CD27/CD70 interactions in effector and memory cell formation

Tesselaar, N.A.

Citation for published version (APA):
Tesselaar, N. A. (2001). CD27/CD70 interactions in effector and memory cell formation
Chapter 5

LETHAL IMMUNODEFICIENCY INDUCED BY PERSISTENT COSTIMULATION VIA CD70

Kiki Tesselaar1, Ramon Arens51, Gijs M.W. van Schijndel1, Paul A. Baars1, Martin A. van der Valk1, Jannie Borst6, Marinus H.J. van Oers5 and René A.W. van Lier1.

1Clinical Immunology Laboratory and 5Department of Haematology, Academic Medical Centre, Amsterdam
1Department of Immunobiology, CLB, Sanquin, Amsterdam
1Divisions of Experimental Animal Pathology and 5Cellular Biochemistry, The Netherlands Cancer Institute, Amsterdam, The Netherlands

1These authors contributed equally to this paper.
It has been proposed that human immunodeficiency virus (HIV-1), in addition to directly infecting and killing CD4+ T cells, leads to T cell dysfunction and T cell loss by chronic immune activation\textsuperscript{134,135}. We studied the effects of continuous immune activation in mice that constitutively express CD70, the ligand for the tumour necrosis factor receptor (TNF-R) family member CD27\textsuperscript{134,137}. On B cells, Young CD70 transgenic (TG) mice have increased numbers of interferon (IFN)-\(\gamma\)-secreting effector CD4+ and CD8+ T cells in the secondary lymphoid organs.\textsuperscript{92} In spite of this apparently hyperactive immune system, most CD70TG mice die at the age of 6-7 months from Pneumocystis carinii infection, a hallmark of T-cell immunodeficiency.\textsuperscript{132,138} We here show that in CD70TG mice T-cell proliferation is approximately 3 times higher than in wild-type (WT) mice. Furthermore, an accumulation of effector T cells in spleens and a progressive decline of naive-T-cell numbers in spleens and lymph nodes of CD70TG mice is observed. In older mice thymic cellularity drops, which may further contribute to depletion of the naive T cell pool. Thus persistent delivery of costimulatory signals, as may occur during chronic active viral infections, functionally and physically exhausts the T cell pool and is sufficient to induce lethal immunodeficiency.

Occupation of CD27 by its ligand CD70 augments T-cell expansion and effector cell formation in vitro\textsuperscript{34,47,48}. Studies in CD27 deficient mice have recently shown that this receptor-ligand interaction is necessary for adequate expansion of antigen-specific T cells upon infection with influenza virus.\textsuperscript{46} To study the impact of dominant CD27 signalling on immune reactions TG mice were generated that continuously express CD70 on B cells.\textsuperscript{93} Initial analyses of these mice showed a strong increase in the number of both CD4+ and CD8+ effector, IFN-\(\gamma\)-secreting T cells in the secondary lymphoid organs. Furthermore, a progressive B-cell depletion was observed, which could be attributed to the effect of IFN-\(\gamma\)-producing T cells. We concluded that CD27/CD70 interactions promote effector T cell formation in vivo.\textsuperscript{93}

During the first months of their life CD70TG mice appear healthy, show enhanced DTH reactions and mount normal primary antibody responses to protein antigens (chapter 6). However, as mice age they progressively fail to thrive, resulting at 20 weeks in a body weight of approximately 80% of that of WT animals (figure 1A). Strikingly, most CD70TG mice die at young age (24-28 weeks) from Pneumocystis carinii pneumonia, an opportunistic infection (figure 1B) usually seen in situations of severe T-cell immunodeficiency. Cachexia and opportunistic infections are core symptoms of AIDS. Impairment of the T cell system in HIV-1 infected individuals was initially believed to be caused by cytopathic effects of the virus.\textsuperscript{134,135} Recently it has been emphasised that continuous activation of the immune system by high level replicating HIV-1 might be a decisive factor for the demise of the T cell compartment.\textsuperscript{134,135} The clinical similarities between CD70TG mice and patients in late stage HIV-1 infection prompted us to examine T-cell turnover and competence in CD70TG mice by measuring T-cell division, distribution and function. To measure T-cell turnover, in vivo BrdU pulse-chase labelling experiments were performed.\textsuperscript{139} After a 10 day period on BrdU containing water, CD70TG mice showed an approximately 3-fold increase in the percentage of BrdU-labelled CD3+ T cells both in peripheral lymph nodes (PLN, represented by axillary, brachial and inguinal lymph nodes) and spleen (figure 2A) as compared to WT. The percentages of labelled cells rapidly dropped.
**Figure 1:** Clinical symptoms in CD70TG mice (Founderline 13(F13))

(A) Cachexia in CD70TG mice. Male WT (○) and TG (●) mice were weighed at 4, 8, 13 and 20 weeks of age. The graph shows the mean weight (n>6) ± standard deviation. Significant differences (Student’s t-test, p<0.05) between mean values of WT and CD70TG are denoted by *. (B) Pneumocystis carinii infection in CD70TG mice. Histochemical staining with hematoxylin and eosin was performed on formalin fixed lung tissue sections derived from WT (left panel) and CD70TG (right panel) mice. Original magnifications: 40 and 200 fold (insert).

