Dissection and manipulation of antigen-specific T cell responses

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Summary

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Given the importance of T cells in the immune system and their potential as immunotherapeutic agents (see scope of this thesis and chapter 1) the aim of this thesis was 1) to obtain more insight into antigen-specific T cell responses and 2) to study how antigen-specific T cell responses can be improved. For this purpose, we generated new tools that allow the visualization of antigen-specific T cell responses (chapter 2) and used these to analyze both physiological antigen-specific T cell responses (chapter 2) and antigen-specific T cell responses that were manipulated by using the costimulatory molecule CD70 (chapter 3), or by *in vitro* generation of antigen-specific CD4+ T cells by retroviral gene transfer (chapter 4). In addition, we developed a novel technique that allows the analysis of how antigen-specific T cells subsets arise from a pool of naïve T cells (chapter 5).

With the aim to obtain more insight into antigen-specific T cell responses, we developed and used MHC class II and MHC class I tetramers to directly visualize antigen-specific CD4+ and antigen-specific CD8+ T cell responses in mice that were intramuscularly injected with Moloney murine sarcoma/leukaemia virus (MoMSV) (chapter 2). In addition, we examined the importance of CD4+ T cells in the resolution of MoMSV-induced lesions by depleting CD4+ cells using CD4-specific antibodies. Mice that are intramuscularly challenged with MoMSV develop tumors that spontaneously regress. We showed that this regression is largely dependent on CD4+ T cells. MHC tetramer analysis revealed that significant numbers of antigen-specific CD4+ T cells are detected both in lymphoid organs and in retrovirus-induced lesions early during infection. Importantly, CD4+ T cells were found not only to contribute to the generation of virus-specific CD8+ T cell responses, but also to promote an inflammatory environment within the tumor via the attraction and activation of macrophages. Furthermore, comparison of the kinetics of the MoMSV-specific CD4+ and CD8+ T cell responses revealed a pronounced shift toward CD8+ T cell immunity at the site of MoMSV infection during progression of the immune response. Together, these data confirm the known role of CD4+ T cells in improving antigen-specific CD8+ T cell responses, and stress the importance of CD4+ T cells in the (early) effector phase of the immune response at the site of infection.

With the aim to study whether providing constitutive costimulation can augment antigen-specific T cell responses, we analyzed antigen-specific CD8+ T cell responses in mice that constitutively express CD70 on B cells (chapter 3). CD70 is the ligand of CD27, which is a costimulatory molecule that is constitutively expressed on T cells. We found that transgenic mice that constitutively express CD70 not only show increased antigen-specific T cell numbers, but also have increased effector functions on a per cell basis upon challenge with influenza virus or tumor cells. Importantly, these mice also show increased protection against tumor formation after subcutaneous tumor challenge, indicating that providing increased costimulation can enhance protective immunity. Together, these results suggest that

vaccination strategies to enhance antigen-specific T cell responses can be improved by targeting CD27 on the T cell. However, the previous finding that constitutive CD27 stimulation can also lead to lethal immunodeficiency indicates that it is essential to regulate the amount and duration of CD27 stimulation *in vivo* to avoid detrimental effects.

In addition to vaccination, the adoptive transfer of antigen-specific T cells is an attractive method to enhance antigen-specific T cell responses. As CD4+ T cells play an important role in both the priming and effector phase of CD8+ T cells (see chapters 1 and 2), co-transfer of antigen-specific CD4+ T cells together with antigen-specific CD8+ T cells could potentially increase the therapeutic effectivity. However, the number of well-defined tumor-specific CD4+ T cell epitopes that can be exploited for adoptive immunotherapy is limited. Therefore, we aimed to determine whether helper T cell responses can be generated, by redirecting CD4+ T cells to MHC class I ligands. For this purpose, MHC class I-restricted TCR genes were introduced into peripheral CD4+ T cells by retroviral transduction. *In vitro* experiments showed that upon engagement of the MHC class I-restricted TCRs, CD4+ T cells that are transduced with MHC class I restricted TCRs produce IFN-gamma and IL-2, upregulate CD40L, and are able to induce the maturation of immature dendritic cells (chapter 4). In addition, these CD4+ T cells were able to proliferate and to enhance antigen-specific CD8+ T cell responses *in vivo*. Together, these data provide proof of principle for the generation of high numbers of functional antigen-specific CD4+ T cells using the transfer of MHC class I restricted TCR genes. Importantly, all MHC class I-restricted CD4+ T cell helper functions were found to be highly dependent on CD8 coreceptor function, in particular the function of the CD8β chain. This indicates that in cases where CD8-dependent TCRs are used, cotransfer of both the α and β chain of the CD8 coreceptor is essential.

Over the past years it has become increasingly clear that both antigen-specific CD4+ and CD8+ effector T cell pools consist of subsets that can differ in functional and migratory capacities (see chapter 1). However, how these distinct T cell subsets arise from a pool of naïve T cells remains largely unclear. To study this, we developed a novel approach, called cellular barcoding, in which individual cells are retrovirally labeled with a molecular barcode consisting of a semi-random stretch of DNA. In chapter 5 we show that combining this labeling of individual cells with unique identifiers with a microarray-based detection system allows the analysis of family relationships between the progeny of such cell populations. Subsequently, this cellular barcoding technique was used to study the effect of imprinting on T cell migration. Previous studies in which T cells are activated *in vitro* with DCs derived from different lymphoid organs, suggested that migration properties of effector T cells may be controlled by imprinting that occurs during the interaction of naive T cells with antigen-bearing DC. By determining the lineage relationships between antigen-specific T cells present at two different effector sites (i.e. lung and tumor tissue), we aimed to determine the impact of imprinting on T cell migration *in vivo*. We found that the majority of T cells present in inflamed lung and in tumor tissue consist of T cells that are derived from the same

precursors, whereas only a small pool of precursors give rise to T cells that preferentially migrate to the tumor. These data suggest that the DC-mediated imprinting has only little impact on T cell migration *in vivo*. Further analysis should allow us to determine whether this is a general rule or is dependent on the type of tissue in which antigenic challenge occurs. Together, these data demonstrate that cellular barcoding is an effective tool to study how different populations of T cells arise from a pool of naïve precursors. Furthermore, we think it is likely that this technique also allows the study of lineage relationships between subsets of other cell types.

In conclusion, this thesis offers new insights in the development of antigen-specific T cell responses and shows that provision of increased costimulation or MHC class I restricted CD4+ T cells can augment antigen-specific CD8+ T cell responses. In addition, it provides us with new tools to study antigen-specific T cell responses that should allow us to further improve our knowledge on antigen-specific T cell responses, thereby increasing the chance of successful immunotherapeutic interventions in humans.