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Author: Amir, Avital
Title: Biology and clinical relevance of T-cell allo-HLA reactivity
Date: 2012-06-28
6

SUMMARY AND GENERAL DISCUSSION
SUMMARY

Allo-HLA reactive T-cells can mediate graft versus host disease (GVHD) after HLA mismatched stem cell transplantation (SCT) and donor lymphocyte infusion (DLI). In addition, these T-cells can mediate graft rejection after HLA mismatched solid organ transplantation and SCT. Both GVHD and graft rejection can have detrimental consequences for patients. It has been demonstrated that in the HLA matched setting GVHD and graft versus tumor response can be separated by selection of T-cells directed against minor histocompatibility antigens (miHAs) exclusively expressed on hematopoietic cells. After HLA mismatched SCT, GVHD is often accompanied by graft versus leukemia response, indicating that allo-HLA reactive T-cells could potentially be useful for beneficial purposes. In this thesis we characterized the composition of an allo-HLA class I directed immune response occurring in vivo during GVHD and investigated which cells were possibly responsible for activating the response. We investigated which T-cells are responsible for allo-HLA reactivity and how these T-cells recognize allo-HLA. Finally we investigated whether allo-HLA reactive T-cells could potentially be used for adoptive T-cell therapy.

In chapter 2 we characterized an allo-HLA class I directed immune response in a patient who developed severe graft versus host disease (GVHD) after the administration of HLA-A2 mismatched donor lymphocyte infusion (DLI) in the absence of inflammatory conditions. A previously administered DLI from the same donor did not lead to an immune response, excluding the presence of a substantial pool of CD8 T-cells crossreactive against HLA-A2 within the memory T-cell compartment of the donor, and suggesting a requirement for CD4 T-cell activation for initiation of the response. Using single cell sorting we isolated CD8 and CD4 donor derived T-cells activated at the time of the GVHD. Analysis of the clonal diversity, alloreactivity, HLA restriction and specificity of the isolated CD4 and CD8 T-cell clones demonstrated that a polyclonal CD8 T-cell response directed against the mismatched HLA-A2 as well as a polyclonal CD4 T-cell response recognizing peptides derived from HLA-A2 presented in HLA class II were present during the GVHD. Investigation of patient blood and bone marrow collected at the time of the two DLIs demonstrated that leukemic blasts were the only patient derived HLA class II positive cells present at the time of the second DLI leading to GVHD, and that these cells were absent at the time of the first DLI not leading to an immune response. Stimulation of alloreactive CD4 and CD8 T-cells clones with the leukemic blasts demonstrated that these cells were able to activate both the alloreactive CD8 and CD4 T-cells. The results in this study indicate that the GVHD was mediated by a coordinated CD4 and CD8 response directed against the mismatched HLA-A2 and suggest that leukemic blasts possibly activated the CD8 as well as the CD4 T-cell response.

Which T-cells are responsible for allo-HLA reactivity and whether this is a property of a specific subgroup of T-cells or of all T-cells is unknown. Although allo-HLA reactivity was shown to be present within naïve and memory T-cell populations, the ability of T-cells to exhibit allo-HLA
reactivity could especially have serious consequences when exerted by memory T-cells, since memory T-cells can be efficiently triggered by non-professional antigen presenting cells based on their lack of requirement for co-stimulation. In chapter 3 the ability of virus specific T-cells to exhibit allo-HLA reactivity was investigated. Screening a large number of virus specific T-cells lines and clones against a panel of EBV transformed B-cells (EBV-LCLs), together covering almost all prevalent HLA class I and II molecules, demonstrated that 80% of the virus specific lines and 45% of the virus specific clones were allo-HLA reactive. This alloreactivity was demonstrated for Epstein Barr virus (EBV), cytomegalovirus (CMV), varicella zoster virus (VZV) and influenza virus (Flu) specific T-cells. Testing the alloreactive virus specific T-cell clones against single antigen transduced K562 cells confirmed that the alloreactivity was based on allo-HLA reactivity. TCR gene transfer demonstrated that the virus specificity and the HLA cross-reactivity were mediated by the same TCR. Three T-cell clones derived from the same individual and with the same specificity, but with different TCR usage demonstrated recognition of three different allo-HLA molecules, indicating that allo-HLA reactivity cannot be predicted on the basis of viral specificity. To extrapolate the results obtained with the EBV-LCLs and K562 cells to recognition of normal cell subsets in vivo, we tested virus specific T-cell clones against activated and non-activated blood derived cells and fibroblasts. Results demonstrated that virus specific T-cells can also be alloreactive against relevant normal cell subsets. The results in this study demonstrate that T-cells specific for different viruses exert cross-reactivity to allo-HLA molecules, and illustrate the high frequency of T-cells able to exert allo-HLA reactivity.

