CD27/CD70 interactions in effector and memory cell formation
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Chapter 1

Introduction
IMMUNITY

The immune system serves to defend our bodies against infectious micro-organisms. Any immune response involves, firstly, antigen recognition and, secondly, antigen neutralisation and or elimination. Because micro-organisms come in many different forms, multiple mechanisms to recognise and eliminate the invading micro-organism have evolved. Based on the capacity to recognise and adapt the response to an antigen, the immune system in vertebrates can be divided in an innate (or natural) and a specific (or acquired) arm. Innate immunity is mediated by different cell types, which recognise a limited number of common antigens or molecular patterns present on various classes of micro-organisms. Innate responses do not change upon repeated exposure to antigen. Specific immunity is mediated by T and B lymphocytes of which each individual cell expresses an unique antigen receptor that recognises epitopes specific for singular pathogens. Due to the presence of large number of lymphocytes this results in an enormous diversity of the repertoire for antigen recognition. The specific immune system adapts upon encounter with antigen. Memory formation guarantees a quicker, larger and more specific response in case of reencounter with a micro-organism. Innate and specific mechanisms of the immune system do not function as separate entities but actively influence each other. Therefore any immune response is a combined, interactive effort of the innate and specific immune systems to neutralise and/or eliminate the antigen.

THE SPECIFIC IMMUNE RESPONSE

The unique specificity of the antigen receptors of T and B lymphocytes severely reduces the possibility that lymphocytes will encounter their specific antigen by chance. To overcome this limitation lymphocytes recirculate via the bloodstream and lymph between the different secondary lymphoid organs. In secondary organs antigens derived from the site of infection, either by trapping or delivered on mature dendritic cells, are presented to the lymphocytes. Antigen recognition by itself is not sufficient to activate a lymphocyte. To ensure that immune responses only develop when needed, antigen in-experienced or naive lymphocytes need a second or costimulatory signal to become fully activated\(^1\). Under physiological conditions this signal is only given by antigen presenting cells under infectious circumstances. After full activation, proliferation and differentiation results in the generation of large numbers of antigen-specific effector cells and effector molecules\(^2\), and the generation of memory T and B cells. Terminal-differentiated effector B cells or plasma cells secrete soluble forms of their antigen receptor. These so-called antibodies are the mediators of the specific immune system to neutralise extracellular pathogens. The germinal centre reaction is a specialised process in which memory B cells are formed\(^3\). To increase the affinity of their antigen receptor for the antigen, activated B cells genetically change the molecular structure of their antigen receptor. This process elegantly demonstrates the unique adaptive potential of the specific immune response.

Effector T cells can have a variety of functions. CD4\(^+\) effector T cells can help phagocytotic cells to destroy vesicular and intracellular pathogens and help B or T cells in their response to
antigen. CD8⁺ T cells can recognise and kill infected cells and in this way contribute to the specific immune response against intracellular pathogens. In marked contrast to B cell memory, T cell memory is not acquired at a specific site nor genetically defined. However, with respect to cell surface phenotype and functional properties, memory T cells are different from naive T cells⁴.

**REGULATION OF THE IMMUNE RESPONSE**

As for all biological processes, a beneficial outcome of the immune response strongly depends on its regulation. The efficiency by which a particular type of antigen will be neutralised/eliminated will depend on the quantity and quality of the immune response that is induced. Adjustment to the amount of antigen requires quantitative control of the immune response. The distinct stages of an immune response, i.e. lymphocyte activation, proliferation, differentiation, migration, death and execution of effector cell function, are subject to regulation. Activating and inhibitory receptors, like Toll-like receptors and Fc-receptors, locally produced soluble mediators, such as cytokines and chemokines, and direct cell-cell interaction mediated by e.g. integrins and costimulatory molecules all participate in this process.

Two families of proteins represent the main co-stimulatory molecules for T cells: the CD28 family⁵ and the TNF receptor family⁶. CD28 is the classic costimulatory molecule and upon interaction with either of its ligands (B7-1 or B7-2) will enhance T cell proliferation, IL-2 synthesis and survival. The receptor CTLA-4 is the counter-actor of CD28. This protein can also bind B7-1 and B7-2 but will give inhibitory signals to the T cell. The balance between CD28 and expression CTLA-4 is thought to determine whether or not a T cell will becomes fully activated⁷. The activating receptor ICOS and the inhibitory receptor PD-1 are the other members of the CD28 family⁵. Like CD28, ICOS can enhance the expansion of T cells in vitro⁸.

