Modulating T cell homeostasis via TNF and TNFR superfamily members: characterization and function of effector & regulatory T cells
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Chapter 1

General Introduction
General Introduction

The tumor necrosis factor receptor (TNFR) superfamily can be divided into two distinct groups: death domain and TNF Receptor-associated Factor (TRAF) binding receptors. The death domain containing receptors can induce apoptosis via the initiation of caspase cascades by death domain signaling intermediates. In contrast, TRAF-binding receptors can regulate inflammation, lymphoid organization and activation of antigen presenting cells (APC’s). Furthermore, TRAF binding receptors can directly influence B and T cell activation, differentiation and survival. The availability of TNFR-ligands is tightly controlled and this regulation is critical for normal immune function since modulation of the expression of either receptor or ligand results in abrogated responsiveness or rather hyper-responsiveness of the immune system. This thesis focuses on the role of the TRAF-binding receptors CD27 and GITR in immune activation. Transgenic mouse models have been used to determine the direct effects of increased ligand availability prior to and after deliberate immunological challenges.

The immune system

The immune system functions as a protection mechanism that defends the host against invading pathogens and malignant cells. The immune system is build on the basis of a layered system. Pathogens first have to penetrate physical barriers such as skin prior to activating cells of the immune system. Hereafter, these pathogens and distressed cells induce the activation of the immune system.

Cells and products of the immune system can be separated into two distinct categories, namely innate and adaptive. Although an interplay exists between cells of the innate and adaptive immune system, innate immunity is generally considered the first line of defense as it provides a direct but nonspecific response against pathogens. In contrast, the adaptive immune system improves a response for a (subsequent) better recognition of the pathogen.
The resulting increase in pathogen recognition and elimination is exploited to combat a secondary infection.

**Innate immunity**

Cells of the innate immune system have evolved to respond against non-self antigens (pathogens) and signals from distressed cells. Both plants and animals have elaborate innate immune systems, suggesting an origin prior to the division of these lifeforms. Generally, during an inflammatory response cells of the innate immune system (e.g. macrophages, granulocytes, mast cells and dendritic cells) directly convert to highly activated short lived effector cells which attempt to clear the invading pathogen. Activation of these cells occurs through the identification of pathogens (pathogen-associated molecular patterns, PAMPs) via pattern recognition receptors (PRR). These PRR include membrane bound, intracellular and soluble receptors (e.g. mannan-binding lectin, C-reactive protein, and serum amyloid protein), which exert their function through opsonization, activation of complement and coagulation cascades, phagocytosis, activation of proinflammatory signaling pathways, and the induction of apoptosis of infected cells. Furthermore, upon pathogen encounter antigen presenting cells such as DC’s upregulate co-stimulatory molecules (CD80, CD86, TNFR superfamily members and/or their ligands) which may activate cells of the adaptive immune system, thereby complementing the innate immune response. In addition, inflammatory cytokines (e.g. IL4 and TNFα by mast cells and IL12 by DC’s) and chemokines (e.g. TARC, MDC, MIG, IP-10, ITAC by DC’s) produced upon activation of an inflammatory response may facilitate the activation of the adaptive immune system.
Adaptive immunity

The adaptive immune system consist of lymphocytes (NK, B and T cells) capable of generating a memory population for the recognition of specific pathogens, thereby providing a faster and stronger response each time the pathogen is encountered. T cell activation starts when naïve T cells passing through lymph nodes recognize antigens presented by activated dendritic cells on major histocompatibility complex (MHC) molecules. Pathogens present in the cytosol (endogenous pathogens; viruses/ bacteria) are generally digested by host cell enzymes and coupled to MHC class I. In addition, dendritic cells have the capacity to process exogenous proteins derived from other cells on MHC class I. This process, termed cross-presentation, promotes immunity against tissue-specific viruses which do not infect DC’s. The resulting complex promotes CD8\(^{+}\) T cell activation, clonal expansion and induces cytotoxic T lymphocyte (CTL) differentiation. CTL’s function through the production and release of perforins and granzymes which are effector molecules involved in the actual killing of infected cells. In contrast to endogenous pathogens, exogenous pathogens are usually displayed on MHC class II molecules, and induce CD4\(^{+}\) T cell activation.