T-cell allo-HLA reactivity is assumed to be less peptide specific than conventional T-cell reactivity, based on the ability of allo-HLA reactive T-cells to recognize antigen processing deficient cells without exogenously loaded peptides and multiple different synthetic peptides loaded on target cells. However, peptide specificity of allo-HLA reactive T-cells against endogenously processed and presented peptides was not investigated. In addition, most of the studied allo-HLA reactive T-cells were in vitro activated and expanded. In chapter 4 the degree of peptide specificity and avidity of allo-HLA reactive T-cells mediating biological relevant allo-immune responses was studies by investigating the peptide specificities of 50 different allo-HLA-A2 reactive T-cell clones activated and expanded in vivo during GVHD. Testing the allo-HLA reactive T-cell clones against HPLC fractions of peptides eluted from HLA-A2 and loaded on antigen processing deficient T2 cells demonstrated that each clone recognized a single or two subsequent fractions, indicating that the in vivo derived allo-HLA reactive T-cell clones were single peptide specific. Allo-HLA reactive T-cell clones recognizing T2 without exogenously loaded peptides were tested against peptides eluted from HLA-A2 of T2 cells loaded on HLA-A2 expressing drosophila cells and also demonstrated to be single peptide specific. The peptides recognized by the allo-HLA reactive T-cell clones, including one T2 reactive clone, were identified using multidimensional HPLC fractionation and mass spectrometry. One of the clones recognized two different HPLC fractions. Identification of the
two peptides recognized by this clone demonstrated sequence similarity between the two peptides and alanine substitution of the identical amino acids suggested that recognition of the two peptides was based on structural similarity. Finally, downregulation of the expression of the antigens recognized by the allo-HLA reactive T-cell clones using silencing RNA confirmed their single peptide specificity.

Since in vivo activated and expanded allo-HLA reactive T-cells were demonstrated to exert high avidity single peptides specific recognition, allo-HLA reactive T-cells directed against tumor associated antigens (TAAs) could be used for adoptive T-cell therapy. In chapter 5, the search for TAAs specific allo-HLA reactive T-cells within an allo-HLA-A2 directed immune response occurring during GVHD resulted in the isolation of two high avidity PRAME specific T-cell clones. These T-cells were demonstrated to exert single peptide specificity and recognition was strictly correlated with PRAME expression. The allo-HLA restricted T-cell clones were, in contrast to the self-restricted PRAME specific T-cell clones, highly reactive against multiple different tumor cell-lines as well as freshly isolated metastatic melanoma and primary leukemic cells, whereas no reactivity against a large panel of non-malignant cells was observed. However, the clones exerted limited reactivity against mature dendritic cells (mDCs) and kidney epithelial cells. Recognition of the different target cells correlated with PRAME expression, as was demonstrated by quantitative RT-PCR, and by downregulation of PRAME using silencing RNA the recognition of mDCs and kidney epithelial cells was demonstrated to be based on on-target toxicity. Finally, PRAME-TCR transduced T-cells were demonstrated to exert high PRAME specific tumor reactivity, indicating that the PRAME-TCR can potentially be used in TCR gene therapeutic strategies. Clinical studies will determine the effectiveness of tumor reactivity in vivo and the significance of the potential toxicity observed against mature DCs and kidney epithelial cells.

