CD27/CD70 interactions in effector and memory cell formation
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Chapter 7

Discussion
CD27 AND CD70 IN THE HUMAN SYSTEM

The TNF-receptor family members form with their respective TNF family members receptor/ligand pairs of which the interaction has important functions in the regulation of immune responses\textsuperscript{11}. Human CD27 is a lymphocyte lineage specific member of the TNF receptor family, which is expressed on CD3\textsuperscript{hsh}CD4\textsuperscript{+}/CD8\textsuperscript{−} single positive thymocytes and on T, B and NK cells\textsuperscript{25}. In the last decade substantial data has been gathered about the expression and function of CD27 in the human system. In peripheral blood (PB), CD27\textsuperscript{+} and CD27\textsuperscript{−} T and B cell subpopulations can be distinguished. Comparison of the CD27\textsuperscript{+} and CD27\textsuperscript{−} T cell populations has shown that CD27\textsuperscript{−} T cells express high levels of tissue-specific homing receptors but lack expression of CD62L, which is necessary for homing into the lymph nodes\textsuperscript{28,165}. Within the CD4\textsuperscript{+}CD27\textsuperscript{+} population increased frequencies of IL-4 and IL-5 producing T cells are found\textsuperscript{165}. The CD8\textsuperscript{+}CD27 population is enriched for T cells expressing the cytotoxic mediators granzyme A/B, perforin and CD95L and moreover CD8\textsuperscript{+}CD27\textsuperscript{−} T cells have, directly ex vivo, cytolytic function\textsuperscript{28}. CD27 T cells thus seem to phenotypically and functionally represent effector T cells. Also for B cells, phenotypical and functional differences are observed between the CD27\textsuperscript{−} and CD27\textsuperscript{+} subpopulation. Functionally CD27\textsuperscript{−} B cells, but not CD27\textsuperscript{+} B cells can be induced to produce Ig in a T cell independent culture system in vitro\textsuperscript{151}. Analysis of somatic mutations in rearranged V genes showed that only CD27\textsuperscript{−} PB B cells carry mutation in their V genes, thereby identifying CD27 as the general marker for memory B cells\textsuperscript{31,32}.

CD27 exerts its function after ligation by its ligand CD70\textsuperscript{18,47}. CD70 expression is found in the thymic medulla\textsuperscript{38}, on mature dendritic cells (chapter 3) and on T and B lymphocytes\textsuperscript{17,37,71} (chapter 3). On T and B lymphocytes, triggering of the antigen receptor regulates CD70 expression\textsuperscript{36,37} (chapter 3). Resting lymphocytes do not express CD70, but after activation via the antigen receptor CD70 expression is induced. CD70 expression is only sustained by continuous antigen triggering\textsuperscript{19,36,37} and CD70 expression on PB lymphocytes thus reflects recent antigenic priming. Besides antigen, cytokines and co-stimulatory molecules also regulate CD70 expression. CD28 and CD40 can enhance antigen receptor induced expression on T and B lymphocytes respectively\textsuperscript{19,36}. The cytokines TNF-\(\alpha\), IL-1\(\alpha\) and IL-12 have been shown to enhance CD70 expression on T cells and IL-4 decreases induction of CD70 on T and B cells.

The first measurable consequence of CD27/CD70 interaction is shedding of the membrane bound form of CD27\textsuperscript{35}. After ligation by CD70 the membrane bound form of CD27 is shed by a metalloprotease (K. Tesselaar, unpublished data), resulting in a soluble form of CD27. In synergy with antigenic triggering, ligation of CD27 on T cells has been reported to enhances proliferation\textsuperscript{34,42,45}, cell survival\textsuperscript{46}, TNF-\(\alpha\) production\textsuperscript{47} and the generation of cytolytic T cells\textsuperscript{34}. On B cells CD27 ligation has only limited effects on the expansion of B cells\textsuperscript{29} but has been shown to promote plasma-cell formation\textsuperscript{29,49,50}. The combined data in the human system imply that during an immune response CD27 is instrumental in regulating the size and function of the antigen primed lymphocyte pool\textsuperscript{25,62}. The transient expression of CD70 suggests that the presence of CD70 will define when CD27 function has its effect and thereby couples CD27's influence on the antigen-primed pool to the amount of antigen.
CD27 AND CD70 IN THE MURINE SYSTEM

