Immune parameters affecting maternal tolerance towards the fetus in normal and aberrant pregnancies

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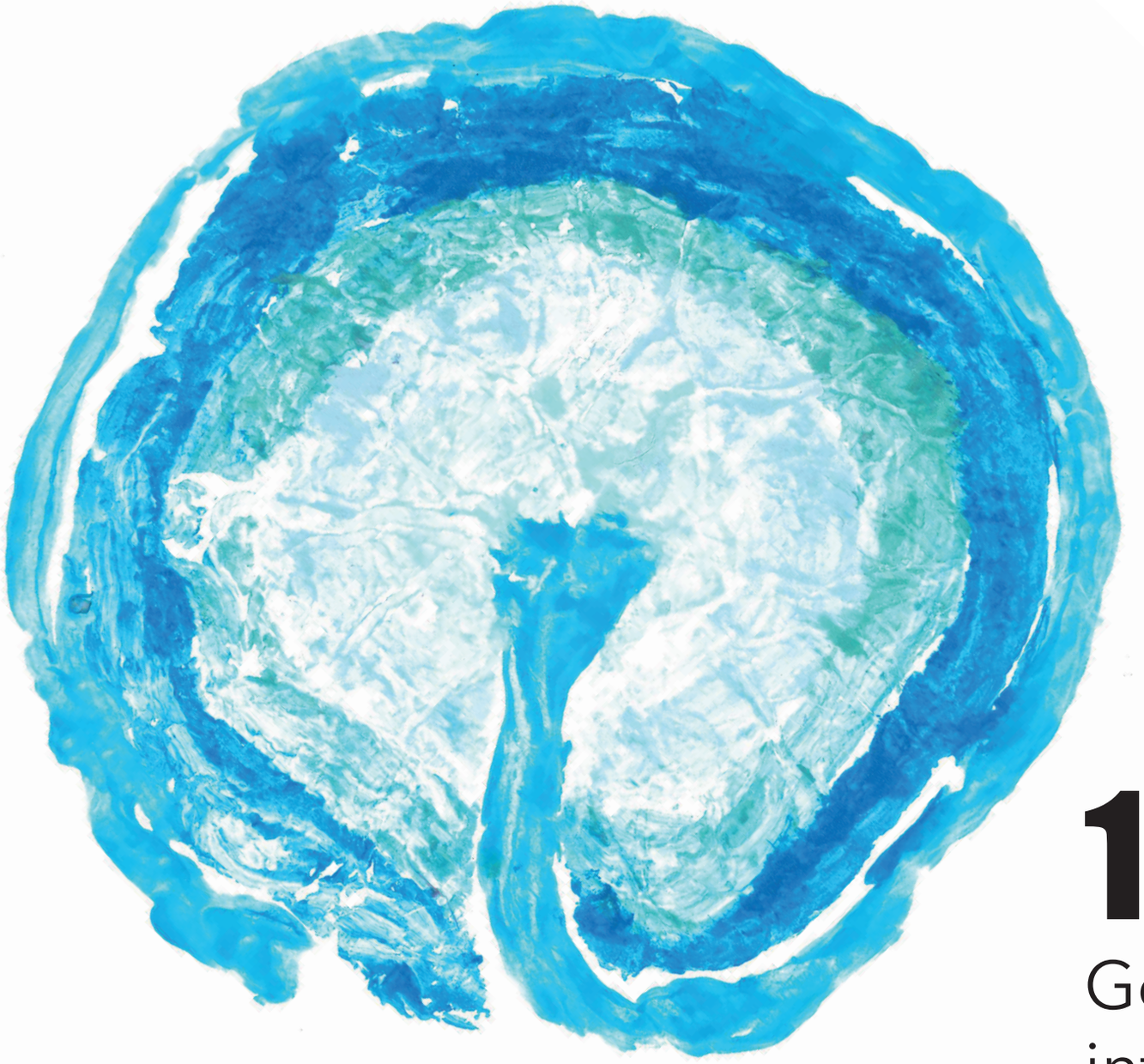

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General introduction

Basics of the immune system

The immune system is a collection of organs, tissues, cells and molecules that protects against microorganisms trying to infect the human body. Microorganisms can reproduce and evolve very rapidly during the course of an infection, and they can cause disease if not controlled by the human immune system. Defense against these microorganisms is mediated by early reactions of the innate immune system and later responses of the adaptive immune system.

