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A Role for CD5 in TCR-Mediated Signal Transduction and Thymocyte Selection

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CD5 is a transmembrane protein that is expressed on the surface of T cells and a subset of B cells. The absence of CD5 rendered thymocytes hyperresponsive to stimulation through the T cell antigen receptor (TCR) in vitro. Selection of T cells expressing three distinct transgenic TCRs was also abnormal in CD5-deficient mice. These observations indicate that CD5 can influence the fate of developing thymocytes by acting as a negative regulator of TCR-mediated signal transduction.

Signal transduction through the TCR determines the outcome of thymic selection (*1*). The T lymphocyte transmembrane protein CD5 is part of a receptor complex that also comprises the TCR-CD3 ζ chain and protein tyrosine kinases (PTKs) (*2*). Activation of T cells results in a rapid phosphorylation of tyrosine residues within the cytoplasmic domain of CD5 (*3*), which is thought to bind Src homology 2 (SH2) domain–containing proteins (*2*).

Previous studies of peripheral T cell activation in vitro revealed a costimulatory effect of antibodies specific for CD5 on TCR-mediated proliferative responses, suggesting that in the absence of CD5, TCR-mediated proliferation of T cells may be reduced (*4*). In contrast, we observed that TCR-mediated proliferative responses of single positive (SP) $CD4^+$ or $CD8^+$ thymocytes, but not peripheral T cells, from CD5-deficient mice (*5*) were greater than those of the corresponding cells from wild-type mice (Fig. 1A) (*6*). However, both control and $CD5^{-/-}$ cells responded equally to stimulation by phorbol 12-myristate 13-acetate (PMA) and ionomycin, which are known to bypass TCR-dependent signal transduction (*7*).

Surface expression levels of the TCR-CD3 complex and CD4 or CD8 coreceptors on thymocytes from $CD5^{-/-}$ and control mice are similar (*5*). Hence, CD5 deficiency likely influences signaling downstream of the TCR. The hyperresponsiveness of $CD5^{-/-}$ thymocytes in terms of proliferation was accompanied by moderate, but stably reproducible, increases in Ca^{2+} mobilization associated with thymocyte activation induced by antibodies to CD3, either alone or in combination with antibodies to CD4 (Fig. 1B) (*8*). The increase in Ca^{2+} mobilization in $CD5^{-/-}$ thymocytes was consistent with a three- to fivefold increase in the phosphorylation of phospholipase C–γ1 (PLC-γ1) and the PLC-γ1–associated phosphoprotein pp35/36 (Fig. 1C) (*9*), which control the TCR-mediated activation of protein kinase C and mobilization of Ca^{2+} (*1, 9, 10*).

Interaction of the TCR with peptide-loaded major histocompatibility complex (MHC) proteins induces the phosphorylation of TCR subunits and activation of PTKs important in signaling downstream of the TCR (*1, 11*). The association of the phosphorylated TCR-CD3 ζ chain with the PTK ZAP-70 plays a critical role in T cell activation (*12*). We performed immunoprecipitation from whole-cell lysates of resting and in vitro–stimulated $CD5^{-/-}$ and control thymocytes with antibodies to ZAP-70 and subjected the immunoprecipitates to immunoblot analysis with antibodies to phosphotyrosine (*9*). Unstimulated and activated $CD5^{-/-}$ and control thymocytes contained equal amounts of similarly phosphorylated ZAP-70 protein. One or two phosphoproteins with molecular sizes of 21 kD (pp21) and 23 kD (pp23) coprecipitated with ZAP-70; these proteins are known isoforms of the CD3 ζ chain (*12*) (Fig. 1C). The identity of these phosphoproteins was confirmed with antibodies to the CD3 ζ chain. The extent of phosphorylation of the pp21 ζ isoform was lower in $CD5^{-/-}$ than in control cells. In $CD5^{-/-}$ cells, but not in control cells, cross-linking of CD3 resulted in the induction of the pp23 isoform (Fig. 1C). This isoform of the ζ chain predominated in lysates of $CD5^{-/-}$ thymocytes activated with the combination of antibodies to CD3 and CD4, whereas in control thymocytes the pp23 ζ isoform was only slightly more phosphorylated on tyrosine than the pp21 ζ isoform (Fig. 1C). Both pp21 and pp23 ζ isoforms are induced by TCR ligands that stimulate T cell proliferation and interleukin-2 secretion (that is, agonists), whereas anergy-inducing TCR ligands (that is, antagonists) induce predominantly the pp21 ζ isoform (*12*). It is possible that CD5 regulates the ratio between pp21 and pp23 ζ isoforms by competing with CD3 for common PTKs (*2*), thereby defining the amplitude of TCR-mediated stimulation.

