Molecular mechanisms underlying CD27-CD70 costimulation
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Citation for published version (APA):
Peperzak, L. (2010). Molecular mechanisms underlying CD27-CD70 costimulation
Chapter 8

Summarizing Discussion
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CD27-CD70: A window for therapeutic intervention

The ultimate goal for fundamental immunological research, such as the work presented in this thesis, is to find suitable clinical applications. The CD27-CD70 interaction can act as a mixed blessing for intervention during disease (1). For example, various tumor types are associated with high and continuous CD70 expression, such as multiple myeloma (MM), glioblastoma, thymic carcinoma, T-cell leukemia and renal cell carcinoma (2). The expression of CD70 by these tumors can be either beneficial for the clinical outcome; for example, it can promote the anti-tumor response by locally stimulating tumor specific cytotoxic T lymphocytes (CTL), or, in contrast, regulatory T cells can be fully activated and suppress anti-tumor responses. Also, CD27 expression has been reported on malignant B cells or in soluble form in sera from patients with chronic lymphocytic leukemia (CLL) and non-Hodgkin lymphomas. In these cases, the level of CD27 expression strongly correlated with tumor load, suggesting that CD27-CD70 interactions promote development of these tumor types (3).

Humanized anti-CD70 antibodies have been created and reveal an antitumor effect in some tumor models in mice (4,5). Furthermore, in certain autoimmune diseases, CD70 expression promotes the severity of autoimmune disease (6,7). In these cases it may be beneficial to dampen immune responses by blocking CD27-CD70 interactions. CD27 signaling has also been implicated in several chronic viral infections. Matter et al. revealed that lymphocytic choriomeningitis virus (LCMV) infections in mice were eliminated by infusion of CD70 blocking antibodies (8). In addition, high CD70 expression has been documented in HIV-infected individuals and contributed to the high levels of immune activation seen in these patients (9).

On the other hand, stimulating T cell responses during acute infections, or certain types of cancer, might promote pathogen or tumor clearance and therefore be desirable for clinical outcome. In this light, infusion of soluble CD70 is shown to significantly promote the formation of CTL in vivo (10). Moreover, infusion of dendritic cells (DC) with transgenic CD70 expression strongly promotes CTL responses and are capable to convert tolerizing conditions into immunity (11,12). Similarly, transgenic expression of CD70 by B cells improves antigen-specific T cell responses during influenza virus infection and tumor burden (13). Vaccines could be optimized to ensure CD70 expression on DC.

Taken together, it is not the question whether we should exploit our knowledge of the CD27-CD70 interaction for therapeutic intervention, the question is how and when we should do so. Therefore it is crucial first to understand the exact mechanisms of CD27-CD70 function and to determine exactly when CD27-CD70 interactions are most prominent. These two aspects are addressed in this thesis and further discussed below.

Mechanisms of CD27-mediated CD8+ effector T cell accumulation

CD8+ T cells, or cytotoxic T lymphocytes (CTL), play a crucial role in the removal of virus-infected cells and malignant cells. In the case of influenza virus infections, CTL inhibit viral replication and shorten the duration of viral shedding in mice (14,15). In humans, the levels of virus-specific CTL before infection strongly correlated with the speed of viral clearance from the respiratory tract (16). CTL can kill target cells by two independent cytolytic mechanisms. The first includes the polarized secretion of granule-stored proteins such as perforin and granzymes, causing target cell death through membrane and DNA damage. Second, interactions of Fas ligand on the CTL
with its receptor (Fas) on the target cell can lead to apoptotic cell death (17). Transcription factors like T-bet and Eomesoderm have been shown to be instrumental in the development of cytolytic effector function of CTL (18,19). Taken together, the ability to control viral infection depends on the functionality of virus-specific CTL (quality) and the absolute numbers of CTL participating in the immune response (quantity).

Previously, we have shown that CD27 stimulation promotes the accumulation of CTL upon influenza virus infection in mice (20,21). On the other hand, we have no indication that the functionality of CTL is affected by CD27 signaling. Concomitantly, microarray analyses of CD27 stimulated CD8+ T cells performed in this thesis did not identify CD27 target genes directly associated with cytolytic effector functions, such as perforin, granzymes or Fas ligand. In our in vivo mouse models, we observed that CD27-mediated CD8+ effector T cell accumulation is not caused by improved cell division but, at least in part, depends on improved cell survival (22). In line with these findings, no genes were observed among the transcriptional targets of CD27 that directly affect cell cycle activity. In contrast, for human CD8+ T cells it has been shown that triggering of CD27 can promote T cell proliferation in vitro (23). In our extensive analyses discussed in this thesis, we found several molecules induced by CD27/CD70 interactions in CD8+ T cells that are known to play a role in the survival of activated T cells.

