The immune compartment at the maternal-fetal interface throughout human pregnancy

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Chapter 08

Summarising discussion

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1. A dynamic network of maternal immune cells at the maternal-fetal interface

Pregnancy is unique in that despite a natural immunogenetic conflict between two individuals (mother and baby), who have a distinct HLA make-up, the outcome is predominantly successful. A network of (immune) mechanisms is at play to maintain mutual tolerance at the maternal-fetal interface. Numerous studies, in both mice and humans, have investigated fragments of this network to get a better understanding of the tolerance mechanisms and cell types involved. Our still incomplete understanding of healthy human pregnancy has hampered our comprehension of complicated pregnancies. In this thesis, several players of the maternal immune system were investigated during healthy pregnancy, with a specific focus on the functionality and specificity of decidual CD4+ and CD8+ T cells.

The presence of a heterogenous group of myeloid subsets in early pregnancy as described in **Chapter 7** suggests an important role for antigen-presentation at the beginning of pregnancy, likely resulting in priming, activation and instruction of other decidual immune cells to ensure their proper functioning throughout pregnancy. Induction of Treg by decidual macrophages is an example of one such instruction provided by antigen-presenting cells (APC) **(Chapter 2)**. Innate lymphoid cells (ILCs), primarily decidual NK cells (dNK), are the most predominant immune cell type in early pregnancy where they play a role in vascularization by remodeling of the spiral arteries, promoting migration of extravillous trophoblasts (EVT) and providing immunity to invading pathogens. dNK can interact with HLA-G and HLA-C expressed by EVT and several subsets of ILCs have been identified in the decidua (1, 2) **(Chapter 7)**. Crosstalk between dNK and decidual CD14+ myeloid cells results in the induction of Treg (3). In addition, Treg can be induced by EVT **(Chapter 2)**, decidual stromal cells (DSC), and granulocytic myeloid-derived suppressor cells (4, 5). Peripheral blood neutrophils exposed to pregnancy hormones are also capable of inducing T cells with a regulatory phenotype (6). Distinct induction mechanisms result in a diverse population of Treg. Apart from the classical CD4+CD25+FOXP3+ Treg that have been widely characterized in healthy and complicated pregnancies, we identified two additional functional Treg subtypes with distinct gene expression profiles that control T cell responses at the maternal-fetal interface. Of interest here are the $PD1^{HI}$ Treg that suppress T cell proliferation in an IL-10 dependent manner and whose induction by EVT may involve antigen-specificity for HLA-C. This Treg subtype resembles Tr1 cells with the exception of lacking co-expression of the markers CD49b and LAG-3 **(Chapter 2)** (7). The complexity within the Treg compartment however appears more extensive as is illustrated in **Chapter 7**. A population of CD4+CD25+ T cells co-expressing FOXP3, Helios, and co-inhibitory receptors, conceivably nTreg, accompanied by cell types expressing the co-inhibitory receptors CD39, ICOS, TIGIT and PD-1 but lacking FOXP3 expression,

conceivably iTreg, advocates for a mixture of (self-specific) nTreg and (fetus-specific) iTreg at the maternal-fetal interface. A decrease of nTreg and increase of iTreg throughout gestation is suggested by diminished expression of FOXP3 and Helios in term parietalis, as is shown in **Chapter 2**. An interesting Treg-like subset with high expression of CD25 and no CD127, FOXP3 and Helios expression was observed in the flow cytometry data in **Chapter 7**. Along with the other identified Treg-like subsets, this subset requires further investigation into its suppressive capacity and its presence and function in relation to complicated pregnancies and is a focus for future studies. CD4+CD25+FOXP3+ Treg were able to suppress both CD4+ and CD8+ effector T cells, whereas $PD1^{HI}$ and TIGIT+ Treg subtypes only revealed suppression of CD4+ effector T cells. The mechanisms of induction, means of suppression, and thereby cellular targets of distinct Treg subtypes are inherently different and a balance between the Treg subtypes appears necessary to maintain healthy pregnancy. An imbalance between nTreg and iTreg with a failure of iTreg induction and non-affected nTreg has been described in pre-eclampsia (PE) and may also play a role in other pregnancy complications (8).

Treg suppress among others the effector functions of CD4+ T cells that have been initiated by their interaction with APC. In **Chapter 7**, mass cytometry revealed previously unexplored heterogeneity within the effector and memory decidual CD4+ T cell (CD4+ dT) compartment. Distinct CD4+ dT populations differed not only between early and term pregnancy, but also between term basalis and parietalis. Knowledge on the pro-inflammatory (Th1 phenotype) and anti-inflammatory (Th2 phenotype) profiles of these CD4+ dT cell subsets needs to be broadened, also to relate their dynamics and functions throughout gestation to the hypothesis of a Th1-Th2 shift in pregnancy (9, 10). An important observation that requires further exploration is the increase of activated effector CD4+CD25+CD127+ T cells complemented by an increase in regulatory-like CD4+CD25+CD127- T cells towards the end of pregnancy, mostly apparent in term parietalis. Strengthening this observation is an increase in CD4+CD25bright T cells from second trimester to term decidua parietalis that has previously been shown by Tilburgs et al. (11). Interestingly, several CD4+CD25+CD127+ T cell subsets were positive for FOXP3, a marker that has not only been described for CD4+ Treg, but also as an activation-induced marker on effector CD4+ T cells in humans (12, 13). This expression of FOXP3 is transient and protects conventional T cells from restimulation-induced cell death during activation-induced proliferation (14).

We hypothesize that activated CD4+ dT may partake in the inflammatory processes towards the end of pregnancy that are associated with parturition. To ensure that these activated CD4+ dT are kept under control, CD4+ Treg also increase in numbers towards the end of pregnancy. Therefore, an imbalance between activated CD4+ dT and CD4+ Treg, especially in term pregnancy, may trigger pregnancy complications such as pre-term birth and their interactions require further investigation. Of interest are also the CD4+ NKT-like

cells that were predominantly observed in early pregnancy and distinct CD8+ NKT-like cell subsets observed in early and term pregnancy **(Chapter 7)**. Increased numbers of NKT cells were observed in women with unexplained recurrent miscarriage (15), implying that these cells may be targets of Treg for their regulation.

Another significant cell type at the maternal-fetal interface that needs to be kept under control are the decidual CD8+ T cells (CD8+ dT). On the one hand, their effector functions appear to be reduced by their expression of gene signatures associated with T cell dysfunction together with increased protein expression of co-inhibitory receptors such as PD-1, TIGIT, and CD39, and low expression of the cytolytic molecule perforin. On the other hand, CD8+ dT appear to simultaneously express gene signatures of T cell activation together with increased protein expression of stimulatory receptors such as CD69, ICOS, and HLA-DR and have the ability to upregulate perforin and granzyme B expression, secrete pro-inflammatory cytokines, proliferate, and degranulate upon stimulation. The possible role that posttranscriptional modifications mediated by miRNAs may play in this increase of cytolytic capacity upon stimulation requires further exploration. This 'strategic' mixed state that CD8+ dT appear to reside in suggests that they are capable of remaining dysfunctional towards the semi-allogeneic fetus yet can be simultaneously activated in order to respond to invading pathogens **(Chapter 3 and Chapter 7)**. Not all CD8+ dT simultaneously express co-inhibitory and stimulatory receptors and it remains to be investigated how the different CD8+ dT cell subsets are in balance with each other, whether they have distinct functions and antigen-specificity, and the role that Treg play in this dual function of CD8+ dT. For example, tissue-resident memory CD8+ dT were more frequent in early pregnancy and also showed co-expression of stimulatory and inhibitory receptors. Their function in early pregnancy may be taken over by different subsets of CD8+ dT in term pregnancy. Investigation into the proliferative and self-renewal potential of CD8+ dT, as hypothesized by the high expression of CD27 and co-expression of CD45RO and CD45RA, will be valuable in understanding the role of CD8+ dT during pregnancy **(Chapter 7)**.

