How costimulation directs regulatory T cell responses

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Appendices

English summary

The immune system protects the body from infectious diseases caused by invading microorganisms. Many different cell types collectively shape innate and adaptive immunity. By immediate action after infection, cells of the innate immune system prevent the fast spread of pathogens throughout the body. Cells of the adaptive immune system respond by clonal expansion and functional maturation of the cells that can specifically recognize the infectious agent. This response is slower, but much more precise and robust. The adaptive immune system can memorize responses to specific pathogens, leading to stronger and quicker responses to future re-encounters.

T cells are key players of the adaptive immune system. Each T cell expresses a unique T-cell receptor (TCR) on its cell surface that is used for antigen recognition. Antigens are peptides (protein fragments) that can be presented on major histocompatibility complex (MHC) molecules on the cell surface of other cells. T cells of the $CD4^+$ and $CD8^+$ lineages bind different MHC molecules, and the activation of T cells depends on whether the peptide-MHC complex is recognized. In general, self-antigens do not evoke a T-cell response, whereas foreign antigens do. Foreign antigens can be derived from pathogens, but also from tumor cells that express mutated proteins. Dendritic cells (DCs) are professional antigen-presenting cells that play a key role in the initial activation of both $CD4^+$ and $CD8^+$ T cells. DCs engulf and degrade pathogens and dead cells in tissues, migrate to lymph nodes and present antigens to T cells. When DCs have been activated by pathogens or danger signals, they express high levels of costimulatory molecules and release cytokines that are required for optimal T-cell activation. Next, naïve T cells undergo rapid expansion and differentiation into specialized effector cells. Effector $CD4^+$ T cells cooperate with other immune cells to facilitate immune responses, whereas effector $CD8^+$ T cells become cytotoxic and can directly eliminate target cells.

Immunity is accompanied by immunological tolerance to self, and to harmless non-self-materials such as food. Immunological tolerance can be divided into two branches: central and peripheral tolerance. Central tolerance involves the negative selection (i.e. elimination) of self-reactive T cells during their development in the thymus. Peripheral tolerance ensures that self-reactive T cells that escape central tolerance are made unresponsive in other tissues. DCs play an important role in this process. Without pathogen-derived or danger signals, DCs remain in a tolerogenic state with low expression of MHC molecules and costimulatory molecules. A T-cell response to self-antigens is then prevented by lack of costimulation. Moreover, tolerogenic DCs can give rise to a regulatory T (Treg) cell response. Treg cells are a specialized subset of $CD4^+$ T cells that suppress undesired and excessive immune responses and are essential to prevent autoimmune and inflammatory diseases. In addition, Treg cells contribute to tissue repair and homeostasis.

Research in the past decades has provided great insights into Treg cell biology, from their discovery to their role in health and disease. Treg cells can populate tumors in high numbers, facilitating a suppressive microenvironment that hampers antitumor immunity.

To release this brake, it is desired to inhibit or deplete tumor-infiltrating Treg cells. On the other hand, the function of Treg cells can be utilized to treat autoimmune diseases, transplant rejection and graft-versus-host disease (GvHD). To improve the treatment of the diseases above, we need to understand the signals that direct Treg cells, also in comparison with conventional T (Tconv) cells. Both Treg and Tconv cells are activated by TCR triggering, which drives their responses together with costimulatory signals and cytokines. Treg and Tconv cells have a different intracellular configuration, which likely coincides with differential signal dependencies and responses. As introduced in **chapter 1**, this thesis focuses on the effects of costimulation on the responses of human $CD4^+$ Treg and Tconv cells.

In **chapter 2**, we first lay out different types of Treg cells. Thymus-derived (t)Treg cells develop in the thymus, have a TCR repertoire geared towards self-antigens, and are important suppressors of systemic and tissue-specific autoimmunity. Peripherally induced (p)Treg cells are converted from activated Tconv cells in the presence of specific factors, such as TGF-β, and recognize foreign antigens. pTreg cells are thus far found only at specific locations in the body, such as the intestinal tract. A common protocol to study Treg cells in vitro is to stimulate Tconv cells in the presence of TGF-β, leading to in vitro-induced (i)Treg cells that acquire Treg cell-like properties. There are currently no strategies to isolate human effector tTreg and pTreg cells. However, it is possible to purify naïve tTreg cells. Therefore, we performed a side-by-side phenotypic analysis of tTreg, iTreg and Tconv cells, using published benchmarks that represent core Treg cell characteristics. By protein expression profiling, we conclude that tTreg and iTreg cells are highly distinct and that iTreg cells do not express these core Treg cell characteristics, whereas tTreg cells do. This limits the credibility of iTreg cells as an in vitro model for Treg cells.