When pathogens gain entry to the human body the innate immune system can react quickly, because it consists of defense mechanisms that are in place even before infection. These mechanisms are specific for structures that are common to groups of related microbes and remain essentially the same in repeated infections. When cells of the innate immune system sense the presence of pathogens or products from an injured cell, they start to secrete proteins called cytokines that interact with other cells to trigger the innate immune response. Cells that can engulf the invading microorganism or kill infected cells are brought rapidly and in large numbers into the infected tissue. This induces a state of inflammation in the infected tissue, causing symptoms like heat, pain, redness, and swelling.

Sometimes pathogens are able to withstand innate immunity and their elimination requires the more powerful and more specific mechanisms of adaptive immunity. When this happens, the innate immune response helps to slow the spread of the infection while lymphocytes become activated that vastly increase the power and focus of the immune response. Lymphocytes and their secreted products are the main components of the adaptive immune response. They express membrane receptors that have an extraordinary capacity to distinguish between different microbes and molecules. When a pathogen is recognized, lymphocytes start to proliferate and differentiate, producing large numbers of effector cells specific for that pathogen. Some of the lymphocytes that recognize the pathogen persist

in the body and provide long-term immunological memory. These memory cells respond more rapidly and vigorously upon a subsequent encounter with the same pathogen.

Cells of the immune system

The cells of the immune system consist mainly of white blood cells or leukocytes. Different types of cells with different characteristic morphological features and functions exist. The main immune cells covered in this thesis are explained here:

Innate immune system:

- **Monocytes** circulate in the blood and travel to tissues, where they mature into macrophages or dendritic cells and take up residence.
- **Macrophages** are large, irregularly shaped cells, which can capture, engulf, and kill microorganisms. Macrophages are characterized by an extensive cytoplasm with numerous vacuoles often containing engulfed material.
- **Dendritic cells (DC)** are resident in the body's tissues and have a distinctive star shaped morphology. They can act as cellular messengers mediating an adaptive immune response when it is needed. For this, they will capture microbial antigens, transport these antigens to lymphoid organs, and present the antigens to naïve T lymphocytes to initiate immune responses.
- **Natural killer (NK) cells** are the killer lymphocytes of the innate immune response. They migrate from the blood into infected tissues, where they prevent the spread of infection by killing virus-infected cells and secrete cytokines that slow the progress of viral replication in infected cells.

Adaptive immune system:

- **B lymphocytes or B cells**: small lymphocytes with cell-surface receptors called immunoglobulins. B cells can be activated to become plasma cells, which are effector cells that secrete soluble forms of immunoglobulin called antibodies that bind to pathogens.
- **T lymphocytes or T cells**: small lymphocytes that have membrane-bound receptors for the recognition of peptides derived from foreign proteins. T cells can be subdivided into CD8+ cytotoxic T cells and CD4+ helper T cells according to their effector functions. Cytotoxic T cells kill cells that produce foreign antigens, such as virus infected cells, whereas helper T cells secrete cytokines that help other cells of the immune system to become fully activated effector cells. CD4+ cells can be subdivided again into Th1, Th2, Th17 or regulatory T cells.

These immune cells can produce cytokines, which mediate and regulate aspects of the immune response. One cell can synthesize different kinds of cytokines and one cytokine can be produced by different kinds of cells. These cytokines can have multiple biologic effects, thereby stimulating or inhibiting the production of others. Therefore, the function of a cytokine can be greatly influenced by other cytokines secreted together with it. These are the main cytokines covered in this thesis:

- **IL-2** drives the growth, survival and differentiation of T cells and is involved in the maintenance of regulatory T cells. IL-2 is mainly produced by activated CD4+ T cells, but also by activated CD8+ T cells , NK cells and DC.
- **IL-12** is a pro-inflammatory cytokine that promotes the differentiation of Th1 cells. It is produced by DC, macrophages and B cells. IL-12 induces the

production of IFN-γ by NK cells and T cells, which stimulates additional antigen presenting cells (APC) to produce IL-12.

- **IFN-γ** is a major pro-inflammatory cytokine, functioning mainly as an activator of effector cells of the immune system. It is produced by CD4+ Th1 cells, CD8+ T cells and NK cells and it is an important mediator of macrophage activation and effector function, resulting in increased ingestion of microbes and the destruction of the ingested pathogens.
- **TNF-α** is a powerful inducer of inflammation. It is mainly produced by macrophages, but can also be produced by many other cell types, such as T cells, NK cells and DC. TNF-α helps recruiting immune cells to the inflammation site and promotes macrophage differentiation.
- **IL-10** is involved in controlling the immune response. It is produced by many immune cell populations, including activated macrophages and DC, B cells, regulatory T cells, and Th1 and Th2 cells. IL-10 inhibits the expression of co-stimulatory molecules and class II major histocompatibility complex (MHC) molecules on DC and macrophages, and it inhibits their production of IL-12.
- **TGF-β** inhibits proliferation and effector functions of T cells to provide regulation of cellular immunity. It is produced by various cell types, including T cells and monocytes. It can inhibit the development of Th1 and Th2 subsets and is involved in the development of regulatory T cells.

