Chapter 8

Summarizing Discussion

SUMMARIZING DISCUSSION

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 suiting 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 en 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 Eomesodermin 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-x_L (26) and Pim-1 (27,28), thereby promoting T cell survival. In **Chapter 4** we revealed an induction of both Bcl-x_L 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-x_L 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-x_L 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-x_L 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-x_L 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-x_L 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-x_L 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-x_L 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).

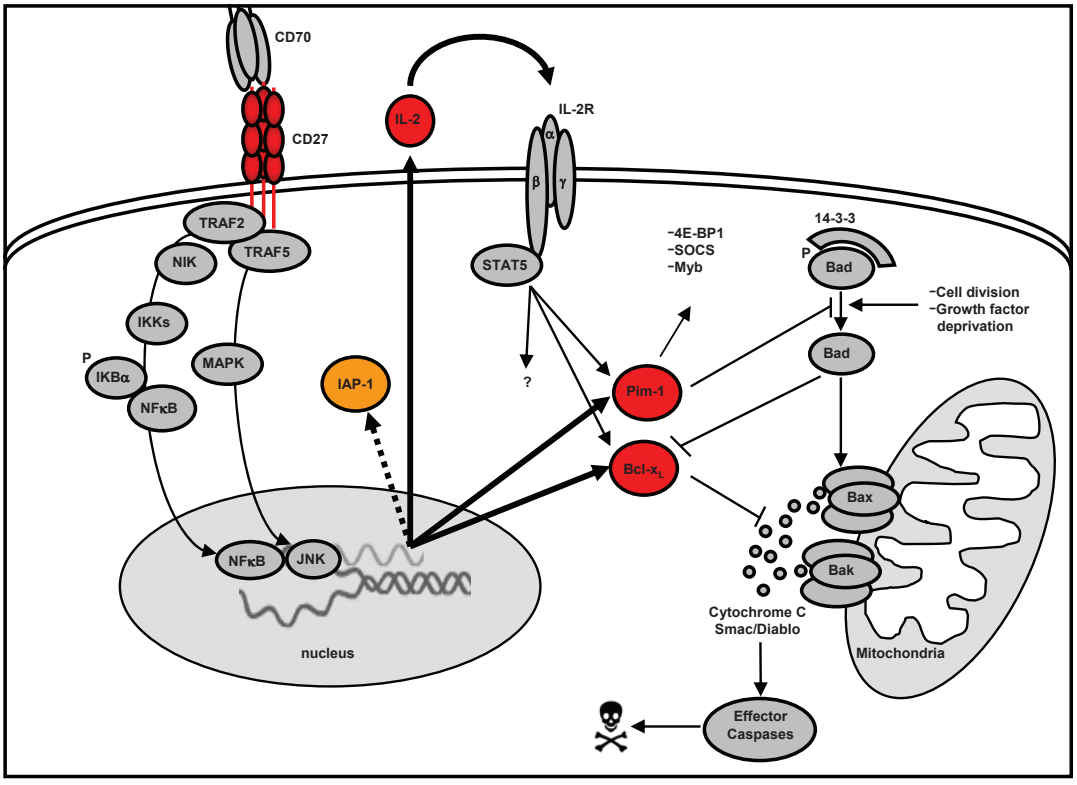

Figure 1. Mechanism of CD27-mediated $CD8^+$ T cell survival.
CD27 stimulation results in gene transcription via the NFκB or JNK pathways. Molecules induced by CD27 are depicted in red (IL-2, Pim-1 and Bcl-x_L) and a potential CD27 target gene (IAP-1) is depicted in orange. Illustrated is the method 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 focussed 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 lymphotoxin 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 T_H1 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