Recently, the focus on the contribution of CD4+ Treg subtypes in the maintenance of self-tolerance and prevention of auto-immune disease has expanded to an additional contribution of CD8+ Treg. Suppressive CD8+CD25+FOXP3+ regulatory T cells have been identified in both mice and humans and they are involved in infectious diseases, reveal reduced levels and function in auto-immune diseases, contribute to immune response evasion against tumors, and reduce the risk of transplant rejection in solid organ transplantation and graft-versus-host disease in hematopoietic stem cell transplantation (16, 17). It remains to be determined whether functional CD8+ Treg are present in mPBMC and decidua during healthy pregnancy and whether their levels are different during complicated pregnancies.

Other cell types that are present at the maternal-fetal interface, and that have mostly been phenotypically described with little functional data, are B cells, TCRγδ T cells, NKT cells and MAIT cells. A role for anti-HLA-C antibodies, relevant for the local placental environment, has been suggested in recurrent miscarriages [18]. This observation, accompanied by the presence of several decidual CD20+ B cell subsets, suggests a (indirect) role for B cells at the maternal-fetal interface. Moreover, as contributions of TCRγδ T cells to transplantation outcomes and their role in HIV controllers (19, 20) have been described, further studies into the functionality, antigen-presentation, and interactions of these heterogenous cell types **(Chapter 7)** with other maternal immune cells and EVT during the course of pregnancy is essential.

Maternal immune cells cannot be seen separate from EVT given how intermixed they are at the three maternal-fetal interfaces. Their interaction is crucial for the maintenance of immune tolerance and inconsistency in their combined efforts can result in a disadvantageous outcome. Besides a heterogenous population of maternal immune cell subsets, several subtypes of trophoblasts (cytotrophoblasts and EVT) and patterns of differentiation have recently been identified with single-cell RNA sequencing (RNA-seq) of placental cells obtained from first, second, and third trimester (21, 22). It remains unknown whether distinct trophoblast subtypes are established by an intrinsic differentiation program of the placenta, influence of the decidual microenvironment or a combination of both (23).

2. Migration and differentiation of maternal immune cells

The relationship between two components of maternal immunity during pregnancy that are nearly impossible to study in humans are the local, decidual immune compartment and the systemic, peripheral blood immune compartment. t-SNE analyses exemplified in **Chapter 7** showed clear separation between the decidual and peripheral blood immune cell subsets, yet their functions are likely not independent from each other. CDR3 spectra typing fragment analysis of the CD4+ T cell TCRVβ repertoire revealed a more oligoclonal TCRVβ repertoire in CD4+ dT when compared to peripheral blood CD4+ T cells. This restricted CDR3 length distribution suggests local, clonal expansion of memory CD4+ dT by locally presented antigens, as has recently also been shown for decidual Treg (24). Here, a lack of clonal expansion by Treg was associated with PE. The presence of distinct subsets of myeloid cells, CD4+ T cells, CD8+ T cells, and ILCs in term basalis and parietalis also supports the concept of local expansion and differentiation of maternal immune cell subsets dependent on antigen availability and recognition in the different anatomical placental locations.

In addition to local expansion, influx of immune cells from the peripheral blood into the decidua may occur simultaneously. Before implantation, contact with seminal fluid induces immune cell recruitment into the endometrial stroma with consequences for the receptivity of the blastocyst and the decidual response (25). After implantation and

during early pregnancy, factors produced by the embryo and trophoblasts, such as progesterone and human chorionic gonadotropin (hCG), induce migration of circulating NK cells and Treg to decidual tissues (26, 27, 28). Furthermore, increasing numbers of circulating neutrophils during pregnancy (29) result in an influx of granulocytes into the placenta towards the end of pregnancy **(Chapter 7)**, where they likely play a role in parturition. The CDR3 spectratyping fragment analysis also revealed overlap of a few CD4+ T cell TCRVβ subfamilies between mPBMC and decidua, reflecting a maternal origin of these clonally expanded cells in the decidua. Despite epigenetic chemokine gene silencing that is suggested to prevent the influx of T cells into the decidua in healthy murine pregnancies (30), placental infections may reduce this barrier to allow recruitment of T cells to the maternal fetal interface (31, 32) **(Chapter 5)**. Other possibilities are the differentiation of immune cells from CD34+ hematopoietic progenitors present in the decidua or from precursor cells present in the uterine mucosa, as has been described for dNK (33, 34).

Another aspect of cell migration is microchimerism where the interaction between mother and fetus during gestation remains visible years after pregnancy. This microchimerism involves the prolonged persistence of fetal progenitor cells and cell-free DNA in maternal blood and persistence of maternal cells in offspring well into adult life (35, 36). There is controversy about whether microchimerism has advantageous, such as clearing of tumors, or disadvantageous, such as auto-immunity, outcomes. To get closer to a consensus on this matter, it will be important for future studies to determine what cell types traffic between mother and child and what their function and specificity is.

3. Placental infections

T cells get activated by non-self antigens, such as viral and bacterial antigens, presented as peptides in self HLA. The most common congenital infection is human cytomegalo-virus (HCMV). HCMV sero-positive woman were shown to have a 1.5-fold increased risk to develop PE (37). Furthermore, clinical associations have been found between bacterial infections and pre-term birth (38). We have shown that primary EVT can get infected with HCMV in vitro **(Chapter 2, 3)** (39). In addition, EVT are susceptible to infection with Zika virus (ZIKV), Listeria monocytogenes, Toxoplasma gondii, and human immunodeficiency virus (HIV) (40, 41). Viral infections can alter the dynamics of peripheral blood CD8+ T cells during pregnancy (42), yet little evidence exists for a role of CD8+ dT to clear infections. HLA-A and -B-restricted virus-specific CD8+ T cells are present at higher percentages in the decidua compared to peripheral blood (43) and the ratio of perforin and granzyme B expression is significantly different between HCMV-specific CD8+ dT and peripheral blood CD8+ T cells. The data presented in **Chapter 3** imply a general suppression of perforin protein expression in all CD8+ dT, including virus-specific CD8+ dT, that may be overcome by T cell activation during viral infections of the placenta. During placental infections, EVT can only present pathogen-derived

antigens to T cells in the context of HLA-C. The presence of decidual HLA-C-restricted pathogen-specific CD8+ T cells has yet to be determined. Given that HLA-C-restricted cytotoxic CD8+ T cell responses comprise 54% of the total CD8+ T cell response in peripheral blood during HIV and HCMV infections (44, 45), it is crucial for future studies into the development and thereby clearance of pathogen-induced placental pathology to include investigation into HLA-C-restricted pathogen-specific CD8+ T cell responses **(Chapter 5)**.