Both murine CD27 and CD70 have been cloned\textsuperscript{44,74} and were shown to have high homology in their primary protein sequence to their human counterparts. Recombinant soluble forms of murine CD27 and CD70 can bind to human CD70 and CD27 respectively, showing that also the tertiary structure of CD27 and CD70 are well preserved\textsuperscript{44}.

Expression of murine CD27 is not completely lymphocyte specific. Besides T, B and NK cells hematopoietic stem cells also express CD27\textsuperscript{43} (R. Arens, unpublished data)\textsuperscript{41}. As for human T cells, CD27 expression on T cells is attained in the thymus\textsuperscript{43,76}. However, in contrast to the human situation, in mice the pre-TCR and not the TCR regulates CD27 expression\textsuperscript{76}. Consequently, CD27 expression is found on virtually all thymocytes. In the periphery the majority of murine T cells express CD27 and up-regulation is induced by activation via the TCR. Small percentages of CD27\textsuperscript{+} T cells can be found in spleen and peripheral lymph nodes of mice kept in a specific pathogen free environment. Although the limited phenotypical data does not conclusively describe the differentiation state of these cells they appear, as evidenced by high CD44 expression, to be antigen experienced (P. Baars, unpublished data). The fact that, compared to peripheral lymph nodes (PLN), higher percentages CD27\textsuperscript{+} T cells are found in the spleen might reflect that CD27\textsuperscript{+} T cells have lost their capacity to re-enter the lymph nodes and are effector cells. Ligation of CD27 on murine T cells co-stimulates T cell expansion as shown by the enhanced \[^{3}H\]-thymidine incorporation of anti-CD3 mAb stimulated T cells\textsuperscript{43,45}.

Within the murine B cell compartment only 2 to 10\% of the cells express CD27\textsuperscript{43}. This is in marked contrast with the situation in humans were about 30\% of PB are CD27\textsuperscript{+}\textsuperscript{31}. It is not known if in the mouse CD27 is a marker for memory B cells. The percentage of memory cells in non-intentionally immunised mice is very low\textsuperscript{166}, but seems to mirror the number of CD27\textsuperscript{+} B cells. Phenotypical analysis of CD27\textsuperscript{+} and CD27\textsuperscript{+} B cells showed that CD27\textsuperscript{+} B cells are enriched for expression of the memory B-cell marker IgG and the GC B-cell marker GL-7 (P. Baars, Y. Xiao, unpublished data). Thus antigen primed B cells seem to be preferentially found in the CD27\textsuperscript{+} population. To date no data on the effects of CD27 ligation on B cells have been described.

Murine CD70 is found in the thymic medulla and on T, B, and dendritic cells\textsuperscript{45,95} (chapter 3). Activation via the BCR induces CD70 expression on B cells\textsuperscript{45} and this expression is enhanced by ligation of CD40. In contrast to the human system, IFN-\(\gamma\) but not IL-4 down-modulates CD70 expression on B cells (chapter 3). On in-vitro generated dendritic cells CD70 expression is found after maturation induced by G-CMSF, LPS or anti-CD40 mAb (chapter 3). For T cells, in vitro activation via the TCR does induce CD70 mRNA expression but no substantial protein expression\textsuperscript{45} (chapter 3.) Notably, CD70 expressing T cells are found in the lung and not in the spleen after infection with influenza virus\textsuperscript{46}. It thus seems that murine T cells do not only need to be activated to express CD70 mRNA, but additively require yet undefined signals to express CD70 protein.
Overall it seems that CD27 and CD70 expression is quite comparable in human and mouse. These similarities suggest that CD27/CD70 interaction may have analogous functions in human and mouse. The data on CD27 function on murine T cells supports this suggestion.