Naïve T cell activation and subsequent polarization proceeds in response to different extracellular signals. The first signal, TCR triggering via the recognition of a specific antigen bound to the MHC complex results in T cell activation and is followed by a second, co-stimulatory signal which licenses T cell expansion. CD28 is a member of the Ig-superfamily and is constitutively expressed on naïve T cells. Ligation of CD28 by its ligands CD80 or CD86 can provide this secondary co-stimulatory signal. In contrast, the absence of a co-stimulatory signal can lead to T cell anergy. In addition to co-stimulatory signals, T cell polarization is determined via the cytokine milieu (the third signal of the three signal hypothesis) during T cell activation. The resulting polarization of T helper progenitor cells results in the commitment of CD4\(^{+}\) T cells to a specific lineage, which include the T\textsubscript{H1}, T\textsubscript{H2},
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TH17 cells and the distinct regulatory lineages (T_{Reg}, T_{H3}, T_{R1}). The development, characterization and function of these different lineages is discussed in more detail below.

**Subsets of differentiated helper T cells**

**T Helper 1 cells**

A T_{H1} response is characterized by the production of interferon-γ (IFNγ), tumor necrosis factor (TNF) and interleukin-2 (IL-2) and prototypically promotes cell mediated immunity. Cell mediated immunity is generally induced to combat viral infections and intracellular pathogens. This response maximizes expansion of cytotoxic CD8^+ T cells and promotes macrophage activation and differentiation. In a positive feedback loop IFNγ potentiates T_{H1} lineage commitment through increasing IL12 production by macrophages and dendritic cells. IL12 induces the transcription factor T_{Bet} in T cells and thereby T_{H1} commitment and IFNγ production. Conversely, IFNγ lowers the production of IL4 and thus limits T_{H2} lineage commitment^{12, 13}.

**T Helper 2 cells**

In contrast to T_{H1} reactions, T_{H2} responses result in the secretion of IL4, IL5, IL6, IL10 and IL13, and promote humoral immunity. Humoral immune responses are characterized by the production of antibodies, which bind to invading pathogens (e.g. viruses and bacteria) and mark these pathogens for removal by the innate immune system. The cytokines associated with a T_{H2} response are capable of promoting further T_{H2} commitment. For instance, IL-10 is capable of downregulating IL12 production by macrophages and dendritic cells and lowers IFNγ production by helper T cells. In addition, IL4 promotes T_{H2} cytokine secretion and T_{H2} lineage commitment via upregulation of the transcription factor GATA-3, thereby preserving the humoral immune response^{14}. The recognition of T_{H2} promoting pathogens by antigen
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presenting cells results in the upregulation of NOTCH ligands\textsuperscript{15}. This promotes NOTCH activation and cleavage of its intracellular domain on T cells, thus initiating transcription of the IL4 gene and generating a $T_H2$ response\textsuperscript{15}.

$T$ Helper 17 cells

In contrast to the $T_H1$ and $T_H2$ lineages, $T_H17$ cells have only recently emerged as a new T helper lineage. $T_H17$ cells are recognized as IL17 producing cells\textsuperscript{16} and have been implicated as important players in autoimmunity. IL17 induces specific cytokines (IL6, IL1β, TNFα) and chemokines (CXCL1,2,8) associated with activation and migration of neutrophils. Following the identification of the $T_H17$ lineage, the factors resulting in the generation of $T_H17$ cells have been further characterized. Naïve T cells commit to the $T_H17$ lineage after TCR triggering in the presence of TGFβ and IL6 (or IL1β in humans) due to the induction of the transcription factor RORγ\textsubscript{t}\textsuperscript{17-21}. $T_H17$ cells are capable of producing IL17, IL21, IL23 and IFNγ. The upregulation of the IL23 receptor by IL21 and IL6 and the subsequent positive feedbackloop through IL23 production results in $T_H17$ cell proliferation and stabilization\textsuperscript{17-19, 22, 23}. In this respect, formation of $T_H17$ and $T_{Reg}$ are mutually exclusive, but do share similar growth factors for their differentiation. The production of IL6 (murine) or IL1β (human) during inflammation blocks $T_{Reg}$ commitment and promotes $T_H17$ cell formation\textsuperscript{24}. In addition, IL21 inhibits FoxP3 transcription further promoting $T_H17$ cell formation\textsuperscript{25, 26}.