**GENERAL DISCUSSION**

**AML blasts may have acted as antigen presenting cells in the initiation of the HLA class I directed immune response**

In Chapter 2 we demonstrated that a coordinated response of CD8 T-cells directed against mismatched HLA-A2 and CD4 T-cells recognizing HLA-A2 derived peptide presented in HLA class II was likely to have been responsible for severe acute GVHD. In addition, we demonstrated that leukemic blasts were the only HLA-A2 and HLA class II positive cells present in blood and bone marrow at the time of emergence of GVHD which were able to activate the CD8 and CD4 T-cell clones. The patient had received a SCT and two DLIs from the same donor, of which the first DLI did not lead to an immune response and the second DLI led to a severe acute GVHD. The leukemic blasts were absent at the time of the first DLI and relapsed before the second DLI. The remaining non-leukemic HLA-A2 positive cells present in patients
blood and bone marrow at the time of the GVHD, consisted mostly of patient T-cells which were present at the time of both DLIs but showed a marginal expression of HLA class II. We therefore hypothesize that the leukemic blasts acting as antigen presenting cells may have been required for initiation of the immune response responsible for GVHD. The absence of an immune response after the first DLI indicated that there was not a substantial pool of CD8 T-cells crossreactive against HLA-A2 within the memory T-cell compartment of the donor and thus suggests that the CD8 immune response required priming by activated antigen presenting cells for initiation. Investigating the leukemic blasts for the expression of co-stimulatory and adhesion molecules demonstrated that these cells did not express the surface molecules CD80, CD86 and CD54 previously shown to be relevant for initiation of primary immune responses. However, even professional antigen presenting cells, such as B-cells and dendritic cells need to be activated in order to express co-stimulatory and adhesion molecules and to be able to efficiently prime naïve T-cells. The activation of professional APCs can occur via the inflammatory signals of pathogens. Alternatively, in the absence of inflammatory signals, CD4 T-cells can activate APCs by CD40-CD40L interaction and cytokine production. This indicates that recognition of antigen by naïve CD4 T-cells on non-activated antigen presenting cells can lead to activation of APCs, which can subsequently prime other CD4 T-cells and CD8 T-cells. Stevanovic et al. demonstrated that also leukemic blasts can be activated by CD4 T-cells, by showing that leukemic blasts which in a non-activated state, did not express co-stimulatory and adhesion molecules and did not function as APCs, differentiated into APCs with expression of appropriate surface molecules after infusion of allogeneic CD4 T-cells in mice. In chapter 5 we demonstrated that the PRAME specific T-cell clones, which were derived from the same patient experiencing GVHD as is described in chapter 2, showed no or low reactivity against HLA-A2+ AML blasts but high reactivity against the same AML blasts activated by cytokines. The high reactivity against activated AML blasts by the PRAME specific clones could indicate that the leukemic blasts expressed PRAME higher after activation, but it could also be due to a more efficient antigen presentation and higher expression of co-stimulatory and adhesion molecules by the activated AML blasts. We speculate that part of the CD4 T-cells recognizing HLA-A2 derived peptides presented by HLA class II expressed on the leukemic blasts activated the leukemic blasts and thereby enabled them to prime both the CD4 and CD8 T-cell response.

Allo-HLA reactivity is a common property of all T-cells

In chapter 3 we used virus specific T-cells as a model for T-cells with a known specificity to investigate which T-cells are responsible for allo-HLA reactivity. 45% of the virus specific T-cells clones were demonstrated to be allo-HLA reactive, indicating that at least a substantial part of all T-cells is able to exert reactivity against specific allo-HLA molecules. However, since T-cells were analyzed for allo-HLA cross-reactivity against an EBV-LCL panel covering all prevalent HLA class I and class II molecules, missing all infrequent HLA molecules as well
as all tissue specific peptides presented in allo-HLA molecules, we speculate that virtually all T-cells may be allo-HLA reactive. It was previously hypothesized that each TCR needs to be able to react with structurally different peptide-HLA (pHLA) ligands. This property of T-cells is thought to permit the recognition of a universe of potential antigenic peptides which is estimated to be much larger than the number of T-cell clones present in an individual at a given moment. In addition, HLA cross-reactivity is thought to be essential for enabling the TCR to briefly dock and ‘scan’ the peptide contents of many different HLA molecules. Combining these hypotheses with our results in chapter 3 we conclude that the ability to form high avidity interactions with structurally different pHLA complexes is not an aberration of a certain subgroup of T-cells, but a normal property of T-cells.

