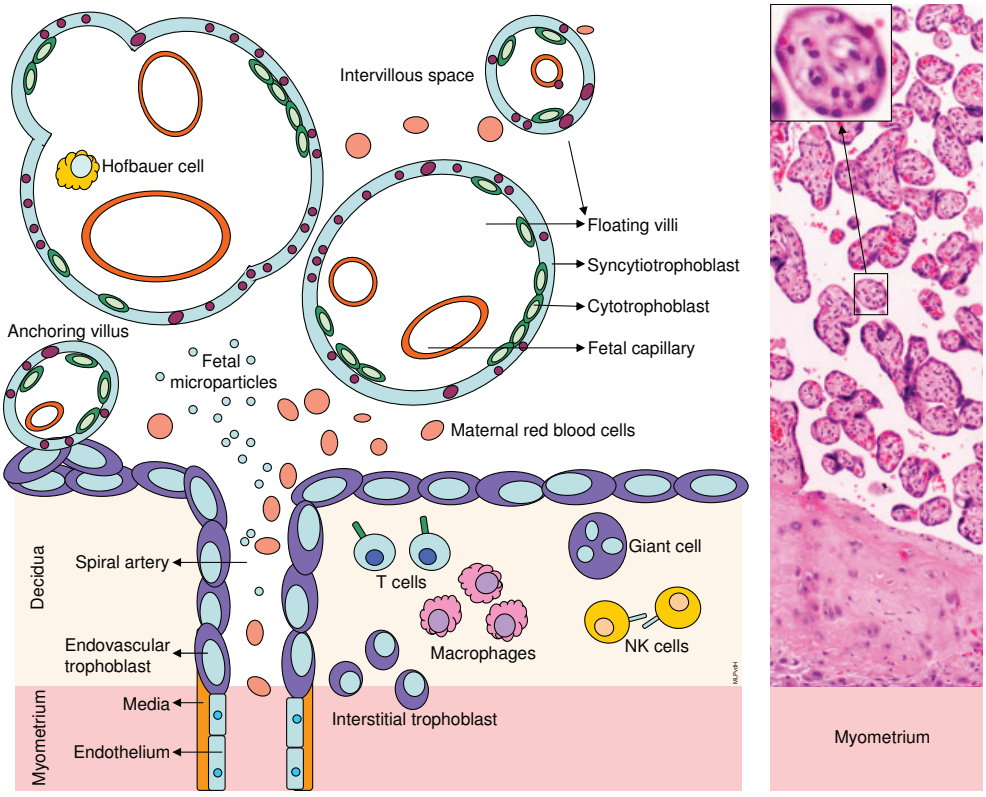

Figure 4 The fetal-maternal interface of the placenta. The left panel schematically illustrate the cells present at the fetal-maternal interface. The villi consist of different cell types and blood vessels. Microparticles are shed from the syncytiotrophoblast layer and enter the maternal blood which surrounds the villi in the intervillous space. The decidua basalis is invaded by different immune cells and spiral arteries. The decidua is adjacent to the maternal myometrium. The right panel shows a histological picture (H&E staining), with an enlargement of a single villus.

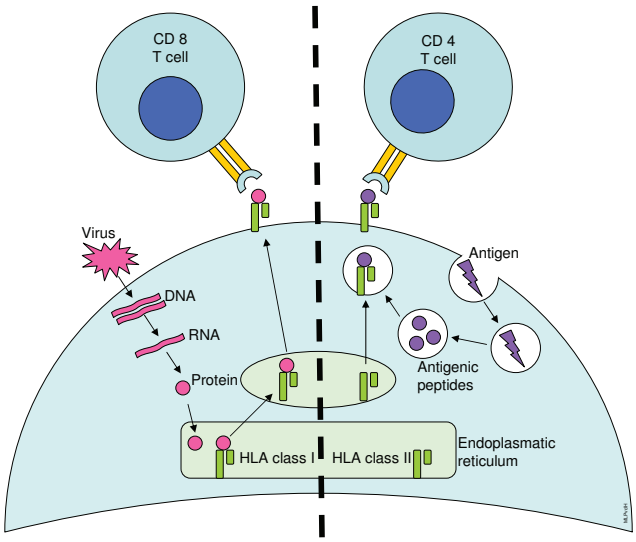

Figure 5 Antigen presentation. Two different routes of antigen presentation by HLA class I and II. On the left side the CD8 T cell is able to recognize peptides presented by HLA class I (HLA-A, -B and -C). The peptides derived from pathogens-derived proteins are processed by the endoplasmatic reticulum and presented by HLA class I molecules. The right side shows a peptide presented by HLA class II (HLA-DP, -DR and -DQ) recognized by CD4 T cells. The peptides are derived from the extracellular environment.

2.5 Cytokines

Cytokines are small proteins secreted by cells to mediate and regulate immune responses, inflammation and hematopoiesis. After an immune stimulus cytokines are produced and secreted, which then will act on a specific membrane receptor. Their expression profile has been used to categorize immune responses and the functional status of the immune system. Many cytokines have been discovered, and the ones relevant for this thesis, are highlighted here.