Development of lymphocytes

Lymphocytes develop and mature to the stage at which they are able to respond to a pathogen in the primary lymphoid tissues, i.e. the bone marrow and the thymus. B and T lymphocytes both arise from stem cells in the bone marrow, but

whereas B cells complete their maturation here, T cells leave the bone marrow at an immature stage to mature in the thymus. After maturation, these naïve lymphocytes migrate to the secondary lymphoid tissues, where they may respond to invading pathogens. Here, the few B and T cells expressing receptors that bind to the foreign antigen will be activated to proliferate and differentiate into effector cells and memory cells. DC are the most effective APC for activating naïve T cells and initiating T cell responses. They are specialized in the uptake and breakdown of pathogens. Resting DC capture microbial antigens and transform into mobile cells, migrating from the infected tissue to the secondary lymphoid tissue that drains the infected site. Here they present the antigens to the T cells and activate them. Following activation, effector cells then migrate to the infected tissues, where they collaborate with cells of the innate immune system to control the infection.

Generation of T cell subsets

Precursors that express both CD4 and CD8 differentiate into either CD4+ or CD8+ T cells within the thymus. CD8+ T cells can differentiate into cytotoxic T lymphocytes whose major effector function is to kill infected target cells. Naïve CD4+ T cells can be activated by antigens to differentiate into helper T cells, synthesizing cell surface molecules and soluble cytokines that activate and help other types of cells – mostly macrophages and B cells – to participate in the immune response.

The differentiation pathway that an activated naïve T cell will take is decided at an early stage of activation. T cell activation requires signals provided by molecules on APC, called co-stimulatory molecules, in addition to antigen-induced signals. Co-stimulation is called the second signal for T cell activation, because it functions together with antigen, the first signal, to stimulate T cells. The best characterized co-stimulatory pathway involves the T cell surface receptor CD28 and the co-stimulatory molecules CD80 (B7-1) and CD86 (B7-2) expressed on APC. Interaction

of these receptor-ligand pairs mainly results in T cell proliferation and the secretion of cytokines, such as IL-2. Receptors homologous to CD28 and their ligands homologous to B7 have been identified. Some of these, e.g. CTLA-4 and PD1, have inhibitory effects, whereas others provide activating signals. The balance between stimulation of activating and inhibitory receptors of the CD28 family influences the outcome of T cell activation. The third signal for T cell activation is provided by the cytokines produced by APC and other immune cells present at the site of the immune response. These cytokines make the differentiating T cell become gradually committed to one specific pathway (Figure 1). Naïve T cells activated in the presence of IL-12 and IFN-γ will become Th1 effector T cells, which induce macrophage activation and inflammation. T cells that become activated in the presence of IL-4 will differentiate into Th2 effector cells, which induce B cell differentiation and eosinophil activation by the production of IL-4 and IL-5. Naïve T cells activated in the presence of IL-23 will differentiate into a third subset of effector cells: Th17 cells. These cells secrete cytokines, such as IL-17, that lead to the recruitment of neutrophils to the site of infection. All three subsets produce cytokines that promote the development of this subset and inhibit differentiation toward other CD4+ subpopulations.

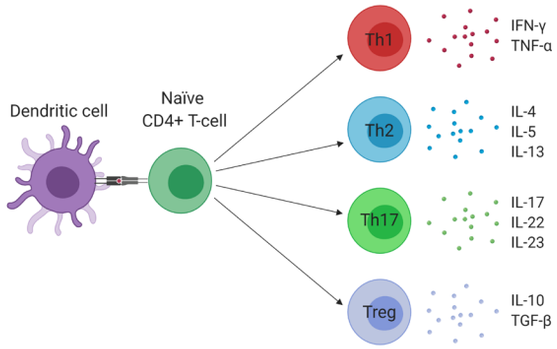

Figure 1. Naïve T cells get activated and differentiate into one of several T helper cell lineages, including Th1, T2, Th17, and regulatory T cells, as defined by the secretion of a specific set of cytokines and function, that subsequently modulates the immune response. Created with BioRender.com

Regulatory T cells are another distinct population of T cells. These can be subdivided into natural and induced regulatory T cells: natural regulatory T cells develop in the thymus and then migrate elsewhere, whereas inducible regulatory T cells are generated from naïve T cells in the periphery under various tolerogenic conditions. Regulatory T cells are involved in preventing an active immune response by restricting the function of effector T cells, immunoglobulin production by B cells, cytotoxic activity of NK cells and maturation of DC. They do so by secreting cytokines, such as IL-10 and TGF-β, and expressing molecules, such as CTLA-4, all of which are hallmark mediators of regulatory T cell suppression. In this way, they modulate the immune system and maintain immune homeostasis in the body. This active form of tolerance mediated by regulatory T cells is very important for maintaining self tolerance and protecting the integrity of the body's tissues and organs.