**Figure 2:** T-cell turnover in WT and CD70TG mice (F138).

(A) Increased T cell expansion, (B) T cell division and (C) increased T cell death in CD70TG mice. Flow cytometry was performed to determine the percentage of (a) BrdU⁺, (B) Ki-67⁺ or (c) Annexin-V⁺ and PI⁺ cells within the CD3⁺ population derived from WT (left panels) or TG (right panels) spleen (primary axis, dots and solid lines) or PLN (secondary axis, triangles and dotted lines) derived from mice sacrificed at the indicated age. (A,B,C) Each symbol represents data from an individual mouse; lines represent the mean value of 2 to 6 mice. (A) Four-week-old mice were given drinking water containing BrdU (0.8 mg/ml) for 10 days. Mice were sacrificed at 5.5, 6.5, 8.5 and 10.5 weeks of age (i.e. 0, 1, 3 and 5 weeks after stopping BrdU administration). (A, B) After cell-surface staining, intracellular BrdU and Ki-67 staining was performed following the manufactures instruction using a BrdU (FITC) Flow kit and a Ki-67-PE set, respectively (Pharmingen, San Diego, CA). (C) Annexin-V and PI staining was performed with an APOPTTEST-FITC kit (Nexins Research BV, Kattendijk, The Netherlands).
Figure 3: T-cell cellularity in CD70TG mice (F1393).
Absolute numbers of total ((A,B) lines), naive (black bars) or memory/effecter T cells (grey bars) of (A) spleen or (B) PLN were determined. T cell numbers were calculated by multiplying the number of mononuclear cells with the percentage of CD3-(total), CD3-CD62L-CD44+naive or CD62L-CD44+memory/effecter cells as determined by flow cytometry. (C) Thymic cellularity. Lymphoid organs were derived from WT (left panels) or CD70TG (right panels) mice sacrificed at 4, 8, 13, and 20 weeks of age. Shown is the mean value of 4 to 6 mice +/- the standard deviation. Differences between mean values of WT and CD70TG at the indicated age are considered significant if p<0.05 (Student's t-test). (A) * Denotes significant differences in mean naive-T-cell numbers. Naive-T-cell numbers also differed significantly at 20 weeks. (B) * Denotes significant differences in the mean total, naive and memory/effecter T cell numbers. (C) * Denotes significant differences in thymic cellularity.

Figure 4: Proliferative capacity of T cells from CD70TG mice in vitro (F1393).
[^H]-Thymidine incorporation was measured to determine the capacity of the T cell compartment to clonally expand. T cells were purified from mesenteric lymph nodes derived from mice at 4, 8 and 13 weeks of age and stimulated with PHA (+, 1 µg/ml) (10^5 cells/well) or anti-CD3, mAb (α(clone 145-2C11, immobilised, 10 µg/ml)).[^H]-Thymidine was added for the last 16 hours of a 96-hour culture period. The stimulation index is defined as the mean [^H]-thymidine incorporation of 2 to 3 TG mice divided by the mean [^H]-thymidine incorporation of 2 to 3 WT mice. The graph shows the mean value of two experiments +/- standard error of the mean ([^H]-thymidine incorporation range: 1396-10219 cpmp (PHA stimulation) and 1975-32966 cpmp (anti-CD3 mAb stimulation)).
when BrdU feeding was stopped which suggested that the increased labelling was not due to impaired apoptosis, but rather reflected increased cell division. To address this we measured the percentages cycling and apoptotic cells within the T cell pool of PLN and spleen. CD70TG mice had increased percentages of T cells in cycle (figure 2B) in PLN (3.3% in TG vs. 1.3% in WT) and spleen (14.5% in TG vs. 3.3% in WT), as measured by Ki-67 expression. FACS analysis of Annexin V and propidium iodide (PI) staining was performed to estimate the percentage of apoptotic cells (Annexin V' PI'). T cell death rates appeared to be relatively constant in WT mice and no differences were observed with CD70TG mice of 4, 8 and 13 weeks of age. In 20-week-old CD70TG mice, an increase in percentages of apoptotic cells was seen in PLN (figure 2C, 40% in TG vs. 27% in WT, p<0.05 student's t-test) as well as spleen (42% in TG vs. 23% in WT, p<0.05 student's t-test).