We have shown in Chapter 3 of this thesis that CD27-induced IL-2 expression strongly increased the survival of CD8+ effector T cells in infected tissue. These findings were completely in line with the findings of D’Souza et al who showed that autocrine IL-2 signaling was dispensable for initiation of CD8+ T cell cycling, but was required for sustained CD8+ T cell expansion in non-lymphoid tissue (24,25). However, the downstream mechanism of IL-2 signaling in this context remains unclear. It has been shown that IL-2 signaling via phosphatidylinositol 3-kinase (PI3K) and/or the transcriptional activator STAT5 can promote the expression of Bcl-\(\lambda\) (26) and Pim-1 (27,28), thereby promoting T cell survival. In Chapter 4 we revealed an induction of both Bcl-\(\lambda\) and Pim-1 upon CD27 stimulation in CD8+ T cells. In theory, one could hypothesize that CD27 induces transcription of these genes indirectly via IL-2 signaling. But, since CD27-mediated transcription of Pim-1 and Bcl-\(\lambda\) is observed after 2 and 4 hours of stimulation respectively and since the differential IL-2 transcription is not seen before 4 hours after CD27 engagement, we propose that CD27 directly initiates transcription of Bcl-\(\lambda\) and Pim-1. Nonetheless, it is very well possible that first CD27 promotes the transcription of these genes directly and that, at later time points, CD27-induced IL-2 promotes their further transcription to guarantee a continuous availability of these anti-apoptotic molecules throughout the immune response. This hypothesis fits with our observations in vivo, where CD27 mediates CD8+ effector T cell survival in the priming organs directly in an IL-2 independent manner, but is dependent on autocrine IL-2 signaling when the CD8+ effector T cells reach the infected tissue.

We revealed in Chapter 4 that induction of Pim-1 contributes significantly to CD27 mediated CD8+ T cell survival in the lung draining lymph node (DLN), spleen and lung upon influenza virus infection. Pim kinases can inhibit apoptosis by phosphorylating Bad (29-31) but also stimulate aerobic glycolysis and the efficiency of translation (32). Furthermore, they induce suppressor of cytokine signaling (SOCS) family proteins (33,34), the transcriptional repressor eIF-4E binding protein 1 (4E-BP1) (35) and/or the transcription factor Myb (36). A combined overexpression of the anti-apoptotic Bcl-2 family members Bcl-\(\lambda\) and Mcl-1 should neutralize all pro-apoptotic BH3-only proteins, such as Bad, Bim, Bid, Puma and Noxa, and block Bax/Bak mediated mitochondrial permeabilisation
and apoptosis. However, in our model, overexpression of Bcl-xL alone, or together with Mcl-1, promoted CD8+ T cell accumulation to a certain extent but was insufficient to rescue CD27− CD8+ effector T cells from apoptosis. There can be two different explanations for this observation; it might be possible that the expression levels of Bcl-xL and Mcl-1 are not high enough to neutralise BH3-only proteins, or there is an additional level of regulation that does not involve the mitochondrial route of apoptosis induction. Since signaling via Pim kinases can provide alternate survival signaling, overexpression of both Bcl-xL and Pim-1 in CD27− CD8+ effector T cells might completely rescue their impaired survival. This experimental setup will be instrumental for understanding the contribution of CD27 targets Bcl-xL and Pim-1 to CD27 mediated T cell survival and will be performed in the near future. Another possible alternate mechanism is that of the inhibitor of apoptosis proteins (cIAPs). We have indications that cIAP-1 may be involved in the function of CD27, since it is moderately, but significantly, induced upon CD27 stimulation in CD8+ T cells. cIAP-1 has been shown to inhibit TNFα mediated signaling and subsequent activation of Caspase 8 and apoptosis (37). However, the contribution of cIAP-1 induction to CD27 function needs to be examined further (paragraph summarized in Figure 1).

