The power of help: mechanistic insights into CD4⁺ T cell differentiation in vaccination and cancer

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Appendix

English Summary

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The immune system consists of a wide variety of different cell types and molecules that work together to protect the host against pathogens like viruses or bacteria. It consists of two arms; an innate arm that responds quickly but relatively nonspecific, and an adaptive arm that responds slowly but with much greater specificity and forms immunological memory to protect the host from subsequent reinfections.

In this thesis, I focus on T cell biology, a critical component of adaptive immunity. Multiple T cell subsets exist, but the most prominent and well-studied are CD4$^+$ T cells and CD8$^+$ T cells. T cells use their T cell receptor (TCR) to recognize small peptide antigens, which can be derived from various sources, such as pathogens, food or the host itself, in the context of major histocompatibility complex molecules (MHC); MHC-I for CD8$^+$ T cells or MHC-II for CD4$^+$ T cells, respectively. MHC-I molecules are expressed by almost all cells, whereas MHC-II molecules are much more restricted, and are mainly expressed by immune cells, particularly antigen presenting cells (APCs). As a consequence, CD4$^+$ T cells primarily interact with immune cells.

T cells are activated in the secondary lymphoid organs (SLOs) such as draining lymph nodes (dLN) by APCs, and most notably by conventional dendritic cells (cDCs). Two main subtypes of cDCs exist, namely cDC1 and cDC2. cDC1 cells excel at cross-presentation of cell-associated antigens, mainly to CD8$^+$ T cells, while cDC2s are optimized for antigen presentation in MHC-II to CD4$^+$ T cells. Through antigen presentation, costimulation and cytokine production, APCs shape the subsequent T cell response.

CD4$^+$ T cells can acquire various differentiation states depending on the type of immune challenge. In this thesis, I focus on DNA vaccination and cancer, which in the mouse strain used raises a type-I immune response. In type-I immune responses, CD4$^+$ T cells differentiate to T-helper 1 (Th1) and T follicular helper (Tfh) cells which help CD8$^+$ T cells and B cells, respectively. During an immune response, Th1 and Tfh cells are generated simultaneously, but currently it is unclear how CD4$^+$ T cells 'decide' whether they become Th1 cells or Tfh cells, and how this decision process takes place during T cell priming. Gaining insight in this differentiation process can help us optimize intervention strategies, for example to boost CD8$^+$ T cell help by Th1 cells. Chapter 1 provides the background and the scope of this thesis, where I highlight prior knowledge, as well as gaps in our understanding of CD4$^+$ T cell differentiation and function.

In Chapter 2, I describe that a mouse vaccination model used by our group to study $CD8^+$ T cell differentiation, enables tracing of the differentiation trajectory of functional, polyclonal $CD4^+$ T cells to Th1 and Tfh cells. We identify by flow cytometry and single cell mRNA sequencing that prior to commitment to Th1 and Tfh cell fate, $CD4^+$ T cells adopt a Th1/Tfh precursor differentiation state that is characterized by both Th1 and Tfh cell associated molecules including chemokine receptors and transcription factors. Using TCR-sequencing, we demonstrate that these Th1/Tfh precursors, Th1 cells and Tfh cells share TCRs and are thus clonally related, which implies that they can be formed from the same naïve $CD4^+$ T cell. Antibody-mediated blockade of costimulatory molecules revealed that Th1/Tfh precursor cell formation is dependent on CD28 signaling via either CD80 or CD86, which is likely linked to activation and clonal expansion. The subsequent differentiation of Th1/Tfh precursors to Th1 cells depends on CD40-CD40L interactions, and differentiation to Tfh cells depends on ICOS-ICOSL interaction. Additionally, using a genetic knockout model for cDC1 and antibody-based depletion of B cells, we show that these two APC types are not required for formation of Th1/Tfh precursors, but mediate differentiation of these cells to Th1 or Tfh cells, respectively. This chapter increases our understanding of $CD4^+$ T cell differentiation and highlights potential therapeutic targets for steering $CD4^+$ T cell differentiation towards either Th1 or Tfh in clinical settings.

