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

van Olffen, R.W.

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Chapter 7

Timing and tuning of CD27-CD70 interactions:
The impact of signal strength in setting the balance between adaptive responses and immunopathology.

Ronald W. van Olffen, Martijn A. Nolte, Klaas P.J.M. van Gisbergen,
and René A.W. van Lier.

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Summary

After binding its natural ligand CD70, CD27, a TRAF-binding member of the tumor necrosis factor (TNF) receptor family, regulates cellular activity in subsets of T, B and NK cells and hematopoietic progenitor cells. In normal immune responses, CD27 signaling appears to be limited predominantly by the restricted expression of CD70, which is only transiently expressed by cells of the immune system upon activation. Studies performed in CD27-deficient and CD70-transgenic mice have defined a non-redundant role of this receptor-ligand pair in shaping adaptive T cell responses. Moreover, adjuvant properties of CD70 have been exploited for the design of anti-cancer vaccines. However, continuous CD27-CD70 interactions may cause immune dysregulation and immunopathology in conditions of chronic immune activation such as during persistent virus infection and autoimmune disease. We conclude that optimal tuning of CD27-CD70 interaction is crucial for the regulation of the cellular immune response. We provide a detailed comparison of costimulation through CD27 with its closely related family members 4-1BB, CD30, HVEM, OX40 and GITR and we argue that these receptors do not have a unique function *per se*, but that rather the timing, context and intensity of these costimulatory signals determines the functional consequence of their activity.
Dimensions of costimulation

The ability of T cells to detect virtually any pathogenic invader is granted by its extraordinarily diverse receptor repertoire, which allows the T cell pool to recognize a vast number of peptides upon presentation by major histocompatibility complex (MHC) molecules. Still, signaling through the TCR (also referred to as “signal 1”) is not sufficient for adequate T cell activation, as costimulatory molecules provide indispensable signals for proliferation, survival and differentiation (“signal 2”). In fact, naïve T cells that only receive “signal 1” without “signal 2” are rendered anergic or die through apoptosis. The integration of “signals 1 and 2” is required for full T cell activation and the strength of these signals shapes the size of the ensuing T cell pool. Moreover, full differentiation into effector T cells is generally dependent on a third signal, which is supplied by the antigen-presenting cell (APC) in soluble form (as a cytokine, e.g. IL-12) and provides instructive signals for the type of effector T cell that is required.

This “three signal” concept depicts a model for the activation of naïve T cells and the subsequent formation of effector T cells. Yet, the immune system provides a plethora of diverse costimulatory molecules and these various types of “signal 2” all contribute in their own unique manner to the quality of the T cell response. Indeed the term “signal 2” reduces the complexity of the intricate regulatory circuits that control tailored T cell expansion and differentiation. Costimulatory signals can act on particular aspects of T cell activation, such as survival, cell-cycle progression, and differentiation to either effector or memory cell. The function of a particular costimulatory molecule is strongly related to the timing of its action, since early costimulatory signals are functionally distinct from those that act late during the T cell response. It is for this reason that the expression of each costimulatory molecule and/or its ligand is tightly regulated and dependent on the activation status of the cell.
The best-characterized costimulatory receptor is CD28, a member of the immunoglobulin superfamily of costimulatory molecules, which is already expressed on naïve T cells and can therefore play an important role in the initial phase of T cell stimulation. Upon interaction with one of its two ligands CD80 and CD86, which are rapidly expressed on APCs upon activation, CD28 induces upregulation of survival genes in the naïve T cell, facilitates its cell-cycle progression and enhances the production of IL-2 (reviewed in (1)). Other important costimulatory molecules on T cells are members of the TNF-R superfamily, such as CD27, 4-1BB, CD30, herpes virus entry mediator (HVEM), OX40 and glucocorticoid-induced TNFR family related gene (GITR). As new insight into the function of CD27 has been gained during recent years, we will focus our attention on this unique receptor in regulating immune responses and compare its function with its closely related family members.

**Molecular aspects of CD27 evoked signals**

CD27 is expressed as a disulphide-linked dimer and upon interaction with its unique ligand CD70 a truncated form of CD27 is released, most probably by a membrane-linked protease (2). Increase in soluble CD27 has been documented in situations of immune activation such as in autoimmune disease and during viral infection (reviewed in (3)). CD70, the only CD27 ligand that has been identified, is a membrane expressed homotrimeric type II membrane molecule. Analogous to other members of the TNF-R family, the first event after ligand engagement is most likely the trimerization of CD27, which forms the first step in the initiation of intracellular signaling. In line with this notion, it has been demonstrated that the intracellular tail of CD27 couples to TNFR receptor-associated factor (TRAF)-2 and TRAF5 (4;5) and deficiency of TRAF5 impairs CD27-mediated co-stimulation (6). Ligation of CD27 by CD70 induces strong ubiquination of TRAF and the activation of both canonical and non-canonical NF-κB pathways (7) (see also Figure 1). Additionally, CD27 has been shown to
result in the initiation of the jun-N-terminal kinase (JNK) signaling cascade (4;5). Finally, it has been reported that CD27, as well as GITR, can bind Siva-1, an intracellular mediator of apoptosis (8;9), but the role of this interaction for the function of CD27 has yet to be resolved as ligation of CD27 generally does not limit but rather contributes to the expansion of activated lymphocytes (see below).

