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CD5 deficiency results in a hyper-responsive phenotype to Ag receptor stimulation. Here we show that the development and responses of CD4 lineage T cells are regulated by the function of CD5. Thymocytes expressing the I-Ab-restricted DO11.10 TCR undergo abnormal selection without CD5. In H-2d mice, the absence of CD5 causes deletion of double-positive thymocytes, but allows for efficient selection of cells expressing high levels of the DO11.10 clonotype. By contrast, there is enhanced negative selection against the DO11.10 clonotype in the presence of I-Ab. T cell hybridomas and DO11.10 T cells are more responsive to TCR stimulation in the absence of CD5. Such hypersensitivity can be eliminated by expression of wild-type CD5, but not by a form of CD5 that lacks the cytoplasmic tail. Finally, CD5 deficiency partially suppresses the block of CD4 lineage development in CD4-deficient mice. Taken together, the data support a general role for CD5 in negative regulation of Ag receptor signaling in the development and immune responses of CD4 lineage T cells.

Changes in the expression of cell surface molecules define the course of thymocyte development (1). Many of these changes have obvious effects on the capacity of thymocytes to receive developmental cues from surrounding stromal cells. For example, precursor and mature forms of the TCR deliver crucial signals that can engage differentiation and/or proliferation programs in the developing thymocytes. Without these signals, development is abortive, and there is no selection for mature MHC-restricted T cells (2, 3). Similarly, the CD4 and CD8 coreceptor molecules are crucial for productive recognition of thymic peptide/MHC ligands by thymocytes undergoing selection (4, 5).

The developmental significance of many other cell surface structures that are induced during thymocyte development is less obvious. An example of this is the CD5 molecule, a 68-kDa glycoprotein that is substantially up-regulated soon after thymocytes receive the pre-TCR signal (6). CD5 is expressed on all thymocytes and T cells and is also found on B-1 cells. Interestingly, the level of CD5 that is expressed on thymocytes and T cells is in part determined by the magnitude or form of signals delivered by the TCR-αβ (6). Thus, CD5 expression is typically lower on CD4+CD8− immature thymocytes than on mature CD4−CD8+ or CD4−CD8− single-positive cells that have received an TCR-αβ signal. Various potential ligands for CD5 have been described on B cells (7–9), but the true identity of the physiologically relevant ligand(s) remains to be established (10).

When cross-linked with mAbs, CD5 delivers a signal to T cells that augments their proliferation in response to TCR stimulation (11–14). This type of effect suggests that CD5 could perform a costimulatory or amplification role during certain types of T cell interactions. For instance, there is evidence that B cell responses may be influenced by binding between CD5 on T cells and a ligand on B cells (9). Paradoxically, however, the absence of CD5 confers a hyper- rather than hyporesponsive phenotype to thymocytes when they are stimulated through their TCRs (15). Moreover, CD5-deficient mice have normal numbers of T cells and B cells and can mount robust immune responses to a variety of antigenic challenges (16). These and other findings have implicated CD5 as a negative regulator of Ag receptor signaling during thymocyte repertoire selection.

In this paper we present the results of studies addressing the impact of CD5 on the selection and responses of CD4 lineage T cells. Whereas previous work had focused primarily on CD8 lineage thymocytes (15), the new data show that CD5 is also a negative regulator of TCR signaling in the CD4 lineage. Furthermore, we show that in addition to thymocytes, CD5 affects TCR responses in mature T cells. Cumulatively, the data support a general role for the CD5 molecule in negative regulation of Ag receptor signal transduction.

Materials and Methods

Antibodies

The mAbs used in the experiments included unconjugated or fluorochrome-conjugated anti-CD3ε (145-2C11), anti-CD69 (H1.2F3), and anti-CD24 (M1/69) from PharMingen (San Diego, CA); anti-CD4 (CT-CD4), anti-CD8α (CT-CD8α), anti-CD90 (CT-Thy1), and anti-CD5 (Ly-5.2), from Caltag (Burlingame, CA); and anti-BrDU mAb from Becton Dickinson (San Jose, CA). The mAb specific for the DO.11.10 clonotypic receptor (KJ-126) (17) was purified from culture supernatant and biotinylated according to standard procedures. Biotinylated mAbs were detected...
with streptavidin-TriColor (Caltag) or streptavidin-Spectral Red (Southern Biotechnology Associates, Birmingham, AL).