**Peptide specificity of allo-HLA reactive virus specific T-cells**

In chapter 4 we demonstrated that biologically relevant allo-HLA reactivity, as defined by reactivity against endogenously processed and presented antigen in the context of an allo-HLA molecule, is based on high avidity single peptide specificity. We assume that the allo-HLA reactivity of virus specific T-cells, as demonstrated in chapter 3, is high avidity and single peptide specific, since allo-HLA reactivity was demonstrated against EBV-LCLs expressing endogenously processed and presented antigens in the context of allo-HLA molecules. In addition, for some of the alloreactive virus specific T-cell clones dependency on recognition of endogenous peptide for allo-HLA reactivity was demonstrated by showing reactivity against EBV-LCLs and PHA blasts expressing the recognized allo-HLA molecules and absence of reactivity against K562 cells transduced with the same allo-HLA molecules. Moreover, testing the virus specific T-cell clone, specific for EBV-BRLF/HLA-A3 and cross reactive against HLA-A2, against peptides eluted from HLA-A2 and loaded on T2 cells, demonstrated reactivity against one HPLC fraction, indicating peptide specific recognition by this clone. By multidimensional HPLC fractionation and mass spectrometry, as described in chapter 4, the single peptide recognized by the BRLF/A3 specific T-cell clone in the context of allo-HLA-A2 could be identified (data not shown in this thesis). The CMV-IPS/HLA-B35 specific T-cell clone cross reactive against HLA-DR4 also demonstrated recognition of a single peptide when tested against a recombinant bacteria cDNA library expressed by HLA-DR4 positive target cells (data not shown in this thesis).

In the future more peptides recognized in the context of allo-HLA molecules by virus specific T-cells may be identified using multidimensional HPLC fractionations and mass spectrometry or by bacteria cDNA library screening. The identification of peptide/allo-HLA complexes recognized by virus specific T-cells offers the possibility to compare the TCR interactions with the viral peptide in self HLA and with the endogenous peptide in allo-HLA. Comparing the two interactions on a molecular level using crystalstructures may lead to more insight into the molecular mechanism of TCR cross reactivity. In addition, crystalstructures of the two TCR-pHLA interactions of the CMV-IPS/HLA-B35 specific T-cell clone cross reactive against
HLA-DR4, may give more insight into TCR interaction with HLA class I and class II and into co-receptor dependency and independency of TCR/pHLA interaction.

The potential risk of allo-HLA reactivity of HLA mismatched virus specific T-cell lines

The results in chapter 3, demonstrating that allo-HLA reactivity of virus specific T-cells is common, suggest that administration of virus specific T-cells over HLA barriers may induce GVHD and therefore virus specific lines should be tested for alloreactivity against the patient prior to administration. However, Melenhorst and colleagues recently reported that partially mismatched virus specific T-cell lines did not lead to de novo development of GVHD in any of the 73 studied patients. As we demonstrated in chapter 3, the reactivity exerted by each of the allo-HLA reactive virus specific T-cell clones was specifically directed against one or a few allo-HLA molecules. It is therefore possible that the virus specific T-cells described by Melenhorst et al. were not alloreactive against the HLA molecules expressed by the recipients, especially since a selection criterion for infusion was a low or absent reactivity exerted by the virus specific line when tested against recipient APCs. In an attempt to exclude that the lack of GVHD was due to fortuitous choice of CTLs lacking recipient-specific allo-HLA reactivity, they demonstrated alloreactivity of the lines by testing them against panels of APCs expressing different HLA molecules. However, the alloreactivity observed against APCs expressing recipient allo-HLA molecules was either marginal of absent. In addition, it is not confirmed that the alloreactivity observed was directed against allo-HLA molecules, since reactivity against single antigen expressing cells or even against a few APCs sharing the same allo-HLA molecule is not described. Another possible explanation for the lack of GVHD in vivo could be that the T-cells lines are immunogenic targets for the immune system of the patient, based on their HLA mismatch, and are therefore quickly lost after transfusion. However, it was demonstrated that these lines were effective against viruses in vivo, indicating that they were at least not immediately lost and were able to establish an effective immune response. In contrast to the results of Melenhorst et al., other groups using partially HLA mismatched virus-specific T-cells lines did report GVHD after CTL infusion. In an extension of a previously reported study of Micklethwaite et al., 10 of 33 adults receiving bivirus-specific T-cells after allogeneic transplantation developed GVHD, de novo in 9 patients. Three patients developed grade III or IV GVHD. Peggs et al. reported similar results with 11 of 30 patients developing acute GVHD after CMV-specific T-cell infusions with grade III disease in 3 of the patients. Randomized studies of both prophylactic and preemptive CMV-specific T-cells currently running in the United Kingdom will provide further information on the risk of GVHD related to virus-specific T-cells in patients receiving uniform conditioning.
Difference in degree of peptide specificity and affinity between in vivo and in vitro activated and expanded alloreactive T-cells