**Figure 1: CD27 signalling induces multiple intracellular signalling pathways that contribute to immune regulation.** CD70-induced triggering through CD27 on T cells is mediated by TRAF2 and TRAF5, which leads to the activation of canonical and non-canonical NF-κB pathways, as well as the JNK pathway. Either directly or indirectly, these signaling events can induce molecules involved in pro-inflammatory effector functions of T cells (in green) and anti-inflammatory regulatory functions (in red).
With the aim to identify genes that are induced as a consequence of CD27 triggering, it has recently been shown by means of an elegant in vivo immunization model in which transcriptomes of WT and CD27 deficient murine T cells are compared, that CD27 signaling contributes to the establishment of a T-helper-1 (Th1) type gene expression profile in CD4 T cells (10). Interestingly, MS4A4B, a tetraspan surface molecule homologous to CD20 that has previously been implicated in the induction of Th1 responses (11), is strongly induced as a consequence of CD27 signaling (10). These murine data indicate that CD27 costimulated responses induce Th1, which is in line with data obtained in the human system showing that CD27-CD70 interactions sensitize naive CD4 T cells for IL-12-induced Th1 cell development (12). In addition, CD27 ligation on these cells induces the upregulation of anti-apoptotic Bcl-xL, an established target of NF-κB signaling (12).

The interpretation of the in vivo consequences of CD27-CD70 interaction is complicated by the fact that CD70 can function as signal transducing receptor itself. Stimulation of CD70 with an agonistic anti-CD70 antibody initiates a signaling cascade that regulates expansion and differentiation of both murine and human T and B cells (13-15). Although the AKT/PKB pathway may play a role in these functional responses, the membrane proximal signaling events remain to be defined. Intracellular tails of murine and human CD70 show a low level of sequence homology and CD70-associated molecules have not been defined until now.

**Expression of CD27 and CD70**

Expression characteristics of both CD27 and CD70 are highly similar between mouse and man. The receptor CD27 is expressed on early thymocytes, as well as on naïve CD4 and CD8 T cells. Upon T cell activation, the expression of CD27 is increased, but it is downregulated when T cells have undergone several rounds of division and differentiate towards effector cells (16;17). Analysis in various infection models in mice and men has revealed that effector
CD8 T cells indeed have low to no expression of CD27 (18-21). Interestingly, central memory T cells, which reside in secondary lymphoid organs do express CD27, which indicates that expression of this receptor is highly dynamic (20).

Expression of CD70 is highly restricted and activation dependent, as it is only transiently expressed on activated T cells, B cells and dendritic cells (DCs) (22-26). Antigen receptor stimulation on T and B cells and Toll-like receptor triggering on both B cells and DCs induces CD70 expression, which is further enhanced by CD40 triggering (24;27). Depending on the cell type and culture conditions, CD70 expression on human lymphocytes can be enhanced by cytokines such as IL-1α, IL-12, GM-CSF and TNFα, while IL-4 and IL-10 can decrease CD70 expression (24;28). Provision of high doses of IL-2 in vitro or in vivo induces CD70 expression on human T cells, which results in a concomitant loss of cell surface expression of CD27 on CD8 T cells (29).

**Impact of the CD27 costimulatory signal on T cell function**

Although CD27 is highly expressed on thymocytes and naïve T cells, these cells do not seem to depend on CD27 for their generation and maintenance, as CD27-deficient mice have normal T cell development in the thymus and similar numbers of naïve T cells in secondary lymphoid organs as WT controls (30). However, deletion of CD27 does hamper the generation of T cell immunity, since CD27-deficient mice infected with influenza virus have a reduced number of CD4 and CD8 effector T cells that infiltrate the lung. The formation of memory T cells also depends on CD27, since a memory T cell response to a secondary challenge with influenza is greatly reduced in these mice. Importantly, CD27 is not required for entry into cell cycle upon T cell activation and neither for the differentiation towards interferon-gamma (IFN-γ) producing or cytolytic effector T cells (30). Instead, CD27 promotes survival of activated T cells throughout successive rounds of division and thereby
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contributes to the accumulation of effector T cells (31). It has also been found that CD27 on CD8 T cells can induce proliferation in the absence of IL-2, which does not lead to effector cell differentiation (32). These studies indicate that CD27-triggering itself is not required, nor sufficient to induce effector cell formation, but that CD27 contributes to the formation of the effector cell pool by inducing proliferation and survival. Moreover, adoptive transfer experiments with WT or CD27-deficient ovalbumin (OVA)-specific CD4 T cells into mice that are subsequently immunized intranasally with OVA show that CD27 expressed on CD4 T cells promotes both the primary CD8 T cell response and the secondary expansion of memory CD8 T cells. In this model, CD27 instructs CD4 T cells to provide help to effector/memory CD8 T cells by inducing a Th1-type gene expression profile and a subsequent increase in the frequency of IL-2 and IFN-\(\gamma\) producing effector CD4 T cells (10). Since IL-2 is important to program memory CD8 T cells for secondary expansion (33), this might indicate that CD27-induced IL-2 production is the mechanism through which CD27 on CD4 T cells helps the memory CD8 T cells response, but this hypothesis remains to be confirmed.

Apart from studies with CD27-deficient mice, a lot of insight in the role of CD27-CD70 interactions has been gained by the *in vivo* use of blocking anti-CD70 antibodies. Several studies have shown that CD70 expression can be rapidly induced on DCs by stimulation of CD40 or Toll-like receptors (34-36) and in particular when these stimuli are combined (27). Subsequent *in vivo* blockade of CD70 is sufficient to inhibit priming of CD8 T cells (27;34-36). Treatment with anti-CD70 is even able to inhibit priming of splenic CD8 T cell responses upon infection with vaccinia virus, Listeria monocytogenes and vesicular stomatitis virus (37). Blockade of CD70 during the late, but not the early phase of a primary response to influenza virus prevents apoptosis of antigen-specific CD8 T cells and decreases the quality of the memory CD8 T cell response (38). Moreover, anti-CD70 can also block the priming of CD4 and CD8 T cells localized in the gut mucosa that respond to oral infection with Listeria
monocytogenes. It was found that the priming of these T cells depends on a unique population of APCs in the lamina propria that expresses high levels of CD70 (39). In the spleen, CD70 plays an important role on a specific DC subset that expresses the uptake receptor CD205 (40). When antigen is targeted specifically to this DC subset with an anti-CD205 antibody, priming and IFN-γ production of cognate CD4 T cells is independent of IL-12 and fully depends on CD70 expression by this DC subset. When the same antigen is targeted to a distinct, DCIR2-expressing DC subset, Th1 differentiation does not depend on CD70, but rather on IL-12 production (40). This indicates that CD70 expression is even differentially regulated between APC subsets and can play an instructive role in CD4 T cell differentiation. Importantly, these findings imply that signaling through CD27 (“signal 2”) is in particular circumstances sufficient to induce differentiation to effector T cells and can thus overcome the requirement for “signal 3”.