CD8+ T cells are not the only players present at the maternal-fetal interface that may be involved in the clearance of placental infections. NK cells may provide protection where their killing of a target cell depends on the balance between activating ligands on infected cells binding to activating KIR resulting in cytotoxicity and the presence of MHC molecules that provide inhibitory signals resulting in tolerance. A skewing of KIR expression by dNK towards recognition of HLA-C is suggested in pregnancy (46) **(Chapter 5)**. dNK are able to clear HCMV-infected DSC but fail to clear HCMV-infected EVT (47, 39). The expression of activating KIR2DS1 by dNK, but not pNK, enhanced the clearance of HCMV-infected HLA-C2-expressing DSC (39). dNK may utilize degranulation-independent mechanisms such as the secretion of the anti-microbial peptide granulysin to provide immunity to placental infections, yet at the same time maintain immune tolerance to invading trophoblasts (48, 49). Furthermore, HCMV-infected EVT can induce $CD25^{HI}$FOXP3+ and $PD\text{-}1^{HI}$ Treg to the same extent as non-infected EVT, suggesting that under infectious conditions EVT are capable of maintaining immune tolerance **(Chapter 2)**. The protective mechanisms utilized by EVT are also apparent under non-infectious conditions, where T cells do not degranulate **(Chapter 3)** and produce IFN-γ in response to EVT. In addition, decidual macrophages show no alterations in cytokine secretion when co-cultured with EVT (50). How EVT are able to prevent their own killing by decidual NK and T cells, even upon infection with a virus or bacteria, resulting in immune tolerance at the possible expense of clearing pathogens is work for future studies and hints may lay in the escape mechanisms used by tumor cells.

Placental infections provide a pro-inflammatory environment that may alter the stability, activation and function of Treg, dNK and CD8+ dT. While dNK have an abundance of intracellular granules containing cytolytic molecules, they fail to polarize these granules to the immune synapse with their target cells in the absence of pro-inflammatory signals. Decidual CD8+ T cells reveal suppressed expression of perforin yet are fully functional upon activation. What signals are required to activate dNK and CD8+ dT at the maternal-fetal interface in case of placental infections, the interplay between these signals, and whether a threshold is in place to regulate this conflict between fetal and maternal responses to prevent allo-reactivity are important questions that need to be addressed to understand the maternal immune response against infections during pregnancy and how this may

lead to complications. Lastly, the controversial subject of whether the placenta is a sterile organ or not should be considered when investigating placental infections (51, 52). If microbiota are indeed present in the placenta, interactions between trophoblasts and placental microbiota may contribute to tissue homeostasis and alterations in their relationship may result in a pro-inflammatory environment involving maternal immune cells, as is proposed in pre-term birth (53).

4. Fetal antigen-specificity

The fetus expresses inherited paternal antigens and is hereby considered a semi-allogeneic graft that is immunologically foreign to the mother. T cells can get activated by the presence and recognition of non-self (allogeneic) HLA molecules. To maintain immune homeostasis during pregnancy and prevent a response against the semi-allogeneic fetus, T cell-mediated immune responses need to be tightly regulated. In pregnancy research, a lot of focus has been on the immune-modulatory HLA-G molecule [54]. This thesis has shed further light on the importance of HLA-C, the only polymorphic classical HLA class I molecule expressed by EVT. HLA-C molecules are major ligands for the KIR expressed by NK cells and the interactions between dNK and fetal HLA-C and its role in pregnancy complications have been described in several studies (46, 55, 39).

The presence of differentiated effector-memory CD8+ T cells at the maternal-fetal interface **(Chapter 3)** are an indication that CD8+ dT may recognize fetal antigens, such as paternal HLA-C or minor histocompatibility antigens (mHags). CD8+ dT proliferate in response to fetal tissues and HLA-A and -B-restricted CD8+ T cells specific for the mHags HY, HA-1 and HA-2 are present in mPBMC and decidua. Furthermore, paternal HLA antigens that had induced HLA-specific antibodies in mothers gave rise to elevated cytotoxic CD8+ T cell frequencies when compared with the response to third party antigens (56, 57, 58, 59, 32). Whether the recognition of placental and fetal antigens by CD8+ T cells is via direct recognition of foreign HLA molecules or occurs via “indirect” antigen presentation where T cell engagement requires the uptake of placental/fetal antigen by maternal APC, as has been described in mice, remains to be determined (60). Thus, future studies should focus on investigating whether HLA-C-restricted CD8+ dT with specificity for paternal HLA-C exist at the maternal-fetal interface. We partly addressed this question in **Chapter 4** by determining the cross-reactive potential of virus-specific CD8+ T cells against allo-HLA-C. Cross-reactivity was observed for an HLA-B*08:01-restricted EBV-specific T cell clone against HLA-C*01:02, and HLA-C*06:02-restricted HCMV-specific T cell lines and clones displayed cross-reactivity against HLA-C*03:02.

We concluded that cross-reactivity against allo-HLA-C can occur and this potential is more common in HLA-C-restricted virus-specific T cells compared to HLA-A and -B-restricted virus-specific T cells. A crucial follow-up step is to determine whether

cross-reactivity against primary EVT expressing allo-HLA-C also occurs, or whether EVT possess protective mechanisms.
CD4+ dT also revealed fetus-specific proliferation (56) and in **Chapter 2** we observed a possible involvement of HLA-C specificity when PD1HI Treg are induced by EVT. Evidence for aberrant expression of HLA-DR in syncytiotrophoblasts in PE (61) together with possible specificity for fetal HLA-C addresses the importance of including examination into heterologous immunity in CD4+ dT against MHC class I and II in future studies. An experiment that would complement the work presented in **Chapter 2 and 3** is to perform single-cell TCR sequencing of the effector-memory CD8+ and CD4+ T cell populations, and CD4+ Treg subsets to better map T cell dynamics and clonotypes. When combined with single-cell RNA-seq this can provide valuable information on the association between transcriptional signatures and the diversity of the T cell response repertoire in order to understand the extent of clonal overlap between T cell subsets, possible TCR selection due to exposure to similar antigens and thereby T cell plasticity at the maternal-fetal interface. Insights into the possible clonal relationships between T cell subsets may provide indirect information into whether memory CD4+ dT and CD4+ Treg subsets experienced similar antigen-specific interactions.

To identify the exact antigens that are recognized, the above-mentioned experiment could be combined with a very recent described genome-wide method for the systematic discovery of T cell epitopes, named T-Scan (62). Kula et al. describe a platform where target cells express a library of lentivirally-delivered candidate antigens that are processed and presented endogenously on MHC molecules. These target cells are subsequently co-cultured with T cells of interest, and target cells that are functionally recognized by T cells are isolated using a reporter for granzyme B activity, and the antigens mediating recognition are identified by next-generation sequencing. Bulk memory CD8+ T cells isolated from the decidua could be expanded, followed by a screening of a genome-wide and/or virome-wide library of peptides in target cells only expressing HLA-C. This approach may aid in the discovery of virus-specific and self-specific T cell epitopes at the maternal-fetal interface.
EVT do not only express HLA-G and HLA-C, but also the non-polymorphic HLA-E molecule. HLA-E was first described as the ligand for CD94/NKG2A (inhibitory) and CD94/NKG2C (activating) NK cell receptors. It binds nonameric leader peptides of the signal sequences of HLA-A, -B (not relevant for trophoblast recognition), -C and -G molecules. dNK express high levels of CD94/NKG2A and their interaction with HLA-E on EVT may have essential implications on placental implantation (63). Emerging evidence reveals that HLA-E can also be recognized by the TCR of CD8+ T cells. HLA-E-restricted CD8+ T cells may have a role in infectious diseases, cancer and auto-immune diseases, and may present as an additional player in the decidua (64, 65).