![Figure 1. Mechanism of CD27-mediated CD8+ T cell survival.](image)

CD27 stimulation results in gene transcription via the NFκB or JNK pathways. Molecules induced by CD27 are depicted in red (IL-2, Pim-1 and Bcl-xL) and a potential CD27 target gene (IAP-1) is depicted in orange. Illustrated is the method by which these molecules can contribute to the survival of activated CD8+ T cells.
Dolfi and co-workers infected mice with influenza virus and showed that CD27 stimulation inhibited Fas-dependent apoptosis of effector CD8\(^+\) T cells at day 10 after infection, a late timepoint that marks the start of T cell contraction (38). Although we have focused on the role of CD27 at the peak of the acute immune response against influenza virus (day 8); by looking at the nature of CD27 targets using microarray or by examining the expression of Fas or Fas ligand throughout infection, we have not found any indication that Fas signaling is involved in CD27 mediated T cell accumulation. On the other hand, under conditions of chronic immune activation, Arens et al. revealed that CD27 stimulation sensitized T cells for Fas-induced apoptosis (39,40). This indicates that CD27 signaling can act as a double edged sword; stimulating effector T cell survival during acute infections when antigen specific T cells are necessary to limit the rate of infection and sensitize T cells for Fas-induced cell death to prevent autoimmunity during chronic infections.

In addition to T cell survival and proliferation, the specific migration of T cells can also contribute to the local accumulation of effector T cells during infection. In Chapter 5, we revealed a contribution of the chemokine CXCL10 to CD27-mediated CD8\(^+\) effector T cell accumulation in the lung draining lymph node (DLN) after infection with influenza virus. We hypothesized that CD27 stimulates the expression of CXCL10 in CD8\(^+\) effector T cells in the DLN, but also in the spleen and lung, which accumulates in the basal lamina of HEV. As a resultant, naïve CD8\(^+\) T cells, that express CXCR3, will be selectively attracted to the DLN and participate in the ongoing immune response. To test whether this is the case, we will inject naïve CD8\(^+\) T cells during the ongoing immune response against influenza virus in mice that received CD27\(^-\) influenza virus-specific T cells transduced with either CXCL10 or empty vector. Several hours after injection of naïve CD8\(^+\) T cells, we will isolate the DLN and examine the number and nature of the injected CD8\(^+\) T cells that have migrated into the DLN. If we observe a selective migration of CXCR3\(^+\) naïve CD8\(^+\) T cells into the DLN in mice that received CXCL10 expressing CD8\(^+\) flu-specific T cells, we can confirm our hypothesis. Besides CXCL10, we also revealed a significantly induced expression of the chemokines XCL1 and CCL4 and an inhibition of CCL7 expression in CD27 stimulated CD8\(^+\) T cells. XCL1 is a chemokine expressed by CD8\(^+\) T cells and can selectively attract cross-presenting CD8\(^+\) DC in vivo (41). Local attraction of CD8\(^+\) DC could be beneficial for primed CD8\(^+\) T cells by providing additional costimulatory signals to enhance their accumulation. The roles of CCL4 and CCL7 in the context of CD8\(^+\) T cell stimulation remain unclear.

Spatial and temporal regulation of CD27 stimulation

Stimulation of the Ig-like co-stimulatory receptor CD28 promotes the initial survival and cell division of activated CD8\(^+\) T cells (42), whereas CD27 signaling supports their subsequent survival during the expansion phase (22). CD27 is expressed on the vast majority of naïve T cells and can be engaged by CD70 immediately (43,44). In contrast, TNF receptors 4-1BB and OX40 are expressed only on activated T cells (45). In line with this notion, Hendriks et al revealed that the co-stimulatory role of 4-1BB is most prominent later in the expansion phase. The contribution of OX40 to the CD8\(^+\) T cell response was revealed even later during T cell contraction and formation of CD8\(^+\) memory T cells (21). In the analyses of CD27 induced genes performed in this thesis we found 4-1BB to be significantly induced upon CD27 stimulation in CD8\(^+\) T cells. This finding points to a role for CD27 in the propagation or amplification of co-stimulatory signals via the induction of 4-1BB. We did not
observe a CD27-mediated induction of OX40, but in addition revealed an increased expression of glucocorticoid-induced tumor necrosis factor receptor (GITR) and TNF ligand lymphotixin beta (LTβ) after CD27 stimulation, which could further aid ongoing T cell responses (46).