The aforementioned studies indicate that the CD27-CD70 axis plays an important role for the priming of T cells in a variety of immunization and infection models. Interestingly, the role of CD27 in protection against infection with lymphocytic choriomeningitis virus (LCMV) appears to be slightly different. Using CD27-deficient mice, it was shown that CD27 is not required during a primary infection with regards to effector cell formation and viral elimination (41), which was confirmed in WT mice using blocking antibodies against CD70 (37). However, CD27 is critically important for clonal expansion of memory cells and protection upon re-infection (41). It was found that LCMV-specific memory cytotoxic T lymphocytes (CTLs) retain CD27 expression, which depends on CD70 expressed by polyclonally activated B cells during the retraction phase (41) and on help provided by CD4 T cells during the primary infection (42). Ligation of CD27 on these memory CTLs during restimulation strongly enhances autocrine IL-2 production and thereby the secondary expansion (42). Whether a similar mechanism applies for the memory response during
influenza virus infection is not yet known and would be interesting to investigate, since retained CD27 expression can be found on memory CTLs induced by LCMV infection, but not by infection with vaccinia virus or immunization with tumor cells (41). This could suggest that the expression as well as the function of CD27 depends on the type of immune response. Alternatively, it could be that LCMV rather takes advantage of the CD27-CD70 axis, since CD27 signaling on CD4 T cells during LCMV-infection also induces immunopathology and suppression of neutralizing antibodies due to the enhanced production of IFN-γ and TNF-α by CD4 T cells. In fact, blockade of CD27 signaling is sufficient to eliminate an otherwise persistent strain of LCMV (43). These data indicate that CD27 can serve as a potent costimulatory molecule for T cell activation, but that its activity should be carefully maintained to prevent collateral damage (44).

**Induction of strong CD27 activity *in vivo***

The potency of costimulation through CD27 also becomes evident in mice that constitutively express CD70 on all B cells: these CD19-CD70TG mice develop increased numbers of effector T cells, both in the CD4 and CD8 T cell compartment (45). The advantage of this enhanced signaling through CD27 is that these mice develop augmented CD8 T cell responses to influenza virus infection and are protected against a lethal dose of poorly immunogenic EL4 tumor cells (46). Antigen-specific CD8 T cells in these mice expand more rapidly, produce more IFN-γ and have a greater cytotoxic potential on a per cell basis. Thus, although CD27 triggering might not be necessary for effector CD8 T cell differentiation (30), constitutive stimulation of CD27 clearly potentiates this differentiation pathway. Still, these CD19-CD70TG mice also demonstrate the downside of this mechanism of enhanced effector T cell differentiation for the immune system, since the increased conversion of naïve T cells into effector cells culminates in the depletion of naïve T cells from lymphoid organs (47).
Moreover, also the differentiation and maintenance of B cells is severely compromised in these mice. Although part of the B cell differentiation defect is caused by CD27 stimulation on stem- and/or progenitor cells in the bone marrow (48), the major part of this B cell depletion is caused by IFN-γ production by effector T cells (45). The loss of B cells itself has severe implications for the architecture and function of the spleen, as it results in the gradual depletion of the splenic marginal zone (49). As a result of this dramatic phenotype, CD19-CD70TG mice die around 6-8 months of age from opportunistic lung infections with Pneumocystis carinii (47). This clearly indicates that continuing CD27-CD70 interactions, which can occur during chronic human immunodeficiency virus (HIV)-1 infection and chronic autoimmune diseases (50-52), not only enhance effector T cell formation, but can also exhaust both the T cell and the B cell pool and thereby contribute to the morbidity and mortality.

To further examine the role of CD27 triggering in the direct context of antigen presentation, the group of Dr. Janny Borst has recently described the phenotype of a transgenic mouse model in which CD70 is constitutively expressed on DCs. These CD11c-CD70TG mice have a phenotype that is comparable to the CD19-CD70TG mice, in that they also show a rapid CD27-mediated conversion of naïve CD4 and CD8 T cells to effector cells and develop a concomitant immunopathology (53). Interestingly, intravenous administration of MHC-I restricted peptide in the absence of adjuvants, which induces deletional tolerance in WT mice, induces high numbers of functional, long-lived effector CD8 T cells in CD11c-CD70TG mice, and to a lesser extent in CD19-CD70TG mice (53). Similar effects are seen in WT mice when, together with TCR stimulation, CD27 is triggered through a soluble recombinant form of CD70 (sCD70) (54). These data indicate that upon TCR triggering, costimulation through CD27 is sufficient to promote strong CTL responses \textit{in vivo}. In fact, CD27-driven immunity by CD70 expression on DCs is even strong enough to break a pre-exisiting state of LCMV-
specific CD8 T cell tolerance (53). To what extent these mice are still able to maintain
tolerance to self-antigens and prevent autoimmunity remains to be addressed.