Next, I investigated how $CD4^+$ T cell activation shapes monocyte responses in Chapter 3. Using a DNA vaccination strategy wherein $CD4^+$ T cells are activated or not, we identify that the DNA vaccine results in attraction of monocytes to the dLN. In the dLN, these monocytes subsequently upregulate DC associated molecules MHC-II and CD11c and therefore we refer to them as MoDCs, in agreement with studies by others. Using extensive spectral flow cytometry, we highlight that MoDCs are not related to cDCs. Interestingly, presence of activated $CD4^+$ T cells amplified this MoDC response. Using antibody-mediated depletion of monocytes, we show that MoDCs are not involved in the initial activation of $CD4^+$ and $CD8^+$ T cells, but are required for $CD4^+$ Th1 and $CD8^+$ T cell effector differentiation. MoDCs in presence of activated $CD4^+$ T cells upregulated costimulatory molecules CD40 and CD80, as well as PD-L1 that can be both costimulatory and coinhibitory, which may explain how MoDCs aid in enhancing T cell effector differentiation. Subsequently, we highlight that $CD4^+$ T cell effector molecules CD40L and IFNγ are the main drivers of enhanced MoDC differentiation. Thus, these data highlight a novel feedforward loop between MoDCs and $CD4^+$ T cells which enhances T cell differentiation, suggesting a potential strategy to counteract immune-suppressive myeloid differentiation, for example in cancer.

$CD4^+$ T cell help for $CD8^+$ T cells is critical for $CD8^+$ T cell effector differentiation and memory formation. In Chapter 4, we investigate how $CD4^+$ T cell help shapes $CD8^+$ T cell differentiation. We identify that $CD8^+$ T cells that are primed in the absence of help cannot fully complete their effector differentiation, and remain in a stem-like $CD8^+$ T cell differentiation state. In the presence of $CD4^+$ T cell help, these stem-like cells are also formed, but they can subsequently differentiate to effector cells. Using adoptive transfer, we show that non-helped $CD8^+$ T cells can still progress to effector cells if help is provided later. Stem-like cells are of great interest as they are the main responders to anti-PD-1 immune checkpoint blockade (ICB) in cancer. We further examined how expression of $CD4^+$ T cell epitopes in tumor cells influences $CD8^+$ T cell differentiation but found that, despite antigen expression, help was not delivered, leading to $CD8^+$ T cell exhaustion due to chronic stimulation. These data argue that for effective tumor clearance by $CD8^+$ T cells, $CD4^+$ T cell effector functions need to be optimized and warrant further research into how help can be delivered in the tumor context.

The immune system also has regulatory mechanisms that prevent autoimmunity or overt activation. A key component of this regulation are Tregs, which dampen immune responses. In Chapter 5, we studied how radiotherapy (RT) affects immune responses in a lymphocyte-depleted cancer model. We found that RT can result in immune cell activation and tumor clearance in a $CD8^+$ T cell dependent fashion, but this is dampened by a simultaneous activation of Tregs. In clinical settings, RT is often combined with anti-PD-1 or CTLA-4 ICB, but in this tumor model, ICB in combination with RT enhanced Treg priming thereby promoting immune inhibition. We demonstrate that the Treg response depended on CD28 costimulation via CD86 and not CD80, and that blockade of CD86 improved tumor control, potentially via reduced Treg priming, improved cDC costimulatory status and $CD8^+$ T cell activation. These data are clinically relevant, as RT and ICB are commonly used anti-cancer therapies and underscore the importance of rational design of combined modality treatment of lymphocyte-depleted cancer.

Finally, in Chapter 6, I integrate the findings from chapter 2-5 with existing literature. I discuss how $CD4^+$ T cell differentiation is regulated in the dLN, the presence and function of Th1/Tfh precursors across various settings such as viral infection and cancer, the parallels between $CD8^+$ T cell differentiation and $CD4^+$ T cell differentiation and how T cell differentiation functions in cancer. I also explore future therapeutic perspectives and potential combinatorial strategies Together, my findings provide mechanistic insight into $CD4^+$ T cell differentiation, $CD4^+$ T cell-based optimization of myeloid and $CD8^+$ T cell responses, and the costimulatory signals deciding $CD4^+$ T cell and Treg responses in vaccination and cancer.