In chapter 4 we demonstrated that in vivo activated and expanded alloreactive T-cells exert high avidity single peptide specific reactivity. In contrast, other research groups studying in vitro activated and expanded alloreactive T-cells demonstrated a large variation of avidity and degree of peptide specificity between the different T-cells. The difference between our findings and those of other groups could possibly be explained by the differences between in vivo and in vitro induced immune responses. It has been shown that during in vivo immune responses only T-cells with high avidity are selected. Zehn et al. demonstrated that low avidity T-cells stop expanding, exit lymphoid organs and start contraction earlier than high avidity T-cells, possibly explaining the mechanisms by which selection of high avidity T-cells occurs. Several factors might influence the selection of high avidity T-cells during in vivo immune responses. First of all, the quantity of antigen could play a role, since it has been shown that in vivo, in the absence of excessive antigen low avidity T-cells undergo apoptosis, whereas high avidity T-cells survive and expand. In addition, the number of activated APCs presenting the antigen could be of influence. Kedl et al. demonstrated that in vivo T-cells have to compete for APCs presenting the antigen, indicating selection of T-cells forming high avidity interactions with APCs. Finally, cytokines like IL2 and IL7 have been shown to play a role in T-cell expansion and survival during contraction phase, suggesting that the available amount of cytokines, which is most likely limited in most in vivo immune responses, also influences the selection of high avidity T-cells. In contrast to the priming conditions of in vivo induced immune responses, during in vitro activation and expansion antigen specific T-cells are usually in the presence of an abundance of antigen, antigen presenting cells and cytokines. Due to this lack of stringent selection, not only high avidity but also intermediate and low avidity T-cells are able to activate and expand during in vitro stimulations, explaining the documented findings of research groups studying in vitro induced alloreactive T-cells. Since in these studies recognition of low avidity T-cells was not confirmed by testing the T-cells against endogenously processed and presented antigen, we suspect that the reported low avidity reactivity directed against synthetic peptides loaded target cells was not biologically relevant recognition. In other terms, these T-cells would probably not have been activated and expanded and therefore not effective in vivo.

