Design of novel TCR gene transfer strategies for the treatment of hematological malignancies

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Summary & General discussion

Summary

This thesis focused on the characterization of novel T cell populations that may serve as host cells for TCR gene transfer and to determine their possibilities and restrictions as TCR engineered immunotherapeutic effector cells for the treatment of hematological malignancies.

$CD8^+$ T cells are believed to be more suitable for the execution of cytolytic reactivity than $CD4^+$ T cells. However, for immunotherapy for leukemia $CD4^+$ T cells may have some advantages over $CD8^+$ T cells. HLA class II molecules are mainly expressed by hematopoietic cells, while other tissues only upregulate HLA class II expression under inflammatory conditions. Therefore, immunotherapy directed to HLA class II restricted antigens under non-inflammatory conditions may induce graft versus leukemia (GVL) reactivity without graft versus host disease (GVHD). In addition, the hematopoiesis specific expression of HLA class II molecules under noninflammatory conditions may circumvent the requirement of hematopoiesis specific minor histocompatibility antigens (mHags) and may enable the targeting of mHags with a broad tissue expression. **Chapter 2** describes the retroviral transfer of an HLA class II restricted TCR to $CD4^+$ and $CD8^+$ T cells to generate HLA class II restricted T cells capable of exerting antigen specific cytolytic activity and producing high amounts of cytokines. The DBY-TCR transduced $CD4^+$ T cell clones showed antigen specific cytolytic activity, produced high amounts of cytokines and had proliferative capacity. While the cytolytic activity and proliferation of the DBY-TCR transduced $CD8^+$ T cells and $CD4^+$ T cells was comparable, the DBY-TCR transduced $CD8^+$ T cells were inferior to the $CD4^+$ T cells in their production of the cytokines IFNγ and IL-4, even with cotransfer of the CD4 coreceptor. Thus, targeting HLA class II restricted antigens by TCR gene transfer to $CD4^+$ T cells may be a novel strategy to induce GVL without GVHD under non-inflammatory conditions.

In **chapter 3** we describe the retroviral transfer of $\alpha\beta$TCRs to $\gamma\delta$ T cells rather than to $\alpha\beta$ T cells. The transfer of TCR chains to $\alpha\beta$ T cells generates T cells with unknown specificity due to the formation of mixed TCR dimers between the endogenous and introduced TCR chains. Mixed TCR dimers have recently been shown to be capable of inducing autoreactivity. By TCR engineering of $\gamma\delta$ T cells mixed TCR dimer formation is prevented, since the introduced TCRα and TCRβ chains cannot form dimers with the endogenous TCRγ and TCRδ chains. The suitability of the $\alpha\beta$TCR transduced $\gamma\delta$ T cells as immunotherapeutic effector cells was assessed in an *in vitro* study. Purified peripheral blood derived $\gamma\delta$ T cells were retrovirally transduced with a TCR recognizing the hematopoietic mHag HA-2 in the context of HLA-A2, with a TCR specific for cytomegalo virus (CMV) pp65 in the context of HLA-B7, or with the HLA class II restricted DBY-TCR. Since most $\gamma\delta$ T cells lack coreceptor expression, we also studied the contribution of the coreceptor on the functional activity by retroviral cotransfer of CD4 or CD8. The $\gamma\delta$ T cells engineered with the HLA class I or HLA class II restricted TCR in combination with the relevant coreceptor were capable of antigen specific cytolytic activity and produced high amounts of cytokines upon antigen specific stimulation. The $\gamma\delta$ T cells transduced with the therapeutically interesting HA-2 TCR showed strong cytolytic activity against primary leukemic cells. In conclusion, $\alpha\beta$TCR gene

transfer to γδ T cells can generate antigen specific effector cells for immunotherapy of hematological malignancies, without the expression of mixed TCR dimers.

