Adoptive transfer of tumor- and minor antigen-specific T cell reactivity in mouse models

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Chapter 1

General introduction

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Although the prevention of infectious diseases by means of vaccination has been one of the major (immunological) contributions to public health in the last 100 years, its role in immunotherapy for most tumor types has been limited[1,2]. This can be explained by the notion that the vast majority of tumors only express self-antigens, to which the T-cell (and B-cell) repertoire is immune tolerant. In this thesis various strategies to induce T cell immunity towards these tumor-associated antigens (TAAs) will be addressed, hence the introduction will focus on the cellular arm of the adaptive immune system. However, many parallels can be drawn to the humoral arm of the immune system, where high affine antibodies recognizing TAAs such as Her2-Neu (on breast cancer cells) and CD20 (on B-cell lymphoma) are now successfully used in the clinic[3].

T cells recognize antigens with their T cell receptor (TCR), a dimer consisting of an α and a β chain, which can engage with peptide fragments presented by major histocompatibility complex (MHC) molecules. These peptides are either derived from endogenous (self) proteins or from pathogens. Upon binding to a peptide MHC (pMHC) complex, a T cell can become activated, resulting in proliferation and the acquirement of effector functions[4]. During T cell development in the thymus, each thymocyte will assemble an unique TCR, which is the result of random gene rearrangements of the variable (V), diversity (D) (only β chain) and joining (J) segments of the TCR locus. This process of genetic recombination creates a pre-selection T cell repertoire with a theoretic diversity of up to $\sim 10^{14}$ different receptors[5], including TCRs specific for foreign peptides, but also TCRs which cannot bind to MHC at all, or which can recognize self-pMHC complexes. To avoid the release of either 'useless' or 'destructive' T cells in the periphery, developing thymocytes undergo positive and negative selection. Thymocytes with no affinity for MHC will die by neglect[6], and thymocytes with a high affinity for any of the large collection of MHC-associated self-peptides presented in the thymus will undergo apoptosis[7,8]. As a consequence, the mature peripheral T cell compartment consists of T cells with low affinity for MHC associated fragments of self-proteins but with potential high-affinity for non-self. In addition, peripheral tolerance mechanisms such as anergy after encountering antigen in absence of co-stimulatory signals[9] or suppression by regulatory T cells[10] further reduce the risk of a T cell mediated auto-immune attack. Due to these stringent tolerance mechanisms, eliciting immune responses towards self-antigens by means of active vaccination is expected to be difficult.

This can for example be illustrated by experiments performed in transgenic mouse models, where tumor development is induced by the transforming protein SV40 large T (REF[11,12] and chapter 5). Whereas in non-transgenic (and thus non-tolerant) mice immune responses against SV40 Large-T can be easily elicited, active vaccination with one or more of these same antigens in SV40 Large-T transgenic littermates neither results in profound antigen specific immune responses nor in tumor suppression. However, when vaccination is supplemented with T cells derived from non-transgenic

animals, immune responses can be induced, resulting in a decrease in tumor development[13,14]. Studies like these show that the absence of high-affinity T cells is an important limitation to strategies which aim to target self-antigens by active vaccination, but that tolerance can be circumvented by infusion of exogenous, non-tolerant T cells.

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) in combination with donor lymphocyte infusion (DLI) as a treatment for chronic myeloid leukemia (CML) (~60% success rate) is one of the most striking analogies in men[15]. Evidence that also in this situation allogeneic T cells are key players in targeting the tumor comes from observations in which T cell-depleted allo-HSCT resulted in an increased leukemia relapse rate[16], whereas infusion of donor lymphocytes was associated with an improved clinical outcome[17]. This Graft-versus-Leukemia (GvL) reactivity of donor lymphocytes is mediated by differences in minor histocompatibility antigens (MiHAgs), peptide antigens that are derived from highly polymorphic genes. In cases when a MiHAg is present on leukemic cells but absent on donor cells, the infused T cells (which are non-tolerant towards this MiHAg) can recognize this specific MiHAg and treat the tumor cells as 'foreign'[18]. The major drawback of this treatment protocol is that the T cell reactivity against self antigens is not specifically directed towards the tumor, but is determined by the available MiHAg differences between donor and recipient. As a consequence, expression of MiHAgs on non-malignant cell types may result in Graft-versus-Host Disease (GvHD), a common complication of allo-SCT/DLI with severe morbidity and mortality[19]. Furthermore, allo-SCT/DLI has been reported to be effective for a relatively small number of malignancies and therefore alternative forms of adoptive T cell therapy may be desirable.

In this thesis we investigated in preclinical animal models how T cell grafts with a defined tumor specificity can be generated. To this end we chose to study two approaches:

1. Use allogeneic donor lymphocytes of which potential harmful T cell specificities are removed.
2. Generate tumor specific T cells by a novel technique called TCR gene transfer.

