An engineering approach to decode immune responses

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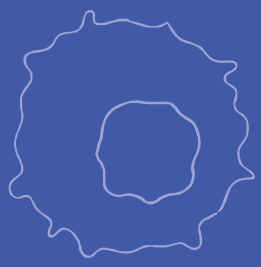
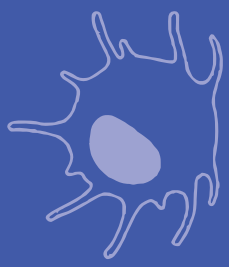
Chapter 1.
Scope of the thesis

OR: “How I scienced the science.”





Throughout this thesis, I have endeavored to apply an engineer's mindset in my pursuit to better understand the marvelously convoluted immune system. In doing so, my colleagues and I have generated a number of new *'hardware'* (i.e., genetically engineered) tools and '*software'* modules (i.e., custom analyses and models) that enable the investigation of several otherwise difficult-to-study concepts. Although we have used these modules here to study immune responses, I hope they may be utilized as tools and approaches to crack outstanding questions in other fields of research. As the work described in this thesis focuses on various aspects of the immune response, I will first briefly touch upon the general organization of the immune system, and subsequently elaborate on the topics relevant for each individual chapter.

The immune system may be viewed as a sophisticated apparatus that is tasked with the identification and eradication of foreign entities. This process occurs through meticulous collaboration between the innate and adaptive branches of the immune system. The innate branch consists of a large number of different cell types (e.g., macrophages, granulocytes and natural killer cells) that collectively recognize a wide variety of common pathogen- and danger-associated molecules. Innate immune cells are generally able to respond quickly upon pathogen encounter, providing a first layer of protection to a nascent infection. In addition, a specialized subset of innate cells, referred to as antigen-presenting cells, are able to leave the site of infection and travel to lymphoid tissues where they can trigger the second branch of the immune system. This adaptive branch comprises T and B cells that are able to recognize foreign antigens in a highly specific manner. During their development, each newly minted T or B cell is endowed with a unique antigen receptor, which determines its antigen specificity. Although the approximate diversity of these antigen receptors present in any given individual is still unresolved, it is likely to be in the order of billions. As a result of this immense diversity, the collective repertoire of T and B cells is able to recognize and respond to any pathogen that is encountered during a lifetime. In addition, due to this massive antigen receptor diversity, T and B cells of a given antigen specificity initially exist in low frequencies. When such 'naïve' lymphocytes recognize their cognate antigen, they transition to an activated 'effector' state and progressively differentiate into various distinct functional subsets that can combat the pathogen. When the pathogen has been subdued, the adaptive immune response enters its final stage, in which the activated lymphocytes will transition to a 'memory' state. Memory lymphocytes are long-lived, potentially persisting for a lifetime, and provide enhanced protection when the same pathogen is encountered later in life. Collectively, through the combined action of all these cell types, the immune system is able to provide tailored responses against many different pathogens.

The recognition and rejection of foreign entities that enter the body is essential to maintain homeostasis. However, in a variety of biomedical studies it is desired to introduce genetically modified cells (e.g., transgenic cells that carry genes encoding fluorescent proteins) into experimental animals such as mice. Due to the exogenous nature of these proteins, such cell transfer experiments are often

plagued by confounding effects caused by the immunogenicity of the transplanted cells. In **chapter 2**, we develop a mouse model in which immune recognition of a large series of reporter proteins is abrogated, thereby providing a solution to this issue. We offer this model (and the methodology through which it was generated) as a tool to the community, and hope it will allow others to perform experiments that would otherwise be impossible.

The antigen presentation machinery continuously samples the intracellular proteome, ultimately leading to the presentation of peptides bound to HLA class I at the cell membrane. These surface-presented peptide-HLA class I complexes are collectively referred to as the HLA class I ligandome, and provides a 'snapshot' of the cellular proteome for scrutiny by T cells. Spontaneous alterations to the proteome—as would be the case during viral infections or through genetic mutations accumulated during tumorigenesis—can result in the addition of foreign peptides to the surface-presented HLA class I ligandome. In turn, such peptides can be recognized by antigen-specific T cells, resulting in their activation and subsequent destruction of the aberrant cells. In **chapter 3**, we set out to better understand the process of peptide selection by the antigen presentation machinery. In this effort we demonstrate that genetically encoded sequence features inform on the likelihood of proteins to yield HLA class I ligands. Importantly, these sequence features can be integrated into a classification model, thereby improving the prediction of HLA class I ligands. The improved predictive models that we generate in this chapter may be of value in studies in which the precise identification of an HLA class I ligandome is required, such as the selection of (neo)antigens for cancer immunotherapy.