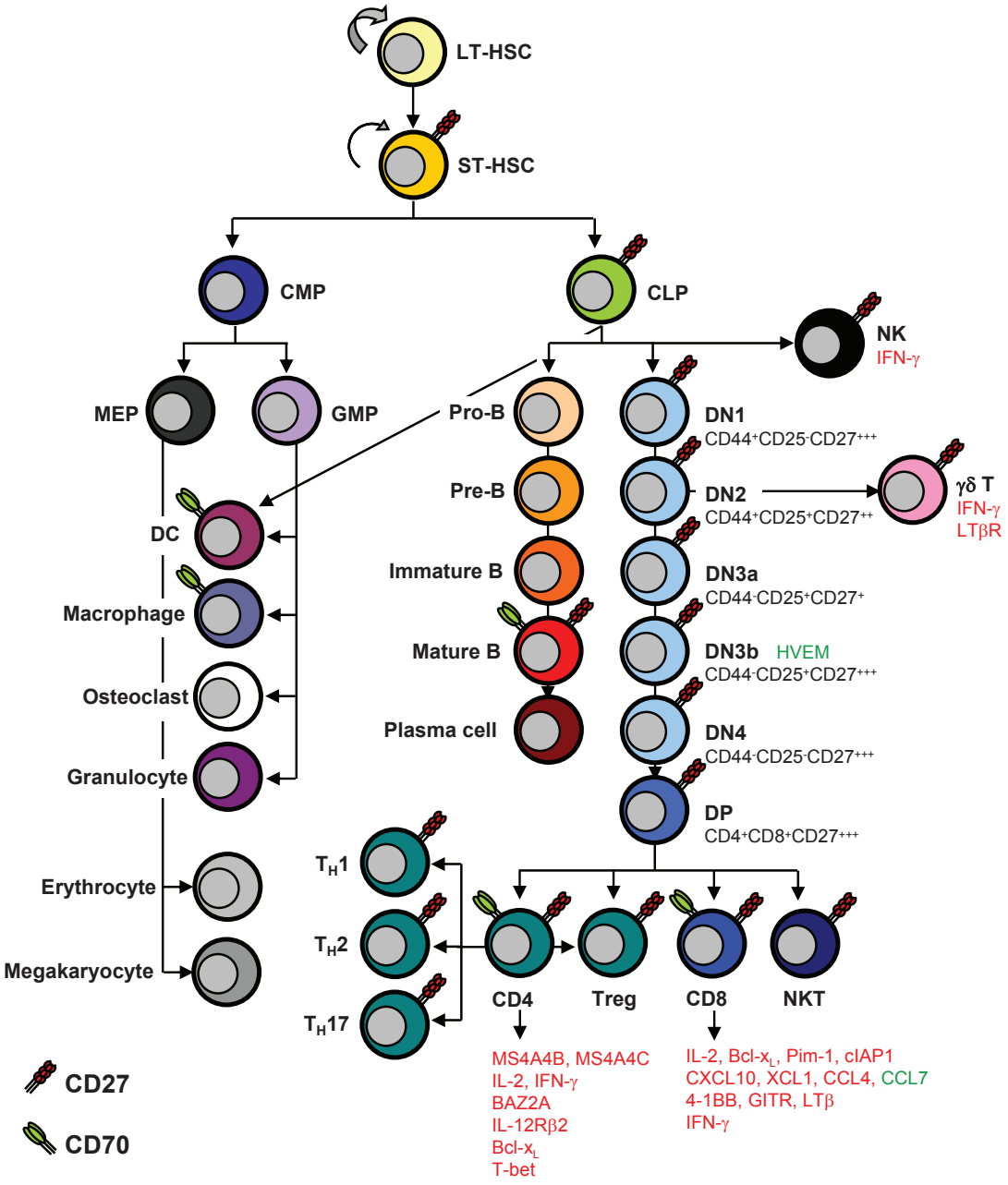

Figure 2. CD27 induced genes.
Cell surface expression of CD27 and CD70 is shown on the different cell types during hematopoiesis. Molecules that are induced (in red) or inhibited (in green) by CD27 signaling reported in this thesis, or by others, are depicted in this illustration.