Cell lines
The 17.1 T cell hybridoma recognizes a hen egg lysozyme (HEL) peptide (NLANPASALLSDL, residues 74–88) presented by I-A\(^d\) (18). It does not express detectable CD5 by flow cytometry. The T cell hybridoma 2A4.3 was generated by immunizing CD5\(^-\) mice s.c. in the hind foot pads using a 1/1 emulsion of OVA (1 mg/ml) in CFA (Life Technologies, Grand Island, NY). Popliteal lymph nodes were harvested 10 days after immunization and activated in vitro with 20 \(\mu\)g/ml OVA and 100 U/ml rIL-2 (Genzyme, Cambridge, MA) for 4 days with syngeneic splenocytes before polyethylene glycol-mediated fusion to the BW5147 cell line. Hybridomas were selected in hypoxanthine-aminopterin-thymidine for 7 days before testing for reactivity to OVA, as determined by IL-2 secretion, and for loss of CD5 expression. The CD5\(^+\) hybridoma 2A4.3 lost responsiveness to OVA after several months of subcloning, but maintained the ability to secrete IL-2 upon anti-CD3 mAb stimulation.

Retroviral gene transfer
A mismatch primer (5'ctgacctgctattctgcacg-3') was used to insert a termination codon in a CDS cDNA in place of the codon for lysine 389 of the mature CD5 polypeptide. The 389\(\Delta\) form of CD5 encoded by the mutant cDNA lacks all but the 11 membrane-proximal residues of the cytoplasmic tail. The cDNAs for the green fluorescent protein (GFP), CD5-WT, or 389\(\Delta\) were cloned into the Moloney murine leukemia retroviral vector LZRS\(^{RMN-Z}\) in place of luc\(\beta\)e (gift from Dr. Garry Nolan, Stanford University, Stanford, CA). Viral supernatants were produced by transient transfection in 293T cells with vectors expressing retroviral gag and pol proteins and the vesicular stomatitis virus glycoprotein (gifts from Dr. Derya Unutmaz, New York University, New York, NY), and these were used to infect either 171.1 or 2A4.3 cells for 6 h in the presence of 8 \(\mu\)g/ml polybrene. Infected cells were matched for expression of CD3, CD4, and CD5 by electronic cell sorting on a FACScan (Becton Dickinson, San Jose, CA) or a MoFlo cytometer (Cytomation, Ft. Collins, CO).

Flow cytometry
Single-cell suspensions were prepared from lymph nodes and thymuses and incubated with the appropriate Abs for 30 min at 4°C. Cells were washed twice in 1× PBS/0.3% BSA/0.01% NaN\(_3\) between staining steps and before analysis. BrdU-labeled cells were detected by staining with an antibody specific for BrdU (PharMingen, San Diego, CA) and before analysis. BrdU-labeled cells were detected by staining with fluorescein digalactoside (Becton Dickinson), and for FACS analysis using anti-BrdU, -CD8, and -CD3 mAbs.

T cell assays
Lymph nodes were harvested from DO.11.10 TCR transgenic control or CD5-deficient mice and incubated with a mixture of anti-CD8 and anti-B220 microbeads (Miltenyi Biotech, Auburn, CA). Cells were then purified by magnetic separation according to the manufacturer’s protocol (Miltenyi Biotech). The purity of the resulting populations was assayed by flow cytometry and was typically 80–90%. CD4\(^+\) T cells (1 \(\times\) 10\(^5\)) were incubated with 2.5 \(\times\) 10\(^5\) T cell-depleted syngeneic splenocytes in the presence of varying concentrations of the OVA\(_{323-339}\) peptide. Cultures were incubated at 37°C for 72 h in 5% CO\(_2\) and pulsed with [\(\text{3H}\)]thymidine (Amersham, Arlington Heights, IL). The lymph node responder cells were incubated in vitro with 5 \(\times\) 10\(^5\) irradiated (2000 rad) stimulator spleen cells for 48 h before analysis. BrdU (5 \(\mu\)g/ml) was included in the cultures so that cells undergoing DNA synthesis would be labeled. Cytokinesis was blocked by the inclusion of 1 \(\mu\)M colchicine. Alloreactive CD4 lineage cells were quantified after 3 days by three-color FACS analysis using anti-BrdU, -CD8, and -CD3 mAbs.