The impact of the increased proliferation was analysed by measuring total (CD3+), naive (CD3+CD2L<sup>high</sup>CD44<sup>neg/dull</sup>), and memory/effector (CD3+CD2L<sup>neg/dull</sup>CD44<sup>high/dull</sup>) T cell cellularity in secondary lymphoid organs (figure 3A, B). Comparison of CD70TG and WT splenic T cell cellularity showed moderately (1.5 fold) elevated T-cell numbers in CD70TG mice irrespective of age. Moreover, in agreement with the proposed role of CD27/CD70 interaction in effector cell formation, an accumulation of memory/effector T cells and a concurrent decrease of naive T cells were observed in CD70TG spleens. T cell hypercellularity was also observed in PLN of 4-week-old CD70TG mice. Strikingly however, as mice aged a progressive decrease of CD3+ T-cell numbers occurred in PLN culminating in virtual empty lymph nodes at the age of 20 weeks. The composition of the T cell pool in PLN of CD70TG mice did not change over time, although comparison of CD70TG and WT mice showed a 2 fold increase in percentages of memory/effector cells in the PLN of CD70TG mice (data not shown). Thus, persistent exposure to CD70 augmented effector cell formation and concomitantly naive-T-cell numbers decreased. The increased splenic cellularity and hypocellular PLN in CD70TG mice may at least in part be explained by redistribution of memory/effector cells from PLN to blood and spleen<sup>4</sup>. Apart from the differentiation-related consumption of naive T cells, naive-T-cell numbers may also decrease as a result of thymic impairment<sup>136</sup>. Thymic cellularity was analysed as a measure of de novo T cell production. In comparison with WT mice 4, 8 and 13-week-old CD70TG mice had normal thymic cellularity. However, in 20-week-old mice thymic cellularity collapsed; compared to WT, thymocyte numbers were decreased approximately 30 fold and thymic tissue was practically absent.

Finally, the ability of T cells to undergo clonal expansion was measured (figure 4). Irrespective of age, LN T cells of CD70TG mice had a reduced ability to divide upon stimulation with anti-CD3 mAb in vitro (stimulation index 0.46-0.66 of WT animals). In contrast, proliferation in response to phytohaemagglutinin (PHA) was adequate at young age (SI 1.75) but dropped (SI 0.63) as mice aged. Thus, apart from to the progressive hypocellularity of the lymph nodes, T cell hyporesponsiveness might contribute in undermining immunocompetence.

In CD70TG mice a conspicuous number of phenomena are found that are considered to be key immunopathologic features of HIV-1 induced immunodeficiency: evidence for increased T-cell turnover<sup>140</sup>; initial lymphadenopathy followed by depletion of lymph nodes<sup>141</sup>; increased
apoptosis of both CD4\(^+\) and CD8\(^+\) T cells\(^{142,143}\); diminution of the naive CD4\(^+\) and naive CD8\(^+\) T-cell population\(^{144}\); and a progressive inability of T cells to respond ex vivo to antigen and mitogenic stimuli\(^{145}\). For a long time it has been considered that direct\(^{146}\) or indirect\(^{147}\) cytopathic effects of HIV-1 accounted for the abundant immune abnormalities in HIV-1. More recently, it has been put forward that chronic immune activation, resulting from the inability of the immune system to control HIV-1 replication, might contribute substantially to, and in fact could be the main determinant in the erosion of the immune system in HIV-1 infected individuals\(^{135,148}\). Our studies in the CD70TG mice conclusively show that persistent immune activation per se can result in a state of lethal immunodeficiency.

In 10-week-old CD70TG mice, neutralising anti-CD70 mAb can reverse most features of immune activation (data not shown) showing the dependence of the hyper immune status on persistent CD27/CD70 interaction. Markedly, activated T cells from HIV-1 infected individuals have enhanced CD70 expression\(^{130}\). If CD27/CD70 interactions are instrumental in driving hyperimmune activation in HIV infection, it is of interest to consider targeting CD27/CD70 interactions to avoid detrimental consequences of persistent immune activation.

Acknowledgements: We thank T. Schrouwers and T. Jansen Hendriks for biotechnical assistance; E. Eldering, F. Miedema and I. ten Berge for critically reading the manuscript.