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 extend MS4A4B contributes to $CD8^+$ memory T cell responses.

REFERENCES

1. Nolte, M.A., Olffen, R.W., van Gisbergen, K.P. and van Lier, R.A. 2009. Timing and tuning of CD27-CD70 interactions: the impact of signal strength in setting the balance between adaptive responses and immunopathology. *Immunol. Rev.* 229:216-231.
2. Grewal, I.S. 2008. CD70 as a therapeutic target in human malignancies. *Expert. Opin. Ther. Targets.* 12:341-351.
3. Van Oers, M.H., Pals, S.T., Evers, L.M., van der Schoot, C.E., Koopman, G., Bonfrer, J.M., Hintzen, R.Q., von dem Borne, A.E. and van Lier, R.A. 1993. Expression and release of CD27 in human B-cell malignancies. *Blood.* 82:3430-3436.
4. McEarchern, J.A., Oflazoglu, E., Francisco, L., McDonagh, C.F., Gordon, K.A., Stone, I., Klussman, K., Turcott, E., van Rooijen, N., Carter, P., Grewal, I.S., Wahl, A.F. and Law, C.L. 2007. Engineered anti-CD70 antibody with multiple effector functions exhibits in vitro and in vivo antitumor activities. *Blood.* 109:1185-1192.
5. McEarchern, J.A., Smith, L.M., McDonagh, C.F., Klussman, K., Gordon, K.A., Morris-Tilden, C.A., Duniho, S., Ryan, M., Boursalian, T.E., Carter, P., Grewal, I.S. and Law, C.L. 2008. Preclinical characterization of SGN-70, a humanized antibody directed against CD70. *Clin. Cancer. Res.* 14:7763-7772.
6. Lee, W.W., Yang, Z.Z., Li, G., Weyan, C.M. and Goronzy, J.J. 2007. Unchecked CD70 expression on T cells lowers threshold for T cell activation in rheumatoid arthritis. *J. Immunol.* 179:2609-2615.
7. Oelke, K., Lu, Q., Richardson, D., Wu, A., Deng, C., Hanash, S. and Richardson, B. 2004. Overexpression of CD70 and overstimulation of IgG synthesis by lupus T cells and T cells treated with DNA methylation inhibitors. *Arthritis. Rheum.* 50:1850-1860.
8. Matter, M., Odermatt, B., Yagito, H., Nuoffer, J.M. and Ochsenbein, A.F. 2006. Elimination of chronic viral infection by blocking CD27 signaling. *J. Exp. Med.* 203:2145-2155.
9. Wolthers, K.C., Otto, S.A., Lens, S.M., Kolbach, D.N., van Lier, R.A. and Miedema, F. 1996. Increased expression of CD80, CD86 and CD70 on T cells from HIV-infected individuals upon activation in vitro: regulation by CD4+ T cells. *Eur. J. Immunol.* 26:1700-1706.
10. Rowley, T.F., and Al-Shamkhani, A. 2004. Stimulation by soluble CD70 promotes strong primary and secondary CD8+ cytotoxic T cell responses in vivo. *J. Immunol*. 172:6039-6046.
11. Keller, A.M., Xiao, Y., Peperzak, V., Naik, S.H., and Borst, J. 2009. Costimulatory ligand CD70 allows induction of $CD8^+$ T cell immunity by immature dendritic cells in a vaccination setting. *Blood.* 113: 5167-5175, 2009.
12. Keller, A.M., Schildknecht, A., Xiao, Y., van den Broek, M. and Borst, J. 2008. Expression of costimulatory ligand CD70 on steady-state dendritic cells breaks $CD8^+$ T cell tolerance and permits effective immunity. *Immunity.* 29:934-946.
13. Arens, R., Schepers, K., Nolte, M., van Oosterwijk, M.F., van Lier, R.A.W., Schumacher, T.N., and van Oers, M.H. 2004. Tumor rejection induced by CD70-mediated