Since some γδ T cells have been shown to preferentially reside in epithelial sites in skin, tonsil and intestine, homing characteristics may not favor the application of these T cells as immunotherapeutic effector cells for the treatment of leukemia. **Chapter 4** describes the analysis of the homing characteristics of γδ T cells transduced with an αβTCR in a mouse model. Besides homing of the αβTCR-engineered γδ T cells, proliferative capacity, capacity to exert antigen specific effector functions, survival, and their capacity to mount recall responses were investigated. Purified mouse γδ T cells transduced with a TCR specific for the model antigen OVA in combination with the CD8 coreceptor were studied *in vivo*. The TCR and coreceptor engineered γδ T cells proliferated *in vivo* in an antigen specific manner, were able to persist and had the capacity to mount recall responses. The modified γδ T cells were found to reside mainly in peripheral blood, while low numbers were detected in the intestinal epithelial sites. Functional analysis showed that αβTCR and CD8 transduced γδ T cells produced IFN-γ upon antigen specific stimulation directly *ex vivo* and after *in vitro* stimulation and the engineered γδ T cells displayed *in vivo* cytolytic activity. Thus, these data indicate that αβTCR transduced γδ T cells display the desired characteristics *in vivo* and may be a safe and effective alternative for TCR transduced αβ T cells in clinical application.

The T cells selected as host cells for TCR gene transfer influence the functional properties of the generated TCR engineered T cells. Therefore, more insight into the functional properties of various candidate T cell populations is desired. Killer Ig-like receptors (KIR) have been shown to be modifiers of the effector functions of T cells. Inhibiting KIR can down regulate or even completely inhibit the cytotoxicity and cytokine production of T cells[1-5]. The effect of KIR on proliferation is unclear. CMV specific T cells may potentially be selected as host cells for gene transfer of therapeutic TCRs and KIR expression on these T cells may be detrimental for their anti-leukemic reactivity. **Chapter 5** describes the characterization of KIR^+ CMV specific T cells in allogeneic SCT patients and healthy donors. The percentage of KIR-expressing CMV specific T cells was determined and the effect of KIR on their functional activity was analyzed in allogeneic stem cell transplantation (SCT) patients and healthy donors. After allogeneic SCT and subsequent reactivation of CMV, all SCT patients showed KIR expression on CMV specific T cells. In contrast, in only two of six corresponding donors KIR-expressing CMV specific T cells were detected. Functional analysis showed that KIR^+ CMV specific T clones isolated from two patients and a healthy donor were capable of antigen specific cytolytic activity, cytokine production, and proliferation when KIR were not triggered. KIR triggering inhibited the effector functions and proliferation, depending on the strength of TCR stimulation. To compare the results obtained for KIR-expressing CMV specific T cells with the functional characteristics of other KIR-expressing T cells with unknown specificities, TCR gene transfer was applied as investigation tool. KIR-expressing T cells with unknown specificity were equipped with a CMV specific TCR and functionally analyzed. The KIR-expressing TCR transduced T cells were shown to be fully functional T cells in the absence of KIR triggering. The cells showed antigen specific cytolytic activity, cytokine production, and proliferation. KIR triggering inhibited the effector functions and proliferation. In conclusion, the low percentage of KIR-expressing CMV specific T cells and the observation that strong TCR stimulation can overcome KIR-mediated inhibition support the selection of CMV specific T cells as host cells for therapeutic TCRs for the treatment of hematological malignancies.

General Discussion

Immunotherapy targeted at HLA class II restricted mHags

$CD4^+$ T cells are generally regarded as helper T cells supporting the activity of $CD8^+$ T cells, and less frequently as cytotoxic T cells. Therefore, most immunotherapeutic strategies are based on $CD8^+$ cytotoxic T cells. However, it can be hypothesized that immunotherapeutic strategies for the treatment of hematopoietic malignancies based on $CD4^+$ T cells may induce GVL with less GVHD.