5. Future prospects: systems biology

The work presented in this thesis contributes an immunological part to a whole system where the placenta is the key player during pregnancy. Important, but often forgotten, when studying decidual immune cells is that hormones, such as estrogens, progesterone and hCG are known drivers of immune changes during pregnancy. Crosstalk between the immune system and hormones contributes to the outcome of pregnancy (66). To gain further insight into the mechanisms involved in healthy pregnancy and thereby comprehension of how even minor modifications in this system may result in an aberrant outcome, I advocate for the field of reproductive immunology to move towards systems biology. Systems biology is a holistic approach that is based on the understanding that the whole is greater than the sum of its parts. Up until now, researchers in the field have put in immense efforts to take apart the placental system and study the individual parts, thereby significantly increasing our understanding of the immunological paradox of pregnancy and some of the complications that may arise from it. However, when studying a complex, dynamic system such as the placental system during pregnancy and aspiring to predict trophoblast invasion, immune and stromal cell behavior and the possible role of pathogens and a microbiome in order to development diagnostic tools and therapeutic strategies in the future, it is not sufficient to understand only the individual parts. The missing link is to put all these parts together into one system. Moving forward, the data obtained from our mass cytometry study (Chapter 7) with in addition data obtained from pre-eclampsia, recurrent miscarriage and pre-term birth samples must be integrated with data from other technologies such as single-cell RNA-seq, single-cell TCR seq, the use of peptide-MHC tetramers to determine T cell antigen-specificity for epitopes (67), algorithms to predict ligand-receptor interactions (2), and imaging mass cytometry to define the cellular anatomical locations followed by neighborhood analysis to predict patterns in adjacent cells (Fig. 1) (68, 69). Subsequently, differential findings between healthy and complicated pregnancy samples should lead the way for functional analyses in vitro, into cytokine release, proliferation, and differentiation potential of the cell types involved.

An approach where cell biology (both fetal and maternal cells), cell signaling, genomics, transcriptomics, proteomics, the influence of hormones, initial priming of cells by seminal fluid, and microbiota come together will form a platform to gain further insight into how this placental network functions, how cells interact with each and whether plasticity between cell types occurs, under healthy and pathological conditions. Although, decidual and peripheral blood immune cells cluster completely separate in t-SNE analyses **(Chapter 7)**, trafficking of cells between these two entities almost certainly occurs. In pregnancy complications both systems should be studied in parallel as the occurrence of certain cell subsets in the blood may predict what takes place locally in the decidua and may thereby as biomarkers to predict whether complications will occur.

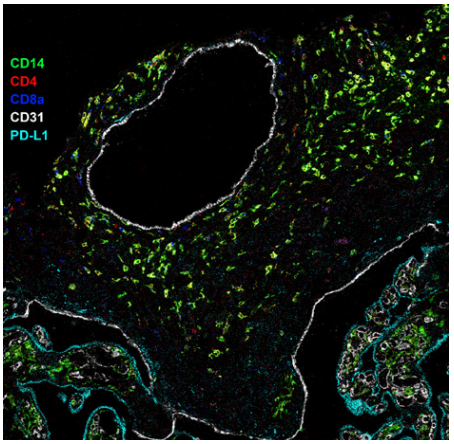

Figure 1. Imaging mass cytometry on human decidual tissue.
A third (term) trimester decidua basalis sample from a healthy pregnancy is visualized. This preliminary data reveals how spatial information on the decidual architecture can be gained with imaging mass cytometry. Colors represent the markers CD14 (lime), CD4 (red), CD8a (blue), CD31 (endothelial cells; white), and PD-L1 (cyan). *Adapted from J. Krop. 2019.*

It is challenging to study all parts of this dynamic network simultaneously without an in vivo model. Pregnancies in rodents are not representative of human pregnancies and for obvious ethical reasons it is impossible to study human pregnancy in vivo. An attempt may involve the use of trophoblast organoids and their co-culture with maternal immune cells with the addition of hormones and microbiota to understand how these entities talk to each other (70). Another way of mimicking this network is the development of a placenta-on-a-chip where fetal and maternal cells interact, and a fluidics system mimics the trafficking of cells to and from the peripheral blood (71). These techniques will also be suitable for the investigation into placental infections and how pathogens are able to cross the placental barrier.
Lastly, implementation of systems biology requires collaboration between cross-disciplinary teams such as clinicians, immunologists, computational biologists, bioinformaticians and physicists. Collaborations with other fields that share immunological commonalities with reproductive immunology, such as cancer immunology, transplantation and auto-immunity, where a balance between tolerance and rejection is also at play, will further enhance our understanding of the placental ecosystem and aid in improving the development of immune-based therapies. In cancer for example, the escape of tumor cells from the host's immune system and the 'escape' of the semi-allogeneic fetus from the maternal immune system may involve similar mechanisms (Fig. 2).

Important to consider in systems biology and the use of multiple novel single-cell technologies, is the computational analysis that accompanies it. We as researchers

increasingly rely on automatic tools to interpret 'big data', thereby creating a gap between the computational output and the raw data. This gap can be minimalized by incorporating extensive quality control measures to ensure reproducibility and reliable interpretation of the data (Chapter 6).

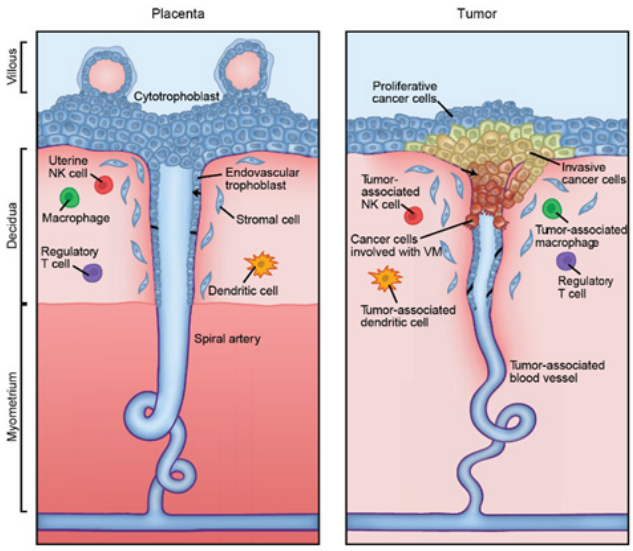

Figure 2. Similarities between the maternal-fetal interface and tumor microenvironment.
Both trophoblasts (left) and cancer cells (right) have the capacity to invade normal tissues and create a tolerant microenvironment, thereby also supporting angiogenesis. *Adapted from Holtan et al. Mayo.Clin.Proc. 2009.*

