8. General Conclusions and Discussion
In order to identify the possible immunologic mechanisms contributing to aberrant pregnancy it is essential to clarify first the mechanisms of fetus specific immune recognition and immune regulation at the fetal-maternal interface during normal pregnancy. To accomplish this, one has to develop and validate methods to assess functional characteristics of isolated leukocyte subsets from the fetal-maternal interface (chapter 7). Besides the analysis of decidual leukocytes, also fetal-maternal HLA differences, clinical pregnancy data, fetal characteristics and maternal genotyping are factors that are crucial for decidual leukocyte activation and induction of regulatory cell subsets.

In this thesis, we demonstrate that CD4+CD25bright T cells which are concentrated in decidual tissue have the capacity to down regulate fetus specific and 3rd party (non-specific) responses. In contrast, CD4+CD25bright T cells in maternal peripheral blood can regulate 3rd party (non-specific) responses, comparable to non-pregnant controls, while the capacity to regulate the fetus specific response is absent. These data suggest a preferential recruitment of fetus specific regulatory T cells from the peripheral blood to the fetal maternal interface (chapter 2 and 3) (1,2). Analysis of the CD8+ T cell pool during pregnancy shows that decidual CD8+ T cells mainly consist of differentiated Effector-Memory T cells, while unprimed Naive cells are almost absent. Decidual Effector-Memory CD8+ T cells contain significantly reduced levels of the cytolytic molecule perforin. These data are suggestive for an alternative CD8+ T cell differentiation and regulation process that may play a crucial role in maintenance of maternal immune tolerance to the fetus (chapter 5). Database analysis of clinical pregnancy data, fetal-maternal HLA mismatches and decidual lymphocyte responses led to the conclusion that a fetal-maternal HLA-C mismatch is crucial for decidual CD4+ T cell activation and required for induction of functional CD4+CD25bright regulatory T cells in decidua. Hereby we provide the first evidence that decidual T cells specifically recognize a fetal HLA-C mismatch at the fetal-maternal interface, possibly using the indirect allorecognition pathway. However HLA-C recognition does not induce a destructive immune response in uncomplicated pregnancies (chapter 4). Besides TCR mediated allorecognition, low frequencies of decidual T cells express NK receptors that can specifically recognize HLA-C allotypes. Engagement of NK receptors on T cells can result in down regulation of TCR mediated T cell activation. Although, no experimental evidence is present so far, NK receptor expression on decidual T cells may provide an alternative way for decidual T cells to recognize allogeneic fetal cells and modulate the decidual immune response (chapter 6). In conclusion, this thesis shows that decidual T cells comprise a very heterogenic subset of T cells that include activated CD4+ and Effector-Memory type CD8+ T cells. However, these highly activated T cells are found together with T cell subsets that are capable to suppress the decidual lymphocyte response. Furthermore, we show that decidual T cells can specifically recognize a fetal-maternal HLA-C mismatch. Hereby we demonstrate that mechanisms of fetus specific allorecognition and T cell regulation are present at the fetal-maternal interface in uncomplicated human pregnancy. Further unravelling of the mechanisms of fetus specific immune recognition and immune regulation may be crucial to understand why some pregnancies are successful whereas others are not.

All experiments in this thesis were performed using lymphocyte isolates from decidua basalis and decidua parietalis tissue as well as maternal PBL samples and control PBL.
samples of non-pregnant donors. Hereby we studied maternal leukocyte responses from 3 important fetal-maternal interfaces and compared the maternal response to the immune response in non-pregnant controls. By analyzing decidua basalis and decidua parietalis tissue we examine fetal-maternal immune interactions locally in the uterus. Decidua basalis forms the maternal part of the placenta at the implantation site and contacts with the invading fetal trophoblasts. Decidua parietalis forms the maternal part of the membranes that contacts the non-invasive trophoblasts of the chorion. Comparison of decidua basalis and decidua parietalis leukocytes shows many differences in leukocyte composition, phenotype and lymphocyte responses. Decidua basalis lymphocytes at term pregnancy contain approximately equal percentages of T cells (50%) and NK cells (48%) while decidua parietalis lymphocytes contain an increased percentage of T cells (65%) compared to NK cells (38%) (Chapter 7). In addition, the proportions of CD4+CD25bright Treg cells and highly differentiated CD8+ EM-2 and EM-3 T cells are increased in decidua parietalis in comparison to decidua basalis. These data show an increased T cells activation and T cell differentiation in decidua parietalis in comparison with decidua basalis. Analysis of lymphocyte function shows comparable proliferation and IFN-γ production by decidua basalis and decidua parietalis lymphocytes. In addition, the CD4+CD25bright Treg cells from both decidua basalis and decidua parietalis are capable of regulating fetus specific UCB and 3rd party UCB responses. This data indicates that the functional capacity of decidua basalis and decidua parietalis to elicit an immune response is comparable. Furthermore, in decidua parietalis but not in decidua basalis a fetal-maternal HLA-C mismatch is correlated with an increased CD4+ T cell activation and the induction of functional CD4+CD25bright Treg cells. This indicates that in TCR mediated recognition of allogeneic HLA-C occurs in decidua parietalis tissue. Whether or not T cells in decidua basalis can specifically recognize HLA-C remains unclear, however as HLA-C is expressed on the invading extra villous trophoblasts it is likely that HLA-C specific T cells are present in decidua basalis but they may not be visible within a more complex array of immune responses or diminished by decidua basalis specific immune regulatory mechanisms. Besides differences in T cell and NK cell responses also differences in macrophage subsets and a differential expression of molecules like TGF-β, FAS and HLA expression profiles may induce divergent immune responses in decidua basalis and decidua parietalis (3-6). In conclusion, all data suggests that decidua parietalis lymphocyte responses consists of a more T cell dominated response while decidua basalis responses may include a more complex immune reaction including both NK cell and T cell responses. Decidual NK cells play a key role in placental development and facilitate trophoblasts invasion (7) whereas decidual T cells play an important role in fetus specific immune recognition and immune regulation. Immune regulation of fetus specific T cells seems essential to prevent a detrimental response at the fetal-maternal interface and may be a requirement for successful pregnancy.