Interleukin-2 – IL-2 is produced after antigen binding to the T cell receptor. This leads to an expansion of IL-2 receptors on the T cell surface, and leads to growth and differentiation of T cells. Normal pregnancy is characterized by a shift towards type 2 immunity and inhibition of cytotoxic type 1 (IL-2) immune responses. An increased production of IL-2 by peripheral mononuclear cells in preeclampsia has been found [6].

Interleukin-6 – IL-6 has important roles in hematopoiesis, acute phase reactions and immune responses. IL-6 is a pro-inflammatory as well as an anti-inflammatory cytokine. It is produced by T cells and macrophages to stimulate immune responses. It acts as an anti-inflammatory cytokines by inhibiting tumor necrosis factor (TNF)-α and IL-1, and it activates IL-10. In contrast, increased concentrations of IL-6 and other pro-inflammatory (IL-1, TNF-α, and IL-8) cytokines are found in the placentas of pregnancies complicated by pre-term premature rupture of the membranes [7]. Furthermore, IL-6 levels in the amniotic fluid are increased preceding uterine contractions [8].

Interleukin-10 – IL-10 is an immunosuppressive molecule, produced by T cells, macrophages, monocytes and B cells. This cytokine is spontaneously produced in high levels by decidual macrophages [9]. It is a type 2 cytokine and appears to be pregnancy protective [10]. IL-10 is seen as a facilitator of successful pregnancy and alterations of the levels of IL-10 may be related to adverse pregnancy conditions [11]. Decreased villous trophoblast staining of IL-10 has been demonstrated in women with preeclampsia compared to normal pregnancy with correlated gestational age [12,13]. IL-10 administration in abortion prone mice significantly abrogated the incidence of spontaneous fetal loss [14]. IL-10 is produced in a gestational age-dependent manner. In first and second trimester the IL-10 levels are significantly higher. This may suggest that IL-10 is downregulated at term to prepare for the onset of labor programmed by the production of an inflammatory milieu [15]. Furthermore, first trimester missed abortion placental samples showed decreased IL-10 production [16].

Interleukin-17 – Th17 cells, the CD4+ cells that produce pro-inflammatory IL-17, is a recently discovered population involved in the maternal immunomodulation [17,18]. These cells are closely related to regulatory T cells and differentiate upon inflammatory signals whereas conditions that promote tolerance favor generation of regulatory T cells [19]. A balance between Th17 and regulatory T cells might be correlated with successful pregnancy; however the role of Th17 in human pregnancy remains to be investigated more substantially.

Transforming growth factor-β – TGF-β has well described immunosuppressive effects. Already during early pregnancy TGF-β might have an important role since it is involved in implantation of the blastocyst by inducing apoptosis of endometrial cells within the uterus. Decidual TGF-β is proposed to act on uterine NK cells to downregulate their cytotoxicity producing the uterine-specific phenotype [20]. TGF-β can stimulate two distinct receptors and thereby it is able to initiate two different SMAD signaling pathways with opposite effects. The TGF-β/ALK1 pathway induces proliferation and migration, while activation of the TGF-β/ALK5 signaling pathway inhibits these responses. Activation of the TGF-β/ALK5 signaling pathway leads to a cascade of reactions eventually leading to the phosphorylation SMAD2. Therefore SMAD2 mediates the signals of TGF-β and thus regulates several cellular processes such as proliferation, apoptosis, tissue remodeling and differentiation. Detection of phosphorylated SMAD2 reveals TGF-β signaling. Endoglin, a co-receptor of the TGF-β receptor, highly expressed during angiogenesis, is essential for ALK1

signaling. In the absence of endoglin, the TGF-β/ALK5 signaling is predominant and maintains quiescent endothelium. High endoglin expression stimulates the ALK1 pathway and indirectly inhibits ALK5 signaling, thus promoting the activation state of angiogenesis [21]. Endoglin is expressed on trophoblast. Increased serum levels of soluble endoglin are found in pregnancies complicated by preeclampsia [22].

Galectin-1 – Galectin-1 is an immunoregulatory glycan binding protein. Galectin-1 is able to modulate immune cell functions in different manners, for example by blocking the secretion of pro-inflammatory molecules [23], apoptosis of activated T cells [24] and antagonizing T cell activation [25]. Galectin-1 deficient mice show increased rates of fetal loss when compared with wild type controls, and injection of Galectin-1 in to the deficient mice rescued the pregnancy, possibly leading to expansion of IL-10 producing regulatory T cells [26].

Vascular endothelial growth factor – Vascular endothelial growth factor (VEGF) is an angiogenic protein. Membrane-bound fmslike tyrosine kinase 1 (Flt-1) is a receptor for VEGF and placental growth factor (PLGF). A splice variant of Flt-1 is soluble Flt-1 (sFlt-1, also known as sVEGFR-1) which antagonizes the VEGF and PLGF receptor. This soluble form prevents interactions of VEGF and PLGF with the functional membrane bound Flt-1 which thereby leads to endothelial dysfunction. In preeclampsia sFlt-1 is expressed in excessive amounts [27]. Hypoxia is considered to be the trigger for the production of sFlt-1 by villous trophoblast cells. VEGF antagonism by sFlt-1 may cause the clinical manifestations of preeclampsia, such as hypertension and proteinuria [28].