6. Conclusion

Insights into the mechanisms that control uterine immune cells, their involvement in placental development and the progression of gestation resulting in a healthy pregnancy are essential to decipher pathological conditions such as pre-eclampsia, recurrent miscarriages, and pre-term birth. At present, the only treatment for pre-eclampsia is birth of the fetus and placenta, and clinical management is focused on postponing delivery and monitoring maternal blood pressure. Women who have suffered from pre-eclampsia have an increased risk for developing kidney and cardiovascular disease later in life (72). In recurrent miscarriages, interest in the use of corticosteroids and treatment with intravenous immunoglobulin (IVIG) to reduce pro-inflammatory activity by immune cells, as well as the administration of progesterone, aspirin, and heparin has not resulted in clear beneficial effects (73, 74, 75, 76). The maternal immune system appears to play an important role in these placental disorders and potential for new treatment options lies in strategies that target the maternal immune compartment in concert with the factors that influences it, such as priming by seminal fluid, hormones and interactions with trophoblasts, DSC, and the microbiome.

In this thesis, several parameters involved in the maternal immune response during

pregnancy are discussed. T cell responses at the maternal-fetal interface are controlled by at least three subtypes of CD4+ Treg, though additional subtypes likely play a role. EVT and decidual macrophages directly increase Treg populations and antigen-specificity for HLA-C may be involved. Decidual CD8+ T cells seem to not only be controlled by Treg but exhibit a mixed transcriptional profile of activation and dysfunction enabling them to provide both tolerance and immunity. Regulation of the cytotoxicity of CD8+ dT is particularly important for virus-specific CD8+ T cells as they have the capacity to cross-react against allo-HLA-C and thereby may have detrimental consequences for pregnancy outcome. Even though EVT maintain protective mechanisms against an attack from maternal immune cells, the pro-inflammatory milieu created by placental infections or any other factors directing an imbalance between CD4+ Treg subtypes, their induction and/or their numbers (77, 78), a reduction in the number and/or an imbalance in the activation state of CD8+ dT and CD4+ dT (79, 80), and the expansion and/or recruitment of virus-specific T cells (shown in mice) (81) that could lead to cross-reactivity, may ultimately affect maternal tolerance resulting in pregnancy complications. Nonetheless, cell clusters from both innate and adaptive immune cell lineages are correlated to each other in gestational-specific networks **(Chapter 7)** and in this integrated placental system is seems unlikely that pregnancy complications are caused by the aberrant function of one cell subset, but by an imbalance between several cell subsets. Future challenges therefore need to unravel which parameters derail this unique dynamic placental microenvironment in order to identify diagnostic markers that will aid in the development of novel therapies to treat pregnancy complications (Fig. 3).

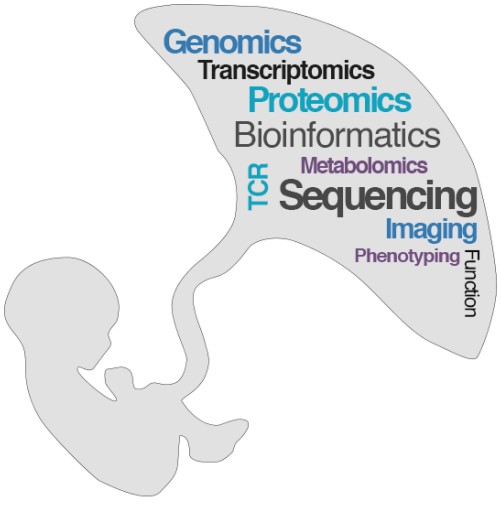

Figure 3. Systems biology in pregnancy.
Multiomics modeling, single-cell sequencing, imaging, and functional characterization of the placental micro-environment during healthy and complicated human pregnancy.

References

1. A. Moffett and F. Colucci. Uterine NK cells: Active regulators at the maternal-fetal interface. The American Society for Clinical Investigation 124:1872-1879, 2014.

2. R. Vento-Tormo, M. Efremova, R. A. Botting, M. Y. Turco, M. Vento-Tormo, K. B. Meyer, J. E. Park, E. Stephenson, K. Polanski, A. Goncalves, L. Gardner, S. Holmqvist, J. Henriksson, A. Zou, A. M. Sharkey, B. Millar, B. Innes, L. Wood, A. Wilbrey-Clark, R. P. Payne, M. A. Ivarsson, S. Lisgo, A. Filby, D. H. Rowitch, J. N. Bulmer, G. J. Wright, M. J. Stubbington, M. Haniffa, A. Moffett and S. A. Teichmann. Single-cell reconstruction of the early maternal–fetal interface in humans. Nature 563:347-353, 2018.

3. P. Vacca, C. Cantoni, M. Vitale, C. Prato, F. Canegallo, D. Fenoglio, N. Ragni, L. Moretta and M. C. Mingari. Cross-talk between decidual NK and CD14+ myelomonocytic cells results in induction of Tregs and immunosuppression. Proceedings of the National Academy of Sciences 107:11918-11923, 2010.

4. T. Erkers, S. Nava, J. Yosef, O. Ringdén and H. Kaipe. Decidual stromal cells promote regulatory T cells and suppress alloreactivity in a cell contact-dependent manner. Stem Cells and Development 22:2596-2605, 2013.

5. X. Kang, X. Zhang, Z. Liu, H. Xu, T. Wang, L. He and A. Zhao. Granulocytic myeloid-derived suppressor cells maintain feto-maternal tolerance by inducing Foxp3 expression in CD4+CD25 - T cells by activation of the TGF-β/β catenin pathway. Molecular Human Reproduction 22:499-511, 2016.

6. S. Nadkarni, J. Smith, A. N. Sferruzzi-Perri, A. Ledwozyw, M. Kishore, R. Haas, C. Mauro, D. J. Williams, S. H. P. Farsky, F. M. Marelli-Berg and M. Perretti. Neutrophils induce proangiogenic T cells with a regulatory phenotype in pregnancy. Proceedings of the National Academy of Sciences 113:E8415-E8424, 2016.

7. N. Gagliani, C. F. Magnani, S. Huber, M. E. Gianolini, M. Pala, P. Licona-Limon, B. Guo, D. R. Herbert, A. Bulfone, F. Trentini, C. Di Serio, R. Bacchetta, M. Andreani, L. Brockmann, S. Gregori, R. A. Flavell and M. G. Roncarolo. Coexpression of CD49b and LAG-3 identifies human and mouse T regulatory type 1 cells. Nature Medicine 19:739-746, 2013.

8. P. Hsu, B. Santner-Nanan, J. E. Dahlstrom, M. Fadia, A. Chandra, M. Peek and R. Nanan. Altered decidual DC-SIGN+ antigen-presenting cells and impaired regulatory T-cell induction in preeclampsia. American Journal of Pathology 181:2149-2160, 2012.

9. M.-P. Piccinni, L. Beloni, C. Livi, E. Maggi, G. Scarselli and S. Romagnani. Defective production of both leukemia inhibitory factor and type 2 T-helper cytokines by decidual T cells in unexplained recurrent abortions. Nature Medicine 4:1020-1024, 1998.