Major histocompatibility complex (MHC)

T cells can only recognize antigen peptides when they are bound by major histocompatibility complex (MHC) molecules. There are two types of MHC molecules, MHC class I and MHC class II. MHC class I is expressed on virtually all nucleated cells and presents antigens from intracellular pathogens to CD8+ T cells, whereas MHC class II is expressed by professional APC that present antigens from extracellular pathogens to CD4+ helper T cells.

To increase the efficiency of antigen presentation, APC express several forms of MHC class I and II molecules, each with a different peptide-binding specificity. In addition, there are many different genetic variants, or alleles, for each of these genes within the human population. Each individual expresses the alleles that are inherited as haplotypes from each of the two parents. This means that most people carry two different alleles of each MHC gene, being heterozygous. This

maximizes the number of MHC molecules available to bind pathogen-derived peptides for presentation to T cells and enables greater variety than would be possible in homozygous individuals, who carry two identical alleles of a given gene. Another word for this allelic variation is polymorphism, and MHC genes are known to be highly polymorphic genes. This variability is maintained in human populations through the need to successfully display a wide range of processed foreign peptides to the T cell antigen receptor. MHC genes that have little or no genetic variation are described as monomorphic and genes having a few alleles are described as oligomorphic.

The human MHC is called the human leukocyte antigen (HLA) complex. There are six HLA class I genes, namely the classical, highly polymorphic HLA-A, HLA-B and HLA-C, and the non-classical HLA-E, HLA-F and HLA-G, which exhibit limited polymorphism. The highly polymorphic HLA-DR, HLA-DQ, and HLA-DP genes reside within the class II region.

Allogeneic immune response

T cells should only recognize foreign peptides presented by that individual's HLA molecules. During thymic T cell development, immature T cells that recognize and bind to HLA molecules will get a survival signal. If these immature cells do not interact strongly enough they will not get the survival signal and die. This is called positive selection. Any cells having T cell receptors that bind with high affinity to HLA molecules with self peptide are eliminated, which is called negative selection. This mechanism prevents a person's T cells from attacking their own healthy tissue and triggering autoimmunity. An extraordinary situation in which foreign HLA molecules are introduced in an individual is transplantation. In the case of organ transplantation it is important that the graft is accepted by the immune system of the receiving party. Transplants of most tissues between any pair of individuals in

the absence of pharmacological immunosuppression, except identical twins, will be rejected due to HLA disparity. Alloreactive T cells in the recipient's circulation can be activated by these allogeneic HLA molecules expressed by the graft. This is called direct allorecognition and would lead to a potent T cell response that attacks the graft. Donor HLA molecules can also be captured and processed by recipient APC that enter grafts. This is called indirect allorecognition. Peptides derived from the allogeneic HLA molecules are presented in association with self MHC molecules and recognized by the host's T cells.

To reduce the probability of graft rejection, donor and recipient are matched for HLA. However, even fully HLA matched individuals undergoing transplantation can experience rejection of the graft, indicating that non-HLA immunity can also contribute substantially to transplant failure. Above all, the recipient needs to be on lifelong immunosuppressants, to prevent that the immune system will mount an immune response and reject the graft.

Immunological paradox of pregnancy

A situation in which there is natural tolerance against a foreign tissue is pregnancy. In case of a successful pregnancy, the maternal immune system does not reject the semi-allogeneic fetus, but lets it peacefully exist in the uterus. In the 1950s, Medawar was already intrigued by this phenomenon and came up with three possible explanations for this immunological paradox: (1) the fetus is physically separated from the maternal periphery, therefore the maternal immune system does not detect the fetus and will not react to it; (2) fetal antigens are not mature and therefore cannot be recognized by the maternal immune system; (3) the maternal immune system is inactive at the time of pregnancy and therefore it will not mount an immune response against the fetus [1]. In the past few decades it became clear that all three hypotheses were incorrect. In contrast to Medawar's first hypothesis,

there is direct contact between maternal blood and fetal trophoblast tissue during pregnancy, and fetal cells can persist in the maternal circulation for decades after pregnancy [2], which is called microchimerism. Furthermore, we know that the maternal immune system can recognize and react to fetal cells. The mother can develop antibodies directed against fetal HLA antigens [3], and *in vitro* tests show that maternal CD4+ and CD8+ T cells can respond to fetal cells [4, 5], ruling out Medawar's second and third hypotheses. However, all of these observations were done with peripheral blood cells of the (pregnant) woman. More locally, at the fetal-maternal interface, cells of the maternal immune system were shown to have a more tolerant phenotype [6, 7].