Interferon-γ – IFN-γ is a pro-inflammatory cytokine which plays a critical role in the initiation of endometrial vasculature remodeling, angiogenesis at the implantation side and maintenance of the decidua [29]. Deviations in these pregnancies are thought to lead to gestational complications like preeclampsia and fetal loss [30]. IFN-γ is involved in the innate and adaptive immunity against virus, intracellular bacterial infections and tumor control. It is predominantly produced by NK cells.

3. Immunology at the fetal-maternal interface

The immunological paradox is a medical enigma that has stimulated research for half a century. In the early days four hypotheses were postulated [31]. The first hypothesis was that the fetus lacked immunogenicity. This hypothesis is abandoned since studies showed that the fetus has immunogenic properties [32]. The second hypothesis was based on a possible diminished maternal responsiveness to pregnancy, leading to acceptance of the foreign fetus. Although peripheral changes during pregnancy are described, this hypothesis can not totally hold since this would make the pregnant women susceptible to harmful infections. The third hypothesis reflects the uterus as an immune-privileged site; however this is not a unique characteristic of the uterus since ectopic pregnancies occur. And the fourth hypothesis states that the placenta is an immune barrier. The immune barrier does not reflect a physical barrier, since fetal and maternal cells indeed come in contact at the location known as the fetal-maternal interface. The acceptance of the immunological foreign fetus is mediated by both maternal and fetal mechanisms. Already during implantation immunological adaptations are necessary, maintaining till the end of a successful pregnancy.

3.1 Immune escape mechanisms by trophoblast

HLA expression – Villous trophoblast (syncytiotrophoblast) expresses no HLA antigens on its surfaces. Extravillous trophoblast expresses a very particular set of HLA. Only four types of HLA class I genes are expressed, HLA-C, HLA-E, HLA-F and HLA-G. These HLA molecules may dampen the immune response by interaction with the leukocyte inhibitory receptors (LIR) on uterine NK cells, macrophages and with the T cell receptor on CD8+ cells [33,34]. This interaction blocks the cytotoxicity of these cells. NK cells have been shown to kill cells which lack HLA expression on the cell surface, therefore, by expression HLA molecules, NK cell mediated cytotoxicity is avoided [35]. HLA-G is mostly restricted in expression to the extravillous trophoblast. Class II HLA molecules are completely absent on extravillous trophoblast cells. Hence, the semi-allogeneic fetus is able to evade immune rejection by the maternal immune system.

B7 family – Second, the co-stimulatory molecules of the B7 family are selectively expressed on the trophoblast cells in human placenta. Activation of lymphocytes in circulating maternal blood is repressed by expression of B7H1 which is uniquely expressed on syncytiotrophoblast [36].

IDO – Indoleamine 2,3-diogygenase (IDO) is an enzymatic protein that catabolises tryptophan [37]. T cells are uniquely sensitive to fluctuations of tryptophan, and by the destruction of tryptophan by IDO the T cells become inactivated. IDO is produced by trophoblast cells and thereby this mechanism may contribute to the reduction or inhibition of immune reactions. Furthermore, IDO is as well produced by macrophages in response to IFN-γ.

Th1/Th2 balance – Uncomplicated pregnancy is considered to be an anti-inflammatory condition with predominantly the production of T helper (Th)-2 cytokines. Th1-type reaction in the placenta generates mainly inflammatory responses and correlate with miscarriage. Th2 cytokines are produced at the fetal-maternal interface and can inhibit Th1 responses, improving fetal survival but impairing responses against some pathogens [38]. Th1 cells produce IL-2 and IFN-γ, and Th2 cells synthesise IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13. Furthermore, the human placenta produces immunosuppressive molecules as progesterone, prostaglandin E2, and anti-inflammatory cytokines as IL-4 and IL-10 [33,39]. In this way trophoblast cells are able to influence the Th1/Th2 balance by the production of cytokines and hormones [10].

Complement system – In the placenta, the complement system helps to protect the mother and fetus against the invasion of pathogens. The fetus is protected by the maternal immune system by the expression of complement inhibitors. Trophoblast cells express complement regulatory proteins, which are important to protect the fetal cells because complement activation leads to destruction of the immunologic target [40]. Uncontrolled complement activation is prevented by decay accelerating factor (DAF), membrane cofactor protein (MCP), and CD59 [41].

Furthermore, tumor necrosis factor (TNF) α, Fas ligand (CD95L), TNF related apoptosis inducing ligand (TRAIL) are ligands identified in or on human trophoblast cells which are able to support the pregnancy host defense by supporting the maternal or fetal antibody production [42-44].

The various strategies of immune evasion may result in the acceptance of the fetus. However, despite these mechanisms the maternal immune system is aware of paternal antigens. Microchimerism is the persistence of a small population of foreign cells in another individual. Microchimerism is present between mother and fetus [45]. Therefore, other additional mechanisms are necessary to tolerate allogeneic cells by the maternal immune system. The microparticles which are shed from the syncytiotrophoblast layer lack HLA expression and therefore they will not be attacked by alloreactive T cells. However, the microparticles are able to bind to monocytes and stimulate the production of inflammatory cytokines, making them potential contributors to altered systemic inflammatory responsiveness in pregnancy [46].

