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The TNF Receptor Family Member CD30 Is Not Essential for Negative Selection

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CD30 is a member of the TNF receptor superfamily that has been implicated in negative selection and some forms of peripheral tolerance. A previous study of CD30-/- mice in a class I-restricted H-Y TCR-transgenic mouse model showed that CD30 is essential for removal of autoreactive thymocytes. During the course of the studies of CD30 in the class II-restricted TCR-transgenic mice, we found that the absence of CD30 has no effect on negative selection. Surprisingly, we also found that the CD30 mutation does not perturb apoptosis of the autoreactive thymocytes in the class I-restricted H-Y TCR-transgenic model. The minimal role of CD30 in negative selection and other recent data are discussed. The Journal of Immunology, 2000, 165: 6170–6173.
Northern blot analysis

Thymi from CD30<sup>-/-</sup> and C57BL/6 mice were removed and homogenized using a VIRTISHEAR homogenizer (Virtis, Gardiner, NY). Total RNA was isolated using the RNase-All method (25). Total RNA was run on a 1.2% formaldehyde gel, 1× MOPS buffer and blotted overnight onto nylon filter. Hybridization was done using the ExpressHyb hybridization solution (Clontech, Palo Alto, CA). Murine CD30 cDNA (a generous gift from Eckhard Podack, Miami University, Miami, FL) was labeled with <sup>32</sup>P by a random priming method and used as a probe for the Northern blot.

Flow cytometry analysis

Thymocytes were isolated from mice at 5–10 wk of age and teased apart in tissue culture media with glutamine (Life Technologies, Grand Island, NY). Cells were stained with mAbs to CD4, CD8 α, αβTCR (Caltag, South San Francisco, CA), Var1 (PharMingen, San Diego, CA), and clonotypic anti-H-2<sup>b</sup> Ab (T3.70) (a generous gift from James Allison, University of California, Berkeley). Cells were stained in 1× PBS (0.2 g/L KCl, 0.2 g/L KH<sub>2</sub>PO<sub>4</sub>, 8.0 g/L NaCl, 2.16 g/L Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O), 2% FCS, and 0.1% sodium azide on ice for 20 min and then analyzed using a Coulter flow cytometer (Coulter Pharmaceutical, Palo Alto, CA).

Results

CD30 expression is completely abrogated in CD30<sup>-/-</sup> mice

Disruption of the CD30 gene has previously been shown to rescue the deletion of self-reactive thymocytes in an H-Y TCR-transgenic model but not in superantigen-mediated negative selection model (13). Our experiments with the CD30<sup>-/-</sup> Nur77FL-transgenic mice have shown that CD30 is not involved in thymic apoptosis caused by Nur77 overexpression (22). In an effort to characterize CD30 further, we undertook a brief study of the CD30<sup>-/-</sup> mice. CD30 knockout mice were previously generated by replacement of the second to last exon of the CD30 gene with the neomycin-resistant gene (13). To rule out the possibility that there may be residual activity from a truncated or aberrantly expressed CD30 molecule, we isolated total thymic RNA from CD30<sup>-/-</sup> and wild-type C57BL/6 mice. We then probed the RNA with the murine CD30 cDNA in a Northern blot analysis. We found that CD30 transcript was absent in the thymus of CD30<sup>-/-</sup> mice (Fig. 1A). In contrast, CD30 expression was visible in the C57BL/6 thymus as had been shown previously (26). These results confirm the lack of CD30 transcript in CD30<sup>-/-</sup> mice.

Negative selection in TCR-transgenic mice does not appear to be impaired in the CD30<sup>-/-</sup> background

We mated CD30<sup>-/-</sup> mice to AND TCR-transgenic animals, a well-studied model of thymic selection. As a control, we also crossed CD30<sup>-/-</sup> mice to the H-Y TCR-transgenic mice. The AND TCR is specific for pigeon cytochrome c and E<sup>k</sup>. In transgenic animals, transgene-expressing T cells are positively selected on an Ab molecule whereas negative selection occurs in the presence of A<sup>a</sup> (23). Negative selection can also be initiated by injection of pigeon cytochrome c peptide with mice in the H-2<sup>kb</sup> background. The alloreactive negative selection, however, is milder than peptide-initiated apoptosis and does not involve killing by TNF secreted by the stimulated transgenic peripheral T cells (27).

The genotypes for CD30 TCR-transgenic mice and their MHC haplotypes were determined by PCR analysis (Fig. 1B and data not shown). Analysis of these mice show that transgenic T cells are positively selected in the H-2<sup>b</sup> background, with skewing toward the CD4<sup>+</sup>CD8<sup>-</sup> lineage as shown before (23). In the CD30 heterozygous, H-2<sup>b</sup> background, negative selection takes place as evidenced by the absence of positive selection and severalfold drop of thymocyte cell number (Fig. 2). In CD30<sup>-/-</sup> H-2<sup>b</sup> animals, however, thymocyte cell numbers and the FACS profiles remained similar to those in the heterozygous littersmates (30 millions, n = 6 vs 37 millions thymocytes, n = 5). No increase in the representation or the number of CD4<sup>+</sup>CD8<sup>+</sup> thymocytes was seen, as would be predicted if CD30 was involved in negative selection (Fig. 2). Cells were also stained with AND TCR-specific Ab (anti-Var1) and again there were no differences in the staining profiles or absolute cell numbers among CD30<sup>-/-</sup>, AND H-2<sup>b</sup> and CD30<sup>-/-</sup>, and AND H-2<sup>b</sup> mice (data not shown).