Mechanisms supporting acceptation of the semi-allogeneic fetus

Increasing evidence suggests that the maternal immune response towards the fetus plays a determinative role in the success of pregnancy. Several mechanisms are involved in the induction of maternal tolerance and immunologic acceptance of the semi-allogeneic fetus during pregnancy (Figure 2). Mechanisms for the evasion of the maternal immune response by the fetus include the absence of the HLA class I antigens A and B and HLA class II on fetal trophoblast cells, preventing allorecognition by T cells and B cells. The fetal trophoblasts do contain HLA-C, HLA-E, HLA-F, and HLA-G to control maternal immune responses, by modulating the activity of decidual natural killer (NK) cells, macrophages, and T cells [8-11].

HLA-G acts on multiple immune subsets by interaction with immunoglobulin-like transcript (ILT) receptors. ILT2/LILRB1 is expressed on monocytes, DC, B cells, and subsets of NK and T cells [12], whereas ILT4/LILRB2 is almost exclusively expressed by cells of the myelomonocytic lineage [13]. Through interaction with ILT receptors, HLA-G can inhibit proliferation and activation of different immune subsets [14-16], preventing a maternal immune response against paternal antigens. By alternative

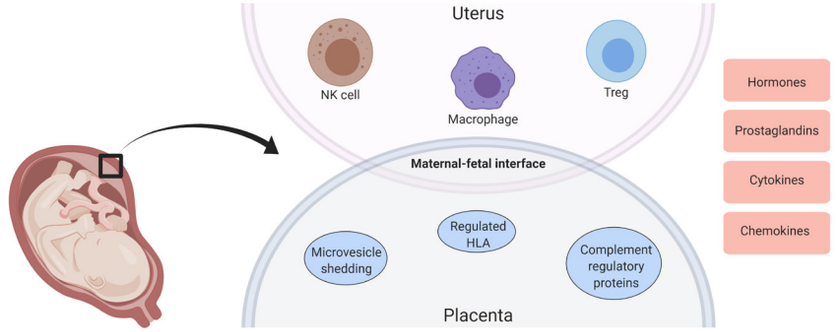

Figure 2. Several mechanisms at both maternal and fetal side are involved to prevent the maternal immune system from rejecting the fetus. Fetal cells express and produce immune regulatory molecules to prevent an attack by maternal immune cells. Maternal immune cells interact to suppress an active immune response towards fetal antigens. Created with BioRender.com

splicing, HLA-G pre-mRNA can give rise to seven different isoforms, of which four are membrane-bound (HLA-G1, -G2, -G3 and -G4) and three are soluble (HLA-G5, -G6 and -G7) [17]. Whereas in healthy tissue membrane-bound HLA-G is only expressed on trophoblasts, the soluble form of HLA-G can be detected in various body fluids, such as amniotic fluid, blood and seminal plasma [18, 19]. Several polymorphisms are present in the 3 prime untranslated region (3'UTR) of the HLA-G gene. Since the 3'UTR is targeted by miRNA that can negatively influence expression, polymorphisms in this region may have an influence on the efficiency of miRNA binding, and consequently on the level of HLA-G expression and on pregnancy outcome.

Another mechanism by which the trophoblast cells may escape attack from maternal immune cells is via the expression of apoptosis-inducing ligands, such as Fas Ligand (FasL) and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) [20, 21]. Expression of Fas is found on decidual leukocytes, suggesting that FasL expression and production by trophoblast cells may be a mechanism protecting the trophoblast against activated leukocytes [22, 23]. Also the programmed death/programmed death ligand (PD1/PDL1) coinhibitory pathway plays a role

in fetomaternal tolerance, by limiting the expansion of alloreactive T cells [24]. Furthermore, trophoblasts display high levels of complement regulatory proteins, such as decay-accelerating factor (DAF, CD55), membrane cofactor protein (MCP, CD46) and MAC-inhibitory protein (MAC-IP, membrane inhibitor of reactive lysis (MIRL), CD59) [25, 26]. These proteins are important for protecting the fetal cells from potential destruction by complement components.