$CD8^+$ T cells recognize antigens presented in the context of HLA class I molecules which are expressed by all nucleated cells. Therefore, administration of donor derived $CD8^+$ T cells to a patient may lead to antigen specific reactivity against the patients malignancy, but also to healthy cells anywhere in the patient's body. In contrast, $CD4^+$ T cells recognize HLA class II restricted antigens, which are expressed mainly by hematopoietic cells[6;7], including hematopoietic malignancies[8-13]. Non-hematopoietic tissues can upregulate HLA class II expression, however this depends on the presence of proinflammatory cytokines[14;15]. Thus, the antigen specific reactivity of $CD4^+$ T cells is, in a non-inflammatory environment, limited to hematopoietic cells. This is supported by the clinical application of $CD8^+$ T cell depleted DLI, which lead to GVL responses in the absence of GVHD[16-18].

Recently, leukemia reactive $CD4^+$ T cells were demonstrated during a clinical response to DLI after a SCT from an unrelated donor mismatched for HLA-DP[19]. *In vitro* characterization of the immune response indicated that different $CD4^+$ T cell clones were present recognizing either allo-HLA-DPB1*0201 or DPB1*0301 molecules on different tissues after treatment with the proinflammatory cytokine IFNγ. Analysis of the reactivity of the $CD4^+$ T cells against various hematopoietic malignancies indicated that allo-HLA-DP restricted selective GVL reactivity can be induced depending on the expression level of HLA-DP by the malignant cells in the absence of proinflammatory cytokines. Another immunotherapeutic target antigen for $CD4^+$ T cells can be the recently identified HLA-DQ restricted mHag LB-PI4K2B-1S[20]. The antigen is also expressed broadly in various tissues, but T cell recognition of the antigen depends critically on the HLA-DQ expression level. The expression of HLA-DQ, and of the mHag, was sufficiently high on B cells, mature DC's, and also on $CD34^+$ CML cells and ALL cells, and resulted in antigen specific recognition by the $CD4^+$ T cells. For male leukemia patients that received a SCT from a female donor there are additional antigens that can be targeted by $CD4^+$ T cells. These target antigens are derived from genes encoded by the Y chromosome, have a broad tissue distribution and can induce antigen specific reactivity by T cells from the female donor. In some male patients female donor T cells were found to contribute to a selective GVL effect, which may have been induced by mHags encoded by the Y chromosome[27]. Examples of such HY mHags are: DFFRY, UTY, SMCY, RPS4 and DBY[21-26]. We previously isolated HY specific cytotoxic $CD4^+$ T cell clones with GVL reactivity from a male patient that received a SCT patient from his HLA identical sister[28]. Thus, HLA class II restricted antigens with a broad tissue distribution may be suitable target antigens for the treatment of hematological malignancies, since the HLA class II molecules are selectively expressed on cells from the hematopoietic system under non-inflammatory conditions.

To acquire large numbers of $CD4^+$ T cells for cellular immunotherapy of hematological malignancies within a short period of time, HLA class II restricted TCRs can be transferred to donor derived T cells. In the study described in **chapter 2**, we investigated whether efficient immunotherapeutic T cells could be generated by transfer of an HLA class II restricted DBY-specific TCR to both $CD4^+$ and $CD8^+$ T cells. The functional reactivity of the $CD4^+$ and $CD8^+$ T cells was compared after retroviral transfer of the DBY-TCR. The antigen specific cytolytic activity for $CD4^+$ and $CD8^+$ TCR transferred T cells was comparable. However, the TCR transferred $CD4^+$ T cells were superior to the TCR transferred $CD8^+$ T cells in the production of cytokines, even after cotransfer of the CD4 coreceptor to the $CD8^+$ T cells.