10. S. Saito, N. Tsukaguchi, T. Hasegawa, T. Michimata, H. Tsuda and N. Narita. Distribution of Th1, Th2, and Th0 and the Th1/Th2 cell ratios in human peripheral and endometrial T cells. American journal of reproductive immunology 42:240-245, 1999.

11. T. Tilburgs, D. L. Roelen, B. J. Van Der Mast, J. J. Van Schip, C. Kleijburg, G. M. de Groot-Swings, H. H. H. Kanhai, F. H. J. Claas and S. A. Scherjon. Differential distribution of CD4+ CD25bright and CD8+ CD28- T-cells in decidua and maternal blood during human pregnancy. Placenta 27:47-53, 2006.

12. S. E. Allan, S. Q. Crome, N. K. Crellin, L. Passerini, T. S. Steiner, R. Bacchetta, M. G. Roncarolo and M. K. Levings. Activation-induced FOXP3 in human T effector cells does not suppress proliferation or cytokine production. International Immunology 19:345-354, 2007.

13. M. Kmieciak, M. Gowda, L. Graham, K. Godder, H. D. Bear, F. M. Marincola and M. H. Manjili. Human T cells express CD25 and Foxp3 upon activation and exhibit effector/memory phenotypes without any regulatory/suppressor function. Journal of Translational Medicine 7:89, 2009.

14. K. Voss and A. L. Snow. Transient FOXP3 upregulation protects human conventional T cells from restimulation-induced cell death during activation-induced proliferation. The Journal of Immunology 198, 2017.

15. J. Yuan, J. Li, S. Y. Huang and X. Sun. Characterization of the subsets of human NKT-like cells and the expression of Th1/Th2 cytokines in patients with unexplained recurrent spontaneous abortion. Journal of Reproductive Immunology 110:81-8, 2015.

16. G. Churlaud, F. Pitoiset, F. Jebbawi, R. Lorenzon, B. Bellier, M. Rosenzwajg and D. Klatzmann. Human and mouse CD8+CD25+FOXP3+ regulatory T cells at steady state and during interleukin-2 therapy. Frontiers in Immunology 6:171, 2015.

17. M. R. Vieyra-Lobato, J. Vela-Ojeda, L. Montiel-Cervantes, R. López-Santiago and M. C. Moreno-Lafont. Description of CD8+ regulatory T lymphocytes and their specific intervention in Graft-versus-Host and infectious diseases, autoimmunity, and cancer. Journal of Immunology Research 3758713, 2018.

18. T. Meuleman, E. van Beelen, R. J. Kaaja, J. M. van Lith, F. H. Claas and K. W. Bloemenkamp. HLA-C antibodies in women with recurrent miscarriage suggests that antibody mediated rejection is one of the mechanisms leading to recurrent miscarriage. Journal of Reproductive Immunology 116:28–34, 2016.

19. O. McCallion, J. Hester and F. Issa. Deciphering the Contribution of γδT Cells to Outcomes in Transplantation. Transplantation 102:1983-1993, 2018.

20. M. F. Chevalier, N. Bhatnagar, C. Didier, M. Lopez-Gonzalez, J. Pavie, D. Bollens, C. Duvivier, L. Collias, C. Jung, D. Scott-Algara, P.-M. Girard and L. Weiss. γδ T-cell subsets in HIV controllers: potential role of Tγδ17 cells in the regulation of chronic immune activation. AIDS 33:1283-1292, 2019.

21. Y. Liu, X. Fan, R. Wang, X. Lu, Y. L. Dang, H. Wang, H. Y. Lin, C. Zhu, H. Ge, J. C. Cross and H. Wang. Single-cell RNA-seq reveals the diversity of trophoblast subtypes and patterns of differentiation in the human placenta. Cell Research 28:819–832, 2018.

22. J. C. H. Tsang, J. S. L. Vong, L. Ji, L. C. Y. Poon, P. Jiang, K. O. Lui, Y.-B. Ni, K. F. To, Y. K. Y. Cheng, R. W. K. Chiu and Y. M. D. Lo. Integrative single-cell and cell-free plasma RNA transcriptomics elucidates placental cellular dynamics. Proceedings of the National Academy of Sciences 114:E7786-E7795, 2017.

23. J. Pollheimer, S. Vondra, J. Baltayeva, A. G. Beristain and M. Knöfler. Regulation of placental extravillous trophoblasts by the maternal uterine environment. Frontiers in Immunology 9:2597, 2018.

24. S. Tsuda, X. Zhang, H. Hamana, T. Shima, A. Ushijima, K. Tsuda, A. Muraguchi, H. Kishi and S. Saito. Clonally expanded decidual effector regulatory T cells increase in late gestation of normal pregnancy, but not in preeclampsia, in humans. Frontiers in Immunology 9:1934, 2018.

25. A. Schumacher, D. J. Sharkey, S. A. Robertson and A. C. Zenclussen. Immune cells at the fetomaternal interface: How the microenvironment modulates immune cells to foster fetal development. The Journal of Immunology 201:325-334, 2018.

26. C. Carlino, H. Stabile, S. Morrone, R. Bulla, A. Soriani, C. Agostinis, F. Bossi, C. Mocci, F. Sarazani, F. Tedesco and others. Recruitment of circulating NK cells through decidual tissues: a possible mechanism controlling NK cell accumulation in the uterus during early pregnancy. Blood 111:3108-3115, 2008.

27. A. Schumacher, N. Brachwitz, S. Sohr, K. Engeland, S. Langwisch, M. Dolaptchieva, T. Alexander, A. Taran, S. F. Malfertheiner, S.-D. Costa et al. Human chorionic gonadotropin attracts regulatory T cells into the fetal-maternal interface during early human pregnancy. The Journal of Immunology 182:5488-5497, 2009.

28. T. Tilburgs, D. L. Roelen, B. J. van der Mast, G. M. de Groot-Swings, C. Kleijburg, S. A. Scherjon and F. H. Claas. Evidence for a selective migration of fetus-specific CD4+CD25bright regulatory T cells from the peripheral blood to the decidua in human pregnancy. The Journal of Immunology 180:5737-5745, 2008.

29. N. Aghaeepour, E. A. Ganio, D. Mcilwain, A. S. Tsai, M. Tingle, S. V. Gassen, D. K. Gaudilliere, Q. Baca, L. Mcneil, R. Okada, M. S. Ghaemi, D. Furman, R. J. Wong, V. D. Winn, M. L. Druzin, Y. Y. El-Sayed, C. Quaintance, R. Gibbs, G. L. Darmstadt, G. M. Shaw, D. K. Stevenson, R. Tibshirani, G. P. Nolan, D. B. Lewis, M. S. Angst and B. Gaudilliere. An immune clock of human pregnancy. Science Immunology 2:2946, 2017.

30. P. Nancy, E. Tagliani, C. S. Tay, P. Asp, D. E. Levy and A. Erlebacher.Chemokine gene silencing in decidual stromal cells limits T cell access to the maternal-fetal interface. Science 336:1317-1321, 2012.

31. D. R. Clark, V. Chaturvedi, J. M. Kinder, T. T. Jiang, L. Xin, J. M. Ertelt and S. S. Way. Perinatal Listeria monocytogenes susceptibility despite preconceptual priming and maintenance of pathogen-specific CD8+ T cells during pregnancy. Cellular and Molecular Immunology 11:595-605, 2014.