Since not only APCs, but also T cells can transiently express CD70 upon activation, we
recently developed a transgenic mouse line in which T cells constitutively express CD70 (55).
This allows us to address whether T cell activation can also be mediated by CD70 provided
by surrounding (activated) T cells. The first interesting finding in these CD2-CD70TG mice is
that CD27-triggering during thymic development does not affect T cell differentiation.
Secondly, CD2-CD70TG mice also generate more effector T cells over time, but the naïve T
cell pool is not lost during aging of the mice. This is a striking difference with the CD19-
CD70TG and CD11c-CD70TG mice, which suggests that CD70 expression on T cells
differently affects the surrounding T cell population than CD70 expression on APCs. Whether
this is related to the finding that the intracellular routing and surface expression of CD70 is
differently regulated in cells that do not express MHC class II (56), such as murine T cells,
remains to be examined. Thirdly, splenomegaly and B cell depletion in CD2-CD70TG mice is
mild compared to CD19-CD70TG and CD11c-CD70TG mice and we find that CD8 T cells
affect humoral immune responses. A fourth important observation in CD2-CD70TG mice is
that CD70 expression by T cells does enhance primary CD8 T cell responses against acute
influenza infection, but severely impairs memory CD8 T cell responses (55). Detailed
analysis after the primary response indicates that antigen-specific CTLs in these mice have an
exhausted phenotype and are unable to be maintained as memory cells. The combined
findings in CD27-deficient and CD70 transgenic mice thus show that not only inhibition of
CD27 activity, but also its constitutive triggering severely impairs memory CD8 T cells. To
what extent this detrimental impact on memory formation is caused by the cell type that
expresses CD70 is currently under investigation.
Regulation of CD27 signals

The potency of in vivo CD27 signaling necessitates negative feedback mechanisms that limit prolonged signaling and/or antagonize the strong cellular responses. One part of the regulation relies on the strict control of CD70 expression that under physiological conditions only occurs in the early phase of immune responses. CD27 appears to be the only receptor for CD70, which makes it unlikely that a negative signal transducer, in analogy to the CD28-CTLA4 system, balances signaling. At the receptor level, prolonged stimulation of T cells either in vitro or in vivo leads to reduced receptor expression, which is not only regulated at the level of transcription, but also by proteolytic cleavage of the transmembrane molecule followed by shedding of the extracellular part of the molecule (2;26) (see Figure 1).

At the cellular level, T cells chronically stimulated via CD70 produce the anti-inflammatory cytokine IL-10 and upregulate PD-1 (55), an inhibitory receptor belonging to the CD28 family. Production of IL-10 by effector T cells is thought to prevent inflammatory pathology in persistent infection (57), while the high expression of PD-1 likely downregulates cellular activation after binding its specific ligands. It can therefore be expected that a blocking PD-1L antibody will even further potentiate immune activation in the CD70 transgenic mice described above, but might thus also increase immune pathology. Additionally, CD70 induces the upregulation of Fas (CD95) expression and sensitivity (58) as well as FasL expression (55). The strong exacerbation of the immune activation phenotype in CD70 transgenic mice deficient for Fas demonstrates that the extrinsic apoptosis pathways contribute to the control of the activated T cell pool, which increases in size as a consequence of strong CD27-CD70 interactions (58). However, as CD27 ligation also results in the upregulation of Bcl-xL (12), it is likely that also intrinsic apoptosis pathways are modulated by CD27-CD70 interactions (see Figure 1).
Finally, at the population level recent observations using either CD70-expressing B cell non-Hodgkins lymphoma’s (59) or chronic lymphocytic B cell leukemias (60) have shown that regulatory T cells can develop in vitro as a consequence of CD27 costimulation. Moreover, it has been argued that proliferating memory T cells can differentiate into regulatory T cells (61), which might imply that immune activation provides its own negative feedback mechanism on a population level in order to restore immune homeostasis. However, it is still unsettled if CD27 plays an important role for regulatory T cells in vivo, as in none of the CD70 transgenic mice described above, nor in CD27 deficient animals, (either on a C57Bl/6 or Balb/c background) conspicuous changes in numbers of regulatory CD4^+ T cells have been found (see Figure 2A). Although the expression of CD27 on regulatory T cells is downregulated in CD19-CD70TG mice due to continuous interactions with its ligand (chapter 6 of this thesis), no significant changes could be observed in numbers of naïve (CD62L^+) and effector (CD62L^-) regulatory T cells (see Figure 2B) (62). Expression of CD103 and CTLA4, which are expressed on highly active regulatory T cells, was also not differentially regulated in these mice, but we did find a significantly higher expression of GITR in CD70 TG mice (see Figure 2C). Interestingly, GITR expression on regulatory T cells was highest in mice with the strongest B cell depletion, i.e. CD19-CD70TG mice (see Figure 2C), which might indicate that GITRL expression on B cells normally suppresses surface expression of GITR on T cells. Conclusively, these data suggest that CD27-triggering does not affect the number nor the activation status of regulatory T cells, which is in sharp contrast to naïve and effector/memory T cells.
Collectively, these studies demonstrate that CD27 can play an important role in the formation of the effector T cell pool by enhancing proliferation and survival of activated T cells. The finding that CD27-deficient mice have a diminished formation of effector and memory cells suggests that CD27 has a non-redundant function in this aspect of T cell biology. However, since CD27 only functions upon interaction with CD70, it can also be inferred that the degree of CD70 expression rather than the presence of the receptor determines the CD27-dependency of the ensuing T cell response. In other words: inflammatory responses that do not induce the

**Figure 2: Numbers of regulatory T cells in genetically modified mice.** The total number of (A) splenic regulatory (FoxP3+ T cells and (B) the number of naïve (CD62L+) and effector (CD62L+) regulatory T cells in WT, CD2-CD70TG, CD19-CD70TG and CD27-/- mice on a C57Bl/6J background (average of 3 mice ± SD).