In conclusion, $CD4^+$ T cells specific for broadly expressed HLA class II restricted antigens generated by TCR gene transfer may be an alternative for $CD8^+$ T cells. The HLA class II restriction of the therapeutic T cells may prevent GVHD in a noninflammatory environment. $CD4^+$ T cells engineered with a TCR specific for a broadly expressed antigen in the context of HLA class II may only recognize the healthy and malignant hematopoietic cells of the patient. In comparison, engineering of polyclonal populations of $CD8^+$ T cells with a TCR specific for a hematopoiesis restricted mHag generates therapeutic T cells that may, besides hematopoietic cells, also recognize unknown antigens on various healthy tissues. GVHD may be induced by the reactivity due to the recognition of antigens via the endogenous TCR of the T cells or via the unknown specificities of mixed TCR dimers that may be generated. TCR gene transfer of HLA class II restricted TCRs to purified $CD4^+$ T cells will also lead to the formation of mixed TCR dimers. However, mixed TCR dimers at the cell surface of redirected $CD4^+$ T cells can induce GVHD only when they recognize HLA class I restricted antigens and function independently of the CD8 coreceptor. Mixed TCR dimers recognizing HLA class II restricted antigens in a noninflammatory environment will only recognize hematopoietic cells. Since the mixed TCR dimers have to be both HLA class I restricted and have to function independently of CD8, the risk for mixed TCR dimer induced pathology is limited after TCR engineering of $CD4^+$ T cells with an HLA class II restricted TCR. Careful design of conditioning regimens, selective use of chemotherapeutic drugs and prevention against high pathogenic loads after transplantation may limit the secretion of proinflammatory cytokines and support the induction of GVL responses by the TCR transferred HLA class II restricted T cells in the absence of GVHD.

TCR gene transfer strategies

The success of TCR gene transfer in a clinical setting is influenced greatly by the host T cells that are selected for TCR gene transfer. One strategy can be the transfer of TCRs to unselected polyclonal populations of both $CD4^+$ and $CD8^+$ T cells. Most TCRs require expression of the correct coreceptor to be fully functional, thus HLA class I restricted TCRs will only function optimal when transferred to $CD8^+$ T cells[29;30]. Coreceptor independent TCRs have been demonstrated to generate functional $CD4^+$ and $CD8^+$ T cells upon transfer to unfractionated lymphocytes, however the functional activity can in most cases be improved by coreceptor transfer[31;32]. Most TCR gene therapies are focused on the generation of cytotoxic T cells capable of direct destruction of malignant cells upon recognition, and therefore based on T cells enriched for $CD8^+$ cells[33-38]. As discussed

above, transfer of HLA class II restricted TCRs to purified $CD4^+$ T cells may be a good alternative for $CD8^+$ T cells for the treatment of hematological malignancies.
Transfer of therapeutic TCRs to polyclonal T cell populations limits laborious selection procedures which may negatively affect the T cell viability. Furthermore, the presence of T cells with various phenotypes may support both direct clinical responses and the formation of long term T cell memory. However, the presence of various phenotypes is also a disadvantage. While TCR transfer to T cells with effector phenotypes and memory phenotypes is desired, TCR transduction of regulatory or anergic T cells with suppressive properties cannot be excluded. Furthermore, the TCR transfer to ignorant self-specific T cells may lead to the generation of autoaggressive T cells after triggering through the introduced TCR. More importantly, TCR gene transfer to αβ T cells can lead to the formation of mixed TCR dimers, due to pairing of the introduced TCR alpha and beta chains with the endogenous TCR alpha and beta chains[39]. TCR transfer to a polyclonal T cell population may lead to large numbers of T cells expressing different mixed TCR dimers. The specificities of the mixed TCRs cannot be predicted, but some mixed TCR dimers may be autoreactive[37;40].

Until recently, no mixed TCR dimer associated autoimmunity was observed in both murine experiments and a first phase I trial[38;41-44]. Recently, however, severe mixed TCR dimer dependent autoimmune pathology was observed in a mouse model under conditions resembling the conditioning regimen applied in the first phase I trial (G. Bendle, personal communication). TCRαβ transferred T cells or T cells transferred with either the TCRα or TCRβ chain showed autoreactivity after lymphodepletion in combination with a high dose of IL-2, while both TCR transgenic and mock-transduced T cells did not induce autoreactivity.