Although MiHAg-specific T cell responses can in theory be directed against a large number of polymorphic differences between donor and recipient[20], it has been shown in murine models that T cell responses against relatively few MiHAgs dominate the immune response[21] and that both the target organ and the severity of GvHD depend on the available immunodominant MiHAgs. In light of these data, it seems possible that depletion of one or a few T cell subsets causing GvHD[21] or enrichment of donor T cells specific for leukemia-associated antigens[18] may improve the clinical outcome of allo-SCT/DLI. MHC tetramers, multimers of pMHC complexes coupled to a fluorescent label, are nowadays routinely used to analyze antigen-specific T cells. This possibility to detect or purify specific T cell populations make MHC tetramer technology a very attractive tool to manipulate T cell grafts prior to adoptive transfer[22]. In **chapter 2** we use a murine HSCT model (B6→BALB.B) to address the feasibility of MHC-based removal of immunodominant T cell responses and study the consequences for GvHD development. These data indicate that antigen-specific graft engineering is

feasible but that parameters other than immunodominance may be required to select T cell specificities that are targeted for removal.

In situations when allo-SCT/DLI is impracticable, TCR gene transfer may become a very attractive alternative. In brief, the concept of TCR gene transfer is based on the notion that the specificity of a T cell solely depends on the TCR. Genetic transfer of allogeneic TAA-specific TCR-genes into autologous T cells may therefore result in high-affine tumor-specific T cells, which can be used for passive immunization (for a more extended overview of TCR gene transfer, see **chapter 3**). Recently, the first 'TCR-gene transfer-clinical trial' has been published, in which adoptive transfer of melanoma-specific TCR-transduced T cells resulted in durable engraftment in 15/17 patients and in a partial response in two of these patients[23]. This study underlines the feasibility and therapeutic potential of TCR gene transfer, but also suggest that clinical efficacy needs to be improved.

To obtain directions for the design of future clinical trials, we study the *in vivo* behavior of genetically modified T cells in animal models to provide. To this purpose, Kessels et al. have developed a protocol to transduce mouse T cells with a TCR of interest, and showed that peripheral T cells genetically modified with a virus-specific TCR were able to expand upon viral infection and home to effector sites[24]. Having established that TCR transduced T cells function upon foreign antigen encounter, the next crucial question to address was whether TCR modified T cells can recognize self antigens and as such be used to circumvent tolerance. In **chapter 4** we have used a transgenic mouse strain (RIP-OVA^{hi}) expressing a pancreas-specific self-antigen (ovalbumin)[25]. Herein we show that adoptively transferred autologous T cells expressing an allogeneic antigen-specific TCR can proliferate upon vaccination, resulting in an auto-immune attack of both the pancreas and a transplantable tumor expressing the same model antigen (B16-OVA)[26]. In **chapter 5** we extend these data to an SV40 Large T-driven spontaneous prostate cancer model (TRAMP)[12], a model considered to more closely resemble human tumor development. Collectively, these studies provide proof of principle that TCR gene transfer can be used to generate high-affine tumor-antigen specific T cells, which can function in immune tolerant recipients, resulting in objective anti-tumor responses. Subsequently we addressed in **chapter 6** which factors determine the *in vivo* effectiveness of TCR modified T cells. Hereto we analyzed the impact of conditioning of the recipient, the composition of the graft and the avidity of the transferred T cells. The result of these analyses have yielded a set recommendations that are likely to improve the potency of TCR gene modified T cells in future clinical trials.

Whereas the data presented in this thesis have focused on some important issues regarding the efficacy of TCR gene transfer, experiments regarding the safety of this form of immune therapy remain largely to be done. As discussed in detail in the 'General Discussion' of this thesis (**chapter 8**), we expect that GvHD is the most likely side effect of TCR gene transfer. In **chapter 4** we screened a large cohort of mice post TCR gene transfer and found no signs of autoimmune pathology. However, two important

reasons why these results should not be taken as evidence that TCR transfer will never be complicated by GvHD deserve to be stressed. First, every TCR is different and the propensity for GvHD depends on TCR-MHC combination. Second, factors such as T cell dose and conditioning regimen have shown to affect the risk of GvHD post allo-SCT[27,28]. With the optimizations of TCR gene transfer protocols it is conceivable (and in fact also suggested by some recent experiments done by Bendle et al.) that the risk for GvHD will increase.

At this point, it is unclear in which situations TCR gene transfer will be complicated with GvHD, and to what extent it will be possible to predict the risk of these side effects. However, it is plausible that in order to exploit the maximum therapeutic potential of TCR engineered grafts, some risk of GvHD may be unavoidable. In such situations, endowing TCR modified T cells with a conditional safety switch may be highly desired. For this reason this thesis ends with a study in which we have examined the *in vivo* efficacy of a conditional caspase-9 based safety switch[29]. The data obtained in a murine model for severe cell therapy-induced type I diabetes indicate that self-reactive T cells expressing this conditional safety switch can be specifically and rapidly eliminated upon triggering (**chapter 7**).

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