The phosphorylation of the Vav protein, which is critical for TCR-mediated proliferation of T cells (*13*), was markedly increased in both unstimulated and stimulated $CD5^{-/-}$ thymocytes relative to control cells (Fig. 1C). The alterations in phosphorylation were selective: There were no other significant differences between $CD5^{-/-}$ and control thymocytes with respect to the general pattern of TCR-induced phosphorylation of intracellular proteins. Moreover, neither the phosphorylation nor the catalytic activity of the TCR-CD4 associated protein kinase $p56^{lck}$ was altered in nonstimulated or stimulated $CD5^{-/-}$ thymocytes.

The changes in TCR-mediated signal transduction in $CD5^{-/-}$ mice might have been expected to influence thymic selection and affect the fate of developing thymocytes. However, an initial analysis of T cell devel-

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opment in CD5-deficient (CD5T) mice did not reveal changes in the thymocyte populations of these mice as defined by CD3, CD4, CD8, CD69, and heat-stable antigen surface marker expression (*5*). This analysis could not exclude the possibility that the CD5T mutation results in changes in thymic selection that are masked by the heterogeneity of the TCR repertoire. To explore this possibility, we analyzed positive and negative selection of T cells expressing transgenic TCRs in the absence of CD5. CD5T mice were crossed with three previously characterized TCR transgenic lines: one producing a receptor specific for the male H-Y antigen (H-Y mice) (*14*), one with a receptor specific for the lymphocytic choriomeningitis virus glycoprotein (P14 mice) (*15*), and the third with a TCR that is specific for the nucleoprotein of influenza virus (F5 mice) (*16*). In all three instances, the selecting peptide is presented by the MHC class I H-2D^b molecule (*14–16*).