$CD4^+$ Regulatory T cells

Regulatory T cells were first described in the early 70’s as suppressor T cells\textsuperscript{27}. These suppressor T cells remained a discredited subset of T cells until the 90’s, when Sakaguchi and colleagues identified $CD4^+CD25^+$ regulatory T cells ($T_{Reg}$) which developed in the thymus and could be isolated from the periphery\textsuperscript{28}. Over the last couple of years several $T_{Reg}$
populations have been identified. The natural occurring $T_{\text{Reg}}$ ($nT_{\text{Reg}}$) inhibit proliferation and/or function of non-$T_{\text{Reg}}$ through a contact dependent mechanism or via the production of IL10 in vivo$^{29}$. In contrast to the $nT_{\text{Reg}}$ which originate from the thymus, inducible $T_{\text{Reg}}$ ($iT_{\text{Reg}}$) are generated after TCR triggering by tolerogenic DC’s or in the presence of tolerogenic cytokines. Inducible $T_{\text{Reg}}$ include the $T_R1$ cells and the $T_H3$ cells, which respectively suppress T cell responses via IL10 or TGFβ production$^{30-32}$. Although characterization of $T_{\text{Reg}}$ has established increased expression levels of CD25, CTLA-4 and GITR on regulatory T cells, it should be noted that these markers are not unique for $T_{\text{Reg}}$ as they are also expressed by activated non-regulatory T cells. Despite variable expression in $T_R1$ and $T_H3$ cells, the transcription factor FoxP3 was found to be exclusively expressed in murine regulatory T cells resulting in the commitment to a regulatory phenotype$^{33,34}$.

The Influence of the microenvironment on helper T cell polarization

The activation of antigen presenting cells by pathogen-associated molecular patterns via pattern recognition receptors results amongst others in the production of cytokines and chemokines. The cytokine microenvironment influences the commitment of naïve T cells to a $T_H1$, $T_H2$, $T_H17$ or a $T_{\text{Reg}}$ lineage upon activation. Interleukin 12 produced by macrophages or activated B cells induce $T_H1$ commitment, whereas IL4 further promotes $T_H2$ lineage skewing. Although both $T_H17$ and $T_{\text{Reg}}$ use TGFβ for lineage commitment, the presence or absence of IL6 determines which lineage will eventually develop.
Interestingly, negative feedback mechanisms via transcription factors result in a sustained lineage commitment (Fig 1)\textsuperscript{35}.

### Figure 1. Representation of cytokine/growth factor influences on murine CD4\textsuperscript{+} lineage commitment.

T\textsubscript{H} progenitor (T\textsubscript{HP}) cells may commit to a specific lineage under influence of the specified cytokines. Induction of specific transcription factors result in negative and positive feedback mechanisms and sustained lineage commitment. Induction of the T\textsubscript{H1} lineage plays an important role in cell mediated immunity, whereas the T\textsubscript{H2} lineage promotes humoral immunity.

The T\textsubscript{H17} lineage is associated with autoimmunity in contrast to the T\textsubscript{Reg} lineage which regulates T cell activation. T\textsubscript{HP} cells signify naïve CD4\textsuperscript{+} T cells. Dashed lines denote a negative feedback mechanism. Straight lines show positive induction.

### The role of TNFR superfamily members in adaptive immunity

For effective T cell activation, T cells require co-stimulatory signals next to TCR triggering. In addition to providing T cell activation via TCR triggering and influencing the cytokine microenvironment, antigen presenting cells upregulate several ligands of the TNFR superfamily. These signals result in effective T cell activation and polarization (Fig 2). Members of the TNFR superfamily have emerged as important mediators of survival, proliferation and differentiation of T cells during all phases of adaptive immune responses. The TRAF-binding co-stimulatory members of the TNFR superfamily, which include CD40, 4-1BB, CD27, OX40, CD30, HVEM and GITR, have distinct effects on T cell expansion.
Below, the effects of these TNFR superfamily members on B cell and CD4$^+$ and CD8$^+$ T cell function will be discussed.