**Costimulation through CD27: how important and unique is it?**

Collectively, these studies demonstrate that CD27 can play an important role in the formation of the effector T cell pool by enhancing proliferation and survival of activated T cells. The finding that CD27-deficient mice have a diminished formation of effector and memory cells suggests that CD27 has a non-redundant function in this aspect of T cell biology. However, since CD27 only functions upon interaction with CD70, it can also be inferred that the degree of CD70 expression rather than the presence of the receptor determines the CD27-dependency of the ensuing T cell response. In other words: inflammatory responses that do not induce the
expression of CD70 are thus not dependent on CD27 and might thrive on other costimulatory signals. This leads to the important question to what extent CD27 has a specific and unique function or whether closely related members of the TNF-R superfamily can do the same job if they are expressed? To address this question, we will compare the role of CD27 with its closely related family members of the TNF-R superfamily.

In an elegant review, Michael Croft has suggested a model in which the function of CD27, 4-1BB, CD30, OX40 and HVEM is dependent on the timing of their maximal expression and that of their respective ligands (63). In the proposed model, HVEM and CD27 play an important role in the early phase of the T cell response, next to CD28, because they are expressed on naïve T cells. Since HVEM expression is rapidly downregulated and CD27 expression is upregulated upon T cell activation, it was suggested that HVEM and its ligand LIGHT (which stands for “lymphotoxin-like, exhibits inducible expression, and competes with HSV glycoprotein D for HVEM, a receptor expressed by T lymphocytes”) have their most important function during initial activation, while CD27-CD70 interactions mainly stimulate the subsequent phase of clonal expansion by stimulating survival and proliferation. In contrast, expression of OX40, 4-1BB and CD30 and their respective ligands is induced on proliferating T cells and activated APCs and it is therefore most likely that these molecules play a role during later stages of the response. Both 4-1BB and OX40 are able to prevent apoptosis by inducing the expression of anti-apoptotic molecules such as Bcl-xL and Bfl1 and thereby contribute to the size of the effector pool (64-66). It is not yet known whether CD30 can act in a similar manner, but parallels with OX40 and 4-1BB have been suggested (63;67).

Thus, rather than having specialized and distinctive functions, the unique contribution of each receptor would actually rely on the timing of its expression and that of its ligand. This would be in line with the rather common signal transduction pathway of these molecules, since all five receptors signal through association with various TRAF family members, which
successively activate the transcription factors NF-κB and in most cases AP-1 (reviewed in (63;68)). Activation of these rather general pro-inflammatory transcription factors can induce a plethora of pro-inflammatory genes, of which many are involved in cell cycle and survival. This supports the notion that not the receptor as such provides unique signals, but that the timing and context of its signaling is most important for its particular function. Conving evidence on the actual contribution of these costimulatory molecules to a T cell response has been obtained in mice that lack one of the receptors (see Table 1; for a detailed overview on the expression of these molecules, see reference (68)). Similar to CD27, mice lacking expression of 4-1BB, OX40 or their ligands have defective primary and secondary T cell responses against several viruses (69-77). Important differences in these models are that 4-1BB typically regulates the CD8 T cell response, while OX40 governs the CD4 T cell response. Interestingly, 4-1BB-deficient mice generate a more enhanced effector CD4 T cell response to a protein antigen, which suggests that 4-1BB might also have a negative regulatory role on T cells (78). In this respect, it is interesting to note that both OX40 and 4-1BB can play an important role on regulatory T cells, possibly in both positive and negative manners (reviewed in (79)). Furthermore, experiments performed with mice deficient in both 4-1BBL and OX40L confirm that 4-1BB and OX40 act independently and nonredundantly to facilitate robust CD8 and CD4 recall responses, respectively (80). Still, other reports indicate that this specialization is not absolute, as OX40 can also contribute to CD8 T cell responses under certain circumstances (81-83). Mice double-deficient for OX40 and CD30 show that these receptors can act synergistically, which is important for the survival of memory CD4 T cells in germinal center responses (84) and effector CD4 T cells during Salmonella infection (67). Studies in mice deficient for either CD30 or CD30L indicate that CD30 is required for adequate effector CD4 T cell responses during mycobacterial infections (85;86) and in the generation of long-lived memory CD8 T cells following infection with Listeria
### 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>
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<tbody>
<tr>
<td>CD27</td>
<td>T &amp; B cells NK cells Progenitors</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>(30;41)</td>
</tr>
<tr>
<td>CD70</td>
<td>T &amp; B cells DCs Macrophages</td>
<td>-</td>
<td>BC 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>(45-47)</td>
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<tr>
<td></td>
<td></td>
<td>-</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>(53)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</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>(55)</td>
</tr>
<tr>
<td>4-1BB</td>
<td>T &amp; B cells NK cells DCs</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>(72;78)</td>
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<tr>
<td></td>
<td></td>
<td>-</td>
<td>T cell-TG</td>
<td>Enhanced T cell proliferation in vitro and increased CHS.</td>
<td>(108)</td>
</tr>
<tr>
<td>4-1BBL</td>
<td>B cells DCs Macrophages</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>(70;73;74;76)</td>
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<tr>
<td></td>
<td></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>(107)</td>
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<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>(85;115;116)</td>
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<td></td>
<td></td>
<td>-</td>
<td>T cell-TG</td>
<td>Enhanced thymocyte apoptosis upon stimulation; splenomegaly.</td>
<td>(114)</td>
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<tr>
<td>CD30L</td>
<td>T &amp; B cells Macrophage</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>(86;87)</td>
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<tr>
<td>HVEM</td>
<td>T &amp; B cells DCs Macrophages</td>
<td>Full KO</td>
<td>-</td>
<td>Reduced CD4 T cell proliferation and effector cell formation to protein immunization.</td>
<td>(96)</td>
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<tr>
<td></td>
<td></td>
<td>-</td>
<td>Soluble form</td>
<td>Resistant to infection with HSV-1, but not pseudorabies virus.</td>
<td>(120)</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>(88;100)</td>
</tr>
<tr>
<td></td>
<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>(117)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>Increased CD4 T cell numbers, enhanced CHS and allogenic stimulation.</td>
<td>(113)</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 protein immunization; reduced CD8 T cell response to allogeneic stimulation.</td>
<td>(69;77)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>Reduced CD4 T cell proliferation and effector cell formation to protein immunization.</td>
<td>(112)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>DC-TG</td>
<td>Increased CD4 accumulation in B cell follicles.</td>
<td>(109)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</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>(110;111)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>All cell-TG</td>
<td>Increased CD4 T cell numbers, enhanced CHS and allogenic response.</td>
<td>(113)</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>(101;105)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</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>(103;106)</td>
</tr>
<tr>
<td>GITRL</td>
<td>B cells</td>
<td>-</td>
<td>-</td>
<td>Enhanced in vivo effector and regulatory CD4 T cell proliferation, delayed disease induction in EAE model.</td>
<td>(146)</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).
monocytogenes (87). Furthermore, in a variety of models with HVEM- or LIGHT-deficient mice it has been shown that the LIGHT-HVEM costimulatory system can play an activating role in both CD4 and CD8 T cell responses (88-92), although expression of LIGHT is dispensable for T cell responses during influenza virus infection (93). Apart from its function as an activating receptor through binding LIGHT, HVEM can also act as a ligand for the inhibitory receptor B and T lymphocyte attenuator (BTLA) and thereby deliver inhibitory signals to T cells (94-96). Another complicating factor in the study of LIGHT-HVEM interactions is that LIGHT can also bind the lymphotoxin (LT)-β receptor and decay receptor 3, while HVEM can also bind soluble lymphotoxin α3 (97-99). The importance of this was revealed in a study using mice deficient for both LIGHT and LTβ, which not only confirmed the costimulatory function of LIGHT in T cell activation, but also revealed a cooperative role for LIGHT and LTβ in lymphoid organogenesis (100). It is thus possible that changes in LIGHT-HVEM interaction also affect the function of other binding partners of these molecules. The last receptor worth mentioning in this series is GITR, which is expressed on activated CD4 and CD8 T cells as well as on regulatory CD4 T cells. Studies using GITR-deficient mice show that costimulation through GITR enhances T cell proliferation and that the absence of GITR is protective in several inflammatory disease models, which is attributed to an impaired effector function of T cells (101-104). This can indeed be a direct effect of less costimulatory signals for the activated T cells, but it can also be an indirect effect due to the fact that GITR-deficient non-regulatory T cells can not escape suppression by regulatory T cells and are therefore less functional than WT controls (105). On the other hand, GITR-deficient mice are less susceptible to infection with Candida albicans and develop more protective effector Th1 cells, which is attributed to the finding that GITR on regulatory T cells can inhibit IL-12 production by DCs and thereby affect Th1 differentiation (106).
In summary, deletion of one of these costimulatory receptors or their ligands generally leads to a mild phenotype in mice and hampers but does not prevent the formation of a functional pool of effector and memory T cells. The consequence of such a deficiency for the course of the T cell response depends on the cell type that is affected, the degree by which expression of the ligand and/or receptor is induced in a particular model and whether other costimulatory molecules are expressed that can take over the function of the deleted molecule.