As mentioned, the availability of CD70 determines the timing of CD27 signaling. In addition to increased CD70 transcription (47), CD70 can also be recruited instantly from MHC class II compartments (MIIC) and traffic in a directed fashion towards the immunological synapse (IS) with a CD4+ T cell (48). Induction of CD70 on the cell surface in dendritic cells (DC) is induced in response to Toll-like receptor (TLR) and/or CD40 stimulation (49). We showed in Chapter 7 of this thesis that it is the MHC class II associated invariant chain (Ii) that chaperones CD70 to MIIC. Hence, this event is crucial for maintaining a pool of CD70 in MIIC to be able to reach the cell surface when needed to stimulate CD27 on the engaged T cell. Considering the findings mentioned, it can be expected that, in the absence of Ii, CD70 recruitment to the IS with a CD4+ T cell is impaired. However, since MHC class II dependent antigen presentation, necessary to stimulate a CD4+ T cell, also relies on the presence of Ii, such a scenario is difficult to test. We showed in this thesis that for CD27 stimulation on CD8+ T cells, the chaperoning function of Ii is not required. Therefore we suggest the following scenario; first a CD4+ T cell engages a DC via interactions between LFA-1 and ICAM-1, MHC class II molecules and the TCR and CD40 ligand and CD40 interactions. As a resultant, CD70 and additional MHC class II molecules are recruited from MIIC to the IS and stimulate the CD27 expressing CD4+ T cell. Subsequently, a CD8+ T cell engages the same DC and benefits from the CD70 that is already present on the cell surface. Therefore, it might not be necessary to recruit CD70 to the IS with a CD8+ T cell. In line with this hypothesis, Keller et al showed that in the case of transgenic expression of CD70 on DC, the CD8+ T cell response was independent of CD4+ T cell help (50).

Generally, CD4+ T cell help during the primary immune response is required for an optimal CD8+ memory T cell response (51). In Chapter 6 we revealed that CD27 stimulation on CD4+ T cells during priming, promoted the secondary expansion of CD8+ memory T cells after re-challenge. We showed it was not the numbers of CD4+ T cells, but the quality of help the CD4+ T cells provided after being stimulated via CD27. After CD27 triggering, CD4+ T cells promoted the expression of the cytokines IL-2 and IFN-γ, but also increased the transcription of BAZ2A (bromodomain adjacent to zinc finger domain 2A), which has been shown to be involved in processes of chromatin remodeling (52). By inducing transcription factors and/or chromatin remodeling factors such as BAZ2A, CD27 stimulation could genetically imprint in CD4+ T cells the capacity to provide optimal CD8+ T cell help. Similarly, signals provided by CD27 proficient CD4+ T cells could genetically imprint into CD8+ T cells the capacity to optimally expand upon re-challenge. Transcription factors or chromatin remodeling factors that might be involved in this process need to be discovered.

In addition, we found that triggering of CD27 on CD4+ T cells increased transcription of the membrane spanning molecule MS4A4B (Ly116, Chandra) and its close relative MS4A4C. MS4A4B is reported to be instrumental in inducing the expression of Th1 cytokines IL-2 and IFN-γ in CD4+ T cells (53,54), but is also enriched in CD8+ memory T cells (55). Concomitantly, we found MS4A4B up-regulated in CD8+ memory T cells that have received CD27 proficient CD4+ T cell help during priming. We therefore propose the molecule MS4A4B as a mediator for CD8+ memory T cells to optimally expand after re-infection. To address this hypothesis we created monoclonal and polyclonal antibodies directed towards MS4A4B and obtained the full length...
Figure 2. CD27 induced genes.
Cell surface expression of CD27 and CD70 is shown on the different cell types during hematopoiesis. Molecules that are induced (in red) or inhibited (in green) by CD27 signaling reported in this thesis, or by others, are depicted in this illustration.
cDNA. By using these antibodies, we will first document when and on what cell types MS4A4B is expressed during primary and memory immune responses. Subsequently, we will introduce the Ms4a4b gene by retroviral transduction in CD27- CD4+ T cells and examine whether this results in improved CD4+ T cell help, read out by CD8+ T cell expansion after secondary challenge. Finally, we will reconstitute CD8+ T cells with the Ms4a4b gene and investigate whether these cells expand properly in the absence CD4+ T cell help. In addition, the reciprocal experiment will be performed where we lower the expression of MS4A4B in CD8+ T cells by RNA interference, and measure the effect on CD8+ memory T cell expansion. To further delineate the role of MS4A4B for the CD8+ memory T cell response we will create mice genetically deficient or with transgenic expression of MS4A4B. These collective experiments will address whether and to what extent MS4A4B contributes to CD8+ memory T cell responses.

REFERENCES
quantitative and qualitative effects on effector CD8+ T cell formation. J. Exp. Med. 199:595-1605.