A strategy that limits the potential number of generated mixed TCR dimers is TCR gene transfer to T cells with defined antigen specificity. In contrast to a polyclonal T cell population the number of different TCR chains is limited in an oligoclonal population with defined antigen specificity, which reduces the number of different mixed TCR dimers that can be formed. We previously demonstrated the transfer of an HA-2 specific TCR to CMV specific T cells, resulting in T cells with both anti-leukemic reactivity and anti-viral immunity[37]. TCR transfer to CMV specific T cells has the additional advantage of selective transduction of T cells with memory or effector phenotypes, but not regulatory, anergic or ignorant self-specific T cells. Furthermore, the TCR engineered CMV specific T cells are likely to have enhanced survival *in vivo* due to transactivation of the endogenous TCR by the latent presence of CMV antigens. Finally, the CMV specificity of the engineered T cells may protect against recurrent CMV infections which are a known complication after SCT. However, although this strategy confines the number of potential mixed TCR dimers formed, mixed TCR dimerization is not prevented. Recently, transfer of TCRs specific for HA-1, HA-2 or CMV to oligoclonal populations of virus specific T cells was found to result in mixed TCR dimer associated auto- and alloreactivity *in vitro* (M. van Loenen, personal communication).

To completely prevent the formation of mixed TCR dimers we designed a novel strategy which is described in **chapter 3**. Instead of transferring αβTCR chains to αβ T cells αβTCR chains were transferred to γδ T cells. γδ T cells express a γδTCR which cannot exchange chains with αβTCRs[45;46]. αβTCRs specific for mHag HA-2, CMV pp65 or mHag DBY were transferred in combination with the relevant coreceptor to peripheral blood derived γδ T cells. The γδ T cells engineered with the αβTCRs were fully functional and γδ T cells

transduced with the HA-2-TCR and CD8 were capable of lysing HA-2 expressing chronic myeloid leukemic cells and acute myeloid leukemic cells *in vitro*.

Besides the lack of mixed TCR dimer formation the transfer of αβTCRs to γδ T cells may have additional advantages in comparison to TCR gene transfer to αβ T cells. Some γδ T cells can recognize certain tumor cells with their γδ TCR. The γδ T cells found most in peripheral blood express a Vγ2 Vδ2 TCR which is specific for nonpeptidic metabolites. In some malignant cells deregulation of particular biochemical pathways leads to the accumulation of these nonpeptidic metabolites, leading to recognition and destruction by the γδ T cells[47-51]. This endogenous anti-tumor recognition may contribute to the elimination of the hematopoietic malignancy. Furthermore, Vγ2 Vδ2 TCR expressing γδ T cells can be restimulated *in vitro* and *in vivo* in a TCR dependent fashion using nitrogen-containing bisphosphonate drugs. Administration of low-dose IL-2 in combination with such compounds was shown to induce significant *in vivo* expansion of Vγ2 Vδ2 T cells in non-Hodgkin lymphoma and multiple myeloma patients[52]. In addition, sufficient numbers of γδ T cells have been shown to be easily generated from peripheral blood by *in vitro* expansion. Recently, up to 8 x 10^9 Vγ2 Vδ2 T cells were generated *in vitro* and infused into patients with renal cell carcinoma[53;54].

However, TCR gene transfer to γδ T cells may also have some disadvantages. The data on the capacity of γδ T cells to persist *in vivo*, mount recall responses and form long term immunological memory are conflicting and incomplete. Since γδ T cells possess both characteristics for the innate immune system and the adaptive immune system it may be possible that γδ T cells are able to mount very fast primary responses, but that these cells have a limited capacity to form T cell memory. In a nonhuman primate model, however, γδ T cells showed a memory type response with rapid expansion after rechallenge leading to clearance of detectable bacteremia[55]. Furthermore, another point of concern may be that γδ T cells not only reside in peripheral blood but that large numbers of γδ T cells can be found in epithelial sites of the intestine, skin, mouth, and genitals. However, the γδ T cells that are found most in the epithelium consist of a subpopulation of γδ T cells that is found less frequent in blood. These γδ T cells expresses a Vδ1 TCR, mainly in combination with Vγ8. For the treatment of hematological malignancies presence of the αβTCR engineered γδ T cells in peripheral blood is desired. By enrichment of Vγ2 Vδ2 T cells before αβTCR gene transfer, homing of the therapeutic T cells to epithelial sites may be limited.