**Figure 2. Schematic representation of APC effects on T helper cell activation and lineage commitment.**

Antigen presenting cells present antigen to naïve CD4$^+$ T cells via MHC class II molecules thereby providing TCR triggering. The recognition of antigen via pattern recognition receptors induce cytokine secretion and can influence lineage commitment. The upregulation of TNFR superfamily member ligands further influence lineage commitment and affect T cell homeostasis.

**CD40 - CD40L**

Upon TCR triggering, CD40L is transiently upregulated on T cells$^{36}$. The functions of CD40L upon interaction with its receptor CD40 include the upregulation of B7 molecules (i.e. CD80, CD86) on APC’s and the induction of IL12, thereby promoting Th1 responses. CD40L triggering plays an important role in germinal centre development, memory B cell formation and production of IgG, IgA and IgE antibody responses$^{37}$. Indeed genetic defects in CD40-CD40L signaling result in hyper IgM syndrome$^{38}$, thus corroborating its pivotal role in B cell
function and humoral responses. Next to this, hyper IgM patients suffer from severe defects in T cell immunity that can be attributed to the function of CD40-CD40L interaction in the activation of APC.

Studies using CD40L−/− mice confirm its role in TH1 skewing as these mice show a defect in TH1 development39. In addition, blockade of CD40L during a typical TH1 mediated autoimmune disease (experimental autoimmune encephalomyelitis, atherosclerosis, arthritis) results in significant protection in disease development40-45. CD40L blockade is associated with a strong modulation of cytokine profile in mice. The TH1 cytokine IFNg is suppressed whereas IL4 is enhanced46. Furthermore, CD40L expression on CD4+ T cells may directly promote memory CD8+ T cell formation47.

Thus CD40L on T cells indirectly skews T cells to a TH1 phenotype, promotes CD8+ T memory cell formation and helps in memory B cell formation.

4-1BB - 4-1BBL

Similar to CD40L, 4-1BB is transiently upregulated on T cells upon TCR triggering. In addition to indirectly promoting TH1 responses by enhancing IL12 production from myeloid DC’s, 4-1BB is mainly associated with CD8 responses48, 49. Inhibiting signaling via 4-1BB on CD8+ T cells does not directly affect the primary CD8 expansion, but does decrease the accumulation of CD8+ effector type T cells at the peak of an immune response50. Furthermore, an agonistic 4-1BB antibody was capable of preventing apoptosis of CD8+ T cells stimulated with superantigen51. Moreover, 4-1BBL−/− mice showed a defective CD8+ T cell response following viral infection52 and the absence of 4-1BB or its ligand had no effect on CD4+ T cell responses during viral challenge with influenza, vesicular stomatitis virus or LCMV52-54. The transgenic overexpression of 4-1BBL under control of the MHC class II I-Eα promoter further revealed a prominent role of 4-1BB – 4-1BBL interactions in humoral
responses as these mice showed a progressive depletion of mature B cells\textsuperscript{55}. Thus, the TNFR superfamily member 4-1BB mainly functions as a costimulatory receptor for CD8\textsuperscript{+} T cells, has only slight effects on CD4\textsuperscript{+} T cell responses and may influence B cell survival.

**CD27 - CD70**

The TNFR superfamily member CD27 is expressed on naïve, activated and memory T cells, hematopoietic progenitor cells, NK cells and a subset of B cells\textsuperscript{56-59}. In collaboration with TCR engagement ligation of human CD27 by its ligand CD70, which is upregulated on B cells and DC’s upon activation, promotes the expansion of IFN\textgamma producing CD4\textsuperscript{+} (i.e. T\textsubscript{H1} cells) and CD8\textsuperscript{+} T cells\textsuperscript{36, 60-62}. In addition, expression of the IL12 receptor is upregulated thereby making CD4\textsuperscript{+} T cells receptive to T\textsubscript{H1} polarization and enhances the transcription of the transcription factor T\textsubscript{Bet}\textsuperscript{63}. Furthermore, studies using genetically modified mice have shown that CD27 ligation is important for memory T cell formation\textsuperscript{61}. Loss of CD27 signaling decreased CD4\textsuperscript{+} and CD8\textsuperscript{+} T cell accumulation in the lung following influenza infection and decreased formation of influenza specific T cells in the periphery. In addition to defects in primary T cell responses, loss of CD27 signaling impairs the formation and optimal secondary expansion of memory T cells\textsuperscript{61, 64, 65}. CD27 signaling promotes T cell accumulation via stimulating survival of activated T cell, and does not affect T cell proliferation\textsuperscript{66}. Therefore, CD27 signaling promotes cellular immunity as this functions as a co-stimulatory receptor for both CD4\textsuperscript{+} and CD8\textsuperscript{+} T cell expansion.