Maternal peripheral blood analysis provides information about the systemic maternal immune response and can be used as a diagnostic tool for monitoring peripheral immune responses during pregnancy. Maternal peripheral blood contacts the syncytiotrophoblast layer during utero-placental circulation and contains trophoblast micro particles that are shed from the syncytiotrophoblast surface. Syncytiotrophoblasts and the trophoblast micro particles do not express MHC class I molecules and therefore they can not directly elicit an alloimmune response by T cells. Previous studies show that maternal
peripheral blood monocytes are primed to produce more TNF-α, IL-12(p70) and IL-18 in comparison to non-pregnant individuals. In addition, trophoblast micro particles induce TNF-α, IL-12(p70) and IL-18 and suppress IFN-γ responses indicative for a systemic activation of the maternal innate immune system during uncomplicated pregnancy (8-10). In this thesis we demonstrate a significant increase of CD4+CD25dim activated T cells and a small but not significant increase in CD8+ Effector T cells in maternal peripheral blood. It is not clear whether T cell activation in maternal peripheral blood is induced by indirect Ag presentation by antologous APCs or direct recognition of fetal cells in maternal circulation. Furthermore, CD4+CD25brightFOXP3+ and CD4+CD25brightHLA-DR+ Treg cells are reduced in maternal PBL whereas maternal PBL CD4+CD25bright T cells are not able to suppress the response to umbilical cord cells of the fetus. In contrast depletion of CD4+CD25bright T cells in maternal peripheral blood decreases the fetus specific response indicating that maternal peripheral blood CD4+CD25bright T cells are activated fetus specific cells. In conclusion, our data shows that fetus specific immune recognition and T cell activation in maternal peripheral blood takes place in uncomplicated pregnancy. Peripheral T cell activation may contribute to the systemic inflammation in normal pregnancy and intensify the maternal inflammatory response during conditions of pregnancy pathology like pre-eclampsia.

In this thesis all experiments and analysis of maternal leukocytes concerned uncomplicated pregnancies. Nevertheless, major variations were observed between individual pregnancies regarding decidual leukocyte yield, leukocyte composition and lymphocyte responses suggesting that each pregnancy concerns a unique mother-child combination that may generate distinct levels of immune activation and requires a distinct combination of immune regulatory mechanisms in order to result in a successful pregnancy. These regulatory mechanisms may include expression of non-specific immune regulatory molecules like IDO, FAS, TGF-β and complement inhibitors or more immune specific mechanisms like HLA expression profiles, NK cell - trophoblast interactions, decidual macrophages, or regulatory T cells. Maternal genotype (like HLA genotype, KIR genotype and cytokine polymorphisms), maternal history (number of previous pregnancies, infection history) and the combination of fetal-maternal HLA matches and mismatches may determine which regulatory mechanisms are most predominant.

It is clear from this thesis that fetal-maternal HLA-C differences significantly influence decidual T cell activation, the decidual lymphocyte response and induction of functional regulatory T cells. Pregnancies containing a HLA-C mismatched child imply a decidual lymphocyte response to fetal cells and the need of functional CD4+CD25bright regulatory T cells in decidual tissue whereas HLA-C matched pregnancies do not. Previous studies have shown that incompatibility of maternal KIR genotype and fetal HLA-C allotype increases the risk for pre-eclampsia and spontaneous abortions (11,12). Although limited experimental data is present on how KIR incompatibilities may influence the decidual lymphocyte response, KIR+ T cells or T cell – NK cell interactions may be involved in the decidual lymphocyte response. The major challenge for future studies is to examine the mechanisms of fetus specific immune recognition and immune regulation by decidual T cells and NK cells in aberrant pregnancy. In conclusion, fetus specific immune recognition and immune activation by T cells takes place in uncomplicated pregnancy and does not lead to a detrimental immune response. The presence of
immune regulatory cells and/or the absence of additional ‘danger signals’ in healthy decidua may explain why maternal T cells which are able to specifically recognize fetal alloantigens do not reject the fetal allograft (13,14).

REFERENCES