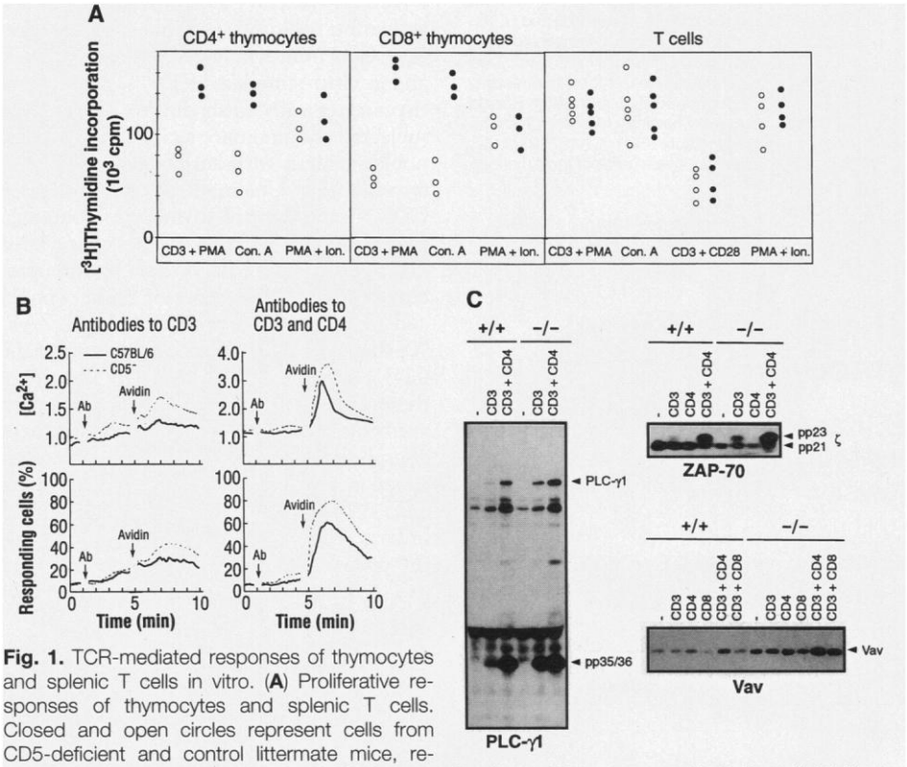

Fig. 1. TCR-mediated responses of thymocytes and splenic T cells in vitro. (**A**) Proliferative responses of thymocytes and splenic T cells. Closed and open circles represent cells from CD5-deficient and control littermate mice, respectively. Cells were stimulated with PMA plus antibodies to CD3, concanavalin A (Con. A), PMA plus ionomycin (Ion.), or antibodies to both CD3 and CD28. (**B**) Ca^{2+} mobilization in CD5$^{-/-}$ and control (C57BL/6) thymocytes. Representative results from one of five independent experiments showing the increase in cytosolic Ca^{2+} concentration ([Ca^{2+}]; indo 1 ratio) after stimulation of thymocytes with antibodies (Ab) to CD3 and CD4 (upper panels) and the percentage of responding cells (lower panels). The amplitude of Ca^{2+} mobilization and the percentage of responding cells were estimated as described (*8*). (**C**) Tyrosine phosphorylation of PLC-γ1, TCR-CD3 ζ chain, and Vav in CD5$^{-/-}$ and control (+/+) thymocytes that were unstimulated (−) or stimulated with antibodies to CD3, CD4, or CD8. Phosphorylated forms of PLC-γ1 and PLC-γ1–associated proteins (pp35/36) (left), TCR-CD3 ζ chain (pp21 and pp23) (coimmunoprecipitated with antibodies to ZAP-70) (top right), and Vav (bottom right) were revealed by immunoblot analysis of immunoprecipitates with antibodies to phosphotyrosine as described (*9*).

Thymocytes expressing the H-Y–specific transgenic TCRαβ receptor are deleted in male mice (*14*). This process of negative selection was not affected in the absence of CD5. In female mice, the interaction of the transgenic TCRαβ with an unknown ligand results in the positive selection of SP CD8$^+$ cells that express large amounts of the transgenic TCR (Fig. 2, A and B) (*17*, *18*). Because of the rearrangement of the endogenous TCRα chain locus in double-positive (DP) thymocytes, a large fraction of DP cells and SP CD8$^+$ cells in H-Y mice expresses surface heterodimers of endogenous TCRα chains paired with the transgenic TCRβ chain and therefore are transgenic TCRα^{lo}β^{hi} (*17*) (Fig. 2B). The CD5T mutation markedly affects this pattern of positive selection. The thymuses of CD5-deficient H-Y TCR transgenic (H-Y × CD5T) females are on average half the size of those of control H-Y mice and contain almost exclusively cells expressing both transgenic TCRα and β chains in large amounts (Fig. 2B). In addition, DP cells of H-Y × CD5T females also showed increased expression of CD69, the expression of which coincides with the onset of positive selection (*19*), and were larger (as determined by forward light scatter) than DP cells of H-Y mice. An in-

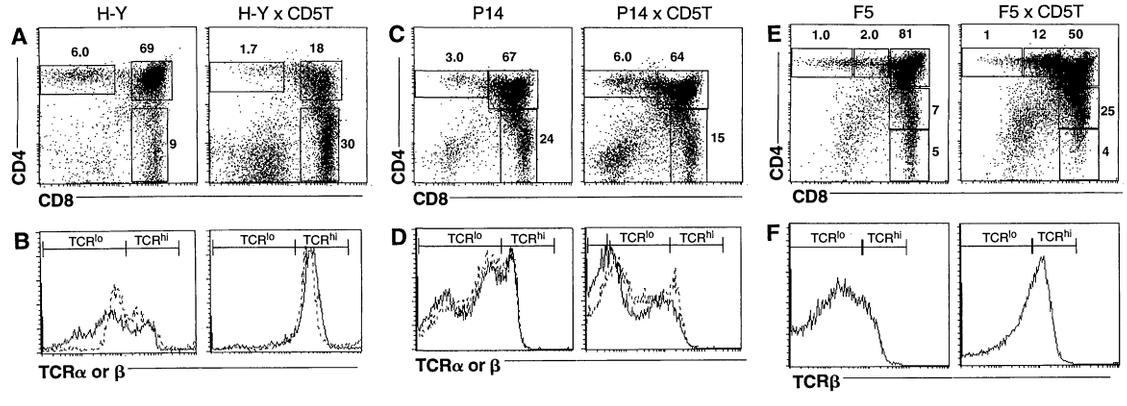

Fig. 2. Thymic selection in CD5-deficient TCR transgenic mice. (**A**, **C**, and **E**) Representative dot plots of the distribution of SP and DP thymocytes within thymocyte populations gated by a combination of forward light scatter and 90° side scatter. Populations of SP CD4$^+$, SP CD8$^+$, and DP thymocytes are framed and the proportion of gated cells (percent) within the total thymocyte population isshown. (**B**, **D**, and **F**) Histograms of the level of expression of the transgenic TCRα and β chains (solid and dashed lines, respectively) (B and D) and transgenic TCRβ chain (F) in the total thymocyte population. Shown in (A) and (B) are control H-Y (left panels) and H-Y × CD5T (right panels) female mice (n = 8); shown in (C) and (D) are control P14 (left panels) and P14 × CD5T (right panels) mice (n = 4); and shown in (E) and (F) are control F5 (left panels) and F5 × CD5T (right panels) mice (n = 10).

crease in the percentage of SP $CD8^+$ cells and a proportional decrease in the fraction of DP and SP $CD4^+$ thymocytes (Fig. 2A) was mirrored in the periphery, where the ratio of $CD8^+$ to $CD4^+$ splenic cells was increased in H-Y × CD5T mice compared to control H-Y females (Fig. 3A). Furthermore, approximately one-half of the $CD8^+$ splenic T cells were transgenic $TCR\alpha^{lo}\beta^{hi}$ in control mice, whereas most such cells expressed large amounts of both transgenic TCRα and β chains in H-Y × CD5T mice (Fig. 3, B and C).