**OX40 – OX40L**

Expression of the TNFRSF member OX40 is restricted to activated T cells, in which OX40 is preferentially expressed on CD4\textsuperscript{+} T cells\textsuperscript{67, 68}. Expression of the ligand (OX40L) is upregulated upon activation of B cells\textsuperscript{69, 70}, T cells\textsuperscript{71} and DC’s\textsuperscript{72}. In addition, OX40L is expressed on epithelial cells\textsuperscript{73}. The function of OX40 triggering on T cells in respect to
lineage commitment does not appear to be specific. OX40 has been implicated in increasing both Th1 as well as Th2 responses and promotes a response subsequent to lineage commitment depending on the cytokine environment\textsuperscript{74-78}.

\textit{CD30 – CD30L}

The TNFRSF member CD30 is expressed on activated T cells, B cells, NK cells and on eosinophils\textsuperscript{79-81}, whereas its ligand is expressed on activated T cells and B cells. CD30 is preferentially expressed on Th2 cells, although naïve T cells and Th1 cells can express CD30\textsuperscript{82}. The cytokine IL4, associated with Th2 skewing, results in upregulation of CD30\textsuperscript{79, 83}. In addition to its importance for the Th2 lineage, CD30 can affect CD8\textsuperscript{+} memory T cell survival\textsuperscript{84}.

\textit{HVEM - Light}

The herpes virus entry mediator (HVEM) is expressed on resting T cells, monocytes and immature dendritic cells. Expression of its ligand (Light) is induced on T cells upon activation and is expressed on monocytes, NK cells and immature DC’s\textsuperscript{85-89}. In contrast to the TNFRSF members described above, HVEM is downregulated upon T cell activation; thereby limiting the time in which HVEM functions as a costimulatory receptor for T cells\textsuperscript{89}. The costimulatory function of HVEM on T cells becomes apparent in Light transgenic mice, which show a clear autoimmune phenotype. CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells are expanded and these mice showed severe inflammation in the intestine. In addition, splenomegaly and lymphadenopathy was observed\textsuperscript{90-92}. Studies using Light KO mice suggest that HVEM-Light interactions have a significant impact on memory CD8\textsuperscript{+} T cell formation, as CTL recall responses were significantly decreased following immunisations with a viral peptide\textsuperscript{93}. Therefore, HVEM can be seen as an important player in T cell expansion and memory formation.
GITR - GITRL

The glucocorticoid-induced TNFR family-related gene (GITR) is highly expressed on activated T cells and regulatory T cells. Studies using recombinant GITRL or agonistic antibodies to stimulate GITR suggest that GITR functions as a costimulatory factor for T cells upon TCR triggering\textsuperscript{94-97}. As of yet, the specific function for GITR on regulatory T cells remains unresolved. Ligation of GITR on T\textsubscript{Reg} has first been associated with neutralization of the suppressive capacity of these cells and later dismissed as non-regulatory T cells escaped suppression upon GITR triggering\textsuperscript{98}. The effects of GITR engagement in vivo on skewing of CD4\textsuperscript{+} T cells to a T_{H1}, T_{H2}, T_{H17} or T_{Reg} phenotype have not yet been determined.

