Contributions of CD27 and relatives to the specific immune response
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Chapter 1

General Introduction
GENERAL INTRODUCTION

To defend us from invading pathogens such as bacteria and viruses and malevolent threats from within, our bodies are equipped with a most formidable defence system: the immune system. The immune system consists of adaptive and innate components, which operate on very different principles. The innate immune system acts as the first line of defence against invading pathogens by causing an inflammatory reaction in response to foreign elements. Cells of the innate immune system such as mononuclear phagocytes, granulocytes and other phagocytic cells, recognise non-self molecular patterns such as lipopolysaccharides on the surface of microbes and destroy them by phagocytosis. The recognition of non-self particles by the adaptive immune system is mediated by antigen receptors, expressed on B and T cells. The specificity of these receptors varies tremendously, which ensures specific recognition of any pathogen by these cells. Cells of the adaptive immune system that recognise foreign patterns with their antigen receptor will multiply to form an army of lymphocytes specific for the pathogen, and will differentiate to become better equipped to eradicate the pathogen from the body. After the pathogen is cleared, immunological memory will have been established, in the form of antigen-specific cells that remain in the body. This ensures that the immune system responds better and faster to rid the body of foreign invaders upon a renewed infection with that pathogen.

The adaptive immune system

B cells provide the humoral arm of the adaptive immune system by secreting soluble forms of their antigen receptor upon encounter of the antigen. These co-called immunoglobulins (Ig) or antibodies facilitate the phagocytosis of extracellular pathogens, prevent intracellular infection and mediate lysis by complement. After activation by particular antigens, which requires T cell help, B cells form germinal centers. Here, B cells proliferate extensively and undergo isotype switching, leading to the expression of IgG, IgA or IgE instead of IgM or IgD. Somatic hypermutations take place to increase the affinity of the antibody for the antigen. The germinal center ultimately produces plasma cells secreting high affinity antibodies and memory B cells, which reside in the body, awaiting possible re-infection with the same antigen (1).

T cells recognise, with their T cell antigen receptor (TCR), short peptides presented in major histocompatibility complex (MHC) molecules at the cell surface of antigen presenting cells (APC) or infected cells. T cells can be divided into two major subsets: CD8+ T cells and CD4+ T cells. CD4+ T cells are activated when their TCR recognises an MHC class II/peptide complex expressed on the surface of APCs. Subsequently, they will develop into helper- or regulatory CD4+ T cells. CD4+ helper T cells aid B cells and CD8+ T cells by direct cell-cell interactions and the secretion of cytokines. CD4+ regulatory T
cells on the contrary suppress the T cell response, limiting the risk of chronic infection or autoimmunity. CD8⁺ T cells, which recognise mostly intracellularly derived peptides presented in MHC class I molecules, serve to kill infected cells or malignant cells, thereby preventing the spread of the pathogen throughout the body and eliminating the source of illness.

The unique feature of the adaptive immune system that guarantees that every short peptide (8-11 amino acids long) derived from non-self proteins is recognised is the fact that every B- and T cell carries a different antigen receptor. The composition of these immunoglobulin-like surface proteins gives rise to a great diversity of antigen specificity. The conventional TCR is a heterodimer of the α and β chains, which are each encoded by a constant gene segment, one of up to 100 variable segments, one of up to 50 joining segments, and 2 joining segments in case of the β chain (2). In addition to the somatic rearrangement of these gene segments, the variety of TCR α and β chains is even more increased by additional random mutation of the Complementarity-Determining Region 3 (CDR3) region, which determines peptide specificity. These mechanisms ensure that each T cell expresses a TCR with unique specificity, leading to a repertoire equipped for the recognition of all non-self peptides.