Originally serving as a control, we also bred CD30<sup>-/-</sup> mice with the H-Y TCR-transgenic line. The H-Y TCR is specific for the H-Y Ag presented in the context of D<sup>b</sup> molecule. In female mice, H-Y TCR-bearing T cells are positively selected on H-2<sup>b</sup> molecules whereas in male mice, transgenic T cells are negatively selected due to the presence of the H-Y Ag. To exclude T cells expressing any endogenous TCR-α, we gated the FACS profiles on thymocytes using the mAb CD30<sup>-/-</sup> and C57BL/6 mice. We then probed the RNA with the murine CD30 cDNA in a Northern blot analysis. We found that CD30 transcript was absent in the thymus of CD30<sup>-/-</sup> mice (Fig. 1A). In contrast, CD30 expression was visible in the C57BL/6 thymus as

![FIGURE 1. Northern blot and genotype analysis of CD30-deficient mice. A, Northern blot analysis of total RNA from wild-type (C57BL/6) and CD30<sup>-/-</sup> thymocytes using CD30 cDNA as a probe. Ethidium bromide gel photograph of the corresponding RNA is shown below. B, Genotype analysis of the wild-type, CD30<sup>+/+</sup>, or CD30<sup>-/-</sup> mice. Two PCR are used to distinguish the CD30 genotypes; k/o, the CD30 knockout PCR product; wt, the wild-type CD30 allele.

![FIGURE 2. Lack of CD30 has no effect on class II-restricted negative selection. Representative plots of CD4 and CD8 expression on thymocytes from AND-transgenic mice in CD30 heterozygous (+/-) or homozygous (-/-) mutant background. The MHC haplotypes of the mice analyzed and their average total thymocyte numbers are indicated.](http://www.jimmunol.org/Downloadedfrom)
T3.70+ cells (T3.70 is a clonotypic Ab specific for the H-Y TCR). Thymocytes were stained with anti-CD4 and anti-CD8 Abs. As shown in Fig. 3, we were surprised to see no difference in the thymocyte profiles of male CD30+/− HY vs CD30−/− HY mice. As expected, the cell number of male H-Y CD30+/− thymocytes is reduced dramatically compared with the nontransgenic littermate or female H-Y (either CD30+/− or CD30−/−) mice (Fig. 3). However, there was no increase in the representation of the double-positive (CD4+CD8+) thymocyte population or in the absolute cell number of thymocytes from male H-Y TCR CD30+/− mice compared with the CD30+/− counterpart. At least six different littersmates were analyzed with similar results. Combined with the AND data above, these unexpected results have led us to conclude that apoptosis by negative selection proceeds normally in CD30−/− mice.

Discussion

CD30 was initially identified as a marker for Hodgkin’s disease. Further studies of CD30 have implicated a role for CD30 in negative selection and peripheral tolerance of CD8+ cells (4, 13, 15). However, our study of two different TCR-transgenic systems, AND and H-Y, points to a conclusion that CD30 plays little or no role in the removal of autoreactive cells in the thymus. The question arises then, as to how to reconcile the results obtained with the H-Y TCR-transgenic model in our study with that of the previously published results by Amakawa et al. (13). One explanation may lie in the differences in the mouse strains used for the experiments. We have used CD30 mutant mice that have been crossed repeatedly to C57BL/6. In contrast, Amakawa et al. (13) might have used strains that were bred only for a few generations to C57BL/6. Another explanation may lie in the conditions under which the mice are housed. Nonetheless, the AND and H-Y TCR-transgenic mice show no defects in negative selection in the absence of CD30. These data argue for a minor role for CD30 in thymic apoptosis.

Recently, overexpressing CD30 in transgenic mice was shown to enhance negative selection but only in the presence of TCR signals (28). The authors conclude that CD30 may act as a costimulatory molecule for negative selection. Using Abs specific for cell surface molecules, however, Kishimoto and Sprent (29) found that anti-CD28 was the only Ab that could provide costimulatory activity for TCR-dependent apoptosis of CD4+CD8+ thymocytes in vitro. Negative results were observed for Ab specific for CD30 (29). Furthermore, CD30 expression is confined to the thymic medulla (14, 30). This is incompatible with the purported role of CD30 in CD4+CD8+ thymocytes, which reside in the cortex. Studies of CD30 signal transduction have revealed TRAF1 and TRAF2 as two signaling proteins that bind to CD30 (16–19). Upon ligand engagement, these TRAF molecules mediate NF-κB activation, which has an antiapoptotic activity. These data, along with our TCR-transgenic experiments, argue for an alternative role of CD30. Further detailed studies of CD30−/− mice are needed to re-examine the role of CD30 in the immune system.

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References