Thymic selection

In chapter 4 we demonstrated that allo-HLA recognition is as peptide specific as conventional T-cell recognition of foreign peptides presented in self HLA. These results appear to be in conflict with a currently accepted hypothesis that allore cognition is less peptide specific than
conventional T-cell reactivity, since allo-MHC molecules are not encountered during thymic development. It is assumed that the prethymic T-cell repertoire contains a large number of MHC and peptide crossreactive T-cells and that thymic selection is responsible for the removal of these T-cells. This assumption is based on the studies of Husbey et al., amongst others, in which T-cells selected in mice expressing MHC linked to a single peptide were demonstrated to exert high peptide and MHC crossreactivity\(^{36,37}\). Thymic selection of T-cells with one pMHC was in these studies interpreted as impaired negative selection and the hereby obtained T-cells repertoire was therefore thought to resemble the prethymic T-cell repertoire. Husbey et al. however ignored the fact that T-cells also undergo positive selection in the thymus. In mice expressing only one pMHC complex, the T-cells have to be able to form a low avidity interaction with this specific pMHC complex in order to be positively selected. It is therefore possible that selection with one pMHC complex does not represent impaired selection, but instead a very stringent selection of TCRs, not representative for the prethymic TCR repertoire. In contrast to Husbey et al. who used expression of a single pMHC as a model for lack of thymic selection, Zerrahn et al. used T-cells selected in MHC negative mice as a model for T-cells without thymic selection and compared them to T-cells selected in normal mice\(^{38}\). With this method Zerrahn et al. demonstrated that prethymic T-cells are as MHC crossreactive as T-cells after normal thymic selection. In addition, TCRs which were self engineered by combining the α-chain and the β-chain of two different TCRs and therefore really representing T-cells which have not undergone thymic selection, were also able to interact with one or a few pHLA complexes, but reactivity against multiple pHLA complexes has not been found\(^{39}\). These results indicate that the ability to specifically interact with pHLA complexes is not a property selected for in the thymus, but is most likely a germline property of TCRs

**Adoptive T-cell therapy**

The single peptide specificity of almost all investigated in vivo activated and expanded allo-HLA reactive T-cells (chapters 4 and 5), opens the door for the use of allo-HLA reactive T-cells in adoptive cell therapy. In contrast to adoptive cell therapy using T-cells directed against miHAs, in which a difference in the single nucleotide polymorphism (SNP) coding for the specific MiHA between patient and donor is necessary for application in a patient, adoptive cell therapy using allo-HLA reactive TAA specific T-cells could be applied in all patients positive for the targeted HLA and suffering from malignancies expressing the targeted TAA. This therapy could therefore potentially be applied in many patients, suffering from many different types of cancer. The TCRs of the in chapter 5 reported PRAME / allo-HLA-A2 specific T-cells could be applied in all HLA-A2+ patients, which are 50% of the West European and North American population, suffering from PRAME expressing melanoma (88% of melanoma's are PRAME positive) renal cell carcinoma, leukemia and other malignancies\(^{40}\). However, the application of allo-HLA reactive T-cells in the clinic could also lead to severe toxicity in patients. Therefore,
Chapter 6: Summary and general discussion

for every TCR potentially useful for adoptive cell therapy, a balance should be made of the expected benefits and risks, prior to application in the clinic.

For adoptive T-cell therapy high avidity TAA specific T-cells are required to mediate an effective anti-tumor response after infusion. High avidity TAA specific T-cells have been discovered before by different research groups. Nevertheless, only a few of these discoveries have actually led to clinical application. Quintarelli et al. and Griffioen et al. reported high avidity T-cell reactivity against PRAME derived peptides (P435-443 and P100-108 respectively) presented in self HLA. Both groups could demonstrate high reactivity against peptide loaded target cells and high peptide affinity in peptide titration experiments. However, in both studies T-cell reactivity against PRAME positive target cells was minimal and unconvincing. In our hands, the previously reported autologous PRAME P100-108/HLA-A2 specific T-cell clone demonstrated high peptide affinity (EC50 = 0.3 nM) in peptide titration experiments, comparable to the peptide affinity of the alloreactive PRAME specific T-cells HSS1 and HSS3 (chapter 5). However, this autologous PRAME specific T-cell clone demonstrated no significant reactivity against endogenously PRAME expressing target cells (data not shown) which were highly recognized by our alloreactive PRAME specific T-cell clones. Since PRAME is a self-antigen, which is, as we demonstrated in chapter 5, expressed by mature DCs, autologous PRAME specific T-cells are most likely deleted in the thymus. This indicates the PRAME derived peptides identified in the two studies are actually not efficiently processed and presented in cells and are therefore not expressed in the thymus, explaining the demonstrated high reactivity against synthetic PRAME peptide loaded target cells but absent reactivity against endogenous PRAME positive target cells by the described autologous PRAME specific T-cells. By using allo-HLA recognition, and thereby circumventing thymic deletion, efficiently processed and presented peptides derived from tumor associated antigens could be targeted despite the fact that these are self-antigens.

