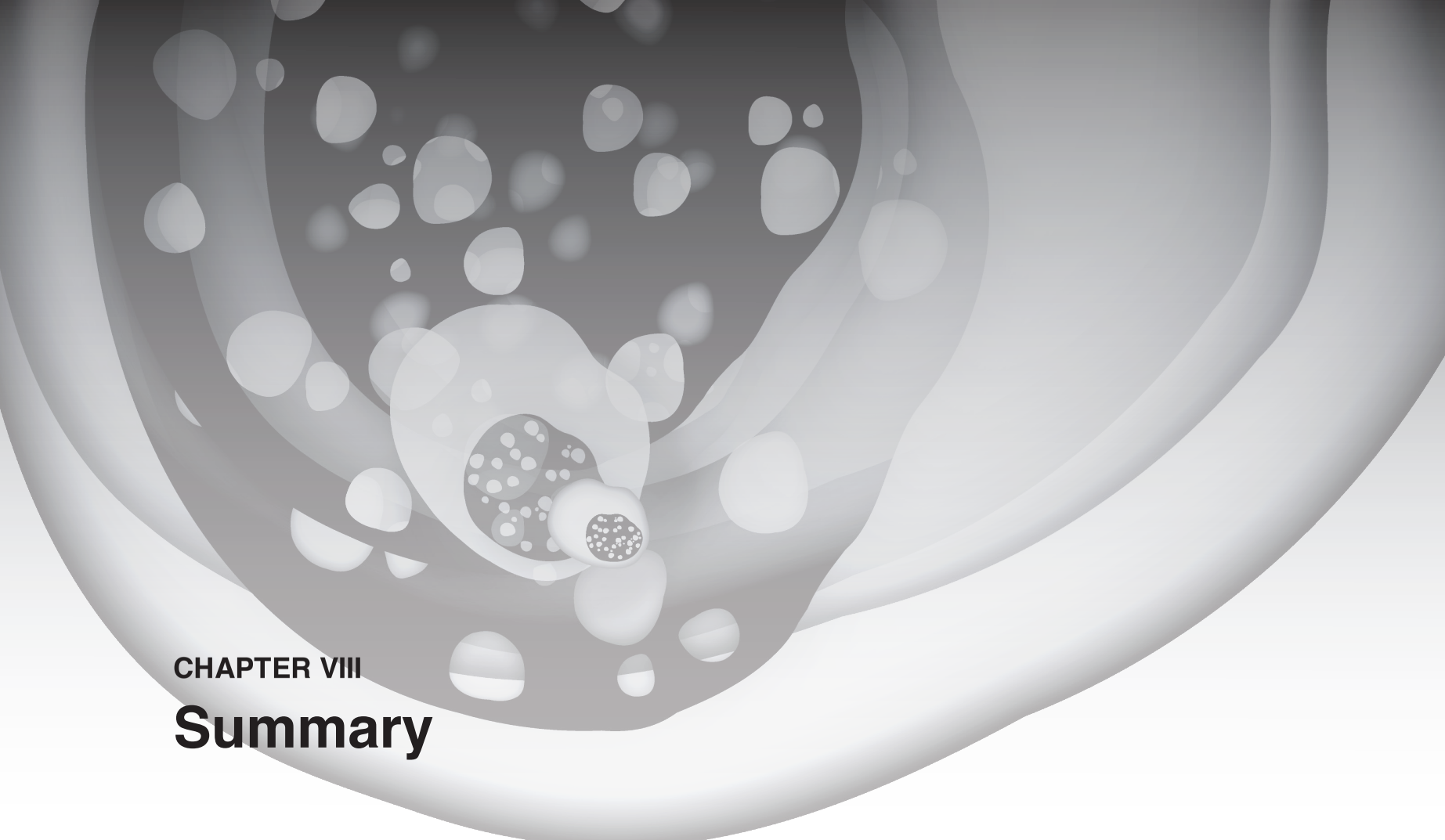

CHAPTER VIII

Summary

In this thesis minor H antigen specific cytotoxic and regulatory immune responses have been studied in health and disease. Minor H antigens are HLA-restricted polymorphic self-proteins. These antigens play an important role after HLA-identical transplantations, performed for hematological malignancies, in both detrimental Graft versus Host disease (GvHD) and beneficial Graft versus Leukemia responses (GvL). Furthermore minor H antigen specific cytotoxic and regulatory responses have been described in the physiological setting of pregnancy in the mother and the child. The responses induced after pregnancy can remain in individuals for decades and might be related to microchimerism.

In the first three chapters several aspects of cell exchange and transmaternal cell trafficking between mother and child during pregnancy were investigated.

In ***chapter II*** the presence of minor H antigen specific cytotoxic T cells against non-maternal antigens in cord blood is described. Umbilical Cord Blood (UCB) is used for hematopoietic stem cell transplantation (HSCT). It is known that UCB can comprise antigen specific T cells. Here it is shown for the first time that UCB not only harbors antigen specific T cells directed against the mother, but also against microchimeric cells present in the mother. Hereto, twenty-three female UCB samples were collected from healthy mothers and analyzed for minor Histocompatibility antigen HY-specific responses. Using flow cytometry HY specific T cells were isolated by tetramer staining and cultured single cell per well. Forty-two out of the obtained 104 tetramer^{pos} T cell clones, isolated from 16 out of 17 UCB samples, showed male-specific lysis in vitro. Male microchimerism was present in 6 out of 12 UCB samples analyzed. These findings underline the sustained presence of microchimeric cells in women after delivery, which can be transferred into the next fetus. Herewith proofing the existence of transmaternal cell flow. Furthermore the isolation of HY specific cytotoxic T cells from female UCB shows that cord blood is not as naïve as is generally believed.