The drawback of a random system of TCR gene rearrangement is the risk to create potentially harmful self-specific TCR molecules. Therefore, all T cells carrying successfully rearranged TCRs are selected during a very tightly regulated process in the thymus. T cells that express a TCR that can interact with MHC molecules are positively selected, while T cells failing to express functional TCR protein die by neglect. To prevent the presence of auto-reactive T cells in the periphery that could cause autoimmunity, T cells that recognise self-antigens are negatively selected and die by apoptosis. T cells are negatively selected for auto-reactivity to ubiquitously expressed proteins, as well as for auto-reactivity to organ-specific antigens (3). Recently, the transcription factor Aire was found to regulate expression of organ-specific proteins, enabling negative selection of auto-reactive T cells specific for antigens only expressed in the periphery (4,5). Still, some auto-reactive T cells may escape negative selection, due to lack of expression of the self-antigen or due to low affinity of the TCR. Several mechanisms in the periphery prevent these potentially auto-reactive T cells from causing harm. First, auto-reactive T cells may be deleted in the periphery by apoptosis. Second, an unresponsive state may be induced in the T cell, named T cell anergy, when an auto-reactive T cell is activated in the absence of the required costimulatory signals. Third, an auto-reactive T cell may, probably due to very low affinity of the TCR, not respond to the self-antigens it is specific for (T cell ignorance). Furthermore, T cell extrinsic factors such as tolerance-modulating dendritic cells (DCs) and regulatory T cells play a role in keeping potentially auto-reactive T cells in check (6).
T cell dynamics

Equipped with a score of naive T cells that in all express a multitude of different TCRs, which are selected to recognise pathogen-derived peptides, the immune system needs only to be activated to start fighting off invading micro-organisms or eliminate malignant cells. Professional APCs like macrophages and DCs constitutively scout the periphery for pathogenic structures. These structures can consist of whole pathogens, infected cells or dead cells (either necrotic or apoptotic), or derived products that are taken up by macro-pinocytosis. Binding of specialized receptors on the surface of DC to specific structures facilitates endocytosis of these structures by immature DC. Pathogen recognition and uptake are often accompanied by activation and maturation of the DC. During maturation the endocytic capacity of the DC is downregulated, which limits the range of antigens presented to T cells. DC can be induced to mature by the direct recognition of antigens through specific pattern recognition receptors or indirectly by sensing inflammatory mediators such as cytokines or receiving signals from other inflammatory cells. Toll-like receptors (TLRs) expressed on DC can recognise bacterial compounds such as lipopolysaccharide, unmethylated CpG motifs and double stranded RNA. Triggering of TLRs results in the maturation of the DC, increasing the expression of MHC/peptide complexes, upregulation of costimulatory molecules and secretion of immunomodulatory cytokines (7). Upon maturation, DC migrate out of peripheral tissues to the secondary lymphoid organs where final maturation takes place. Full maturation of DC by activated CD4+ T cells has been shown to involve CD40-CD154 interactions, which are crucial for effective CD8+ T cell priming (8-10). Mature DC express more adhesion- and costimulatory molecules, such as CD80 and CD86, which are ligands for the costimulatory receptor CD28 on T cells. Mature DC present processed pathogen-derived peptides to naive T cells which, when expressing the appropriate TCR and having received the required costimulatory signals, will be activated.

Activation of the T cell will set a program into motion, leading to proliferation of the T cell, ensuring an enlarged population of antigen-specific T cells (Figure 1). Studies have shown that, once proliferation is initiated, the presence of antigen is no longer needed for proliferation (11). Indeed, 24 hours of T cell stimulation by APC was sufficient for ongoing clonal expansion (12,13). In addition to proliferating, these T cells were also found to differentiate and acquire effector T cell functions such as cytotoxicity. In vivo, T cells could differentiate into memory T cells following a brief stimulation in vitro. The effectiveness of the T cell response is still determined by the duration of antigenic stimulation since overly brief contact with antigen will result in abortive T cell proliferation and function (14).

In contrast, persistent antigenic stimulation, such as chronic infection, does not guarantee optimal T cell immunity, since in some studies T cell responses and memory T cell formation are abrogated (15,16).
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Figure 1. Dynamics of the antigen-specific T cell response. T cells, upon activation during priming, proliferate to expand the antigen-specific T cell pool and differentiate to effector T cells. They then migrate to the site of infection where they exert their effector functions. After the pathogen is cleared from the body, most antigen-specific T cells die during the contraction phase. A few memory T cells survive and reside in the body and upon rechallenge they can mount a secondary response that is faster established, greater in magnitude and is made up of T cells that are effective in eliminating the pathogen.

During expansion, CD8⁺ T cells will acquire properties that enable killing of infected or malignant cells, such as perforin and granzyme B production and the formation of cytolytic granules. Interferon-γ (IFN-γ) production is upregulated, which may be used as a measure of differentiated effector CD8⁺ T cells. CD4⁺ T cells differentiate into one of two major subtypes of cytokine producing helper T cells. Type 1 CD4⁺ T cells produce mainly IFN-γ, tumor necrosis factor (TNF) and interleukin-2 (IL-2) which serve to support CD8⁺ T cell immunity, while type 2 cells aid humoral responses by producing IL-4, -5, and -10 (17).