**The impact of constitutive costimulation**

A more serious phenotype is generally observed when the costimulatory ligands (and in some cases also the receptor) are overexpressed and constitutively provide costimulation to T cells (*Table 1*). Similar to CD70, overexpression of 4-1BB and OX40L on APCs, using the MHC class II I-E\(\alpha\) promoter, induces a profound splenomegaly, B cell depletion in both primary and secondary lymphoid organs and a concomittant reduction of IgG responses upon immunization (107). This phenotype is quite similar to CD19-CD70TG mice (45), but it is not yet known whether the phenotype of 4-1BB-TG mice is equally dependent on the increased IFN-\(\gamma\) production by effector T cells. Overexpression of 4-1BB on T cells does not induce gross phenotypic changes in lymphoid organs, but it does enhance proliferative responses of CD4 T cells *in vitro* and it induces an elevated contact hypersensitivity (CHS) response *in vivo* (108). In contrast, mice that overexpress OX40L on T cells develop a severe autoimmune phenotype, as they develop massive splenomegaly, interstitial pneumonia, inflammatory bowel disease and produce anti-DNA auto-antibodies (109). These mice display a more severe disease intensity during experimental autoimmune encephalomyelitis (EAE) (110) and are more susceptible to infection with Leishmania major, which is accompanied by an excessive Th2 response (111). Moreover, these mice have hyperproliferative CD4 T cells, accumulate high numbers of effector memory CD4, but not CD8 T cells and do not loose their B cells (109) as seen in
CD70TG and 4-1BBL-TG mice (45;107). OX40L-overexpression on dendritic cells induces an accumulation of activated CD4 T cells in B cell areas following immunization, but this does not influence antibody development (112). Finally, mice that overexpress OX40L on all cell types, using a β-actin promoter, have increased numbers of CD4 T cells, which are more responsive in an allogeneic setting and induce an enhanced CHS response (113). Whether these two transgenic models develop an equally severe phenotype as the T cell-specific OX40L-transgenic mice is not described in these papers.

Whereas overexpression of CD70 or OX40L on T cells does not seem to affect T cell selection in the thymus, constitutive expression of CD30 on T cells induces apoptosis of thymocytes, but only after deliberate stimulation (114). This study suggests that CD30 is important in thymic negative selection, which correlates with findings in CD30-deficient mice (115), but which is opposed in another study (116). CD30-overexpression also increases the size of the spleen and mesenteric lymph nodes (114), but the quality of T cell responses in these mice has not been described. Furthermore, overexpression of LIGHT on T cells is sufficient to increase apoptosis of double-positive thymocytes and it is suggested that LIGHT plays a role in negative selection of T cells in the thymus (117). A large proportion of the T cells that make it into the periphery in these mice differentiate into IFN-γ producing effector T cells, which elicit a dramatic phenotype, including splenomegaly and lymphadenopathy, severe intestinal inflammation, glomerulonephritis and destruction of the reproductive organs (118;119). Adoptive transfer of LIGHT-transgenic T cells to RAG-deficient mice is sufficient to induce intestinal inflammation, which is dependent on expression of LTβ receptor in the recipient and HVEM on the donor T cells (96). Whether the rest of the severe phenotype of LIGHT-transgenic mice also depends on both receptors is not clear. Finally, transgenic mice that overexpress a soluble form of HVEM-Ig are resistant to infection with herpes simplex virus type 1 (HSV-1), but not pseudorabies virus (120). HSV-1 uses HVEM as a receptor for
entry into the cell (hence its name) and the transgenic soluble form of HVEM can bind the virus and thereby block its infectivity. Whether HVEM-overexpression also affects anti-viral T cell responses, eg. by increased signaling through BTLA, is not described.