**TNF-RECEPTOR FAMILY AND TNF FAMILY**

Membership of a protein to TNF-receptor family is defined by the presence of cysteine-rich repeats of approximately 40 amino acids in the extracellular domain of a type-1 membrane protein⁹. These cysteine rich repeats provide the motif for binding to the shared structures of the complementary family of TNF ligands¹⁰. TNF-like ligands are trimeric type II membrane or soluble proteins that exert their physiological effect by binding to TNF-receptor family members. Although not confined to interactions between TNFR family members and their respective ligands have important functions in the regulation of the immune response¹¹. Based on their cytoplasmic domains, the TNF-receptor family members can be divided into two groups¹². Signalling via the death-domain containing family members can activate caspases and induces apoptosis of the cell. Triggering of the non death-domain containing family members has been shown to affect expansion, differentiation and survival of different cell-types of the immune system. Although still largely controversial, interaction between a TNF-receptor family member and its ligand might also effect the ‘ligand' expressing cell via reverse signalling¹³-¹⁶.
One of the main determinants regulating receptor function is availability of the ligand. In general, antigenic activation is required for induction of ligand expression and ligand expression is only transient\textsuperscript{12}. Furthermore, soluble forms and signalling incompetent decoy receptors can be generated\textsuperscript{19-22}. These molecules compete with the functional receptors for ligand binding and thereby reduce the accessibility of the ligand. Besides co-stimulatory molecules for T cells, The TNF-receptor family also contains costimulatory molecules for B cells, such as CD40 and BCMA, which are essential for developing humoral immune responses\textsuperscript{23,24}.

**The molecules CD27 and CD70**

CD27 is lymphocyte-specific member of the TNF receptor family. CD27 is expressed by thymocytes, NK, T and B cells\textsuperscript{25}. In man, CD27 expression and its regulation have been thoroughly characterised. On T and B cells, CD27 expression correlates with the antigen experience and differentiation status of the cell. Naive T cells constitutively express CD27 and antigenic triggering upregulates this expression\textsuperscript{26,27}. However, persistent stimulation with antigen and the concomitant terminal differentiation into effector T cells is accompanied by loss of CD27 expression\textsuperscript{26,28}. Naive B-cells do not express CD27 but CD27 expression is induced and, importantly, sustained after antigenic triggering\textsuperscript{29,30}. Consequently, CD27 is a marker for memory B cells\textsuperscript{31,32}. What happens with CD27 expression during terminal differentiation of B cells is not clear. Recently, data showing CD27 expression on in vitro generated plasma cells has been published\textsuperscript{33}, however immunohistochemical data confirming these data are lacking.

CD27 exerts its function after being crosslinked by its ligand CD70\textsuperscript{34,35}. In analogy with CD27, antigenic triggering upregulates CD70 expression on T and B lymphocytes. However, CD70 expression does not mark a differentiation state but identifies recently activated lymphocytes. CD70 expression wanes after the removal of an antigenic stimulus\textsuperscript{36}. Besides antigen, accessory signals, such as costimulatory signals (e.g. CD28 or CD40) and cytokines regulate CD70 expression\textsuperscript{36,37}. In histochemical-stainings of spleen or tonsil sections only a limited amount of CD70 expressing cells can be found\textsuperscript{17,35}, which implies that together these control mechanism very strictly regulate CD70 expression.

Although lymphocytes are responsible for the majority of CD70 expression, CD70 is also expressed on other cell types. Thymic medullar epithelial cells (in human\textsuperscript{38,39}), thymic medullar dendritic cells (in mouse, chapter 3) and peripheral mature dendritic cells\textsuperscript{40} (chapter 3), can all express CD70. Non lymphocyte specific expression has recently also been described for CD27. In the mouse CD27 expression has been found on haematopoietic stem cells and has been suggested to discriminate between self-renewing and more committed developmental stages of hematopoietic stem-cells\textsuperscript{41} (and R. Arens, unpublished data).

**Effects of CD27/CD70 interaction**

Numerous in vitro studies have described the cellular effects of CD27 crosslinking on NK, T and B cells. In synergy with antigenic triggering, ligation of CD27 on T cells increases proliferation\textsuperscript{9,42-45}, cell survival\textsuperscript{46}, TNF-\textgreek{a} production\textsuperscript{47} and the generation of cytolytic T cells\textsuperscript{34,48}.
For B cells CD27/CD70 interaction primarily enhances differentiation rather than proliferation. In T-cell dependent and T-cell independent systems for B cell activation increased numbers of plasma cells and concentrations of Ig are found when CD27 is crosslinked. On NK cells CD27 can costimulate accessory molecule or cytokine induced NK activity, but can also have effects on its own. Human NK cells show enhanced cytolytic activity after crosslinking of CD27; IFN-γ production and proliferation are increased by the sole triggering of CD27 on murine NK cells. "Reverse signalling", i.e. modulation of cellular function through the CD27 ligand, as a consequence of CD27/CD70 interaction has been described for human lymphocytes. Addition of anti-CD70 mAb increased proliferation of T and B-CLL cells, in PHA stimulated cultures and PMA stimulated cell cultures respectively.