In addition, soluble immunomodulators are present at the maternal-fetal interface. Trophoblasts synthesize indoleamine 2,3-dioxygenase (IDO) [27, 28], a tryptophan catabolizing immunomodulatory enzyme that prevents maternal T cell activation. Also transforming growth factor beta (TGF-β), prostaglandin E2 (PGE2), galectin-1, and IL-10 are produced by the human placenta [29-32], all of which can promote the generation of tolerogenic immune cells.

Trophoblast cells can also produce and secrete extracellular vesicles of different size, morphology, and function, which may participate in the maternal–fetal cross-talk during pregnancy [33]. These placenta-derived microparticles, such as nanovesicles and exosomes, can also enter the maternal circulation [33]. The concentration of placenta-derived exosomes increases with gestational age and they are thought to play a role in regulating the maternal immune system during pregnancy [34]. Their composition comprises of placental proteins, mRNA, and microRNAs and reflects the cell type from which the vesicle originates. Previous studies have shown that trophoblast cells secrete functional FasL and TRAIL via exosomes [35]. Also PDL1 and HLA-G can be released from the placenta via exosomes [36].

Taken together, these findings suggest there are multiple mechanisms to prevent maternal immune rejection of the semi-allogeneic fetus. It is possible that a disbalance in the immunological environment in the placenta can lead to pregnancy related problems, such as pregnancy loss.

Recurrent miscarriage

Approximately 15% of pregnant women experience spontaneous loss of a clinically recognized pregnancy. About 1–2% of couples trying to conceive are confronted with recurrent miscarriage (RM) [Unpublished data][37]. Various definitions of RM are being used. Some consider RM as two or more failed clinical pregnancies defined by ultrasonography or histopathologic examination [38]. Others define RM as three consecutive pregnancy losses within the first 24 weeks of gestation, including biochemical pregnancies and non-visualized pregnancies [38]. This discrepancy makes it hard to study underlying causes for this phenomenon and to compare the outcome of different studies.

Several factors influence the risk of miscarriage such as maternal age and previous pregnancy loss. Etiological categories for RM include chromosomal abnormalities, uterine anatomic abnormalities, and antiphospholipid syndrome. However, in a significant proportion of the couples trying to conceive the underlying cause for this recurring problem is unknown (Figure 3) [unpublished data][39]. This burden of continuous uncertainty has a major impact on the lives of these women and their partners.

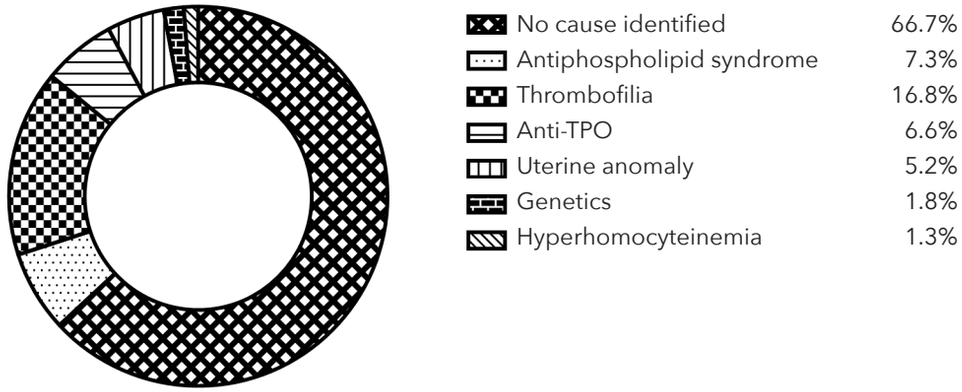

Figure 3. Several etiological factors for recurrent miscarriage have been identified. However, more than half of the couples experiencing recurrent miscarriage do not know the underlying cause.

It has been postulated that immunologic aberrations may be the cause in many of these unexplained cases of RM. Several immune factors have been investigated in women with RM. Defects in complement-inhibitory proteins, maternal regulatory T-cells, tryptophan catabolizing enzymes, and immunoregulatory cytokines at the fetomaternal interface have been implicated to play a role in RM [40-43]. In order to prepare the immune system of a woman against the 'foreign' cells of a future pregnancy, several immunologic treatments have been suggested to induce a proper immunomodulation such as transfusion of paternal leukocytes prior to conception or passive immunization with intravenous immunoglobulin (IVIG) during pregnancy. In 2014 the effect of these immunological treatments on the chance of live births in women with a history of RM was determined in a Cochrane review [44]. It was concluded that immunotherapy did not lower the risk of future miscarriage in women who repeatedly miscarry, and that these therapies should no longer be offered as a treatment.