32. T. Tilburgs and J. L. Strominger. CD8+ effector T cells at the fetal–maternal interface, balancing fetal tolerance and antiviral immunity. American journal of reproductive immunology 69:395-407, 2013.

33. P. Vacca, C. Vitale, E. Montaldo, R. Conte, C. Cantoni, E. Fulcheri, V. Darretta, L. Moretta and M. C. Mingari. CD34+ hematopoietic precursors are present in human decidua and differentiate into natural killer cells upon interaction with stromal cells. Proceedings of the National Academy of Sciences 108:2402-2407, 2011.

34. V. Male, T. Hughes, S. McClory, F. Colucci, M. A. Caligiuri and A. Moffett. Immature NK cells, capable of producing IL-22, are present in human uterine mucosa. The Journal of Immunology 185:3913-3918, 2010.

35. D. W. Bianchi, G. K. Zickwolf, G. J. Weil, S. Sylvester and M. A. DeMaria. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. Proceedings of the National Academy of Sciences. 93:705-708, 1996.

36. S. Maloney, A. Smith, D. Furst, D. Myerson, K. Rupert, P. Evans and J. Nelson. Microchimerism of maternal origin persists into adult life. J Clin Invest 104:41-47, 1999.

37. F. Xie, Y. Hu, L. A. Magee, D. M. Money, D. M. Patrick, M. Krajden, E. Thomas and P. Von Dadelszen. An association between cytomegalovirus infection and pre-eclampsia: a case–control study and data synthesis. Acta obstetricia et gynecologica Scandinavica 89:1162-1167, 2010.

38. R. L. Goldenberg, J. C. Hauth and W. W. Andrews. Intrauterine infection and preterm delivery. New England journal of medicine 342:1500-1507, 2000.

39. Â. C. Crespo, J. L. Strominger and T. Tilburgs. Expression of KIR2DS1 by decidual natural killer cells increases their ability to control placental HCMV infection. Proceedings of the National Academy of Sciences 113:15072-15077, 2016.

40. N. Arora, Y. Sadovsky, T. S. Dermody and C. B. Coyne. Microbial vertical transmission during human pregnancy. Cell host & microbe 21:561-567, 2017.

41. M. León-Juárez, M. Martinez–Castillo, L. D. González-Garcia, A. C. Helguera-Repetto, V. Zaga-Clavellina, J. Garcia-Cordero, A. Flores-Pliego, A. Herrera-Salazar, E. R. Vázquez-Martinez and E. Reyes-Muñoz. Cellular and molecular mechanisms of viral infection in the human placenta. Pathogens and disease 75:ftx093, 2017.

42. D. Lissauer, M. Choudhary, A. Pachnio, O. Goodyear, P. A. H. Moss and M. D. Kilby. Cytomegalovirus seropositivity dramatically alters the maternal CD8+ T cell repertoire and leads to the accumulation of highly differentiated memory cells during human pregnancy. Human Reproduction 26:3355-3365, 2011.

43. A. van Egmond, C. van der Keur, G. Swings, S. Scherjon and F. Claas. The possible role of virus-specific CD8+ memory T cells in decidual tissue. Journal of Reproductive Immunology 113:1-8, 2016.

44. S. Ameres, J. Mautner, F. Schlott, M. Neuenhahn, D. H. Busch, B. Plachter and A. Moosmann. Presentation of an immunodominant immediate-early CD8+ T cell epitope resists human cytomegalovirus immunoevasion. PLoS pathogens 9:e1003383, 2013.

45. A. T. Makadzange, G. Gillespie, T. Dong, P. Kiama, J. Bwayo, J. Kimani, F. Plummer, P. Easterbrook and S. L. Rowland-Jones. Characterization of an HLA-C-restricted CTL response in chronic HIV infection. European journal of immunology 40:1036-1041, 2010.

46. A. M. Sharkey, L. Gardner, S. Hiby, L. Farrell, R. Apps, L. Masters, J. Goodridge, L. Lathbury, C. A. Stewart, S. Verma and A. Moffett. Killer Ig-like receptor expression in uterine NK cells is biased toward recognition of HLA-C and alters with gestational age. The Journal of Immunology 181:39-46, 2008.

47. J. Siewiera, H. El Costa, J. Tabiasco, A. Berrebi, G. Cartron, P. Bouteiller and N. Jabrane-Ferrat. Human cytomegalovirus infection elicits new decidual natural killer cell effector functions. PLoS pathogens. 9:e1003257, 2013.

48. D. Veljkovic Vujaklija, S. Sucic, T. Gulic, M. Dominovic and D. Rukavina. Cell death mechanisms at the maternal-fetal interface: insights into the role of granulysin. Clinical and Developmental Immunology 2012:180272.

49. H. Barman, M. Walch, S. Latinovic-Golic, C. Dumrese, M. Dolder, P. Groscurth and U. Ziegler. Cholesterol in negatively charged lipid bilayers modulates the effect of the antimicrobial protein granulysin. The Journal of membrane biology 212:29-39, 2006.

50. T. Tilburgs, Â. C. Crespo, A. van der Zwan, B. Rybalov, T. Raj, B. Stranger, L. Gardner, A. Moffett and J. L. Strominger. Human HLA-G+ extravillous trophoblasts: Immune-activating cells that interact with decidual leukocytes. Proceedings of the National Academy of Sciences 112:7219-7224, 2015.

51. K. Aagaard, J. Ma, K. M. Antony, R. Ganu, J. Petrosino and J. Versalovic. The placenta harbors a unique microbiome. Science translational medicine 6:237ra65, 2014.

52. M. C. de Goffau, S. Lager, U. Sovio, F. Gaccioli, E. Cook, S. J. Peacock, J. Parkhill, D. S. Charnock-Jones and G. C. S. Smith. Human placenta has no microbiome but can contain potential pathogens. Nature 572:329-334, 2019.

53. G. Mor and J.-Y. Kwon. Trophoblast-microbiome interaction: a new paradigm on immune regulation. American journal of obstetrics and gynecology 213:131–137, 2015.

54. L. Pazmany, O. Mandelboim, M. Vales-Gomez, D. M. Davis, H. T. Reyburn and J. L. Strominger. Protection from natural killer cell-mediated lysis by HLA-G expression on target cells. Science 274:792-795, 1996.

55. F. Colucci. The role of KIR and HLA interactions in pregnancy complications. Immunogenetics 69:557-565, 2017.

56. R. M. Powell, D. Lissauer, J. Tamblyn, A. Beggs, P. Cox, P. Moss and M. D. Kilby. Decidual T cells exhibit a highly differentiated phenotype and demonstrate potential fetal specificity and a strong transcriptional response to IFN. The Journal of Immunology 199:3406-3417, 2017.

57. R. M. Verdijk, A. Kloosterman, J. Pool, M. Van De Keur, A. M. I. H. Naipal, A. G. S. Van Halteren, A. Brand, T. Mutis and E. Goulmy. Pregnancy induces minor histocompatibility antigen–specific cytotoxic T cells: implications for stem cell transplantation and immunotherapy. Blood 103:1961-1964, 2004.