Of the previously reported allo-HLA reactive TAA specific T-cells, most were derived from in vitro activations and expansions. Since, in the majority of these studies, single peptide specificity of the TAAs specific T-cells was not confirmed, we suspect that at least some of these T-cells could also recognize other peptides, and thereby could possibly lead to off-target toxicity when applied in the clinic. The use of in vivo activated and expanded allo-HLA reactive T-cells could, based on their single peptide specificity, limit the risk of off target toxicity. However, as we demonstrated in chapter 4, also in vivo activated and expanded T-cells could be specific for more than one peptide (as clone HSS11 in chapter 5). Recognition of another look alike peptide with a broader expression pattern than the targeted peptide would most likely lead to severe toxicity. Therefore, single peptide specificity should in any case be confirmed before the use of allo-HLA reactive T-cells for clinical purposes.

Another obvious risk of the use of high avidity tumor associated self-antigen specific T-cells in the clinic is the occurrence of on-target toxicity, by the potential recognition of non-malignant cells expressing the tumor associated antigen. Several research groups who
applied adoptive cell therapy have indeed found organ specific toxicity. Infusion of high avidity T-cells directed against the RCC antigen carboxy anhydrase IX (CAIX) resulted in severe cholestasis, based on the CAIX expression by bile duct epithelial cells and patients who received high avidity MART-1 or gp100 T-cells developed uveitis and hearing loss due to expression of MART-1 and gp100 by melanocytes in eye and ear. The one patient who received T-cells expressing chimeric antigen receptor (CAR) specific for the TAA HER-2/neu died of respiratory distress based on HER-2/neu expression in the lungs. The disabling or mortal on-target toxicity observed in these studies suggests the need for validated cell type specific screening of the high avidity TAs specific T-cells before application in the clinic. As described in chapter 5, we screened the high avidity PRAME specific T-cells against multiple different cells derived from different organs, and found unexpected and not yet described reactivity against kidney tubular epithelial cells and mature DCs. Downregulation of PRAME by siRNA demonstrated that this reactivity was indeed based on on-target recognition. Based on these results, we propose that high avidity tumor associated self-antigen specific T-cells destined for use in the clinic, should be thoroughly screened against multiple cell lines or primary cells derived from different organs, in order to assess the on-target toxicity that can be expected when these T-cells are infused in patients. Additionally, expression arrays including the targeted tumor associated antigen could be performed on tissue samples derived from multiple different organs.

The discovered reactivity of the high avidity PRAME specific T-cells against kidney tubular epithelial cells and mature DCs could possibly lead to nephrotoxicity and to reduced immunity, respectively. Co-transduction of the PRAME-TCR engineered T-cells with a suicide gene could resolve the temporary loss of mDCs. In addition, the low reactivity observed against cultured PTECs in vitro does not necessarily indicate nephrotoxicity in vivo, as indicated by absence of renal dysfunction in the patient. Therefore, since the toxicity of high avidity PRAME specific T-cells could also be very limited or absent, and because the large potential benefits of this therapy, we decided to continue our development of clinical applicable PRAME-TCR viral constructs and aim at applying adoptive cell therapy using high avidity PRAME specific TCR transduced T-cells in the clinic.

Besides the PRAME specific T-cells, other allo-HLA reactive TAs specific T-cells could be searched for in PBMCs derived from patients undergoing GVHD and / or GVL response after HLA mismatched transplantations. Using tetramers of all known TAs, in vivo activated and thereby probably single peptide specific allo-HLA reactive TAs specific T-cells could be searched for. Alternatively, allo-HLA reactive T-cells activated in an in vivo immune response leading to GVHD or GVL could be screened against non-malignant and malignant cells, and the specificities of those cells only recognizing malignant cells could be identified. Thus, a whole arsenal of high avidity TAs specific T-cells could be created.
Chapter 6: Summary and general discussion

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