Similar to the situation in H-Y females, the interaction of the P14 TCR with an unknown thymic ligand results in the positive selection of SP $CD8^+$ cells (Fig. 2, C and D) (*15*, *20*, *21*). In contrast to thymuses of control P14 mice, in which 20 to 30% of thymocytes express the transgenic TCRα and β chains at a high level (*22*) (Fig. 2D), the thymuses of CD5-deficient P14 TCR transgenic (P14 × CD5T) mice are one-third to one-half the size of those of control mice and are comprised largely of transgenic $TCR\alpha^{lo}\beta^{lo}$ cells (Fig. 2D). Consequently, the proportion of SP $CD8^+$ transgenic $TCR\alpha^{hi}\beta^{hi}$ thymocytes and peripheral $CD8^+$ T cells in P14 × CD5T mice was ~10 to 20% of that in control mice, suggesting a partial negative selection of the transgenic TCR-expressing cells attributable to the absence of CD5.

Finally, in CD5T mice expressing the F5 transgenic TCR (F5 × CD5T), the distribution of cells among the various thymic subpopulations differed from that in control F5 transgenic mice (Fig. 2, E and F). The most marked feature was a large increase in the representation of coreceptor-skewed $TCR^{hi}CD4^+CD8^{lo}$ or $TCR^{hi}CD4^{lo}CD8^+$ cells (Fig. 2E). However, the thymuses of F5 × CD5T mice did not show an obvious decrease in cellularity compared to F5 controls. Although the lack of an F5 clonotype-specific reagent precludes accurate determination of the retention of transgenic TCRα expression, the high ratio of peripheral $CD8^+$:$CD4^+$ T cells was equivalent for F5 and F5 × CD5T mice, reflecting the persistence of positive selection in the absence of CD5.

Our results with the three transgenic lines are consistent with CD5 being a component of a system that regulates TCR-mediated thymocyte activation. CD5 may influence the fate of developing thymocytes by depressing the responsiveness of DP cells to selection signals. In this manner, expression of CD5 may influence the selection of individual TCRs in either a positive (for example, P14) or a negative (for example, H-Y) manner depending on initial differences in TCR-ligand affinity. The increase in the proportion of $CD4^+CD8^{lo}$ coreceptor-skewed cells in F5 × CD5T mice indicates that CD5 exerts its effect at the DP stage during lineage commitment, before the completion of coreceptor down-regulation. Overall, thymocytes are likely to be most sensitive to CD5 function at the DP stage during the course of positive selection. Coincidentally, it is at this point that thymocytes usually up-regulate expression of several cell surface molecules, one of which is CD5 (*23*).

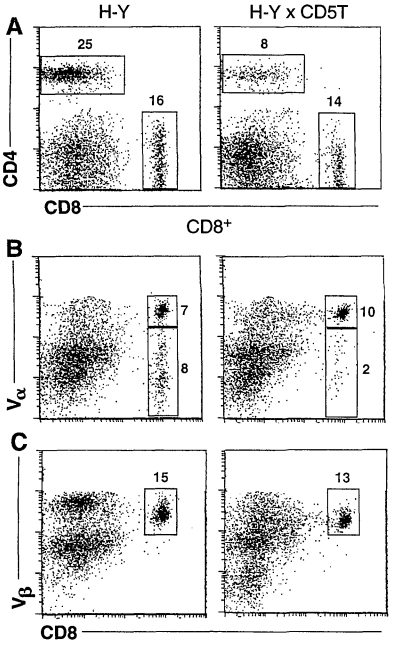

Fig. 3. Transgenic TCRαβ expression in splenic T cells of H-Y (left panels) and H-Y × CD5T (right panels) female transgenic mice. (**A**) SP populations of splenic T cells (n = 8). Populations of SP $CD4^+$ and $CD8^+$ are framed, and numbers show the percentage of gated cells within the total splenocyte population. (**B** and **C**) Expression of the transgenic TCRα (B) and TCRβ (C). Subpopulations of SP $CD8^+$ cells were gated according to the expression levels of TCRα and β chains. Numbers show the percentage of gated cells within the total splenocyte population.

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