3.2 Maternal cells

The decidua is populated by a variety of leukocytes during pregnancy [47,48]. Levels of lymphocytes are relatively low. During implantation the leukocytes mainly consist of NK cells. Macrophages form, after the uterine NK cells, the largest population of decidual leukocytes in early pregnancy (20-30%). Their numbers remain relatively constant throughout gestation [49]. In contrast, the numbers of NK cells decrease during pregnancy being absent at term [50]. This suggests that the innate immune system plays an important role in fetal-maternal immune adjustment. Macrophages as the main cells of the innate immune system are key players in the local regulation of maternal immune responses toward the fetus. The presence of both macrophages and dendritic cells at the fetal-maternal interface permits modulation of the immune response to protect the mother and fetus. Figure 6 summarize the leukocyte densities at the fetal-maternal interface during gestation.

Antigen presenting cells

An antigen has the capacity to trigger the adaptive immune response through several steps. The antigenic particles or proteins must be captured, processed and presented to T cells. These activities are performed by antigen presenting cells (APCs). Three kinds of APCs are defined: B lymphocytes, macrophages and dendritic cells. APCs sample the environment for potentially harmful extracellular particles. They are able to present components of antigenic particles on their cell surface via an intracellular breakdown mechanism. T cells can recognize the membrane bound components. To come in contact with the T cells, APCs transport antigens from the tissues to the peripheral lymphoid organs.

B cells

Only a few B cells can be detected in the endometrium and decidua. Their number does not vary during pregnancy. Uterine B cells are able to respond to antigenic challenges in for example pregnancies complicated with intrauterine infections.

Macrophages

The origin of the macrophages is in the bone marrow where myeloid progenitors differentiate into promonocytes and then into circulating monocytes which migrate transendothelially into the various organs to become macrophages. These macrophages are very effective in presenting antigenic peptides to T cells. They occur in almost all organs of the body. Upon fertilization, macrophages flux into the decidualized endometrium, and are found in close association with trophoblasts populations which secrete chemotactic molecules [51]. Macrophages comprise at least 10% of total decidual leukocytes [52]. In the decidua parietalis the trophoblast cells are scarce and also the macrophages are found in few numbers [50]. Macrophages are pluripotent, especially near the end of pregnancy, therefore it is hypothesized that their relative number increase at the end of gestation [52]. The close association of macrophages and extravillous trophoblast cells suggest an early recognition of fetal tissue by the immune system and a role in placental development, possibly by connection with HLA-G. Two types of macrophages populate the decidua, pro-inflammatory CD163- type 1 macrophages and immune modulatory CD163+ type 2 macrophages. Type 1 macrophages produce high levels of IL-12 and have a T cell stimulating potential. Type 2 macrophages do not have the T cell stimulating potential, do have a phagocytosis potential, and produce high levels of IL-10. Several studies show that decidual macrophages may have an immunoinhibitory function at the fetal-maternal interface since these macrophages are not able to differentiate into dendritic cells. Furthermore, they produce IL-10 and IDO and express low levels of the T lymphocyte co-stimulatory molecules CD80 and CD86 [9]. IL-10 can, by blocking the expression of co-stimulatory molecules on APCs, reduce the T cell activity against the fetus [53].

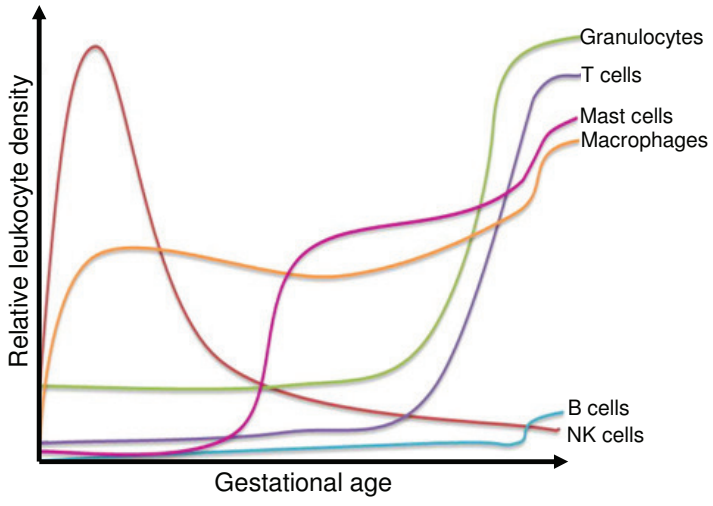

Figure 6 Leukocytes in human decidua. The relative leukocyte density at the fetal-maternal interface and the relation with gestational age is shown. Adjusted from [52].

Dendritic cells

Dendritic cells are closely related to macrophages. Dendritic cells have the power to induce primary immune responses and occur in mucosal sites such as skin, airways, gut and decidua. These cells transform information to the adaptive immune system. They also play a role in the induction of immunological tolerance by regulation of T cell mediated immune responses. Dendritic cells comprise approximately 1-2% of decidual leukocytes.

Two types of dendritic cells are reported in the literature. Myeloid dendritic cells are the major subpopulation of human blood dendritic cells and express the BDCA-1 (CD1c) antigen. These cells are efficient in antigen uptake and presentation. Plasmocytoid (or lymphoid) dendritic cells express the antigen BDCA-2 (CD303) and have the ability to induce T cell differentiation into Th2 cells. Thus dendritic cells are able to modulate the immune system in a stimulatory or tolerogenic way. This makes them suitable cells to exert regulatory functions in pregnancy. Consequently, decreased levels of plasmocytoid dendritic cells can be involved in the impairment of differentiation into Th2 cells in preeclamptic pregnancies. This has been shown in peripheral blood [54]. Furthermore, in the decidua of preeclamptic pregnancies a dense infiltration of immature and mature dendritic cells has been demonstrated [55].

