An engineering approach to decode immune responses

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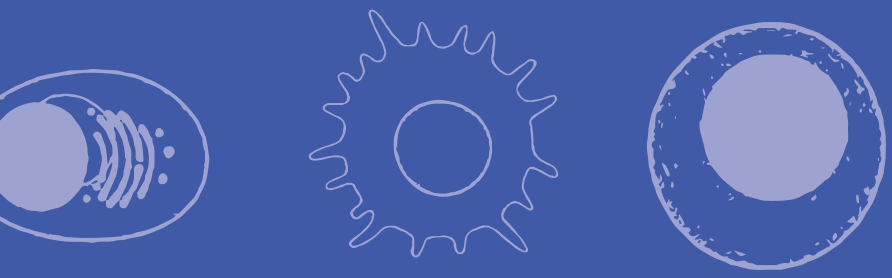
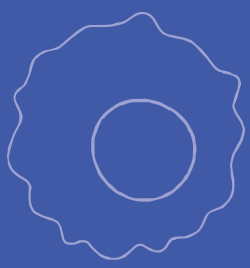
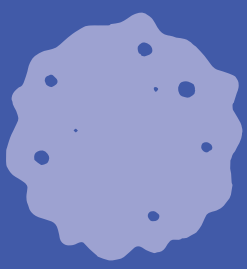


Chapter 7. Appendices

OR: "The part that people actually read."





English summary

In my (perhaps somewhat subjective) judgement, the immune system can be considered to be the most fascinating part of the body. This system encompasses a large diversity of cell types and an unbelievably large number of interactions and processes, all of which are the result of an age-old evolutionary arms race against the pathogens that surround us. While compiling this thesis, I have had the pleasure of studying various components of the immune system, and making small contributions to various topics within the field of immunology. Through this summary, I will provide some general background information on the immune system, and then briefly highlight the findings made in each chapter.

The central task of the immune system is to detect and then destroy 'foreign entities' that are potentially harmful to the body. For example, the detection and recognition of pathogens (e.g. bacteria or parasites) is based on the principle that these organisms look highly different from us. This recognition occurs mainly in two ways: (1) A *non-specific* manner, involving a large group of immune cells collectively termed the 'innate' immune system. These cells often recognize common patterns associated with pathogens, for example certain sugars that are prominently produced by a large family of bacteria and that are not produced by the human body. (2) A *specific* manner, mainly due to the action of T and B cells, collectively referred to as the 'adaptive' branch of the immune system. These T and B cells can recognize foreign proteins via specialized receptors that are present on the surface of these cells, the so-called T and B cell receptors, respectively. During the development of T and B cells, those receptors are assembled through a semi-random process, in which each new T or B cell acquires a unique receptor. Due to this process, there is always at least one T and B cell in the body that can *specifically* recognize a protein of a pathogen that is causing an infection. Once those pathogen-specific cells recognize their target, they become activated and begin dividing at a high rate to generate a large number of daughter cells, all of which can attack the pathogen in a targeted manner: B cells through the production of antibodies, and T cells by the production of cytotoxic molecules or substances that alert other cells to the ongoing danger. Ultimately, in most cases, the foreign entity is defeated and cleared through a coordinated collaboration between the many cells that operate in these two branches of the immune system.

Tricking the immune system

While the recognition of foreign proteins by the immune system is normally a great asset, it complicates in biomedical studies that investigate the physiological 'behavior' of various cell types. In these experiments, cells are frequently studied in the context of a living organism, such as mice (Mus musculus), where it is often crucial that the cells of interest can be distinguished from all other cells. The technological solution generally applied in these cases is to label the cells of interest with a fluorescent protein. This is done by inserting a gene that encodes a fluorescent

protein (often from some type of jellyfish or coral) into the DNA of the cells of interest, after which these cells start producing this protein themselves. After this genetic modification, the cells can be readily distinguished from other cells by their fluorescence. However, when such modified cells are transplanted into a mouse with a competent immune system, those cells are rejected from the host. Not entirely unexpected, since the modified cells now contain large amounts of a foreign protein (e.g., from a jellyfish). For this reason, many types of experiments are difficult to perform, if not completely impossible.

In **chapter 2**, we created a novel genetically modified 'transgenic' mouse model to solve this problem. Our approach is based on the following reasoning; modified cells are rejected because the fluorescent protein is foreign to the body. By ensuring that this protein is already present from birth, it will be recognized as part of the organism, and hence rejection may be prevented. To achieve this, we inserted the genetic code of several frequently used fluorescent proteins into the DNA of mouse embryonic stem cells, which we then used to create a transgenic mouse. For obvious reasons, we did not want all cells in this animal to become fluorescent, and we therefore modified the genetic code of the introduced genes such that they were no longer functional. This approach is described in **chapter 2**. In addition, we show that these transgenic animals have indeed become tolerant to a number of fluorescent proteins, allowing cells that are labeled with these proteins to be transplanted without complications. We have made this transgenic mouse strain available to the academic community.

