Orchestrating the immune system to initiate adaptive anti-tumor immunity

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Thesis Summary

Our immune system has evolved to protect ourselves from threats and harm from both outside (pathogens like bacteria) and inside (damaged cells or tumors). The innate immune system is able to respond quickly to predefined pathogen-associated molecular patterns (PAMPs), while the adaptive immune system can be taught to respond to a specific part (antigen) of a pathogen. While arisen in separate events during evolution, the innate and adaptive immune system function in a highly coordinated fashion to ensure an immune response specifically tailored in both space and time when pathogens are encountered. Teaching of the adaptive immune response by the innate immune system also enables quicker response times upon a second encounter of the same antigen, leading to long term protective immunity. Understanding the principles of protective immunity and the underlying innate-adaptive immunity crosstalk may guide rational approaches to vaccine design. Currently, successful vaccines are based on the generation of protective antibodies directed against pathogens, also known as humoral immunity. However, vaccines aimed at generating antigen-specific T lymphocytes to combat viral infections or tumors (cellular immunity) are notoriously difficult to develop. In Part I of this thesis we focus on vaccine compounds that are hypothesized to enhance T cell mediated adaptive immune responses. Using *in vitro* co-culture systems of dendritic cells and TCR-specified T cells, as well as *in vivo* immunization strategies, we test the ability of our novel compounds and vaccination strategies to induce antigen-specific T cell responses. In Part II we explore the modes of immune suppression employed by tumor cells that impede adaptive anti-tumor immune responses.

We hypothesized that in order to rationally formulate vaccine compounds with the capacity to induce adaptive immune responses, the interplay of innate and adaptive immune cells needed to be investigated. In this thesis we explored several vaccine compounds in their ability to induce innate immune activation and antigen-specific T lymphocyte responses. In Chapter 2, we describe the use of outer membrane vesicles (OMVs), derived from genetically engineered bacteria, as PAMP-rich immunogenic vaccine compound. OMVs induced MyD88-dependent dendritic cell activation, antigen processing and antigen presentation to $CD8^+$ T cells. A different bacterial component (inclusion bodies; IBs) was explored in Chapter 3, showing high potential of antigen presentation to $CD8^+$ and $CD4^+$ T cells *in vitro*, although for effective immunization *in vivo*, IBs required additional adjuvants. Protective immunity not only requires innate activation, but also antigen specific to the pathology, preferably delivered to the dendritic cell. In Chapter 5 we report the modification of antigens with a single lipid tail, leading to membrane loading of DCs and potent cross-presentation to $CD8^+$ T cells. To deliver antigen to the dendritic cell efficiently, DC-specific endocytic receptors can be targeted using antigens attached to receptor-specific antibodies or natural ligands. In Chapter 4, we have investigated the mouse orthologue of the endocytic receptor, DC-SIGN. Mouse DC-SIGN (mDC-SIGN) was shown to exhibit overlapping functionality with its human counterpart, allowing antigen targeting for vaccination purposes. By conjugating a model-antigen to an anti-mDC-SIGN antibody, mDC-SIGN-expressing DCs could be targeted *in vivo* for the initiation of adaptive immune responses. Alternatively, the natural ligand of DC-SIGN, the glycan Lewis Y, can be added to a trimeric antigen (Antigen MAtriX; AMAX). The resulting glycosylated AMAX allowed the *in vivo* targeting of DC-SIGN in mice expressing the human form of the receptor on dendritic cells (Chapter 7). DC-SIGN is an endocytic receptor expressed by DCs differentiating from the monocytic lineage. We hypothesized that antigen delivery could be enhanced by first recruiting and differentiating monocyte-derived dendritic cells expressing DC-SIGN to the vaccination site before targeting with a DC-SIGN-specific antigen-antibody conjugate. In chapter 6, we describe the recruitment of DC-$SIGN^+$ monocyte-derived dendritic cells to the skin after sterile adjuvant-induced inflammation. Interestingly, targeting skin-infiltrating moDCs *in situ* using DC-SIGN targeting antibodies led to increases in antigen-specific antibody production and a reduction in antigen-specific $CD8^+$ T cells. Hence, timing local vaccine site inflammation with the antigen affects adaptive immune responses. Concluding Part I, in Chapter 8 we discuss our findings in light of the evolving science of vaccinology. Additionally, we address the question whether vaccine design can be rationally approached, considering the current gaps in basic knowledge on the generation of

protective immunity. Also, we discuss the position of the public, academic science and pharmaceutical companies in the search and support for successful vaccines. Finally, we explore the motivation of academic scientists and how the development of vaccines fits in this motivational scheme.

In Part II we explore the modes of evolutionary escape that allow tumors to evade immune destruction. Using mouse models of tumorigenesis we investigate what forms of immune suppression exist, where they are employed and how they can be blocked to enhance anti-tumor immunity. First, we examine the innate and adaptive immune response in the tumor microenvironment upon PD1 immune checkpoint blockade (ICB; Chapter 10). We show that during PD1 ICB, monocytes infiltrate the tumor and follow a bimodal differentiate pathway, of which the DC-like path correlates with successful anti-tumor immunity in both mouse models and human melanoma patients. Next, we describe the immune finger print of a mouse model of glioblastoma, the most malignant form of brain tumor (Chapter 11). We show that the immune system is changed in the hemisphere contralateral to the bulk tumor and shows an immune suppressed phenotype. We hypothesize that the tumor cells infiltrating the contralateral hemisphere can be targeted by immune checkpoint blockade due to brain-wide efficacy. In Chapter 12 we switch gears to the field of glycobiology, arguing that changes in glycosylation in the tumor microenvironment mark distinct immune suppressed environments (glyco-code). We anticipate that targeting the specific glyco-code in cancer will bolster the recent efforts to increase anti-tumor immunity. To concretize this train of thought, we describe the glyco-code of pancreatic ductal carcinoma (PDAC) in Chapter 13. Using multi-plex microscopy imaging techniques, we show that the aberrant glycosylation changes (increased sialic acid expression) in PDAC are associated with stromal-derived extracellular matrix. Using a variety of PDAC mouse models, we show that host-derived stromal cells express high levels of sialic acid and are key to the fibrosis that is so characteristic of PDAC. We conclude in Chapter 14 that immunotherapy of cancer should ideally be composed of two components: targeting of convergent phenotypes (shared among patients) and unique immunogenic genotypes (unique to individual patients). A multi-dimensional approach to tumor eradication will drive individualized treatment strategies and eventually long-term survival across the many patients groups.

The research described in this thesis is the accretion of five years of research within the framework of a personal search to understand disease and cure. As most of science, the conclusions made and explanations given will be refuted within my lifetime. However, the data is genuine and is expected to withstand the test of time. Such is the fundamental fate of scientific conduct and the personal motivation to repeatedly venture into uncharted scientific territory, in an arguably romantic search of the unknown.

If any man is able to convince me and show me that I do not think or act rightly, I will gladly change; for I seek the truth, by which no man was ever injured. But he is injured who abides in his error and ignorance.
- Marcus Aurelius -