CD27/CD70 interactions in effector and memory cell formation
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Citation for published version (APA):
Tesselaar, N. A. (2001). CD27/CD70 interactions in effector and memory cell formation

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Summary

An immune response is the combined effort of the different components of the immune system to neutralise antigen. As for all biological processes this reaction is quantitatively and qualitatively regulated. Interactions between TNF-receptor family members and their respective ligands, the TNF family members, have important functions in the regulation of the immune response. The molecules CD27 and CD70 are members of the TNF-receptor and TNF family respectively and form a receptor ligand pair that can control human and murine T-cell expansion and T and B cell differentiation in vitro. However the function of CD27/CD70 interaction in an immune response is still unclear. We therefore set out to study the role of CD27/CD70 interaction in an in vivo mouse model.

To this end we first cloned murine CD70 (mCD70)(chapter 2). Murine CD70 protein is a 195 amino-acid type-II membrane protein that is 62% homologous with human CD70. Biochemical analysis of recombinant mCD70 shows a 29-kDa protein that is presumably present on the cell membrane as a trimeric molecule. In accordance with the co-mitogenic potential of human CD27/CD70 interaction, transfectants expressing recombinant mCD70 costimulate murine T-cell proliferation. CD70 mRNA is hardly detectable in resting splenocytes or thymocytes, but as in human lymphocytes, is present early after activation of these cells. Taken together, the above-presented data show that the characteristics of murine and human CD70 are highly similar.

Using recombinant mCD70 transfectants, monoclonal antibodies directed against mCD70 were generated. These mAbs were used to define the expression of the mCD70 protein (chapter 3). As for CD70 mRNA, mCD70 protein is not expressed on resting lymphocytes. Lymphocyte activation in vitro induces mCD70 expression on B cells but not, despite the presence of mRNA, on T cells. Next to antigen, cytokines also regulate mCD70 protein expression on B cells. Murine CD70 is also expressed on in vitro generated mature dendritic cells. Immunohistochemistry showed mCD70 expression on thymic medullar dendritic cells. In peripheral lymphoid organs only a very limited number of dispersed mCD70 expressing cells can be found, even during an ongoing immune response. However, clusters of mCD70 expressing cells could be found at the site of infection after influenza infection. Thus analogous to the observations in the human system, tight regulation highly restricts mCD70 expression in vivo. As for murine CD70, the limited data on murine CD27 expression and function reflects the data of obtained in the human system. In the peripheral organs the majority of T cells and a small population of B cells express CD27. Activation of T and B cells via the antigen receptor enhances/induces CD27 expression. Ligation of the CD27 on T cells co-stimulates T cell expansion. The observed similarities between human and mouse CD27 and CD70 expression and function are of importance because they justify the use of the mouse as a model system to evaluate the general importance of CD27/CD70 interaction in vivo.

After establishing the analogy between human and mouse CD70-characteristics, a mouse model in which CD70 is present as dominant co-stimulatory ligand for T and B lymphocytes was
generated. The mCD70 gene was cloned behind the human B cell specific CD19 promoter and transgenic mice were produced (chapter 4). All B cells, and only B cells, in these mice constitutively express CD70. In wild-type mice naive T-cells express CD27. However, interaction between T and B cells in CD70TG mice induces the downmodulation of CD27 and implicates a functional interaction between CD27 and CD70. In 4-week and 8-week-old CD70TG mice increased numbers of T cells are found in spleen and lymph nodes. Furthermore, alterations in the percentages of cells expressing the surface molecules CD43, CD44 and CD62L, and the cytokine IFN-γ indicate that CD27/CD70 interaction is instrumental in the development of CD4ε+ and CD8ε+ memory/effector cells. In contrast to the increased cellularity of the T cell compartment, the cellularity of the B cell compartment progressively declines in CD70TG mice. Analysis of the different development stages of bone-marrow derived and peripheral B cells revealed a relatively increased reduction in the percentage of immature B cells and only modest differences in the different peripheral B-cell fractions. Increased CD27/CD70 interactions thus most likely reduce B cell numbers by negatively regulating B cell-development in the bone marrow. Crosses of CD70TG with CD27ε mice and adoptive transfer of effector-molecule-deficient T-cells into CD70TGxCD27ε mice show the mechanism that is responsible for the B cell depletion. CD70TGxCD27ε mice have a normal B cell compartment. Adoptive transfer of WT, CD95Lεε, perforinεε or TNF-αεε T-cells in these mice leads to a significant reduction of B numbers cells in BM and spleen. However, IFN-γεε T cells are not able to induce the B cell depletion, indicating that T cells mediate the B cell depletion by an IFN-γ dependent mechanism. Crosses with TCRαεε and IFN-γεε mice confirm this conclusion.