Dendritic cells have several mechanisms to induce immune tolerance in absence of inflammation or infection. First, dendritic cells present antigens in lymph nodes and in response T cells proliferate and are then destroyed. Second, dendritic cells can induce IL-10 production. Dendritic cells express IDO, which is involved in inhibiting T cell proliferation [56]. These mechanisms may operate to prevent maternal T cell activation to the trophoblast. In absence of infection dendritic cells have an immature phenotype. They capture antigens generated by dying, infected or allogeneic cells. The presentation of these antigens to T cells induces antigen-specific T cell tolerance. Antigen capture by dendritic cells in an infectious environment drives dendritic cells to draining lymph nodes. Here the dendritic cells will transform into mature dendritic cells. This cell functions as a potent APC, capable of activating naive and memory helper T cells, cytotoxic T cells and B cells [57]. Relating these different functions to the decidua, immature dendritic cells present fetal antigens from invading trophoblast cells and present these to maternal T cells which are locally in attendance. This interaction induces tolerance to these antigens. However, in the midst of an infection mature dendritic cells are able to capture fetal antigens and migrate to lymph nodes which could result in maternal T cell reactivity to the conceptus.

T cells

The numbers of decidual T cells increase during pregnancy, starting with 5-20% of all CD45+ decidual lymphocytes in early pregnancy samples, till 40-80% at term [58]. Decidual T cells encompass a very heterogenic subset of T cells that include activated CD4+ and effector memory type CD8+ T cells. These activated T cells are found together with T cells subsets that are capable to suppress the decidual lymphocyte response [59]. Suppression of T cells may lead to acceptance of the allograft. Therefore, T cell research has dominated research in the immunology of pregnancy in the past years. Furthermore, T cells play an important role in the immunology after solid organ transplantations. CD4+ T cells can respond directly or indirectly to antigens of the semi-allogeneic allograft. Extravillous trophoblast cells only express HLA-C as the HLA class I molecules, and no HLA class II molecules. Therefore direct presentation is unlikely to be very important. In indirect presentation, allogeneic HLA molecules are taken up and processed by recipient APCs and these processed T cells are presented to recipient T cells in the context of self HLA. In the decidua dendritic cells and macrophages are present to fulfill this role.

Regulatory CD4+CD25bright T cells are present in human decidua in higher numbers compared to peripheral maternal blood [59], suggesting an important role at the fetal-maternal interface. It has been shown that fetus specific CD4+CD25bright T cells are recruited to the maternal decidua where they are able to suppress the local immune response [60]. T cells produce a variety of type 1 and type 2 cytokines and thereby may contribute to the local regulation of the fetus-specific responses within the decidua.

Alterations in the distributions of T cells may lead to pregnancy complications. Decreased numbers of regulatory T cells in peripheral blood have been found in preeclampsia and recurrent spontaneous abortions [61,62]. These results postulate that a sufficient number of regulatory T cells is necessary to maintain an uncomplicated pregnancy. The exact mechanism how regulatory T cells are activated and induce tolerance during pregnancy remains to be elucidated.

NK cells

NK cells are the predominant cell type of the decidua during implantation. Every menstrual cycle uterine NK cells are activated and expanded in to the decidua. High numbers are found in the stroma and clustered around glands and spiral arteries. When trophoblast invasion is complete, after the twentieth week, the number of NK cells will decrease. NK cells interact with extravillous trophoblast cells, this interaction is thought to be essential for the control of implantation [63]. In tubal pregnancies, which are characteristic for excessive trophoblast invasion, NK cells are absent [64]. In preeclampsia abnormal implantation occurs as a result of increased NK cell activity. NK cells express a variety of receptors which are able to recognize HLA class I molecules. Decidual NK cells are different compared to peripheral NK cells. Decidual NK cells express perforin, granzyme A and B and, unlike peripheral NK cells, they contain reduced cytolytic activity to HLA class I negative targets [65], secrete proteins with immunomodulatory potentials [66] and produce angiogenic factors like VEGF and PLGF [67]. Furthermore, decidual NK cells may recognize fetus HLA-C1 and HLA-C2 by the expression of killer immunoglobulin like receptor (KIR) [68].

It seems that mother's immune suppression is restricted to responses directed against the fetus. The fetus as well as the mother is dependent on the maternal immune system during the pregnant state. Even beyond birth the fetus is protected from harmful pathogens by passive immunization by the transfer of maternal antibodies through the colostrum and milk [69].