Results
CD4 lineage development in CD5\(^-\) mice expressing an MHC class II-restricted TCR
To study the influence of CD5 on the development and function of CD4 lineage cells, we crossed CD5-deficient mice to transgenic mice expressing the DO11.10 TCR. This TCR is specific for a peptide from chicken OVA presented by I-A\(^d\), but it is also weakly alloreactive against I-A\(^b\) (22). In H-2\(^d\) mice, there is positive selection of thymocytes bearing this TCR into the CD4 lineage (23, 24). In contrast, TCR transgenic H-2\(^d\) mice show decreased thymic cellularity, reduced numbers of peripheral CD4\(^+\) T cells, downregulation of TCR levels, and an overabundance of double-negative T cells. H-2\(^d\) DO11.10 TCR transgenic mice have an intermediate phenotype, with sizeable thymuses, increased numbers of double-negative T cells, and slight down-regulation of TCR levels (Fig. 1) (24, 25).

The absence of CD5 caused a marked reduction in the representation of CD4\(^+\) CD8\(^-\) thymocytes in DO11.10 TCR transgenic mice that were homozygous for the H-2\(^d\) haplotype (Fig. 1, A and C). This apparent enhanced depletion of thymocytes did not block the development of CD4 lineage cells. Rather, there was a trend toward more CD4\(^+\) CD8\(^-\) cells in the thymuses of these mice than in CD5-expressing control mice (Fig. 1, A and C). There were also abundant clonotype-expressing CD4\(^+\) T cells in the lymph nodes of the CD5\(^-\) TCR transgenic mice (Fig. 1B). Thus, CD5 deficiency in H-2\(^d\) mice leads to pronounced negative selection of many DO11.10 TCR transgenic thymocytes, while still allowing for efficient positive selection of clonotype-bearing cells.

In contrast to the H-2\(^d\) phenotype, CD5 deficiency appeared to enhance selection against thymocytes with high levels of the DO11.10 TCR in both homozygous and heterozygous H-2\(^b\) mice. This enhancement was evident in at least two ways. First, the absence of CD5 correlated with lower expression of CD3 and the transgenic clonotype on CD4 lineage thymocytes and T cells (Fig. 1A). This down-regulatory effect was most pronounced in H-2\(^b\) mice, but was also apparent in H-2\(^b\) mice. Second, there were significantly more double-negative clonotype-expressing peripheral T cells in H-2\(^d\) CD5\(^-\) mice (Fig. 1B and data not shown). Such double-negative T cells have been previously observed in several other TCR transgenic settings that feature prominent negative selection (26). Thus, by these criteria, the negative selection phenotype characteristic of H-2\(^b\) DO11.10 TCR transgenic mice appeared to be more severe in the absence of CD5.

Cumulatively, the above observations on DO11.10 TCR transgenic mice indicate that CD5 influences the selection of CD4 lineage T cells. Moreover, the data are consistent with a role for CD5 in negative regulation of TCR signal transduction. In both H-2\(^d\) and H-2\(^b\) mice, the loss of CD5-mediated negative regulation enhances negative selection. In the latter, but not the former, the impact of CD5 deficiency is sufficient to preclude the selection and/or survival of cells that retain high levels of the transgenic TCR heterodimer. These data are similar to those previously reported for the variable impact of CD5 deficiency on the selection of CD8 lineage T cells (15).

