Dissection of spontaneous and therapy-induced T cell immunity in mice

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Summary
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Cytotoxic CD8⁺ T cells have the capacity to clear pathogen-infected cells from the body. They specifically recognize small fragments of microbial proteins that are displayed at the cell surface by Major Histocompatibility Complex (MHC) class I molecules. T cells bind to these MHC-peptide complexes with the peptide-specific T cell receptor (TCR). TCRs are somatically generated during T cell development, which ensures a broad range of antigen specificities within the T cell population. In order to prevent the generation of auto-aggressive T cells during T cell development, self-specific T cells are selectively removed through a process called negative selection. Yet, in some cases self-specific T cells can escape this selection procedure. Because of this incomplete negative selection, spontaneous T cell responses can in some cases be observed against self-antigens that are (over)expressed by tumor cells. For instance, spontaneous T cell responses to tumor-derived self-antigens have been observed in melanoma patients. How these tumor-specific T cell responses are induced, however, remains unclear. Many tumors grow at a restricted site outside the lymphoid system. Yet, a prerequisite for T cell activation is that tumor antigens reach the lymphoid organs where naïve T cells exclusively reside. For the induction of tumor-specific T cell responses, tumor cells may migrate to the T cell priming site and directly present the antigen to the naïve T cells within the lymphoid organs, a process called direct priming. Alternatively, professional antigen presenting cells (APCs) take up dying cells in the periphery and transport the antigenic material to the lymphoid organs where the activated APCs cross-present the antigens to naïve T cells, a process termed cross-priming.

To determine the contribution of direct priming and cross-priming in the induction of tumor-specific CD8⁺ T cell responses, we developed a mouse model that allowed us to study tumor-specific T cell induction directly ex vivo (chapter 5). By disruption of either T cell priming pathway, we demonstrated that both direct priming by tumor cells and cross-priming through professional APCs can lead to the induction of tumor-specific CD8⁺ T cell responses.

We next examined if all epitopes can equally well enter the cross-presentation pathway. We speculated that the location of an epitope within a protein could affect the efficiency of antigen cross-presentation. To address this issue, we introduced antigens either in the signal peptide, or in the mature protein (chapter 6). When these fusion genes were introduced into murine tumor cells, direct antigen presentation was independent of antigen location. In contrast, when we analyzed the capacity to induce CD8⁺ T cell responses through cross-priming, antigens located within the mature protein induced massive CD8⁺ T cell responses, but the signal peptide-derived epitopes failed to do so. Our results show that antigen location greatly influences the capacity to induce antigen-specific T cell responses, indicating that cross-presentation is a very efficient but biased pathway for T cell induction. Signal peptides are believed to be rather short-lived whereas mature proteins can be very stable. Therefore, we hypothesize that the antigen stability (i.e. the capacity to accumulate in tumor cells) determines the efficiency of cross-priming.

Antigen-specific T cell induction generally results in the formation of T cell memory that can rapidly become reactivated upon antigen encounter. To determine whether T cell memory also provides protection to antigenic...
variants that contain mutations within the sequence, we studied the cross-reactive capacity of the CD8$^+$ T cell population in an influenza model (chapter 3). We show that restimulation with an antigenic variant resulted in the exclusive expansion of a cross-reactive T cell population that recognized both the original antigen and antigenic variant. This cross-reactivity could also be observed upon rechallenge with several other antigenic variants. Importantly, the cross-reactive T cell population provided at least partial protection from a tumor expressing an antigenic variant, indicating that T cell memory indeed can provide cross-protection towards antigenic variants.

Since CD8$^+$ T cell responses can be elicited towards at least some tumor-associated antigens, vaccination towards these antigens is a promising strategy to treat cancer patients. In the past years, many tumor-associated antigens have been identified, allowing the design of epitope-directed vaccines. One strategy to induce tumor-specific T cell responses is DNA vaccination. Upon administration, somatic cells take up the DNA, transcribe and translate the delivered gene product, which ultimately leads to antigen presentation to the immune system. One aspect that may greatly influence the efficacy of epitope-directed DNA vaccines for T cell induction is the context in which the epitope is expressed. Therefore, we set out to determine several guidelines that help to optimize epitope-directed DNA vaccination (chapter 4). We show that genetic fusion of the T cell epitope to a carrier protein can greatly improve the efficacy of T cell induction by DNA vaccination. In addition, carboxy-terminal fusion to the carrier protein is favored above amino-terminal fusion. Furthermore, we demonstrate that T cell induction by DNA vaccination is critically dependent on CD4$^+$ T cell help, and that the efficacy of vaccination is significantly enhanced when the fusion partner of the epitope is of foreign origin.

In those cases where the T cell repertoire is tolerant towards the tumor antigen or where the immune system is impaired, vaccination is not applicable. In these cases, adoptive T cell transfer of antigen-specific T cells has been shown to be a potent tool to induce tumor regression. This strategy comprises the injection of a large amount of \textit{in vitro} generated tumor-specific CD8$^+$ T cells into the host. Recently, a new strategy has been developed termed TCR gene therapy. This approach comprises gene transfer of the antigen-specific TCR into T cells, thereby introducing a novel antigen specificity into these T cells. In chapter 7, we set out to determine the functionality of these redirected T cells in a mouse model. We show that redirected T cells expressing the novel TCR expand and migrate to the site of infection. In addition, they have the capacity to reject tumors, and in an immunodeficient setting they provide protection for at least two months.

This thesis provides new insights in the fundamental question how T cell responses towards locally expressed antigens are induced. In addition, guidelines are presented to improve epitope-directed vaccines for efficient tumor-specific T cell induction. Furthermore, the feasibility of TCR gene transfer is addressed as a powerful therapy in cases where the tumor-specific T cell population is absent.