To gain more insight in the *in vivo* persistence, homing and *in vivo* functional reactivity of αβTCR engineered γδ T cells, these modified γδ T cells were studied in a murine model, as described in **Chapter 4**. The αβTCR-modified γδ T cells had the capacity to persist *in vivo*, proliferated upon antigen specific stimulation, exerted *in vivo* antigen specific cytolytic activity, produced cytokines and were able to mount recall responses. The *in vivo* proliferative capacity and *in vivo* effector function of the αβTCR-transduced γδ T cells appeared to be reduced in comparison with the proliferation and effector function of αβTCR-transduced αβ T cells observed in earlier experiments[43;56]. An explanation can be the lower number of γδ T cells used in most experiments in comparison with the previous experiments with αβ T cells. Furthermore, the viability of the isolated murine γδ T cells may be negatively influenced by the requirement of multiple cell sorting steps. In contrast, the previous studies with TCR transduced αβ T cells required no cell sorting procedures[38;43]. However, it still cannot be excluded that γδ T cells are inferior to αβ T cells in mounting primary and secondary responses and exerting antigen specific cytolytic activity.

Thus, αβ TCR gene transfer to γδ T cells generates therapeutic effector cells that do not express potentially autoreactive mixed TCR dimers. TCR gene transfer to polyclonal populations of αβ T cells may generate these detrimental mixed TCR dimers, however in contrast to γδ T cells the biology, immunotherapeutic potential and limitations of αβ T cells has already been thoroughly documented. Codon optimization, generation of improved retroviral gene transfer vectors, manipulation of the TCR chain interface and selective linkage of the TCR chains to intracellular components of the TCR signaling pathway may enhance the expression level of the therapeutic TCRs while limiting mixed TCR dimer formation. However, mixed TCR dimer formation is likely to be not completely prevented and manipulation of TCR chains may have a negative influence on ligand recognition and signaling properties of the TCR. At present, CMV specific T cells engineered with therapeutic TCRs seems to be the strategy of choice for further testing in clinical trials. TCR gene transfer to CMV specific T cells reduces the number of mixed TCR dimers, generates TCR engineered T cells with memory or effector phenotypes, and prevents TCR transfer to regulatory, anergic or ignorant self-specific T cells. In addition, TCR engineered T cells may have enhanced survival due to activation of the endogenous TCR by CMV antigens, and may protect against recurrent CMV infections.

Functional characterization of the KIR-expressing CMV specific T cells

Since CMV specific T cells have some advantages as host cells for TCR gene transfer a subpopulation of these T cells which express KIR was functionally characterized as described in **chapter 5**. KIR-expressing T cells can be inhibited in their cytotoxicity and cytokine production upon ligation of the KIR receptor[1-5] and KIR-expressing T cells have been reported to have a general proliferative defect[57-59]. Together, this may indicate that KIR^+ T cells may represent end-stage dysfunctional T cells. Our functional analysis of inhibiting KIR^+ CMV specific T cells showed that in the absence of KIR triggering the KIR^+ CMV specific T cells were capable of antigen specific cytolytic activity, cytokine production, and proliferation. KIR triggering inhibited the effector functions and proliferation, depending on the strength of TCR stimulation. Comparable results were obtained for the KIR-expressing T cells with unknown specificity, thus KIR-expressing T cells are functional T cells that can be inhibited in effector functions and proliferation by triggering of the KIR and do not represent end-stage dysfunctional T cells. While our data on the KIR-mediated inhibition of cytotoxicity and cytokine production were in agreement with data from other studies, we did not find a general proliferative defect of KIR-expressing T cells as reported earlier. Since the activation status of inhibitory KIR-expressing T cells is a dynamic balance between TCR mediated activating signals and inhibitory signals via the KIR receptor, small discrepancies between the different studies may explain the different results. Differences in the KIR^+ T cell populations studied, differences in the method used to stimulate the T cells (i.e. superantigens, immobilized anti-CD3, or peptide loaded cells) and *in vitro* culture conditions can all influence the experimental outcome.