Although the in vitro effects of CD27 triggering on specific cell populations are well established, the responsible intracellular events are much less defined. CD27-induced changes in intracellular Ca²⁺ levels and phosphorylation status have been described, but the interest in these data has waned by the identification of the family of TNF-receptor associating proteins (Trafs). The different domains in these adapter proteins conduct the simultaneous association with TNF-receptors and intracellular (signalling) proteins. CD27 can bind Traf2, 3 and 5. Traf2 was shown to be involved in the CD27-induced activation of the transcription factor NF-κB and the c-Jun N-terminal kinase (JNK). Traf2 also associates with the inhibitors of apoptosis proteins (IAPs). The functions of Traf2 are in line, and compatible with, the functions of CD27 in regulating cell expansion and differentiation. Besides Traf2, the pro-apoptotic protein SIVA can also bind to the intracellular tail of CD27. Yet, the physiological meaning of this finding is unclear, since the description of CD27 as an inducer of apoptosis is very limited. Until now, it is not known if there are any specific genes of which the expression is induced by CD27. However if so, the use of the recently-available DNA chips offer the promising possibility to reveal these genes.

THE PROPOSED FUNCTION OF CD27/CD70 INTERACTION IN THE IMMUNE RESPONSE.

Based on the above described, in vitro expression and functional data we proposed that CD27/CD70 interaction contributes to the determination of the size and function of the antigen primed lymphocyte pool. The limited CD70 expression and its dependence on antigenic stimulation will directly couple CD27's influence on the antigen-primed pool to the amount of antigen. The significance of this hypothesis for the physiological role of CD27/CD70 interaction can be tested in an in vivo model.

MOUSE MODELS

The biological similarity between mouse and man and, more importantly, recent advances in our ability to manipulate the mouse genome has made the mouse the pre-eminent in vivo model to study biological process during health and disease. Knock out and transgenic technology provides the means to study the specific contribution of a molecule of interest in two opposing situations. In KO mice the protein of interest is absent and evidently its biological contribution
nullified. In contrast, in transgenic mice excessive expression of a molecule may lead to a
dominant contribution of the protein in certain biological responses. Functional importance,
redundancy and the proteins expression-level and -pattern will determine which of these
approaches is going to be most informative. Combining both approaches will give
complementary data. For extracellular proteins, administration of agonistic or antagonistic
antibodies can induce similar opposing situations. Although temporality of the effects makes this
an appealing approach, its use in study of immune regulation is limited. Being a component of
the immune system and an antigen, Ab can potentially influence an immune response and
hence obscure the meaning of the experimental result.

Recently, the first studies on the function of CD27/CD70 interaction in mouse models have
been described. Infection of CD27− mice with influenza virus has revealed a function of CD27
for the T-cell compartment. Absence of CD27 leads to reduced expansion and numbers of
antigen specific cells in primary and secondary immune responses. Noteworthy, this effect was
most pronounced at the site of infection. Several studies using antagonistic anti-CD70 mAbs
have been performed. In TCR transgenic mouse systems for LCMV infection administration
of anti-CD70 mAb did not alter CD8+ or CD4+ T cell priming after primary infection with LCMV
(M. van der Broek, K. Tesselaar, unpublished data). Also after infection of mice with Leishmania
major no effects were seen of anti-CD70 mAb. Furthermore, neither in the spontaneous NOD
model for insulin dependent diabetes mellitus (IDDM) (N. Sarvetnick, personal
communication) or the collagen induced model for arthritis (P.P. Tak, K. Tesselaar,
unpublished data) did administration of anti-CD70 mAb ameliorate disease outcome. Only in
experimental autoimmune encephalomyelitis (EAE) blocking CD27/CD70 interaction seemed to
have effect. Induction of EAE was prevented by administration anti-CD70 mAb, presumably by
inhibiting TNF-α production.

AIM OF THIS THESIS
The aim of this thesis was to investigate the role of CD27/CD70 interaction in the regulation of
the immune response in an in vivo (mouse) model. We chose to create an in vivo mouse model
in which CD70 is present as a dominant co-stimulatory molecule. To this end we first cloned and
molecular characterised murine CD70 (chapter 2). Subsequently anti-CD70 mAbs were
generated and expression of CD70 was analysed. As in man, CD70 expression is highly
restricted in the mouse (chapter 3). Transgenic expression of CD70 was thus expected to
create a dominant role for CD27/CD70 interaction. Transgenic mice constitutively expressing
CD70 on all B cells were generated and in chapter 4 we describe the generation and initial
analysis of the phenotype of the T and B cell compartment in CD70TG mice. The analysis of the
T cell compartment and the in vivo function of CD27 on T cells was further elaborated in the
experiments presented in chapter 5. Finally the role of CD27/CD70 interaction in the humoral
immune response was analysed in CD70TG mice (chapter 6).