Surprisingly, the fate of CD70TG mice is determined by the alterations in the T and not the B-cell compartment (chapter 5). Although healthy during the first weeks of life, ageing CD70TG mice fail to thrive. Moreover, most CD70TG mice die at a relative young age of a severe Pneumocystis carinii (PCI) infection. Failure to thrive and PC infections are characteristic-pathological symptoms induced by severe T-cell immunodeficiency, as seen in patients suffering from HIV infection. A detailed longitudinal analysis of the T-cell compartment in CD70TG mice revealed that in CD70TG mice the percentage dividing T cells is approximately 3 times as high as in wild-type mice and initially no differences in the percentages of apoptotic cells are found. This leads to increased T-cell numbers in the spleen. The excessive CD27/CD70 interactions also induce memory/effector cell differentiation and the concomitant loss of CD62L expression. The inability of CD62Lεεε cells to (re)enter peripheral lymph nodes (PLN) might explain why, in spite of increased proliferation, T cell numbers in peripheral lymph nodes progressively decline. Influx of naive thymic emigrants probably counteracts this decline since the most dramatic decrease in PLN T cell numbers is seen at the moment that thymic cellularity collapses. Finally, the in vitro proliferative capacity of CD70TG T-cells is reduced compared to this capacity of WT T-cells. It thus seems that in CD70TG mice the T cell pool deteriorates functionally as well as numerical. Comparing CD70TG mice and HIV patients shows similar clinical symptoms and a large number of analogous alterations in the T-cell compartment. The cause of T-cell depletion in HIV patients is still a matter of debate. However,
the effects of the chronic costimulation in CD70TG mice strongly favour the hypothesis that chronic immune activation is an important driving force in HIV induced T-cell depletion.

In the human system in vitro CD27 triggering on B cells induces plasma cell differentiation. The possible contribution of this function in the course of a humoral immune response was studied in CD70TG mice (chapter 6). Preliminary in vitro experiments with purified CD70TG B cells showed that transgene expression had no effect on the intrinsic capacity of CD70TG B cells to proliferate and differentiate into Ig secreting plasma cells. After induction of T-cell independent humoral responses in TCRαβ−/−CD70TG mice, no influence was found on the number of TNP-specific plasma cells or anti-TNP IgM levels. Due to the absence of T cells these mice have normal B cell numbers and can be compared with their wild-type counterparts. Also in primary T-cell dependent humoral responses in CD70TG mice, despite reduced B cell numbers, normal levels of anti TNP-specific Ig were generated. Markedly however no visible GC were formed. Consequently secondary responses were severely disturbed, as evidenced by reduced anti-TNP IgG1 levels and lack of high affinity anti-TNP IgG1 antibodies. Together, these data suggest that CD27/CD70 interactions regulate B cell differentiation by promoting signals that favour plasma cell formation rather than germinal centre formation, resulting in reduced B-cell memory formation in CD70TG mice.

Recently the analysis of CD27 function in CD27 deficient mice was described. In accordance with the increased numbers of T lymphocytes in CD70TG mice, reduced expansion of virus specific T cells after influenza virus infection were observed. The finding that effector cell formation was not hampered in CD27−/− presumably reflects the potential of other co-stimulatory molecules to compensate for the loss of CD27 expression. Division and survival are the determinants that set the size of antigenic primed lymphocyte pool. The increased Ki-67 expression and BrdU incorporation imply a role for CD27 in costimulation of cell proliferation.

Next to co-stimulatory molecules cytokines are important factors for lymphocyte differentiation. The nature of cytokines, instructive or selective, is still a matter of debate. Likewise CD27’s function can either be instructive, permissive or selective. Deciphering of the intracellular and gene specific effects of CD27 will possibly shed light on this issue.

Besides CD27 the TNF receptor family contains other receptors with co-stimulatory capacity for B and T cells. Similarities in expression regulation (of both receptor and ligand), in vitro function and intracellular signalling effects suggest that there is redundancy in vivo. Indeed in most cases deficiency for a specific TNF member only reduces and not abolishes the immune 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. The total combination of the different signals will determine the differentiation state of an individual cell.

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.