The above described models clearly show that constitutive signaling through one of these costimulatory molecules is generally sufficient to induce a severe phenotype with immunopathology. We recently found that the GITR-GITRL pathway forms an exception to this rule, since transgenic expression of GITRL on B cells induces strong effector CD4 T cell formation without any signs of immunopathology as seen in the above described models (Van Olffen et al., unpublished data). The reason for this protected phenotype is that increased availability of GITRL not only provides costimulation for conventional T cells, but also activates and expands regulatory CD4 T cells. These regulatory T cells are fully functional and are even able to counteract the expanded pool of effector CD4 T cells in an EAE model, thereby delaying disease induction (Van Olffen et al., unpublished data).

In conclusion, these transgenic models demonstrate the necessity of tightly regulated expression of costimulatory receptors and their ligands: even though constitutive costimulation does enhance T cell responses, these cells can also induce severe immunopathology, unless they are kept in check by regulatory T cells. CD27-CD70 interactions can play a potent role in T cell activation and should be tightly controlled, but comparison within the family clearly indicates that close relatives can act in a similar fashion. Therefore, we would like to suggest that although lack of a single costimulatory TNF(-R) superfamily member only moderately inhibits a productive immune response, constitutive stimulation through these receptors is rather detrimental for the host, due to collateral damage induced by excessive immune activation. This implies that the relationship between the amount of costimulation given through these receptors and the productivity of the ensuing immune response is not sigmoid, as one might intuitively have expected, but rather bell-
shaped (see Figure 3). To what extent signal strength rather than signal duration determines the outcome of the response is difficult to assess in these *in vivo* systems and probably depends on the type of immune activation model that is used. We anticipate that the importance of this model is most relevant for chronic infectious diseases and tumours, since these are conditions in which expression of costimulatory ligands can be disproportionate and lasting. It will therefore be important to assess to what extent excessive costimulation contributes to the course of the disease.

Figure 3: The strength of co-stimulatory signals delivered through members of the TNF-R superfamily determines the outcome of immune reactions.

Low level triggering (as in deficient mice or after treatment with blocking antibodies) diminishes, but hardly ever completely blocks immune responses (left side of the figure), whereas continuous and strong activation limits immune reactivity and leads via chronic immune activation to the collapse of the immune system (right side). Optimal responses depend on the proper dosing and timing of costimulatory signals (middle part of the graph).

**Function of CD27 on non T cells**

Although CD27 can play an important costimulatory role on T cells, this receptor is also expressed on a variety of other cell types. In humans, CD27 is induced on B cells through antigen receptor triggering and because its expression is maintained long-term after activation, it is also a typical marker for memory B cells (121-123). Triggering of CD27 on human B cells stimulates immunoglobulin production by promoting the differentiation to
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plasma cells (124-126). Moreover, it was recently shown that interaction between activated CD8 T cells and freshly isolated B cells promotes survival and proliferation of the former, which is dependent on CD27 on the B cells and CD70 on the T cells (127). In mice, CD27 is not a marker for somatically hypermutated B cells as it is only expressed at the centroblast stage, after which expression is rapidly lost (128). On these cells, CD27 triggering promotes germinal center formation, most likely by supporting centroblast expansion. Still, CD27 is not absolutely required for adequate B cell responses, as CD27-deficiency does not affect isotype switch, somatic hypermutation or antibody production (128).

Apart from T and B cells, NK cells have also been reported to express CD27. In humans, CD27 expression was originally found on resting NK cells and activation with IL-2 increases its expression (129). Triggering of CD27 through CD70 in the presence of IL-2 or IL-12 directly enhances NK activity by increasing effector-target conjugate formation (130). Recently, we and others have found that CD27 expression can define phenotypically and functionally distinct human NK cell subsets, as the majority of human NK cells in peripheral blood is CD27$^{\text{low}}$/CD56$^{\text{dim}}$, while a minor population has a CD27$^{\text{hi}}$/CD56$^{\text{bright}}$ phenotype (131;132). The former population contains higher levels of perforin and granzyme B and is correspondingly more cytotoxic, whereas the latter population is better capable in production of the inflammatory cytokines IFN-$\gamma$ and TNF-$\alpha$. We found that CD27 expression on NK cells is controlled by IL-15 and that CD27 down-regulation is specifically induced by CD70 (132). In mice, CD27 is uniformly expressed on immature CD11b$^\text{low}$ NK cells, whereas the population of mature CD11b$^\text{hi}$ NK cells consists of two distinct subsets based on CD27 expression: CD27$^\text{hi}$ cells are more proliferative, produce more IFN-$\gamma$ upon cytokine stimulation and are more cytotoxic than the CD27$^\text{low}$ population (133). Transplantation experiments suggest that the mature and functionally active CD27$^\text{hi}$ population can differentiate into the more resting CD27$^\text{low}$ subset, which indicates a lineage relationship
between these subsets, rather than the presence of two independent types of NK cells (133). In mice, CD27 triggering is sufficient to induce proliferation and IFN-γ production of freshly isolated NK cells, while prestimulation via CD27 also enhances the cytotoxic capacity of NK cells in an IFN-γ dependent manner (134). Little is known about NK cell function in either CD27-deficient or CD70-transgenic mice.