CD4 lineage development in CD4\(^+\)CD8\(^-\) mice.
The development of the CD4 lineage is markedly impaired, but not completely disabled, by the absence of CD4 (27, 28). Because CD4 is a positive regulator of TCR signal transduction, it seemed possible that the loss of CD5-mediated negative regulation might partially suppress the CD4\(^+\) phenotype and rescue the development of some Th cells. Flow cytometric analysis of thymocytes and
peripheral T cells showed a phenotype consistent with this expectation. Specifically, there were typically 3-fold more mature (i.e., CD24low) CD4 lineage (i.e., CD82CD3high) thymocytes (Fig. 2A) and 2-fold more peripheral CD82 T cells (Fig. 2B) in CD4°CD5° mice than in CD4° mice. To confirm that the CD82 thymocytes belonged to the CD4 lineage, we also established crosses between CD4°CD5° mice and mice carrying a β-galactosidase transgene inserted directly into the CD4 locus (20). The CD4/β-galactosidase knockin allele (CD4β) in these mice does not encode a functional CD4 protein, but instead expresses β-galactosidase in place of CD4. β-Galactosidase expression in mature thymocytes and T cells from these mice can therefore be used as a marker for the
**FIGURE 2.** CD5 deficiency rescues CD4 lineage development in CD4° mice. A and B. Thymocytes were gated for low/absent expression of CD24 (heat-stable Ag, expressed on immature thymocytes) (64) as shown at the left. The gated dot plots show the percentages of CD4 and CD8 lineage cells in mice of the indicated genotypes. CD4 lineage cells in mice carrying the CD4/b-galactosidase allele were identified by staining with fluorescein digalactopyranoside (20) as shown in the histograms for CD8^1 and CD8^2 cells beneath the dot plots (C). Lymph node T cells (B) were gated for the expression of CD3 as shown at the left, and then analyzed for expression of CD8 as shown at the right. Percentages of CD8^- T cells (i.e., the population that includes CD4 lineage cells) are shown in the histograms. In A and B the average percentages of cells in the indicated regions are shown for representative experiments involving three mice (6–8 wk old) per group. The data are representative of multiple independent determinations on mice over a broad age range. The percentages of cells shown in C are for representative mice from groups of three. The average percentages of CD3^+CD8^- cells in CD4°CD5° mice and CD4° mice were 46.3 and 15.1%, respectively (p ≤ 0.0003) in the thymus and 47.8 and 26.3% in the periphery (p = 0.0004, by paired Student’s t test). D. The graph shows the rate of production of BrdU-labeled CD3^hiCD69^+ thymocytes over a 6-day period in CD4° (○) vs CD4°CD5° (●) mice. CD69 is induced coincident with positive selection of thymocytes (65). The mice were injected i.p. with 1 mg of BrdU twice, 4 h apart, on day 0. The data are representative of three independent time-course experiments.
CD4 lineage. As shown in Fig. 2C, the absence of CD5 increased the fraction of CD24<sup>low</sup> TCR<sup>β</sup><sup>high</sup> CD8<sup>+</sup> cells in the thymuses of mice that were homozygous for the CD4/<β>-galactosidase knockin allele (CD4<sup>1/2</sup>). CD8<sup>+</sup>, but not CD8<sup>+</sup>, mature thymocytes in CD4<sup>1/2</sup> CD5<sup>+</sup> mice were clearly enriched for expression of the β-galactosidase reporter and therefore belonged to the CD4 lineage.

We performed in vivo BrdU labeling studies to compare the rates of formation of mature thymocytes in CD4<sup>+</sup> vs CD4<sup>+</sup>CD5<sup>-</sup> mice (19). Cycling thymocytes (primarily immature double-negative and double-positive blasts) were pulse-labeled with BrdU on day 0. In the absence of CD5, more of these CD4<sup>+</sup> cells then underwent positive selection to become CD3<sup>high</sup>CD69<sup>+</sup> (Fig. 2D) or CD3<sup>high</sup>CD8<sup>+</sup> (not shown) thymocytes. Thus, the apparent increase in the representation of the CD4 lineage in the CD4<sup>+</sup>CD5<sup>-</sup> mice was due to enhanced development.

To confirm that the peripheral CD8<sup>+</sup> T cells were functional CD4 lineage cells, we compared the frequency of allo-class II-reactive cells in the CD8<sup>+</sup>CD3<sup>+</sup> populations of wild-type, CD4<sup>+</sup>, and CD4<sup>+</sup>CD5<sup>-</sup> mice. As stimulator cells in these experiments, we used irradiated allogeneic spleen cells from C57BL/6 congenic mice carrying either the bm1 or bm12 mutations (in K<sup>b</sup> or I-A<sup>b</sup>, i.e., MHC class I or MHC class II, respectively) (29). Purified CD3<sup>+</sup>CD8<sup>+</sup> T cells from the various mice were incubated in vitro with bm1, bm12, or C57BL/6 stimulator cells in the presence of colchicine and BrdU. Alloreactive cells were then identified by FACS using an anti-BrdU Ab to detect BrdU incorporated into their DNA. Because cell division was blocked by the action of colchicine (21), it was possible to use BrdU incorporation as a direct measure of the frequency of alloreactive cells in the starting population. As shown in Fig. 3B, the CD8 lineage in all mice included T cells with reactivity for the bm1, but not the bm12. Mutually, wild-type CD4 lineage T cells showed the inverse pattern of reactivity (Fig. 3A). Although, the CD4<sup>+</sup> animals showed virtually no reactivity for bm12, the CD3<sup>+</sup>CD8<sup>+</sup> T cells in the CD4<sup>+</sup>CD5<sup>-</sup> mice included a frequency of bm12-reactive cells similar to that observed in the wild-type CD4<sup>+</sup> lineage (Fig. 3A). Importantly, the CD3<sup>+</sup>CD8<sup>+</sup> population did not react to stimulation with bm1 cells (Fig. 3B). These observations are wholly consistent with the interpretation that the CD3<sup>+</sup>CD8<sup>+</sup> population is MHC class II restricted in its reactivity and therefore belongs to the CD4 lineage. Thus, the absence of CD5 potentiates the development of MHC class II-restricted CD4 lineage cells in CD4<sup>+</sup> mice.