4. Preeclampsia

Four hypertensive disorders can occur during pregnancy; preexisting hypertension, gestational hypertension, preeclampsia and superimposed preeclampsia [70]. Preexisting hypertension is defined as systolic pressure of higher than 140 mmHg and/or diastolic pressure higher 90 mmHg before pregnancy, present before the 20th week of pregnancy, or persists longer than 12 weeks postpartum. Gestational hypertension refers to elevated blood pressure first detected after 20 weeks of gestation without proteinuria. Some patients with gestational hypertension will develop proteinuria over time and be considered preeclamptic, while others will be diagnosed with preexisting hypertension because of persistent blood pressure elevation postpartum. Preeclampsia refers to the syndrome of new onset of hypertension and proteinuria after 20 weeks of gestation in a previously normotensive woman or worsening hypertension with new onset proteinuria in a woman with preexisting hypertension (superimposed preeclampsia). Additional symptoms include visual disturbances, headache, epigastric pain, thrombocytopenia and abnormal liver function can occur. Preeclampsia occurs in approximately 3 to 14% of all pregnancies worldwide [71,72]. Abnormal placenta development plays a critical role in the pathogenesis of preeclampsia. Immunological factors are postulated to contribute to this abnormal development, since prior exposure to paternal antigens appears to protect against preeclampsia [73,74]. Preeclampsia is only a disease of pregnancy since it is cured after delivery.

The pathogenesis of preeclampsia starts during implantation and occurs before clinical manifestation. In normal pregnancies the spiral arteries are invaded by cytotrophoblasts and these vessels undergo a transformation from small to large leading to facilitated blood flow to the placenta. This remodeling of spiral arteries begins in the first trimester and is completed by 18 to 20 weeks of gestation. In preeclampsia the trophoblast cells do not have the capacity to migrate into the myometrium part of the spiral arteries. This will result in placental hypoperfusion, since the re-modulation of the vessels does not occur [75]. Ischemia and impaired placentation are thought to be the primary events leading to the release of soluble factors that are able to cause systemic endothelial dysfunction resulting in the clinical symptoms of the disease [76].

4.1 Preeclampsia and immunology

Preeclampsia is seen as an immunological disease. It is a disease of primipara and it is thought to occur in multipara with new parternity since previous studies have shown that partner change increased the risk of preeclampsia or hypertension in pregnancy. However, women who change partners often have a longer birth interval, and a longer interval is associated with a higher incidence of preeclampsia [77]. Artificial donor insemination and ED increase the risk of hypertensive disorders in pregnancy. In contrast, there is a protective role of maternal exposure to seminal fluid of her partner during an extended period [74].

Pregnancy related disorders as preeclampsia, abortions or fetal growth restrictions are a major cause of morbidity and mortality of both the mother and fetus. These disorders are related with increased levels of type-1 inflammatory cytokines, decreased levels of type-2 cytokines and macrophages have been found to be aberrantly activated [78].

In the decidua a specialized population of NK cells are present in high numbers at the implantation side. Direct interaction between invading trophoblast and decidual NK cells results in the production of various cytokines [79]. Hereby, NK cells play a direct role in trophoblast invasion and spiral artery remodeling and hereby disturbance of NK cell functions might be involved in the pathogenesis of preeclampsia. The receptors for HLA-C expressed on NK cells are known as KIRs. Every gestation represents a unique couple-specific interaction between fetal trophoblast HLA-C and maternal KIRs [80]. Specific HLA-C – KIR interactions are strongly associated with

preeclampsia; mothers lacking most or all activating KIR (women with the AA genotype) when the fetus possessed HLA-C belonging to the HLA-C2 group, are at a greatly increased risk of preeclampsia [81]. Furthermore, mothers with KIR AA frequencies have an increased risk of affected pregnancies only when the fetus has more group 2 HLA-C genes (C2) than the mother [82].

In normal pregnancy extravillous trophoblasts are located around the spiral arteries. Macrophages are located next to this layer in the stroma of the spiral arteries. In pathological pregnancies the distribution of macrophages is altered. The macrophages are located within and around the spiral arteries. Extravillous trophoblast cells are separated from the arteries. This creates a barrier between the spiral arteries and the invading trophoblast cells and complicates the transformation of spiral arteries [83]. In the normal situation, macrophages enhance trophoblast survival while in the pathologic situation the macrophages induce apoptosis. Aberrantly activated macrophages could contribute to the etiology of preeclampsia, fetal growth restrictions or abortions by disturbing the placental angiogenesis. Macrophages secrete the angiogenic factor VEGF [84]. Low levels of VEGF and PLGF may contribute to the deficiency in placental angiogenesis. The function of VEGF and PLGF can be inhibited by sFlt-1, which is a splice variant of VEGFreceptor 1 (Figure 7). In pregnancies complicated by preeclampsia the level of sFlt-1 is increased and alters the angiogenic activity of macrophages by binding to its receptors [84].

Fetal and placental growth is dependent on an adequate IL-10 production. A decreased IL-10 expression in trophoblast in preeclampsia compared to normal pregnancy has been observed [13]. IL-10 can promote the differentiation of monocytes into macrophages. Since the level of IL-10 is lower in preeclampsia, it is possible that the number of macrophages is also reduced.

