Modulating T cell homeostasis via TNF and TNFR superfamily members: characterization and function of effector & regulatory T cells
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English Summary

T cell activation requires the recognition of a cognate antigen bound to major histocompatibility complex (MHC) molecules via their T cell receptor (TCR). Hereafter, the clonal expansion, differentiation and polarization of T cells is dependent on costimulatory molecules and the cytokine environment. In chapter 1, T cell activation is described in more detail with a specific focus on the effects of costimulatory molecules, specifically members of the TNFR superfamily, on adaptive immune responses. This thesis focuses on the function of two members of the TNFR superfamily, namely CD27 and GITR, in T cell homeostasis and immune activation.

The glucocorticoid-induced tumor necrosis factor receptor family-related protein (GITR) is expressed on activated T cells and regulatory CD4+ T cells. To gain insight in the function of GITR on these functionally opposing cell types, we developed a mouse model in which GITRL is constitutively expressed on B cells, and assessed the effects of chronic GITR signaling on T cell activation, differentiation and proliferation in vivo (chapter 2). We found that overexpression of GITRL induced an accumulation of both effector and regulatory CD4+ T cells, but it did not affect CD8+ T cells, nor naïve CD4+ T cells. This increase was the consequence of increased proliferation of effector and regulatory CD4+ T cells and not due to enhanced differentiation of naïve T cells. In addition, GITR ligation enhanced the activation state of regulatory T cells, but did not affect their suppressive capacity. Furthermore, the impact of GITRL overexpression on the CD4+ T cell pool was functionally protective in an autoimmune model, i.e. experimental autoimmune encephalomyelitis (EAE), as it significantly delayed the onset of disease. Thus, we conclude that costimulation through GITR provides a functional balance between CD4+ effector and regulatory T cells by enhancing the proliferation of both cell types.
Following recognition of an antigen by the B cell receptor, B cells generally require additional
signals from helper T cells to further differentiate to plasma or memory B cell. We therefore
postulated that the increase in regulatory and effector CD4+ T cells in GITRL TG mice could
affect the humoral immune system (chapter 3). Whereas B cell development and terminal
differentiation of B cells in the spleen was not affected in GITRL TG mice, these mice
showed increased serum IgA titers and decreased IgG3 levels, suggesting modulated B cell
responses. When stimulating splenic B cells in vitro, GITRL TG B cells displayed normal
immunoglobulin production, which indicates that there is no intrinsic B cell defect in these
mice. Interestingly, compared to WT mice the numbers of mucosal B cell subsets were
affected in GITRL TG mice, as peritoneal B1 B cells numbers were decreased and numbers of
B2 B cells were increased, correlating with increased serum IgA immunoglobulin levels. The
increased levels of IgA in GITRL TG mice did not protect these mice against an influenza
infection compared to WT mice. In conclusion, the GITR-GITRL axis of costimulation
modulates the numbers of mucosal related B cell subsets, abrogates IgG3 responses and
promotes IgA responses in vivo.

Similar to effects observed in GITRL TG mice, transgenic overexpression of CD70, the
ligand for CD27, results in expansion of IFNγ-producing CD4+ T cells in vivo. However in
contrast to GITRL TG mice, CD70 TG mice eventually deplete their naïve CD4+ T cell
compartment and show early mortality due to opportunistic pulmonary infections. As
macrophages are key players in defense against pulmonary pathogens and IFNγ is an
important factor in macrophage activation, we assessed the effects of chronic immune
activation via CD70-driven costimulation on the myeloid compartment (chapter 4). CD70
TG mice showed an IFNγ-dependent increase in numbers of activated monocytes, which
expressed high levels of MHC class II, compared to WT mice. These activated monocytes
showed normal phagocytosis and migration characteristics in vitro, but displayed an enhanced
IFNγ-dependent susceptibility to apoptosis. As a consequence, monocytes from CD70 TG mice failed to accumulate at inflammatory sites in vivo and these mice were highly protected against atherosclerosis. These findings reveal that CD70-driven immune activation modulates the myeloid compartment, which on one hand increases the risk of opportunistic infections, but on the other hand protects from atherosclerosis.

Normally, costimulation of T cells via CD27 signaling is dependent on the transient expression of CD70 on cells of the immune system (T, B and DC’s) upon activation. However, during chronic immune activation CD70 is constitutively and highly expressed on activated T cells. Chronic immune activation also results in enhanced formation and exhaustion of effector CD8+ T cells and a failure to develop a memory CD8+ T compartment. To assess the functional consequences of enhanced CD27 triggering on T cell homeostasis during chronic immune activation, we generated mice in which CD70 is constitutively expressed on T cells (CD2-CD70TG). These CD2-CD70TG mice showed increased numbers of CD8+ effector T cells, phenotypically similar to exhausted cells found during chronic inflammation (chapter 5). Interestingly, the formation of CD4+ effector T cells was not affected. CD70-driven costimulation enhanced the primary CD8+ T cells response to influenza A infection, but showed impaired maintenance of memory CD8+ T cells. Thus, we conclude that CD70 driven costimulation deregulates CD8+ T cell homeostasis, reminiscent to events found in chronic inflammation.

Generally, the formation of CD4+ helper T (T_H) cells is dependent on costimulatory molecules and polarizing cytokines. In chapter 6, we investigated whether CD27 signaling influences helper T cell formation by providing instructive, supportive or inhibitive signals for T_H cell differentiation. CD70-driven costimulation enhanced formation of IFNγ-producing T_H1 polarized cells in T_H1 prone C57Bl/6J mice, but did not influence helper T cell formation in T_H2 prone Balb/c mice. In addition, CD27 signaling did not affect the formation or activation
status of regulatory T cells \textit{in vivo}. By stimulating CD4$^+$ T cells \textit{in vitro} in the presence or absence of CD70-mediated costimulation, we found that CD27 signaling supports the formation of IFN$\gamma$, but also IL13 producing CD4$^+$ T cells depending on the cytokine environment and the genetic background, whereas it inhibited T$_{h}17$ polarization. In CD70 TG Balb/c mice the induction of allergic airway inflammation, a typical T$_{h}2$ polarizing pathology, resulted in increased formation of IFN$\gamma$ producing CD4$^+$ T cells, without impairing T$_{h}2$ responses. Furthermore, in line with a supportive rather than an instructive role, CD27 signaling did not modulate transcription factor expression levels. In conclusion, these data indicate that CD27 signaling supports T$_{h}1$ differentiation, permits T$_{h}2$ formation, and inhibits T$_{h}17$ formation.

\textbf{Chapter 7} reviews the current knowledge of costimulation via TNFR superfamily members in general and CD27 in particular and how adaptive immune responses are modulated depending on the timing, context and intensity of these costimulatory signals. In line with the observations presented in this thesis, we propose that excessive costimulation can affect the immunoregulatory balance of the immune system, which can lead to immune pathology.