58. D. Lissauer, K. Piper, O. Goodyear, M. D. Kilby and P. A. H. Moss. Fetal-specific CD8+ cytotoxic T cell responses develop during normal human pregnancy and exhibit broad functional capacity. The Journal of Immunology 189:1072-1080, 2012.

59. G. J. Bouma, P. Van Caubergh, S. P. M. J. van Bree, R. M. C. Castelli-Visser, M. D. Witvliet, E. M. W. van der Meer-Prins, J. J. van Rood and F. H. J. Claas. Pregnancy can induce priming of cytotoxic T lymphocytes specific for paternal HLA antigens that is associated with antibody formation. Transplantation 62:672-678, 1996.

60. A. Erlebacher, D. Vencato, K. A. Price, D. Zhang and L. H. Glimcher. Constraints in antigen presentation severely restrict T cell recognition of the allogeneic fetus. The Journal of clinical investigation 117:1399-1411, 2007.

61. C. Tersigni, C. W. Redman, R. Dragovic, D. Tannetta, G. Scambia, N. Di Simone, I. Sargent and M. Vatish. HLA-DR is aberrantly expressed at feto-maternal interface in pre-eclampsia. Journal of reproductive immunology 129:48-52, 2018.

62. T. Kula, M. H. Dezfulian, C. I. Wang, N. S. Abdelfattah, Z. C. Hartman, K. W. Wucherpfennig, H. K. Lyerly and S. J. Elledge. T-Scan: A Genome-wide Method for the Systematic Discovery of T Cell Epitopes. Cell 178:1016-1028, 2019.

63. A. King, D. S. J. Allan, M. Bowen, S. J. Powis, S. Joseph, S. Verma, S. E. Hiby, A. J. McMichael, Y. Wai Loke and V. M. Braud. HLA-E is expressed on trophoblast and interacts with CD94/NKG2 receptors on decidual NK cells. European journal of immunology 30:1623-1631, 2000.

64. G. Pietra, C. Romagnani, C. Manzini, L. Moretta and M. C. Mingari. The emerging role of HLA-E-restricted CD8+ T lymphocytes in the adaptive immune response to pathogens and tumors. Journal of Biomedicine and Biotechnology, 2010:907092.

65. S. A. Joosten, L. C. Sullivan and T. H. M. Ottenhoff. Characteristics of HLA-E restricted T-cell responses and their role in infectious diseases. Journal of immunology research, 2016:2695396.

66. D. P. Robinson and S. L. Klein. Pregnancy and pregnancy-associated hormones alter immune responses and disease pathogenesis. Hormones and behavior 62:263-271, 2012.

67. E. W. Newell, N. Sigal, N. Nair, B. A. Kidd, H. B. Greenberg and M. M. Davis. Combinatorial tetramer staining and mass cytometry analysis facilitate T-cell epitope mapping and characterization. Nature biotechnology 31:623-629, 2013.

68. C. Giesen, H. A. O. Wang, D. Schapiro, N. Zivanovic, A. Jacobs, B. Hattendorf, P. J. Schüffler, D. Grolimund, J. M. Buhmann, S. Brandt et al. Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry. Nature methods 11:417-422, 2014.

69. A. Somarakis, V. Van Unen, F. Koning, B. P. F. Lelieveldt and T. Höllt. ImaCytE: Visual Exploration of Cellular Microenvironments for Imaging Mass Cytometry Data. IEEE transactions on visualization and computer graphics, 2019:2931299.

70. M. Y. Turco, L. Gardner, R. G. Kay, R. S. Hamilton, M. Prater, M. S. Hollinshead, A. McWhinnie, L. Esposito, R. Fernando, H. Skelton, F. Reimann, F. M. Gribble, A. Sharkey, S. G. Marsh, S. O'rahilly, M. Hemberger, G. J. Burton and A. Moffett. Trophoblast organoids as a model for maternal–fetal interactions during human placentation. Nature 564:263-281, 2018.

71. J. S. Lee, R. Romero, Y. M. Han, H. C. Kim, C. J. Kim, J. S. Hong and D. Huh. Placenta-on-A-chip: A novel platform to study the biology of the human placenta. Journal of Maternal-Fetal and Neonatal Medicine 29:1046-1054, 2016.

72. L. Dai, Y. Chen, W. Sun and S. Liu. Association between hypertensive disorders during pregnancy and the subsequent risk of end-stage renal disease: a population-based follow-up study. Journal of Obstetrics and Gynaecology Canada 40:1129-1138, 2018.

73. S. A. Robertson, M. Jin, D. Yu, L. M. Moldenhauer, M. J. Davies, M. L. Hull and R. J. Norman. Corticosteroid therapy in assisted reproduction–immune suppression is a faulty premise. Human reproduction 31:2164-2173, 2016.

74. L. F. Wong, T. F. Porter and J. R. Scott. Immunotherapy for recurrent miscarriage. Cochrane Database of Systematic Reviews, 2014.

75. A. Coomarasamy, H. Williams, E. Truchanowicz, P. T. Seed, R. Small, S. Quenby, P. Gupta, F. Dawood, Y. E. Koot, R. B. Atik et al. PROMISE: first-trimester progesterone therapy in women with a history of unexplained recurrent miscarriages-a randomised, double-blind, placebo-controlled, international multicentre trial and economic evaluation. Health technology assessment (Winchester, England) 20:1-92, 2016.

76. P. G. de Jong, M. Coppens and S. Middeldorp. Duration of anticoagulant therapy for venous thromboembolism: Balancing benefits and harms on the long term. Br J Haematol 158:433-444, 2012.

77. A. Steinborn, G. M. Haensch, K. Mahnke, E. Schmitt, A. Toermer, S. Meuer and C. Sohn. Distinct subsets of regulatory T cells during pregnancy: Is the imbalance of these subsets involved in the pathogenesis of preeclampsia? Clinical Immunology 129:401-412, 2008.

78. K. Inada, T. Shima, A. Nakashima, K. Aoki, M. Ito and S. Saito. Characterization of regulatory T cells in decidua of miscarriage cases with abnormal or normal fetal chromosomal content. Journal of Reproductive Immunology 97:104-111, 2013.

79. L. Rieger, S. Segerer, T. Bernar, M. Kapp, M. Majic, A. K. Morr, J. Dietl and U. Kämmerer. Specific subsets of immune cells in human decidua differ between normal pregnancy and preeclampsia - a prospective observational study. Reproductive Biology and Endocrinology 7:132, 2009.

80. M. Arenas-Hernandez, R. Romero, Y. Xu, B. Panaitescu, V. Garcia-Flores, D. Miller, H. Ahn, B. Done, S. S. Hassan, C.-D. Hsu, A. L. Tarca, C. Sanchez-Torres and N. Gomez-Lopez. Effector and activated T cells induce preterm labor and birth that is prevented by treatment with progesterone. The Journal of Immunology 202:2585-2608, 2019.

81. C. M. Constantin, D. Masopust, T. Gourley, J. Grayson, O. L. Strickland, R. Ahmed and E. A. Bonney. Normal establishment of virus-specific memory CD8 T cell pool following primary infection during pregnancy. The Journal of Immunology 179:4383-4389, 2007.