**THE IN VIVO OF CONSEQUENCES OF CD27-CD27 INTERACTION ON THE ANTIGEN-PRIMED T LYMPHOCYTE POOL**

In CD27\(^{-}\) mice reduced numbers of lung infiltrating CD4\(^{+}\) and CD8\(^{+}\) T cells were found after intranasal influenza virus infection\(^{46}\). This effect was most pronounced in the secondary response in which not only the numbers but also the kinetics were comparable to the primary response. Furthermore, analysis of antigen specific CD8\(^{+}\) T cells by tetramer staining showed a reduction in numbers of virus specific CD8\(^{+}\) T cells in lung and spleen after primary and secondary infection. A possible role of CD27 on effector-cell differentiation was analysed by measuring the capacity of the lung infiltrating CD4\(^{+}\) and CD8\(^{+}\) T cells to produce IFN-\(\gamma\) and the capacity of splenic antigen specific CD8\(^{+}\) population to exert cytolysis. Neither in the primary or the secondary response, either of these function differed between CD27\(^{-}\) T cells and WT T cells\(^{46}\).

In CD70TG mice, CD70 is present as a dominant co-stimulatory signal\(^{93}\). The potency of CD27 as a co-stimulator of T cell expansion is shown by the strong increase of CD4\(^{+}\) and CD8\(^{+}\) number in spleen and PLN numbers in 4 and 8-week-old mice. However in this model system there also is a striking accumulation of CD62L\(^{neg/dull}\)CD44\(^{high/dull}\) memory/effector cells that produce IFN-\(\gamma\) but not IL-2, which suggest that CD27/CD70 interactions are important in effector T cell formation\(^{93}\).

The data in the CD27\(^{-}\) and CD70TG mice are consistent with a role for CD27/CD70 interaction in controlling the size of the antigen-primed pool. They are however not informative about the mechanism by which CD27 exert its function. Comparison of \(^{3}\)H-thymidine incorporation and CSFE dilution can separate the contributions of cell survival and cell division to expansion. Comparison of CD27\(^{-}\) T cells and WT cells in such an experiment, indicated a role for CD27 in cell survival\(^{46}\). In similar experiments with human cells and CD70 transfectants, cell division and \(^{3}\)H-thymidine incorporation correlated, implying that CD27 co-stimulates T cell proliferation (K. Tesselaar, unpublished data). In CD70TG mice an increase in T cell numbers is seen\(^{93}\). Measurement of KI-67 expression in CD70TG mice shows a 2.5 fold increase of dividing T cells. Concordantly, during an in vivo BrdU pulse-chase experiment approximately 2.5 fold more T cells were BrdU positive after the labelling period (chapter 5). Enhancement of cell proliferation and survival are both compatible with these results.

Effector T-cell formation is not blocked in CD27\(^{-}\) mice\(^{46}\). However, the data in the CD70TG mice clearly point to a role for CD27 in T cell differentiation\(^{93}\). The postulation that in CD27\(^{-}\) mice the differentiation signal is provided by other co-stimulatory receptors is a possible explanation for the discrepancy between these results. Furthermore, the experimental design to measure effector cell differentiation might have influenced the outcome of the results. It can for example be assumed that only terminally differentiated effector cells migrate to the site of
infection and consequently, as seen in CD27⁻ mice, only the numbers and not differentiation status of the T cells will differ at this site.