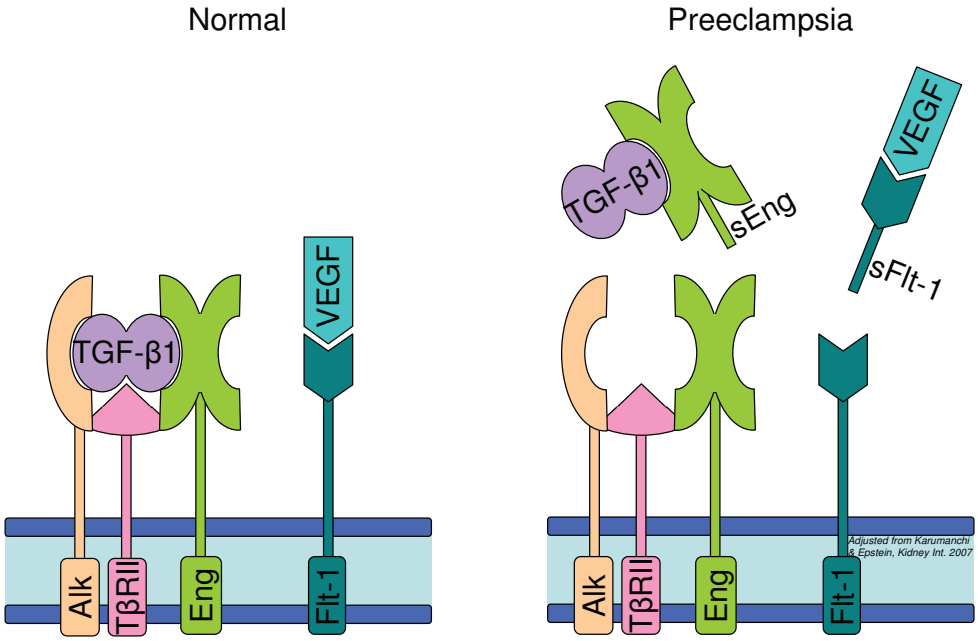

Figure 7 Cytokines involved in preeclampsia. During normal pregnancy vascular homeostasis is maintained by physiological levels of vascular endothelial growth factor (VEGF) and transforming growth factor-β1 (TGF-β1) signaling in the vasculature. In preeclampsia soluble endoglin (sEng) and soluble fmslike tyrosine kinase 1 (sFlt-1) derived from placental tissue are able to inhibit the normal functions of VEGF and TGF-β1, resulting in endothelial dysfunction [88].

Macrophages in the basal plate of the preeclamptic decidua are present in a reduced number compared to normal control decidua [85]. This can be caused by reduced migration of monocytes through the blood vessels into the tissue, or by reduced differentiation after migration of monocytes. However, discrepancy in literature exists whether the number of macrophages in preeclampsia is increased, reduced or unaltered.

Since the risk of preeclampsia is lower in second pregnancies with the same partner, there must be a certain degree of immunological memory. T cells belong to the adaptive immune system, and an activated T cell is able to develop in to memory cells. For this reason, T cells probably have a role in the pathogenesis of preeclampsia. NK cells, macrophages and dendritic cells belong to the innate immunity, which is probably not able to develop immunological memory. However, recently it has been shown that NK cells can demonstrate immunological memory [86,87]. If decidual NK cells are also capable of immunological memory, they might play a role in the pathogenesis of partner specific preeclampsia.

5. Egg donation

ED is the donation of unfertilized eggs to a woman who does not have (appropriate) eggs of herself or to women with genetic disorders. The egg donor receives a hormone treatment followed by an egg retrieval procedure. After retrieval the eggs are fertilized by sperm of the future father. In the meantime the recipient uterus is appropriately prepared to receive the fertilized eggs. After several days the best embryo is transferred in the uterus of the recipient.

The law forbids commercial and anonymous ED in the Netherlands. ED based on non-commercial purposes is allowed. The main reason to perform ED in the Netherlands is premature ovarian failure. This disease is characterized by early onset of ovarian failure by for example radiotherapy, genetic disorders, surgical destruction of the ovaries or an unknown cause. The woman does have a functional uterus. Furthermore, ED is necessary if the ovary can not be reached in an IVF procedure, although women do have correctly functioning ovaries. An additional indication to perform ED is present in women who have a high risk of getting children with high risk genetic disorders. The age on which women gets their first child has increased up till 29.4 years in the Netherlands in 2008. Reasons to postpone pregnancy are the availability of anti-conceptive and better educational and career opportunities for women. Since the year 2000 the number of women who go abroad for egg donation has increased threefold. Couples wishing an anonymous donor or who can not find a (non-profit) egg donor in the Netherlands go abroad. Spain is by far the most popular country, followed by Belgium. Overall, abroad more embryos are transferred per cycle compared to the single embryo transfer in the Netherlands. Although exact numbers are difficult to collect, a maximum of five embryo transfer per cycle has been described in ED pregnancies performed abroad. Multiple pregnancies are potentially hazardous for the gestational carrier and fetuses since they lead to more complications. The costs for ED abroad differ from 3,000 up to 30,000 euro per treatment [89].

Pregnancy conceived after ED, reflects an interesting model to study immunological reactions. ED pregnancies are a result of in vitro fertilization of a donated egg by a relative, or more commonly an unrelated donor. Hereby, neither of the fetal haplotypes matches with the gestational carrier. Progressive knowledge in the field of assisted reproductive technologies and extension of the medical indications leads to an increase of number of ED pregnancies. Nevertheless, it can lead to harmful maternal consequences during pregnancy, which may be related to the allogeneic nature of the fetus. Maternal complications in ED pregnancies include an increased risk of pregnancy induced hypertension, an increased rate of caesarean section deliveries, an increased risk of postpartum hemorrhage and an increased risk of first trimester vaginal bleeding. Although the

maternal complications are higher in ED pregnancies compared to spontaneously conceived pregnancies, there is no increased complication risk for the fetus or newborn [90-93].