To reach the site of infection, T cells acquire the capacity to migrate out of the secondary lymphoid organs and into non-lymphoid tissues (18,19). Although the precise mechanisms are as yet unclear, downregulation of lymphoid homing molecules such as CD62L and upregulation of CD44 and integrins in order to enter peripheral tissues may be involved (20). Loss of the CC chemokine receptor (CCR7) may be part of the mechanism of disengagement from lymphoid tissue (21). After activation, T cells gain the expression of several chemokine receptors (e.g. CCR3, CCR5 and CXCR3) to guide them to peripheral sites of inflammation. More specifically, T cells seem to be instructed during priming on which sites of infection to migrate to. It was found that DC isolated from Peyer’s patches in the gut elicited upregulation of α4β7 integrin on T cells (22), which is involved in CD4⁺ T cell trafficking to the intestinal mucosa (23). In addition, certain types of T cells may specifically upregulate chemokine receptors that support their particular purpose. CCR3 is found to be enriched on type 2 CD4⁺ T cells and thought to play a role in allergic inflammation (24). On type 1 CD4⁺ T cells CCR5 and CXCR3 are preferentially expressed and suggested to be involved in inflammatory lesions of an autoimmune nature (25).

After migration to the site of infection, antigen-specific T cells exert their effector function in order to eliminate the pathogen from the body. CD8⁺ T cells are equipped with cytolytic
compounds such as perforin and granzyme B stored in granules and express the apoptosis-inducing CD95 ligand to eliminate antigen-bearing target cells (26). Thereafter, large populations of antigen-specific T cells are no longer required, so the antigen-specific T cell population involutes to minimal proportions, typically 5-10% of the clonal burst size (27) (Figure 1). This contraction of the antigen-specific T cell pool may be independent of duration of infection and amount of antigen (28). The role of the death receptor CD95 as a mediator of antigen-induced cell death (AICD) during T cell contraction is still unclear. While some studies show a requirement for CD95 in death of activated T cells, others do not (29-31). A second mechanism of T cell contraction involves the withdrawal of cytokines after the inflammation wanes. Here, T cell death is dependent on the pro-apoptotic BH3-only protein Bim which can be suppressed by Bcl-2 overexpression (32,33).

The number of memory T cells remaining depends on the size of initial clonal expansion, however, the selection of memory T cells may not be a stochastic process. Memory T cell survival and formation requires the cytokine IL-7. During the peak of the antigen-specific response, future memory T cells could be identified by the expression of the IL-7 receptor α chain (34). Thus, it was postulated that the formation of memory T cells is not determined by withstanding death during the contraction phase, but rather T cells are instructed to become memory T cells early during T cell priming. Recent studies have shown that CD4⁺ T cells are essential in generating functionally competent memory CD8⁺ T cells (35-37). Primary CD8⁺ T cell responses were generated normally in the absence of CD4⁺ T cells, however, recall responses were abrogated (35,36). The necessary CD4⁺ T cell signal is conveyed early during priming, since depletion of CD4⁺ T cells three days following priming did not affect secondary responses (37). The precise signal that supports memory T cell formation is unknown but may involve the direct interaction of CD40 on CD8⁺ T cells with the ligand on CD4⁺ T cells (38).

Memory T cells do not form a homogenous population, rather two subsets can be distinguished based on their anatomical location, expression of cell surface markers and effector functions (39). Central memory T cells express CD62L and CCR7 which allow homing to lymph nodes. Effector memory T cells reside in non-lymphoid tissue and do not express these markers. Although it was first thought that both populations develop separately, recently a linear relationship of effector memory T cells converting to central memory T cells was suggested (40).

The presence of T cell memory is not only the consequence of higher precursor frequency of antigen-specific T cells compared to the naïve situation, but also lies in the fact that memory T cells are qualitatively better than their naïve counterparts. Memory T cells can respond faster to renewed challenge with the same antigen, since after a shorter lag time, they proliferate faster than naïve T cells (41). In addition, memory T cells differentiate
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faster to fully functional effector T cells as judged by increased production of cytokines such as IL-2 and IFN-γ, and perforin expression (41,42). In fact, memory T cells were shown to lyse target cells in vivo within hours of recognition, exhibiting similar cytotoxic effectiveness as effector T cells (43). Memory T cells are significantly more resistant to apoptosis, ensuring long-lived protection against re-infection (44). Maintenance of memory T cell populations is independent of MHC interaction and is sustained by cytokines such as IL-7 and IL-15 (45-47). Taken together, these properties of memory T cells lead to a T cell response that is faster established and greater in magnitude upon re-infection, causing a quicker elimination of the pathogen and preventing illness (Figure 1).