In CD70TG mice there seems to be a specific increase in cells producing IFN-γ and not IL-2 or TNF-α. Ligation of OX-40, another TNF-receptor family member, specifically upregulates the expression of the chemokine receptor CXCR5. This suggests that co-stimulatory molecules can drive differentiation into a certain direction. The nature of the co-stimulatory signal is however unclear. It could be either, instructive, permissive or selective. Whereas in an instructive scenario CD27 by itself would direct cells to produce IFN-γ, in a permissive situation CD27 would prime cells for differentiation signals like IL-12. A selective signal could involve survival of IFN-γ producing cells. Instruction of T cells by CD27 could entail the transcription factors T-bet and gata-3, which have recently been shown to be sufficient to induce IFN-γ and IL-4 production in T cells. Deciphering of the intracellular and gene specific effects of CD27 will possibly shed light on this issue.

**T CELL CO-STIMULATION**

Next to CD27, other molecules such as CD28, OX-40 and 4-1BB can co-stimulate T cells. CD28 is the classic T cell co-stimulatory molecule and is constitutively expressed on T cells. The ligands for CD28, B7-1 and B7-2 are expressed on APCs, activated B and T cells. Ligation of CD28 enhances T-cell activation, proliferation (importantly by the induction of IL-2 production) and survival. Besides CD28, the B7 molecules also bind to CTLA-4. Ligation of CTLA-4 transduces inhibitory signals that modulate the CD3 and/or CD28 signals and thus counteracts CD28 function. OX-40 and 4-1BB are members of the TNF-receptor family. In contrast to CD27 and CD28 these molecules are only expressed on activated CD4⁺ and CD8⁺ T cells. Like CD70, the ligands for OX-40 and 4-1BB are regulated by antigen and can be found on dendritic cells, activated B cells, macrophages (4-1BBL) and activated T cells (OX-40L). Being members of the non-death-domain containing subfamily of TNF-receptors CD27, OX-40 and 4-1BB show similarity in their signalling pathways. All three can bind Traf2 and 3 and activate the transcription factors NF-κB and the c-Jun N-terminal kinase (JNK) after ligation. In vitro and in vivo experiments have shown that ligation of CD40 as well as 4-1BB enhances CD4⁺ and CD8⁺ T cell differentiation, proliferation and/or survival. Similarities in expression regulation, of both T cell co-stimulatory receptor and their ligands, and in signalling pathways suggest that there is redundancy in vivo. The immune response to LCMV and influenza virus has been studied in CD28⁻, CD27⁻, OX40⁻ and 4-1BB⁻ mice and indeed deficiency for any of the co-stimulatory molecules impairs but not abolishes the T cell response. However these data are also compatible with a linear mode of action in which fine-tuning of the immune response is achieved by consecutive interaction with different TNF-receptor family members. Whereas CD27 deficiency affects the size of the CD4⁺ and CD8⁺ effector pool after influenza virus infection. 4-1BB deficiency in this situation only affects the size of the effector CD4⁺ pool. Likewise it has been shown that 4-1BB specifically regulates the size of the CD6⁺ effector pool after LCMV infection. It is not clear how this divergence
arises but an easy explanation would be the differential expression of the various receptor/ligand pairs.

Setting the size of the antigen-primed pool is not only of importance at the initiation of the T cell response. When antigen has been neutralised, the effector T cell pool has to succumb and T cell memory has to form. Interestingly, in vivo ligation of OX-40 and 4-1BB by antibody can inhibit peripheral T cell deletion\textsuperscript{178,179} and OX-40 promotes long term survival of effector cells in vitro\textsuperscript{180}. Although this approach may not represent physiological conditions it does show the potential of TNF-receptor members to influence the size of the antigen-primed pool. In autoimmunity and cancer the size and/or function of the antigen-primed pool does not meet its demands. Modulating OX-40, 4-1BB or CD27 function could be an interesting therapeutic intervention.