Paternal factors

Programming of the uterine environment for successful implantation in a semi-allogeneic pregnancy may be effectuated by the presence of semen in the woman's genital tract. Semen contains not only paternal HLA antigen but also immunomodulatory factors, such as chemokines, cytokines and prostaglandins [45, 46]. The introduction of seminal plasma at intercourse elicits recruitment of macrophages, DC, and, memory T cells in the female reproductive tract [47].

Besides the classical HLA antigens, seminal plasma contains soluble HLA-G (sHLA-G) [48, 49]. Additionally, seminal plasma contains immunomodulatory factors TGF-β and PGE2. Seminal TGF-β has been shown to be a principal stimulating agent in the post-coital inflammatory response, and could be essential for induction of immune tolerance to paternal antigens [46]. Removal of seminal prostaglandins resulted in a dramatic decrease in immune suppressive activity [50].

Immune recognition of paternal antigens may play a role in pregnancy complications: change of partner is a risk factor for intrauterine growth restriction, preterm birth, low birth weight and infant mortality, and it counteracts the protective effect of multiparity against preeclampsia [51-53]. Additionally, the length of unprotected sexual cohabitation affects the incidence of pregnancy-induced hypertensive disorders [54, 55], and oral exposure to semen is correlated with a diminished occurrence of preeclampsia [45]. Furthermore, preeclampsia occurs more frequently in pregnancies induced by artificial insemination with donor semen [56]. Combined, these findings indicate that exposure to paternal antigens prior to gestation may have a beneficial effect on pregnancy outcome.

Study of reproductive immunology in mice

In mouse models it has been shown that the lack of certain immune cell subsets, e.g. Tregs specific for paternal antigens, leads to a higher incidence of failed pregnancies [57]. Furthermore, injection of interferon (IFN)-γ or IL-2 in mice results in increased abortion rates, whereas injection of IL-10 results in decreased abortion rates [58, 59]. However, murine pregnancies are very different from the human situation [60]. The most remarkable difference is a second placenta type in mice, the inverted yolk sac placenta, which is completely absent in humans. Furthermore, human trophoblast cells show deep interstitial and endovascular invasion, reaching the human myometrium (Figure 4), whereas the murine labyrinth only shows shallow trophoblast invasion.

Additionally, the time of gestation in mice is only three weeks and many of the developmental processes that occur in humans during intrauterine life are

postnatal events in mice. Direct extrapolation from animal models to humans has led to assumptions of mechanisms for which the evidence is incomplete. Therefore, in our research we only use human pregnancies to study parameters, which affect the induction of maternal tolerance towards the fetus.

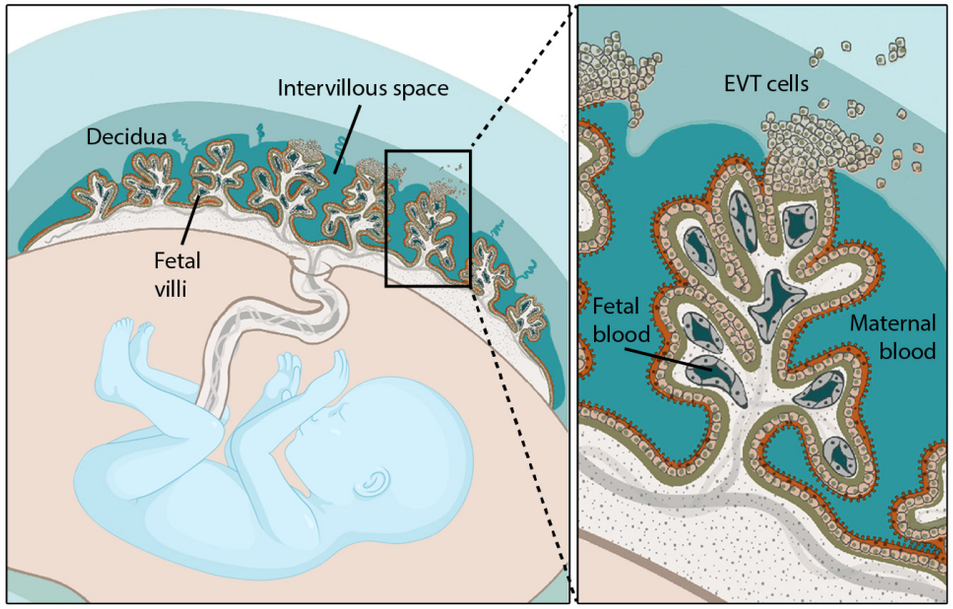

Figure 4. Immediately after implantation, cells forming the outermost layer of the blastocyst give rise to diverse trophoblast cell types. Invasive trophoblasts migrate into the maternal endometrium. Fetal villi will be generated by proliferation and invasion, and throughout pregnancy there will be villous branching and vascularization. Through these villi, nutrients and oxygen can be exchanged ensuring appropriate fetal development and growth. Adapted from V.B. Zeldovich. PLOS Pathogens. 2011.