Treg cells express diverse costimulatory receptors, two of which we studied in great detail. CD28 is a renown costimulatory receptor on T cells in general, while TNF receptor 2 (TNFR2) has gained attention as an important costimulatory receptor on Treg cells in particular. In **chapter 3**, we propose that these costimuli may have different consequences for Treg cell responses. We analyzed their effects on tTreg cell identity and how the resulting cell populations relate to Treg cells found in vivo. Treg cells are present throughout the body: in peripheral blood, lymphoid tissues (e.g. lymph nodes, spleen) and non-lymphoid tissues (e.g. lungs, muscles). While Treg cells are involved in the regulation of immune responses in lymphoid tissues, they play more specialized roles in immune regulation, tissue repair and homeostasis within non-lymphoid tissues. We show that TNFR2 costimulation drives the differentiation of blood-derived naïve tTreg cells into effector Treg cells as found in non-lymphoid tissues. In contrast, CD28 costimulation maintains a phenotype as found in blood- or lymphoid tissue-resident Treg cells. Our results are important for the development of Treg cell-based therapies. Current strategies to create Treg cell products make use of CD28 costimulation, but we show that TNFR2 costimulation endows tTreg cells with non-lymphoid tissue-specific properties that may be more suitable in treating tissue-specific autoimmune and inflammatory diseases.

T-cell responses are shaped by the interplay of immune receptor signaling with nutrient availability in the microenvironment and cell metabolism. Understanding differential dependencies on metabolic programs may reveal vulnerabilities in Treg versus Tconv cells that can be exploited for therapeutic purposes. Upon TCR/CD28 activation, Tconv cells are known to boost the metabolic pathway glycolysis, that converts extracellular glucose into building blocks for rapid proliferation and effector differentiation. Activated Treg cells were thought to disfavor glycolysis, but we show in **chapter 4** that tTreg cells become glycolytic following TNFR2 costimulation, newly identifying TNFR2 as a metabolic regulator. The increase in glycolytic activity is important for their identity and suppressive function. We also describe adaptations in glucose metabolism, as tTreg cells secrete less lactate, the final product of glycolysis, than Tconv cells.

In **chapter 5**, we build on these findings by studying the connection between glucose or lactate metabolism with lipid metabolism in the same cells. The lipid composition regulates cellular function, differentiation and identity. We show that TNFR2-induced glycolysis fuels fatty acid synthesis in tTreg cells. Importantly, we also describe that tTreg cells can use lactate as an alternative source for fatty acid synthesis. We demonstrate that tTreg cells rely on fatty acid synthesis for their proliferation and suppressive function, while Tconv cells are less dependent on fatty acid synthesis to proliferate. Thus, our findings explain how tTreg cells can function in a low-glucose and high-lactate microenvironment, as present in tumors, but also reveal a specific vulnerability of tTreg cells that might be therapeutically exploited.

Increasing evidence indicates that TNFR2 is an important receptor for Treg cells, but it is not uniquely expressed on Treg cells. For instance, Tconv cells also express TNFR2, albeit at lower levels. In **chapter 6**, we review literature regarding the role of TNFR2 on these cell types. Additionally, we present new data showing that TNFR2 costimulation has a much greater impact on gene expression in tTreg cells. However, we also show that TNFR2 costimulation upregulates glutamine metabolism in both tTreg and Tconv cells, which can sustain proliferation and differentiation. We propose that therapeutic strategies targeting TNFR2 should take into account potential consequences for other cell types than Treg cells.

Among others, points of discussion in **chapter 7** include when and where in the body TNFR2 comes into play in Treg cell biology, how TNFR2 can influence Treg cell differentiation, and how TNFR2 can provide metabolic flexibility in challenging microenvironments. Potential roles of other costimulatory receptors are also considered. Finally, the clinical implications of our findings for Treg cell-based therapy and drug-based targeting are discussed. Treg cell products are currently made using CD28 costimulation, but our findings show that TNFR2 costimulation creates more of an equivalent of non-lymphoid tissue-resident effector Treg cells, which may be optimal for cell therapy. Many autoimmune diseases are currently treated with TNF-blocking agents to inhibit the pro-inflammatory effects of TNF receptor 1 (TNFR1). However, these agents have limitations, as they also block TNFR2 signaling and thereby Treg cell responses. The potential of more selective approaches to modulate TNF signaling at the ligand or receptor level are

discussed, including TNFR2 agonism to combat autoimmunity and inflammation, but also TNFR2 antagonism to increase antitumor immunity.