The impact of genetic modification of TNFRSF member expression

The function of TNFRSF members on T cell homeostasis has extensively been investigated using knockout and/or transgenic mice. In table 1 an overview is given on the effects on T cell homeostasis using gene targeted and transgenic mice.
Table 1  Immunological consequences of gene targeting the TNFR superfamily members

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Expression</th>
<th>KO</th>
<th>TG</th>
<th>Phenotype</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD27</td>
<td>T &amp; B cells NK cells</td>
<td>Full KO</td>
<td>-</td>
<td>Reduced CD4 and CD8 T cell expansion and memory formation to influenza; normal primary T cell response, but reduced memory response to LCMV.</td>
<td>62, 99</td>
</tr>
<tr>
<td></td>
<td>Progenitors</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD70</td>
<td>T &amp; B cells DCs</td>
<td>-</td>
<td>B cell-TG</td>
<td>Enhanced CD4 and CD8 effector T cell formation; improved clearance of influenza and tumor; splenomegaly and IFNγ dependent B cell depletion; premature death due to opportunistic infection.</td>
<td>62, 106, 101</td>
</tr>
<tr>
<td></td>
<td>DCs</td>
<td>DC-TG</td>
<td>Enhanced CD4 and CD8 effector T cell formation; lack of CD8 deletional tolerance; splenomegaly and B cell depletion.</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T cell-TG</td>
<td>T cell-TG</td>
<td>Enhanced CD8 effector T cell formation, but impaired maintenance of memory CD8 T cells; only minor splenomegaly and B cell depletion.</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td>4-1BB</td>
<td>T &amp; B cells NK cells</td>
<td>Full KO</td>
<td>-</td>
<td>Reduced CD8 T cell response to VSV; enhanced CD4 T cell response to protein immunization.</td>
<td>54, 104</td>
</tr>
<tr>
<td></td>
<td>DCs</td>
<td>T cell-TG</td>
<td>Enhanced T cell proliferation in vitro and increased CHS.</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td>4-1BBL</td>
<td>B cells DCs</td>
<td>Full KO</td>
<td>-</td>
<td>Reduced CD8 T cell expansion and memory formation upon acute infections; normal CD8 T cell numbers but impaired function during chronic infection.</td>
<td>32, 51, 106, 107</td>
</tr>
<tr>
<td></td>
<td>Macrophages</td>
<td>-</td>
<td>B cell-TG</td>
<td>Normal T cell response, but reduced APC function during allogeneic stimulation; splenomegaly, B cell depletion and reduced IgG responses.</td>
<td>55</td>
</tr>
<tr>
<td>CD30</td>
<td>T cells</td>
<td>Full KO</td>
<td>-</td>
<td>Reduced T cell expansion and IFN-γ production by CD4 T cells upon mycobacterium infection; contradictory reports on thymic negative selection.</td>
<td>99-110</td>
</tr>
<tr>
<td></td>
<td>T cell-TG</td>
<td>T cell-TG</td>
<td>Enhanced thymocyte apoptosis upon stimulation; splenomegaly.</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>CD30L</td>
<td>T &amp; B cells Macrophages</td>
<td>Full KO</td>
<td>-</td>
<td>Reduced CD8 memory T cell formation upon Listeria infection; reduced CD4 effector T cell formation upon mycobacterium infection.</td>
<td>98, 112</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>Soluble form</td>
<td>Resistant to infection with HSV-1, but not pseudorabies virus.</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>HVEM</td>
<td>T &amp; B cells DCs</td>
<td>Full KO</td>
<td>-</td>
<td>Enhanced T cell activation and cytokine production.</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>Macrophages</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Light</td>
<td>T cells DCs</td>
<td>Full KO</td>
<td>-</td>
<td>Reduced CD8 T cell expansion and effector cell formation to SEB or peptide; reduced CD8 T cell proliferation and CD4 T cell IL-2 production in MLR.</td>
<td>98, 115</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>T cell-TG</td>
<td>Enhanced CD4 and CD8 effector T cell formation; splenomegaly, autoantibodies and inflammation of several organs; reduced thymic output.</td>
<td>116, 92</td>
<td></td>
</tr>
<tr>
<td>OX40</td>
<td>T &amp; B cells DCs</td>
<td>Full KO</td>
<td>-</td>
<td>Reduced CD4 T cell proliferation and effector cell formation to infection with influenza and LCMV, but not with L. major, N. brasiliensis or TMEV; reduced formation of effector and memory CD8 T cells to VACV infection.</td>
<td>117-119</td>
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<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>OX40L</td>
<td>T &amp; B cells DCs</td>
<td>Full KO</td>
<td>-</td>
<td>Reduced CD4 T cell proliferation and effector cell formation to protein immunization; reduced CD8 T cell response to allogeneic stimulation.</td>
<td>49, 72</td>
</tr>
<tr>
<td></td>
<td>Macrophages</td>
<td>-</td>
<td>DC-TG</td>
<td>Increased CD4 accumulation in B cell follicles.</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>T cell-TG</td>
<td>T cell-TG</td>
<td>Enhanced CD4 T cell responses, inflammation of lung and intestine; more severe EAE; enhanced Th2 response and impaired clearance of L. major.</td>
<td>121, 122, 123</td>
<td></td>
</tr>
<tr>
<td></td>
<td>All cell-TG</td>
<td>All cell-TG</td>
<td>Increased CD4 T cell numbers, enhanced CHS and allogeneic response.</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>GITR</td>
<td>T cells</td>
<td>Full KO</td>
<td>-</td>
<td>Reduced regulatory CD4 T cell numbers; reduced effector T cell activity and disease intensity during experimental colitis or arthritis; enhanced CD4 effector T cell formation upon Candida infection.</td>
<td>98, 123</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>GITRL</td>
<td>B cells</td>
<td>-</td>
<td>B cell-TG</td>
<td>Enhanced in vivo effector and regulatory CD4 T cell proliferation, delayed disease induction in EAE model.</td>
<td>This thesis</td>
</tr>
</tbody>
</table>