Aims of this thesis

With the studies described in this thesis, we want to get more insight in the immunologic mechanisms that play a role in pregnancy. The results of this research can help to identify underlying etiologies in patients with unexplained pregnancy complications, such as recurrent miscarriage. Identifying these causes is important for providing answers and taking away anxiety in these couples, and eventually for the development of effective therapies. Furthermore, elucidating the mechanism

leading to survival or rejection of the fetal allograft is not only essential for our understanding of processes leading to normal and abnormal pregnancies, but may also result in important concepts in the field of transplantation and autoimmunity.

We start with a literature study to answer the question: what is wrong with regulatory T cells in recurrent miscarriage (**Chapter 2**)? Regulatory T cells play a pivotal role in controlling adaptive immune responses and maintaining self-tolerance. This unique subpopulation of T cells has shown to be involved in preventing autoimmunity, and tolerance to allogeneic organ grafts after transplantation [61, 62]. The suppressive activity of Tregs is mediated either in a cell-cell contact-mediated fashion via cytotoxic T lymphocyte-associated protein 4 (CTLA-4) or by the secretion of cytokines such as TGF-β or IL-10. Dynamic changes in circulating Treg frequencies during pregnancy have been found: a marked increase during early pregnancy, peaking during the second trimester, and a progressive decrease to levels comparable with non-pregnant conditions at term [63-66]. Because data suggest that regulatory T cells (Treg) are involved in the maternal acceptance of the allogeneic foetus, RM could possibly be explained by a disturbance of the Treg network.

In a retrospective observational study, we investigated the role of HLA-DR sharing between mother and child in pregnancy outcome. Children inherit one HLA haplotype from each parent (Figure 5), so a mother will always share one HLA haplotype with her child. Paternally-inherited fetal HLA antigens can induce maternal immune activation to secure and promote the pregnancy. Does HLA-DR incompatibility between mother and child have a positive influence on pregnancy outcome parameters (**Chapter 3**)?

Next, we study HLA-G expression in placentas of women with a history of RM and controls. We study whether HLA-G expression in term placenta is different in women with a history of RM compared to healthy controls

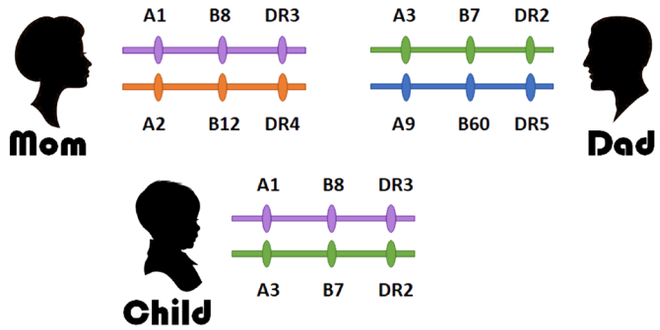

Figure 5. HLA is inherited as a set, which is known as a haplotype. A child inherits one HLA haplotype from each parent. Therefore, there is a 25% chance siblings inherit the same set of HLA.

and analyze whether this is related to HLA-G genotype (**Chapter 4**). In soluble form, HLA-G is also present body fluids. We investigate the role of the man by analyzing their HLA-G genotype and examine whether there is an association with sHLA-G levels in seminal plasma (**Chapter 5**).

To analyze the effect of seminal plasma on the phenotype and function of certain immune cell subsets *in vitro* tests with human cells are commonly used. However, it is very important to take into account under which circumstances these experiments are performed. In **Chapter 6** we study the effect of seminal plasma on human DC, which we culture in the presence of different protein sources (fetal calf serum/human serum). Previous studies suggestive for an immune modulating role of seminal plasma had been performed in the presence of FCS, which is known to affect the vitality of human immune cells in the presence of seminal fluid. We questioned whether the presence of seminal plasma indeed leads to the differentiation of anti-inflammatory DC, when these are cultured with human serum instead of fetal calf serum?

Finally, **Chapter 7** provides a summary of and general discussion of the results found in this thesis.

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