Predicting the 'appearance' of a cell

All proteins in a cell have a certain lifespan, after which they are broken down into smaller fragments, generally referred to as peptides. The majority of these peptides are further degraded to their individual amino acids, and these are then recycled in new cellular processes. However, a small fraction of peptides is transported into the lumen of the endoplasmic reticulum, bound to a specialized protein named HLA (Human Leukocyte Antigen) class I, and finally transported to the cell surface to be presented to the immune system. Cytotoxic T cells continuously scan HLA class I-peptide complexes on the surface of cells with their T cell receptor, and in this way monitor whether something is amiss with the cell. For example, if cells are infected with a virus, or if mutated proteins are present (as is the case with many cancers), foreign peptides will be presented. These foreign peptides can subsequentially be recognized by a cytotoxic T cell, after which the T cell becomes activated and can clear the affected cells.

The peptides presented via HLA class I thus determine how cells are 'seen' by a cytotoxic T cell. Understanding which peptides are presented by HLA class I is therefore of major interest in therapeutic approaches where activation of cytotoxic T cells is desirable, such as vaccination and cancer immune-therapy.

In **chapter 3** we aimed to acquire more insight into this process by directly measuring which peptides were bound to HLA class I in a number of different melanoma lines. Using these data, we compared genes (and proteins) that either did, or did not, yield HLA-bound peptides using a large database of

gene and protein characteristics. This database contained more than 7,000 characteristics, including the occurrence of sequence motifs and potential protein modification sites. We observed that the genes (and proteins) from which presented peptides were derived often contained or lacked certain of these characteristics, with, for instance, a clear predictive power of certain predicted post-translational modifications. We then incorporated the database of protein and gene characteristics into an algorithm that we trained to predict whether or not peptides are presented by HLA. Finally, we were able to show that the incorporation of this information greatly improved the predictive value of such algorithms.

The role of dormant T cells in immunological memory

Once a T cell becomes activated during an infection, it will start to divide at a high rate. The aim of this proliferation is to generate a large number of daughter cells that all share the same T cell receptor, and can thus all specifically recognize and attack cells that present a specific antigen. When the infection has been successfully cleared, the vast majority of these 'effector T cells' are no longer needed and die off. A fraction of the pathogen-specific T cells remains alive for many years as a relatively stable population. During a reinfection, these 'memory T cells' are able to generate a new wave of effector T cells much faster than during the first infection, thereby offering the body long-term protection against the pathogen. This principle is central to the prophylactic activity of vaccination and, for this reason, the formation and function of memory T cells (and B cells) is a widely studied topic in immunology.

As noted above, cell division is a key feature of the T cell response; however, little is known about the relationship between T cell proliferation during an infection and the formation of T cell memory. In **chapter 4** we set out to study this process in vivo. To this end, we developed a synthetic transgenic construct (termed DivisionRecorder) that contains an inactive gene that encodes a fluorescent protein. The DivisionRecorder was designed in such a way that during each cell division there is a small probability that the gene activates, switching the cell to an irreversible fluorescent state. This means that we were able to use the fraction of fluorescent cells within a population as a measure for the amount of proliferation that had occurred in the past. Using this method, we were able to determine the relative number of cell divisions that different groups of T cells had undergone during, and after, infection. From these data it became clear that there is a large degree of heterogeneity in the number of cell divisions that memory T cells have undergone. In addition, we observed that this heterogeneity was associated with distinct cell characteristics. For example, we identified a group of memory T cells that have undergone only few cell divisions during the immune response. Furthermore, these 'dormant' memory T cells were found to have the highest potential to rapidly divide during reinfection, making these cells a crucial component of immunological memory.

Priming tumors

Like pathogens, cancer cells can be recognized by the immune system as foreign. Indeed, during its development, a tumor is 'infiltrated' by many different types of immune cells. In many cases,

however, the immune system is unable to reject the cancer cells. The subsequently established tumor micro-environment contains a large number of immune-supportive and immune-dampening molecules that exist in a stalemate. For example, fibroblasts may secrete proteins named chemokines that act as signaling molecules to attract different types of immune cells, whereas tumor cells can increase cell surface levels of membrane-bound inhibitory proteins (such as CD47 or PD-L1) that prevent immune cells from attacking the malignancy.

Proteins consist of a chain of distinct amino acids that can—due to their sequence—fold into complex three-dimensional structures. The formation of such structures is crucial for the function of a protein, but is often not sufficient. Additional modifications to the amino acid residues may be necessary for the 'maturation' of the protein. An example of such a modification is the cyclization of glutamine or glutamic acid residues located at the start of a protein chain, which is crucial for the function of certain membrane-bound and secreted proteins, including several chemokines and inhibitory protein CD47. The formation of this modification is catalyzed by the enzyme glutaminyl-peptide cyclotransferase (QPCTL), therefore making this enzyme a potentially important regulator of the balance between immune-supportive and immune-dampening molecules in the tumor micro-environment.

In **chapter 5** we used genetically modified mice and melanoma cells to investigate tumor growth and characteristics of the tumor micro-environment in a scenario in which QPCTL is rendered inactive. Using this approach, we observed that tumor growth was unaffected, but that the composition of tumors was significantly altered in the absence of QPCTL. Interestingly, we found that these alterations to the tumor micro-environment where indicative of a more inflamed milieu. This led us to conclude that QPCTL-deficient tumors might be more sensitive to additional immunotherapeutic treatments. We confirmed this by combining QPCTL deletion with an immune activating therapy (anti-PD-L1 therapy), a combination treatment that resulted in slower tumor growth and sporadic tumor regression. These findings suggest that QPCTL is an interesting target in the treatment of cancer, as an addition to existing cancer immunotherapies.