5.1 Transplantation and egg donation pregnancies

Since in ED pregnancies the entire fetal genome is allogeneic towards the gestational carrier, immune mechanisms in successful ED pregnancies might be relevant for the induction of immunological tolerance in solid organ transplantation.

Blood transfusions are the most widespread kind of transplantations in clinical medicine. Compared to solid organ transplantation, blood transfusions have less immunological barriers. Recipients and donors are typed and cross-matched for the ABO and the rhesus erythrocyte antigens. If an ABO incompatible organ is transplanted a hyperacute rejection may occur. Anti HLA antibodies may also be present in prospective transplant patients. Blood transfusions or pregnancies are the source of these antibodies. Fetal cells enter the maternal circulation and antibodies against the paternal HLA antigens or in case of ED, the donor HLA antigens. The presence of HLA antibodies is associated with a reduced chance of a live birth [94]. Besides blood transfusion and pregnancy, anti-HLA antibodies can be developed after previous organ transplants.

Acute rejection of the transplanted graft occurs if donor antigen presenting cells carry complexes of donor HLA molecules on their surfaces. In a secondary lymphoid organ they will enter T cell areas and present their antigens towards them. The recipient T cells become activated by specifically binding to the complexes of allogeneic donor HLA. The effector T cells have the capability to attack the transplanted organ. In (ED) pregnancy this type of immunological rejection possibly plays no role, since maternal T cells do not come in contact with fetal antigen presenting cells. In chronic rejection, indirect antigen presentation plays a major role. The recipient's dendritic cells enodcytose HLA class I and II particles from donor cells. The peptides are then presented by the recipient's HLA and CD4 T helper cells may become activated. Indirect presentation possibly plays a role in the immunology of pregnancy. Fetal derived microparticles are present in the maternal bloodstream and might be taken up by maternal antigen presenting cells [46,95] and present them via the indirect pathway to maternal T cells.

In placentas of ED pregnancies severe chronic deciduitis combined with fibrinoid deposition has been observed [96]. These pathological findings are localized in the basal plate of the placenta, the location where the extravillous cytotrophoblast lines with the maternal decidua. This pathological finding is considered to be immunological modulated.

6. Outline of this thesis

The question why the semi-allogeneic fetus is accepted by the immune system of the mother has already risen in 1953 [97]. Medawar was the first to imply the fetus as a semi-allograft. Ever since then much research has been performed in the field of reproductive immunology. However, until today pregnancy remains an immunological paradox and the exact mechanism leading to the acceptance of the semi-allogeneic fetus remains to be elucidated. Although the mechanism is still not yet well understood in normal pregnancies, immunological knowledge of complicated pregnancies might give insight in the underlying mechanisms of tolerance.

The aim of this thesis is to study the immunological mechanisms in uncomplicated, preeclamptic, ED and non donor IVF pregnancies. Preeclampsia and ED are seen as an additional immunological challenge during pregnancy. Since ED pregnancies are characterized by a higher number of HLA mismatches compared with naturally conceived pregnancies, this thesis hypothesizes that differential immune regulation is necessary to maintain pregnancy.

To investigate the immunological mechanism, placentas of uncomplicated, preeclamptic, ED and non donor IVF pregnancies were collected. They were used to study the local immunological mechanisms by immunohistochemistry analysis of the decidua basalis and parietalis. Blood samples of umbilical cord blood and of the mothers of uncomplicated, preeclamptic, ED, and non donor IVF pregnancies were taken and cells were isolated. Those cells were used to simulate peripheral immune responses. The reaction of peripheral cells from uncomplicated, ED, and IVF pregnancies and non pregnant controls up on stimulation with own umbilical cord blood, allogeneic umbilical cord blood and peripheral blood samples was measured by mixed lymphocyte reactions and by cytokine production. The cells were phenotyped using flow cytometry. Of the pregnancies described in this thesis the number of HLA mismatches was calculated.

Chapter 2 investigates the peripheral immune response in uncomplicated pregnancies compared with non pregnant controls. The specific and non-specific maternal immune response was studied. The aim of Chapter 3 is to study macrophages in the decidua of preterm preeclamptic pregnancies compared with uncomplicated preterm control and control pregnancies by immunohistochemistry. Chapter 4 describes two case reports. The first case describes a woman pregnant after IVF suffering from preeclampsia while the fetuses have severe growth retardation. The second case describes an ED pregnancy with preeclampsia without fetal growth retardation. The question is raised whether preeclampsia in ED pregnancy is based on different pathophysiological mechanism.

The focus of the studies described in the Chapters 5 – 7 is on ED pregnancies. Since the fetus in ED pregnancies is fully allogeneic to the gestational carrier, immune mechanisms in successful ED pregnancies might be relevant for the induction of immunological tolerance in solid organ transplantation. This is discussed in Chapter 5. Chapter 6 gives an overview of the clinical and immunological aspects of ED pregnancies. In Chapter 7 ED, non donor IVF and naturally conceived pregnancies are studied. The expression of several cytokines in the placenta and in serum of the patients is investigated. Furthermore, the phenotype of cells in peripheral blood is analyzed and the reactivity of those cells in response to umbilical cord blood of the own or allogeneic umbilical cord blood is studied.

The conclusions of the different chapters are summarized and discussed in Chapter 8.

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