Costimulating the T cell response

Primary instigator of all the processes making up T cell dynamics is the TCR, interacting with the MHC/peptide complex. In addition to this signal 1, the two signal hypothesis dictates that signal 2 is required to achieve effective activation of T cells. However, one costimulatory signal given at the time of TCR/MHC complex interaction cannot account for the array of events that constitute T cell dynamics. As more knowledge is gathered on the molecules that affect every aspect of the T cell response, this concept may prove obsolete. Instead, the input of different costimulatory signals may be required at different stages of T cell dynamics to ensure effective T cell immunity.

Costimulatory signals given at the moment of T cell activation may act to enhance TCR signalling by inducing accumulation of common signalling factors, or provide unique signals that give rise to distinct effects that are not induced by the TCR. In addition, timing of the costimulatory signal may be crucial. The expression of many costimulatory receptors and their ligands depends on the stage of T cell activation and the inflammatory circumstances. This indicates that these signals are required at distinct time points. Also, certain costimulatory signals may be more widely available in some locations compared to others. Signals supporting T cell expansion may be more copious in lymphoid organs compared to sites of infection. As many costimulatory molecules are found on different cell types, the availability of these molecules depends on the type of cells that are in the vicinity of the T cells at the right locations at the right time.

The ‘classical’ costimulatory molecule CD28 is thought to quantitatively enhance TCR mediated signals (48). The signalling cascade downstream of CD28 leads to activation of the Src-family protein tyrosine kinases (PTKs) Lck and Fyn, phosphatidylinositide-3-kinase (PI3K), the guanosine exchange factor Vav1 and Tec PTKs (49). CD28 costimulation promotes the assembly of the TCR signalling complex by affecting actin-cytoskeleton rearrangement (50). This results in a decrease in the threshold for T cell activation especially at low level TCR stimulation (51). In addition to accelerating cell-cycle progression, CD28 enhances T-cell survival by inducing nuclear factor-κB (NF-κB)-
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dependent expression of the anti-apoptotic protein BCL-X_L (52). Early on, CD28 was shown to convey a potent costimulatory signal in conjunction with TCR triggering that enables T cells to survive better and proliferate more. Mice deficient in the receptor CD28 or the ligands (CD80/CD86) had markedly decreased T cell responses to antigen challenge (53,54). CD28 signals were found to enhance IL-2 production, upregulate the IL-2 receptor and accelerate entry into and progression through the cell cycle (53,55,56). The binding of IL-2 to its receptor actually drives T cell proliferation. In addition, CD28 prevents death of activated T cells (57). Since CD28 is already expressed on the T cell when it encounters the APC expressing CD80/CD86, CD28 is able to signal parallel to the TCR.

While CD28 signals are important during the priming of T cells, the inducible costimulator (ICOS) is involved in regulating T cell responses in the periphery (58). Using ICOS-deficient mice, several groups showed that ICOS plays a role in generating type 2 CD4+ T cell responses in that it supported the production of IL-4 and IL-10 (59-62). In addition, ICOS regulates B cell responses to T cell-dependent antigens (61-63).

In addition to the TCR and costimulatory signals, a third signal may be required to develop cytolytic effector function. Cytokines like IL-12 or adjuvants may deliver this third signal to activated T cells (64).

Protective immunity depends on the level of memory generated which is directly connected to the size of the clonal expansion during the primary response (27). Since clonal expansion of T cells is the net result of proliferation and death during the expansion, molecules that regulate life and death are likely candidates in shaping the T cell response. Some members of the Tumor Necrosis Factor Receptor (TNFR) family have been shown to promote apoptosis while other members counter-act it. Members that are able to induce cell death, such as CD95 or TRAIL receptors, are characterised by a death domain in their cytoplasmic tail, which serves as a docking site for adaptor molecules such as FADD. The activation of caspases ultimately leads to apoptotic cell death (65).