In ***chapter III*** possible consequences of the above described transmaternal cell trafficking in HSCT is explored. It has been hypothesized that due to transmaternal cell trafficking the younger sibling might have come into contact with non-shared antigens of his older sibling in a tolerogenic environment during pregnancy. This tolerogenic immunization against non-shared antigens between siblings might result in better transplantation outcome when the donor is transplanted with its younger sibling. This phenomenon is referred to as the birth order effect. We questioned whether it is pos-

sible to relate this birth order effect to minor H antigen mismatches. This was investigated in an unique international collaborative study, in which we analyzed a group of 311 HLA-identical sibling transplantations. In this cohort the birth order effect was significantly present in adult female donor/female recipient SCT pairs. Therewith proposing that gender might be one of the causal factors. Due to small numbers the effect could not be related to a specific autosomaly encoded minor H antigen. Our current plausible explanation is the intra-uterine exposure to sibling antigens occurring during pregnancy leading to minor H antigen experienced (regulatory) T cell responses in females. Subsequent (re-)exposure to fetal antigens during pregnancy of the donor herself might boost pre-existent immune responses resulting in good transplantation outcome.

In ***chapter iV*** we studied whether there is a correlation between the detectable presence of microchimerism in peripheral blood and minor H antigen specific regulatory responses. To address this question a cohort of 45 women with a fully documented pregnancy and family history was studied. Since gender difference is an established risk factor for HSCT-related complications like GvHD, Y chromosome-encoded minor Histocompatibility antigens (HY) specific immune responses were studied as a proof of principle. The presence of HY-specific regulatory T cells (Treg) was investigated by HY peptide-induced linked suppression, a commonly reported functional feature of $CD4^{pos}$ and $CD8^{pos}$ Treg. HLA class I or class II restricted HY-specific Treg were detected in 26/42 (62%) women eligible for analysis. The prevalence of HY-specific Treg was significantly higher in women who had never given birth to sons than in women with male offspring (p=0.004). Also within the group of women with only female offspring, the incidence of regulation was higher compared to women with male offspring. As source of HY antigens, male microchimerism was analyzed by real-time PCR and defined by the presence of male DNA in at least one purified leukocyte cell type. Male microchimerism could be detected in 24 out of 45 (53%) women but did not correlate with the presence of HY specific Treg. Neither could the presence of male microchimerism be related to male offspring. This is in line with earlier studies in which microchimerism of different sources can be present in an individual.

These first chapters study minor H antigens in relation to or in the context of HSCT. In ***chapter V*** minor H antigen responses are studied in HLA-identical sibling renal transplantation. In collaboration with 16 laboratories of the IHIWS, the role of 15 autosomal, 10 Y-chromosome encoded minor H antigens and 3 CD31 polymorphisms, was

investigated in relation to the incidence of renal graft rejection and graft loss in 444 HLA-identical sibling renal transplantations. Recipient and donor DNA samples were genotyped for the minor H antigens HA-1, HA-2, HA-3, HA-8, HB-1, ACC-1, ACC-2, SP110, PANE1, UGT2B17, C19Orf48, LB-ECGF-1, CTSH, LRH-1, LB-ADIR and HY. Outcome in this cohort of HLA-identical renal transplantations was very good. Only 36 patients experienced a rejection episode of which 8 resulted in graft loss. In this small group no correlation was observed between one or more minor H antigen mismatch(es) and graft rejection and/or graft loss. Furthermore there was no influence of gender mismatch on outcome. From this study we could not draw definitive conclusions regarding the role of minor H antigens in renal transplantation. Nevertheless it seems that the role of minor H antigens either in graft rejection or tolerance, as described before, is minor.

In ***chapter VI*** we studied the T cell receptor of HA-1 specific T cells in more detail. HA-1 specific TCR-transferred T cells are currently under study for clinical application. Therefore we questioned whether the formerly described restricted TCR VBeta usage of HA-1 specific CTL is also applicable for HA-1 specific Treg which is crucial information for the clinical application of HA-1 TCR transferred T cells. HA-1 tetramer staining T cells were isolated by flow cytometry from 6 HA-1^{RR} healthy donors and from a patient after HLA-identical HA-1 mismatched HSCT. The clonally expanded T cells were functionally tested and TCR Valpha and Vbeta chain was determined. We identified three functionally different type of T cell clones. One type recognizing both the natural ligand and peptide loaded target cells, while the other type only recognized peptide loaded target cells. Both these type of T cell clones shared the same TCR TRBV7-9. Before peptide specific T cells have been indicated to have regulatory functions. In this study we could not test regulatory functions of these T cells and we did not found any CD8 Treg associated markers. The third type of T cell clone showed clear tetramer staining intensity but did not show any HA-1 specific cytotoxicity. These T cell clones did not share TRBV7-9. From this we concluded that T cell clones with any cytolytic capacity shared TRBV7-9. However we could not exclude possible regulatory functions of the peptide specific T cell clones. Therefore the different cytolytic capacity among the TRBV7-9 expressing HA-1 specific T cells needs further investigation.

In conclusion, this thesis emphasizes the presence of minor H antigen specific immune responses directly after birth, which will be present throughout life. The presence of minor H antigen mismatched microchimeric cells obtained through pregnancy from a mother or a child play a crucial role herein. Subsequent immunization against minor H antigens can lead to both cytotoxic and tolerogenic responses. Furthermore HA-1 specific T cells can share the same TCR VBeta, yet being functionally different.
The here performed studies enhances our understanding of immune reactions after HSCT and if applicable after renal transplantation, especially regarding the birth order effect and the role of women as transplant donors. Since many women display regulatory functions against minor H antigens, they can no longer be disqualified as possible transplant donors per se. It is crucial to further expand the knowledge about the influence of pregnancy in female donors in order to select tolerant donors over sensitized donors.