The factors responsible for the induction of KIR expression on $CD8^+$ T cells are still unclear. The presence of cytokines at the time of TCR triggering was shown not to induce the inhibitory KIR expression[60]. The expression is stable over time and activation status independent[1;61]. We detected T cells expressing KIR receptors that were specific for

ligands that were not expressed by the individual that the KIR^+ T cells were derived from. The KIR could thus not encounter their ligands *in vivo*, illustrating that KIR expression by these T cells was not based on functional selection but was induced in a stochastic fashion. It has been hypothesized that chronic antigen stimulation promotes KIR expression by T cells to limit their responses[62;63]. This corresponds with the observed memory phenotype of KIR^+ $CD8^+$ T cells and indicates that KIR expression is induced at later stages of T cell differentiation[2;60;64]. T cells recognizing antigens derived from viruses that have the ability to remain latently present within humans and that can reactivate (CMV and EBV), can be expected to be chronically stimulated. In contrast to others[57;65], we observed KIR-expressing CMV specific T cells in 6 of 23 healthy SCT donors and in 6 of 6 SCT patients after allogeneic SCT and subsequent reactivation of CMV. Careful examination of the flowcytometric data of both papers indicated, however, that there might have been some KIR-expressing virus specific T cells, but that numbers were very low.

Conclusion

The studies presented in this thesis show that TCR gene transfer has potential both as a therapeutic modality and a research tool. TCR gene transfer enables the engineering of T cells with a desired antigen specificity and phenotype and may lead to a future off-the-shelf therapy for the treatment of large numbers of patients. The recent clinical phase 1 study in which two out of 15 patients with metastatic melanoma showed objective tumor regression[44] indicates both the strength of TCR engineered T cells to eliminate malignant cells in a clinical setting and the limitations of the current state of the art. Various aspects of TCR gene transfer deserve further evaluation to improve both efficiency and safety. Transfer of HLA class II restricted TCRs specific for broadly expressed antigens to $CD4^+$ T cells may induce high levels of GVL reactivity, while conditioning regimen that maintain a noninflammatory environment prevent the induction of GVHD. Testing of this strategy in murine models may lead the way to future clinical trials. The recent observations of autoreactivity caused by mixed TCR dimers, in both murine models and *in vitro* experiments with human T cells, transformed mixed TCR dimers from a theoretical problem to a important issue which should be dealt with in the design of future TCR gene transfer strategies. CMV specific T cells engineered with therapeutic TCRs may be a strategy that partly deals with this problem. The oligoclonal nature of CMV specific T cells reduces the number of mixed TCR dimers, which may facilitate the screening of the potential autoreactivity. TCRα or TCRβ chains can be transferred separately to test the generated T cells functionally for autoreactivity. Careful selection of therapeutic TCRs which combine a desired antigen specificity with the capacity to compete with the endogenous TCR for cell surface expression may limited mixed TCR dimer formation further. Additional arguments for the clinical application of TCR engineered CMV specific T cells are the selective transduction of T cells with memory or effector phenotypes, the prevention of TCR transfer to regulatory, anergic or ignorant self-specific T cells, their enhanced survival due to activation of the endogenous TCR by CMV antigens, and finally their contribution to protect against recurrent CMV infections. However, the only strategy that can completely prevent the formation of mixed TCR dimers is TCR gene transfer to $\gamma\delta$ T cells. The results described in this thesis showed that human TCR engineered T cells can exert antigen-specific cytolytic activity and can produce cytokines when the relevant

coreceptor is cotransferred. In addition, γδ T cells transduced with an HA-2 TCR in combination with CD8 showed high anti-leukemic reactivity against HA-2 expressing leukemic cells. Furthermore, in a murine model TCR engineered γδ T cells proliferated in an antigen specific manner, were able to persist *in vivo* and had the capacity to mount recall responses. Thus, TCR engineered γδ T cells may solve the mixed TCR problem, however some aspects of these T cells have to be explored further. More knowledge about the characteristics, limitations and possibilities of modified and unmodified γδ T cells should be obtained from murine studies and clinical trials. The successful clinical studies with unmodified γδ T cells[53;54] may support the short term assessment of TCR engineered γδ T cells as therapeutic effector cells for the treatment of hematological malignancies in clinical trials.

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