The T cell pool can be subdivided into 2 major lineages; $CD4^+$ and $CD8^+$ T cells. Whereas $CD4^+$ T cells provide a supportive function during the immune response, $CD8^+$ T cells directly seek and destroy infected cells. Upon recognition of their cognate epitope, $CD8^+$ T cells enter a phase of rapid clonal expansion, resulting in the generation of a large pool of cytotoxic effector T cells that can combat the infection. A key feature of the $CD8^+$ T cell response is the formation of long-lived central memory T cells (T_{CM}) after antigen clearance. This specialized CD8 T cell subset is able maintain itself long-term through homeostatic cell division and possesses a heightened capacity to mount a secondary cytotoxic response upon antigen re-encounter. In **chapter 4**, we study the relationship between the cell state and function of memory $CD8^+$ T cells, and the extent of clonal expansion that those cells undergo during an immune response. To this end, we engineer a genetic reporter system that exploits low-probability mutations that occur during cell division to induce the expression of a fluorescent protein, allowing one to 'record' the extent of prior proliferation within a cell population of interest. Combining this system with single-cell transcriptomics, we find that the T_{CM} pool is comprised of subsets that have either divided little or extensively, and that this extent of prior division is associated with heightened expression of multipotency- or effector-associated transcripts, respectively. Importantly, we show that the capacity to re-expand into a new wave of cytotoxic cells upon antigen re-encounter is skewed toward memory T cells that had divided little

during the primary response. Our observations in chapter 4 are in support of a model in which during the primary response a sub-group of effector T cells adopts a quiescent phenotype and maintain a less differentiated cell state. These 'sleeper T cells' possess superior replicative capacity upon re-infection and therefore represent an important pillar of adaptive immunity.

As I noted above, T cells only constitute a fraction of the total immune response, functioning alongside a large variety of immune and non-immune cells (e.g., fibroblasts and endothelial cells) that collectively determine the progression of the response. The complex interplay between all these cell types is highly apparent in chronic illnesses that the immune system in unable to resolve, as is the case in solid cancers. During tumorigenesis the constantly growing tissue is often infiltrated by immune cells that subsequently engage in a vast network of immune interactions within the tumor microenvironment. In the event that the cancer cells are not eradicated, the tumor microenvironment eventually reaches a state of homeostasis in which immune cells are unable to recognize—or respond to—the foreignness of the tumor. A relatively young field of research, immuno-oncology, has aspired to understand and manipulate these immune interactions in the tumor microenvironment, aiming to tip the scales in favor of the immune system, allowing it to eliminate the mutated cells. In **chapter 5**, we demonstrate that the enzyme glutaminyl-peptide cyclotransferase-like protein (QPCTL) acts as a pleiotropic modifier of the tumor microenvironment. Using syngeneic tumor models, we observe that genetic deletion of QPCTL increases the quantity of tumor-infiltrating macrophages, favors the differentiation of cancer-associated fibroblasts known for inflammatory function, and rewires a TGF-β dominated environment to an IFNγ dominated one. Importantly, we show that the combination of this ablation of QPCTL activity with an immune-activating agent (anti-PD-L1 treatment) can result in delay of tumor growth and enhanced survival in mice. The findings presented in this chapter suggest that abrogation of QPCTL activity can sensitize the tumor microenvironment to immune-activating agents, and provide support for the development of QPCTL inhibitors.

Finally, in **chapter 6**, I will discuss two separate topics that captured my interest while working on this thesis. In the first part of this chapter, I will focus on a concept that has gained a great deal of popularity in the T cell field, the presence of stem cell like behavior in the memory T cell pool. In the second part of this chapter, I will switch gears to a more societal aspect of science, and discuss the manner in which scientific data is recorded and made available to the community.