Other members of the TNFR family do not show overt pro-apoptotic properties, but rather promote activation, proliferation, differentiation and/or survival of immune cells (66). CD40 plays a role in maturation of DC by activated CD4+ T cells, which are then proficient in effective CD8+ T cell priming (8-10). Alternatively, CD40 on CD8+ T cells may be directly triggered by the ligand on CD4+ T cells (38). Receptor activator of NF-κB (RANK) was identified on DC and was shown to increase T cell responses by enhancing DC function (67). Triggering of RANK by its ligand TRANCE promotes survival of DC, thus favoring the expansion of activated T cells (68).

A TNFR family member that acts directly on T cells is herpes virus entry mediator (HVEM) (69). Studies on HVEM indicate a role in activating T cells and supporting clonal expansion. Mice deficient for the ligand of HVEM, LIGHT, have decreased CD8+ and CD4+ T cell responses (70,71). Glucocorticoid-induced TNF receptor (GITR) was shown to
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prevent activation-induced cell death of T cells and enhance T cell responses in vitro (72). Although the function of GITR is not fully characterised yet, it may support the function of regulatory T cells and their function in maintenance of self-tolerance (74).

Recently, TNFR family members OX40 (CD134) and 4-1BB (CD137) have been shown to convey costimulatory signals that affect different stages of T cell dynamics, enhancing expansion, effector function and development of memory T cells (75). Both receptors are absent on naïve T cells, but acquired upon T cell activation. OX40-deficient CD4^+ T cells showed impaired proliferation in later divisions (76). This results in generation of fewer antigen-specific CD4^+ T cells and the development of fewer memory T cells (77). 4-1BB affects mainly CD8^+ T cells. Mice deficient for 4-1BB/4-1BBL interactions generated markedly less antigen-specific effector CD8^+ T cells during primary responses and fewer memory T cells developed (78). Similar to OX40, in absence of 4-1BB signals, early T cell expansion seems unaffected (79).

CD27 is constitutively expressed on naïve and activated T cells, while its ligand, CD70, is expressed on T- and B cells only after antigen receptor triggering and on mature DC (80,81). CD27-CD70 interactions could thus play a role in T cell-DC interactions, directly after initial TCR triggering or, sustain T expansion during T cell -T cell interactions. In vitro, CD27 supported T cell proliferation and cytokine production (82,83). The further elucidation of the role of CD27 in shaping the T cell immune response is subject of this thesis.

TNFR family members signal via an unique set of adaptor proteins; TNFR-associated factors (TRAFs). These TRAFs interact with small, specific motifs in the cytoplasmic tail of the TNFR family members. Signalling via TRAFs leads to the activation of NF-κB, Jun N-terminal kinase (JNK) and the transcriptional complex AP1 (84-86). The involvement of TRAF2 in TNFR family member signalling was shown to be essential since mice with abortive TRAF2 signals had profound defects in T cell responses (87,88). Since TNFR family members all signal via TRAFs, redundancy may be expected. Still, although some functions may overlap, TNFR family members are involved in all stages of T cell dynamics.

Scope of this thesis

The focus of this thesis is the elucidation of the role of CD27 in regulating the immune response. To gain knowledge on the function of CD27 in vivo, a CD27-deficient recombinant mouse was generated in our laboratory by L.A. Gravestein. The work described in this thesis started with studying the consequences of the absence of CD27 for pre-TCR mediated thymocyte development and positive and negative selection of thymocytes (chapter 2). In addition to a suspected contribution of CD27 to T cell development, a role for CD27 in antigen-specific immunity was studied. As a model for
antigen responsiveness, we used intranasal infection with influenza virus. In chapter 3, we focus on the primary and memory T cell response in wild-type mice versus CD27-deficient mice. Since both primary and memory CD8$^+$ T cell responses to intranasal influenza infection were found to be reduced (chapter 3), we wanted to compare the role of CD27 in T cell immunity to that of the 'classic' costimulatory molecule CD28 (chapter 4). Here, we examined the precise mechanism by which CD27 and CD28 contribute to T cell expansion. In chapter 5, we determined the effect of CD27 deletion on the B cell response, including immunoglobulin production and somatic hypermutation. In addition, the contribution to germinal center formation of CD27 in relation to CD28 was examined by making use of adoptive transfer techniques. In chapter 6 the relative impact of CD27 and close relatives OX40 and 4-1BB on the primary T cell response and memory formation is compared in one antigen-specific model side by side. Finally, a general discussion of the preceding chapters and their relation to the relevant literature is presented in chapter 7.
Chapter 1

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General Introduction


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