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

14. Flynn, K.J., Belz, G.T., Altman, J.D., Ahmed, R., Woodland, D.L. and Doherty, P.C. 1998. Virus specific CD8+ T cells in primary and secondary influenza pneumonia. *Immunity.* 8:683-691.
15. Taylor, P.M. and Askonas, B.A. 1986. Influenza nucleoprotein-specific cytotoxic T-cell clones are protective in vivo. *Immunology.* 58:417-420.
16. McMichael, A.J., Gotch, F.M., Noble, G.R. and Beare, P.A. 1983. Cytotoxic T-cell immunity to influenza. *N. Engl. J. Med.* 309:13-17.
17. Lowin, B., Mattman, C., Hahne, M. and Tschopp, J. 1996. Comparison of Fas (Apo-1/CD95)- and perforin-mediated cytotoxicity in primary T lymphocytes. *Int. Immunol.* 8:57-63.
18. Sullivan, B.M., Juedes, A., Szabo, S.J., von Herrath, M. and Glimcher, L.H. 2003. Antigen-driven effector CD8 T cell function regulated by T-bet. *Proc Natl Acad Sci USA.* 100:15818-15823.
19. Pearce, E.L., Mullen, A.C., Martins, G.A., Krawczyk, C.M., Hutchins, A.S., Zediak, V.P., Banica, M., DiCioccio, C.B., Gross, D.A., Mao, C-.A., Shen, H., Cereb, N., Yang, S.Y., Lindsten, T., Rossant, J., Hunter, C.A. and Reiner, S.L. 2003. Control of effector CD8+ T cell function by the transcription factor *Eomesodermin*. *Science.* 302:1041-1043.
20. Hendriks, J., Gravestein, L.A., Tesselaar, K., van Lier, R.A.W., Schumacher, T.N.M., and Borst, J. 2000. CD27 is required for generation and long-term maintenance of T cell immunity. *Nat. Immunol*. 1:433-440.
21. Hendriks, J., Xiao, Y., Rossen, J.W.A., van der Sluijs, K.F., Sugamura, K., Ishii, N., and Borst, J. 2005. During viral infection of the respiratory tract, CD27, 4-1BB, and OX40 collectively determine formation of CD8+ memory T cells and their capacity for secondary expansion. *J. Immunol*. 175:1665-1676.
22. Hendriks, J., Xiao, Y. and Borst, J. 2003. CD27 promotes survival of activated T cells and complements CD28 in generation and establishment of the effector T cell pool. *J. Exp. Med.* 198:1369-1380.
23. Van Oosterwijk, M. F., H. Juwana, R. Arens, K. Tesselaar, M. H. J. van Oers, E. Eldering, and R. A. W. van Lier. 2007. CD27-CD70 interactions sensitise naive CD4+ T cells for IL-12-induced Th1 cell development. *Int. Immunol.* 19:713-718.
24. D'Souza, W.N., and Lefrancois, L. 2003. IL-2 is not required for the initiation of CD8 T cell cycling but sustains expansion. *J. Immunol*. 171:5727-5735.
25. D'Souza, W.N., Schluns, K.S., Masopust, D., and Lefrancois, L. 2002. Essential role for IL-2 in the regulation of antiviral extralymphoid CD8 T cell responses. *J. Immunol*. 168:5566-5572.
26. Gonzalez-Garcia, A., Merida, I., Martinez-A.C. and Carrera, A.C. 1997. Intermediate affinity interleukin-2 receptor mediates survival via a phosphatidylinositol 3-kinase-dependent pathway. *J. Biol. Chem*. 272:10220-10226.
27. Kovanen, P.E., Young, L., Al-Shami, L., Rovella, V., Pise-Masison, C.A., Radonovich, M.F., Powell, J., Fu, J., Brady, J.N., Munson, P.J. and Leonard, W.J. 2005. Global analysis of IL-2 targets: identification of chromosomal clusters of expressed genes. *Int. Immunol*. 17:1009-1021.
28. Rowher, F., Todd, S., and McGuire, K.L. 1996. The effect of IL-2 treatment on transcriptional attenuation in proto-oncogenes pim-1 and c-myb in human thymic blast cells. *J. Immunol*. 157:643-649.
29. Yan, B., Zemskova, M., Holder, S., Chin, V., Kraft, A., Koskinen, P.J. and Lilly, M.. 2003. The Pim-2 kinase phosphorylates BAD on serine 112 and reverses BAD-induced cell death. *J. Biol. Chem*. 278:45358-45367.
30. Aho, T.L., Sandholm, J., Peltola, K.J., Mankonen, H.P., Lilly M. and Koskinen P.J. 2004. Pim-1 kinase promotes inactivation of the pro-apoptotic Bad protein by phosphorylating it on the Ser112 gatekeeper site. *FEBS Lett.* 571:43-49.
31. Macdonald, A., Campbell, D.G., Toth, R., McLauchlan, H., Hastie, C.J. and Arthur, J.S. 2006. Pim kinases phosphorylate multiple sites on Bad and promote 14-3-3 binding and dissociation from Bcl-x_L. *BMC Cell Biol*. 7:1-14.
32. Amaravadi, R., and C. B. Thompson. 2005. The survival kinases Akt and Pim as potential pharmacological targets. *J. Clin. Invest*. 115: 2618-2624.