Expression profile of costimulatory members of the TNF-R superfamily and the phenotype of genetically mutated mice, in which these respective molecules have either been deleted (KO) or transgenically expressed (TG).
Chapter 1

Scope of this thesis

The activation and interplay between both an innate and adaptive immune response is highly regulated though cellular ligands and receptors on cells of the immune system. Here, we assessed the function of two members of the TNFR superfamily in T cell homeostasis and immune activation, namely CD27 and GITR, via modulation of the expression of their respective ligands CD70 and GITRL.

GITR ligation has been implicated in T cell costimulation and abrogation of the suppressive capacity of regulatory T cell in vivo\textsuperscript{94, 95, 128-130}. To assess the effects of constitutive GITR ligation, we have generated mice in which GITRL was constitutively expressed on B cells (chapter 2). GITRL TG mice showed a specific increase in CD4\textsuperscript{+} effector and regulatory type T cells due to enhanced proliferation of these T cells, whereas the naïve T cell compartment was not affected. In addition, GITR triggering did not affect the suppressive capacity of regulatory T cells. Thus, GITR ligation in vivo influences the maintenance CD4\textsuperscript{+} effector and regulatory T cells via enhanced division. In chapter 3, we investigated the effects of GITR-GITRL signaling in humoral immunity. Terminal differentiation of splenic B cells remained unaffected in GITRL TG mice, whereas numbers of peritoneal B1 and B2 B cells were modulated. GITRL TG mice showed increased IgA serum immunoglobulin levels, correlating with an increase in numbers of peritoneal B2 B cells. Chapter 4 describes the indirect effects of CD27 ligation on T cells on innate immune responses. Although CD70 TG mice had a highly activated monocyte compartment, it was found that these monocytes showed a reduced response to sterile induced peritonitis and were protected against atherosclerosis in an atherosclerotic model. In chapter 5 we investigated the effects of CD70 co-stimulation by non-APC’s. To this end, we generated CD70 TG mice which expressed CD70 on T cells. These CD70 TG mice showed increased numbers of CD8\textsuperscript{+} effector T cells and enhanced CD8
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T cell responses to influenza A infection. Due to increased activation induced cell death, CD4$^+$ T cell help was hampered resulting in decreased T and B cell memory responses. Chapter 6 describes the function of CD27 ligation on T cell polarization. We found that CD27 ligation promoted formation of IFN$\gamma$ producing T$_{H1}$ polarized cells in T$_{H1}$ prone C57Bl/6J mice, but did not enhance helper T cell formation in T$_{H2}$ prone Balb/c mice. However, induction of allergic airway inflammation in CD70nTG Balb/c mice showed that CD27 signaling plays a supportive role in T$_{H1}$ differentiation, without modulating the classical T$_{H2}$ response. In conclusion, chapter 7 discusses how immunodeficiency’s may occur through deregulated expression of TNFR and ligand superfamily members.
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General Introduction