Other mice in which co-stimulatory molecules are present in a dominant fashion have been described. In analogy with CD70TG mice, B7-2 TG\textsuperscript{127,128} and 4-1BBL TG\textsuperscript{181} mice show a progressive depletion of B cells, which seems to be caused by disturbed B-cell development\textsuperscript{127}. It seems likely that as for CD70TG mice enhanced generation of IFN-\(\gamma\) producing memory/effector T cells is responsible for this disturbance. Accordingly, increased percentages of CD44\textsuperscript{high} T cells are found in B7-2 TG mice and crossing of B7-2 TG mice with TCR\(\beta\textsuperscript{CD44}\) mice prevents the B-cell depletion.

Only in CD70TG mice effects on T cell numbers have been described\textsuperscript{93} (chapter 5). This dramatic change in the T cell compartment might partially be explained by the expression level of the transgene, but might also reflect the regulatory mechanisms of the co-stimulatory signal. In contrast to CD27, 4-1BB expression is limited to activated T cells. CD28 is expressed on naive T cells but its action is counter-acted by CTLA-4. As stated above in CD70TG mice chronic co-stimulation enhanced T cell proliferation and differentiation, leading to increased T cell numbers in PLN and spleen of young CD70TG mice. However, as mice age PLN T cell numbers progressively decrease. This can be explained by the enhanced T cell differentiation, which will probably change the migratory behaviour of the T cells. While memory/effectort T cells can still migrate into the spleen they have lost the capacity to (re)enter the PLN. In CD70TG mice, the population of PLN by thymic emigrants partially upholds PLN T cell numbers, since at the moment that thymic cellularity collapses, PLN become virtually empty. In this time span the total splenic T cell number does not alter significantly, while the percentage of memory/effectort T cells drastically increases. Old CD70TG mice hence show that chronic T cell co-stimulation can have severe consequences for the size and composition of the T cell compartment. Therefore deliberate manipulation of interaction in disease the manipulation of CD27/CD70 interaction in disease should be carefully controlled.
The in vivo of consequences of CD27-CD27 interaction on the antigen-primed B lymphocyte pool

The analysis of the antigen-primed B cell pool in CD27 deficient mice has been restricted to the measurement of virus specific Ig levels after primary and secondary influenza virus infection. Normal levels of virus specific Ig were formed in CD27" mice indicating that CD27 is not required for antibody production per se. Humoral immune responses were studied in greater detail in CD70TG mice (chapter 6). Analysis of T-cell independent humoral responses was performed in CD70TG/TCRα− mice that have a B cell compartment comparable to their non-transgenic counterpart. After immunisation with TNP-ficoll similar numbers of TNP-specific plasma cells and levels of anti-TNP IgM were observed. This was also seen, despite the reduced B cell numbers, after primary immunisation of CD70TG mice with the T-cell dependent antigen TNP-KLH. Thus in CD70TG mice, there is no indication that CD27/CD70 interaction promotes plasma-cell differentiation. However, in CD70TG mice the humoral immune responses are disturbed. Immunohistochemistry revealed that in CD70TG mice neither in the primary nor in the secondary immune response GCs are present. Consequently, after secondary immunisation reduced serum levels of anti-TNP IgG were present and high affinity anti-TNP IgG was lacking.

The cause of the disturbed GC formation in CD70TG mice is unclear. Both T cells and antigen activated B cell express CD27 and modified T cell help and B cell behaviour might disturb the GC formation. In a normal GC limited CD70 expression is found and the dominant expression of CD70 could lead to aberrant interaction with CD27 on T and/or B cells. Analysis of humoral responses after adoptive transfer of WT T or B cells into CD27"/CD70TG mice will distinguish between these two components.

Concluding remark

In summary we can conclude that, as proposed, CD27/CD70 interaction regulates the size and function of the antigen-primed lymphocyte pool. The fact that the function of CD27 is so prominently revealed in CD70TG mice implies that under physiological circumstances CD70 expression regulates CD27 function. Since antigenic triggering of lymphocytes determines CD70 expression, CD27/CD70 interaction directly couples the size and function of the antigen-primed lymphocyte pool to the amount of antigen.