33. Chen, X.P., Losman, J.A., Cowan, S., Donahue, E., Fay, S., Vuong, B.Q., Nawijn, M.C., Capece, D., Cohan, V.L. and Rothman, P. 2002. Pim serine/threonine kinase regulate the stability of Socs-1 protein. *Proc. Natl. Acad. Sci. USA*. 99:2175-2180.
34. Peltola, K.J., Paukku, K., Aho, T.L., Ruuska, M., Silvennoinen, O. and Koskinen, P.J. 2004. Pim-1 kinase inhibits STAT5 dependent transcription via its interactions with SOCS1 and SOCS3. *Blood*. 103:3744-3750.
35. Fox, C. J., P. S. Hammerman, R.M. Cinalli, S.R. Master, L.A. Chodosh and C. B. Thompson. 2003. The serine/threonine kinase Pim-2 is a transcriptionally regulated apoptosis inhibitor. *Genes. Dev.* 17:1841-1854.
36. Winn, L.M., Lei, W. and Ness, S.A. 2003. Pim-1 phosphorylates the DNA binding domain of c-Myb. *Cell Cycle*. 2:258-262.
37. Dubrez-Daloz, L., Dupoux, A. and Cartier, J. 2008. IAPs, More than just inhibitors of apoptosis proteins. *Cell Cycle*. 7:1036-1046.
38. Dolfi, D.V., Boesteanu, A.C., Petrovas, C., Xia, D,. Butz, E.A. and Katsikis, P.D. 2008. Late signals from CD27 prevent Fas-dependent apoptosis of primary $CD8^+$ T cells. *J. Immunol*. 180:2912-2921.
39. Arens, R., Baars, P.A., Jak, M., Tesselaar, K., van der Valk, M., van Oers, M.H.J. and van Lier, R.A.W. 2005. Cutting edge: CD95 maintains effector T cell homeostasis in chronic immune activation. *J. Immunol*. 174:5915-5920.
40. Tesselaar, K., Arens, R., van Schijndel, G.M.W., Baars, P.A., van der Valk, M., Borst, J., van Oers, M.H.J. and van Lier, R.A.W. 2002. Lethal T cell immunodeficiency induced by chronic costimulation via CD27-CD70 interactions. *Nat. Immunol*. 4:49-54.
41. Dorner, B.G,. Dorner, M.B., Zhou, X., Opitz, C., Mora, A., Guttler, S., Hutloff, A., Mages, H.W., Ranke, K., Schaefer, M., Jack, R.S., Henn, V. and Kroczek, R.A. 2009. Selective expression of the chemokine receptor xcr1 on cross-presenting dendritic cells determines cooperation with $CD8^+$ T cells. *Immunity*. Nov 11. [Epub ahead of print].
42. Acuto, O. and Michel, F. 2003. CD28-mediated co-stimulation: a quantitative support for TCR signaling. *Nat. Rev. Immunol.* 3:939-951.
43. De Jong, R., Loenen, W.A., Brouwer, M., van Emmerik, L., de Vries, E.F., Borst, J. and van Lier, R.A. 1991. Regulation of expression of CD27, a T cell-specific member of a novel family of membrane receptors. *J. Immunol*. 146:2488-2494.