Expression of CD27 is also found on early hematopoietic stem cells in murine bone marrow (48;135), as well as more differentiated precursors, including the earliest lymphocyte precursors and common lymphoid progenitors (136;137). We found that CD27-deficient progenitor cells perform better in differentiation assays both in vitro and in vivo, while CD27-stimulation leads to a decrease in their differentiation capacity and an accumulation of early progenitor cells (48). The molecular mechanism of this effect is currently under investigation. CD27 has not been found on the earliest hematopoietic precursors in human bone marrow (135), but it is present on early B cell progenitors (138). Although the physiological role of CD27 expression on bone marrow precursors is not yet known, we have suggested that it is part of a cellular feedback mechanism, in which activated lymphocytes that express CD70 are able to affect hematopoiesis during immune activation (48).

**CD27/CD70 interactions in pathophysiology**

In a number of chronic clinical conditions that are associated with an enhanced activation of the immune system, an increase in CD70 expression has been documented. First, T cells from HIV-infected individuals express enhanced levels of CD70 upon activation, which contributes to their APC-like properties in vitro (52). Enhanced stimulatory potential of these non-professional APC may contribute to persistently high levels of immune activation in HIV infection and be related to disease progression which is supported by the demise of T cell system observed in CD70 transgenic mice. In patients with rheumatoid arthritis, CD70 is
significantly more expressed on CD4 T cells compared with age-matched controls (50). Interestingly, as CD70 on T cells lowers the activation threshold predominantly of low-avidity T cells, it suggests that high expression of CD70 might contribute to the breakdown of tolerance in this autoimmune disease. Likewise, T cells from SLE patients overexpress CD70 (51). This increased expression might contribute to B cell costimulation and subsequent immunoglobulin overproduction that may contribute to lupus. Although the role of CD27-CD70 interactions in the pathogenesis of human autoimmune disease needs clarification, blocking studies with anti-CD70 antibody show that this treatment can prevent EAE, the mouse model for multiple sclerosis (139). The preventive effect of anti-CD70 mAb was not due to the inhibition of T cell priming and antibody production from B cells, or immune deviation. However, TNF-alpha production was suppressed by treatment with anti-CD70 mAb, indicating that the ameliorating effect of anti-CD70 mAb appeared, at least in part, to be mediated by the inhibition of TNF-alpha production. These results indicate that the CD70-CD27 interaction plays a pivotal role in the development of cell-mediated autoimmune disease.

CD70 expression has been found on human malignancies from different origins including thymic carcinoma, renal cell carcinoma, glioblastoma, chronic lymphocytic leukemia, non-Hodgkin lymphoma and human T cell leukemia virus-1 induced T cell leukemia (reviewed in (140)). Since many of the tumors arising from the hematopoietic lineage also express CD27, a possible role for CD27-CD70 interactions in the regulation of tumor cell expansion and survival might be envisaged. Further, the high expression of CD70 on particular tumors implies that the molecule is an attractive candidate for active immunotherapy. Indeed, a humanized CD70 mAb has been engineered that possesses Fc-dependent antibody effector functions and mediates anti-tumor activity in vivo (141;142).
Finally, the ability of CD70 to induce a strong expansion of effector T cells in vivo (45), breakdown of tolerance (53) and induction responses to non-immunogenic tumors (46) makes CD70 an attractive adjuvant for active immunotherapy. In this respect it is promising that multimeric soluble CD70 is as able as membrane-bound costimulatory activity in vivo (54). Soluble CD70 has the advantage that dose and timing of costimulation can be tuned avoiding the side effects of excessive CD27-CD70 interactions.

**Perspective**

The large variety of costimulatory molecules that can be put into action during T cell activation generally enables an adequate and well-balanced T cell response upon encounter of most antigens and pathogens. As highlighted here, CD27 and its related family members assist activated T cells in their processes of survival, proliferation and acquisition of effector functions. They also seem to play a role in the formation of distinct T cell subsets that are generated during infection, such as effector T cells that target infected cells, effector memory T cells that provide immediate effector function upon re-challenge, and central memory T cells that have the potential to clonally expand upon secondary infection. However, the specific contribution of CD27 and other TNFR molecules in formation of these different T cell subsets is not yet known and it will not be an easy task to examine this, as at present contrasting and mutually exclusive views exist on T cell differentiation (143). Nevertheless, a better understanding on the role of CD27 and related molecules in development of short lived effectors versus long-lived memory T cells has high value, as this knowledge may guide the design of optimal vaccination strategies.

The role of CD27 has been well studied in acute infection models and CD27-driven costimulation seems to be beneficial for T cell responses, leading to enhanced pathogen clearance. Clearly, CD70 is constitutively expressed during chronic infection and in
autoimmune disease, but not much is known on the role that CD70 and CD27 play in chronic infection models. The only exception in this respect is infection with LCMV, but these studies rather indicate that CD70-driven responses are counter-effective on viral clearance (41;43). Transgenic mouse models have clearly revealed the powerful effector T cell differentiation capacity of CD27, but also indicate that CD70 can initiate inhibitory pathways mediated by inhibitory molecules PD-1 and IL-10 and activate apoptosis pathways driven through FasL and Fas. However, the importance of these regulatory events driven by CD27 in chronic diseases remains to be proven.

Although it is evident that transient signaling through CD27 and related costimulatory molecules is beneficial for the immune response, sustained signaling rather results in immune pathology. At present we do not understand the underlying molecular mechanism, but new insight might come from studies on CD40 and TNFR-I, as it has been suggested that downstream signaling pathways through NF-κB and MAPKs are different between sustained and transient triggering of these receptors (144;145). Whether the same concept holds true for CD27 and its relatives and to what extent this could explain the induction of immune pathology is food for future research.

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Ref Type: Generic


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