44. Hintzen, R.Q., de Jong, R., Lens, S.M., Brouwer, M., Baars, P. and van Lier, R.A. 1993. Regulation of CD27 expression on subsets of mature T-lymphocytes. *J. Immunol.* 151:2426-2435.
45. Watts, T.H. 2005. TNF/TNFR family members in costimulation of T cell responses. *Annu. Rev. Immunol*. 23:23-68.
46. Ronchetti, S., Zollo, O,. Bruscoli, S., Agostini, M., Bianchini, R., Nocentini, G., Ayroldi, E. and Riccardi, C. 2004. GITR, a member of the TNF receptor superfamily, is costimulatory to mouse T lymphocyte subpopulations. *Eur. J. Immunol.* 34:613-622.
47. Tesselaar, K., Xiao, Y., Arens, R., van Schijndel G.M.W., Schuurhuis, D.H., Mebius, R.E., Borst, J. and van Lier, R.A.W. 2002. Expression of the murine CD27 ligand CD70 in vitro and in vivo. *J. Immunol.* 169:33-40.
48. Keller, A.M., Groothuis, T.A., Veraar, E.A.M., Marsman, M., Maillette de Buy Wenniger, L., Janssen, H., Neefjes, J. and Borst, J. 2007. Costimulatory ligand CD70 is delivered to the immunological synapse by shared intracellular trafficking with MHC class II molecules. *Proc Natl Acad Sci USA.* 104:5989-5994.
49. Sanchez, P.J., McWilliams, J.A., Haluszczak, C., Yagita, H. and Kedl, R.M. 2007. Combined TLR/CD40 stimulation mediates potent cellular immunity by regulating dendritic cell expression of CD70 in vivo. *J. Immunol.* 178:1564-1572.
50. Keller, A.M., Xiao, Y., Peperzak, V. Naik, S.H. and Borst, J. 2009. Costimulatory ligand CD70 allows induction of $CD8^+$ T-cell immunity by immature dendritic cells in a vaccination setting. *Blood.* 113:5167-5175.
51. Bevan, M.J. 2004. Helping the $CD8^+$ T-cell response. *Nat. Rev. Immunol.* 4:595-602.
52. Zhou, Y., Schmitz, K.M., Mayer, C., Yuan, X., Akhtar, A. and Grummt, I. 2009. Reversible acetylation of the chromatin remodelling complex NoRC is required for non-coding RNA-dependent silencing. *Nat. Cell. Biol.* 11:1010-1016.
53. Venkataraman, C., Schaefer, G. and Schindler,

U. 2000. Cutting edge: Chandra, a novel four-transmembrane domain protein differentially expressed in helper type 1 lymphocytes. *J. Immunol*. 165:632-636.

54. Xu, H., Williams, M.S., and Spain, L.M. 2006. Patterns of expression, membrane localization, and effects of ectopic expression suggest a function for MS4a4B, a CD20 homolog in Th1 T cells. *Blood.* 107:2400-2408.

55. Kaech, S.M., Hemby, S., Kersh, E. and Ahmed, R. 2002. Molecular and functional profiling of memory CD8 T cell differentiation. *Cell.* 111:837-851.