Negative regulation of TCR signaling by CD5 in peripheral CD4 lineage T cells and hybridomas

To examine the influence of CD5 on TCR signaling in mature T cells, we adopted two approaches. The first of these was to compare the peptide-specific responses of DO11.10 CD4<sup>+</sup> T cells from CD5<sup>+</sup> and CD5-deficient mice. As shown in Fig. 4A, purified CD5<sup>+</sup> transgenic CD4<sup>+</sup> T cells responded more strongly to lower concentrations of the OVA peptide than did control T cells. For these experiments, we used cells from mice that were homozygous for the H-2<sup>+</sup> haplotype; such cells do not show the down-regulation of cell surface TCR levels that is evident in mice carrying the H-2<sup>-</sup> haplotype. Thus, this experiment shows that loss of CD5 function correlates with enhanced reactivity for TCR ligands in mature peripheral T cells.

Our second approach for examining the impact of CD5 on T cell responses involved gene transfer of CD5 into CD5-deficient T cell hybridomas. One of these hybridomas, 2A4.3, was derived from CD5<sup>+</sup> mice after OVA immunization. This cell line makes a robust IL-2 response to CD3 ligation, but has lost reactivity to OVA. The other cell line was the previously described 171.1 cell line that makes IL-2 when stimulated with a HEL peptide presented by I-A<sup>b</sup> molecules (18). Both cell lines were infected with retroviral vectors encoding wild-type CD5 or a mutant form of CD5 lacking almost all of its cytoplasmic tail. Control cells in some experiments were infected with a GFP retrovirus. Cells were sorted for equivalent surface expression of CD5 and then stimulated with anti-CD3 or the HEL peptide. The IL-2 secretion was used as an index of T cell activation. As shown in Fig. 4, A and C, the presence of wild-type CD5 in both cell lines correlated with reduced responsiveness to Ag receptor engagement. Cell lines expressing the mutant form of CD5 showed similar responses to the parental cell lines. Thus, gain of CD5 function negatively regulates Ag receptor signaling in these two T cell hybridomas, and this effect depends on the presence of the CD5 cytoplasmic domain.

Discussion

In this paper we show that the development of CD4 lineage cells is regulated by the function of the CD5 molecule. Without CD5, there was enhanced negative selection of DO11.10 TCR<sup>+</sup> thymocytes in both H-2<sup>-</sup> and H-2<sup>+</sup> mice. Interestingly, this enhanced negative selection largely precluded the selection of clonotype-expressing CD4<sup>+</sup> cells in the latter, but not the former, mice. In CD4-deficient mice, the loss of CD5 rescued a significant fraction...
cell-depleted splenocytes that had been pulsed with the OVA 323–339 peptide (E) showed that expression of CD5 suppressed TCR

DO11.10 TCR transgenic thymocytes and gene transfer studies in the absence of CD4. Experiments with mature T cells from the CD4 lineage that would otherwise not develop because of the absence of CD4. In a previous report, mice expressing MHC class I-restricted TCRs showed variable responses to the absence of CD5 (15). Here, the added sensitivity gained through CD5 deficiency was apparently enough to improve selection for the H-Y TCR, but was too great for selection of the P14 TCR. Because the DO11.10 TCR is weakly alloreactive against I-Ak (22), it is perhaps not surprising that the loss of negative regulation by CD5 would augment the slight H-2b negative selection phenotype (24, 25) and reduce the frequency of clonotype-expressing cells.

In contrast to the H-2b phenotype, H-2d DO11.10 TCR transgenic mice showed characteristics of enhanced negative selection in the context of persistent positive selection. Thus, the loss of CD5 caused an obvious reduction in the number of CD4+CD8+ TCR transgenic cells present in H-2d thymuses, but it did not select against high levels of the DO11.10 clonotype. Furthermore, there were often more CD4+CD8− cells present in the CD5− thymuses than in control thymuses (Fig. 1C). One possible interpretation of these data is that, as argued for H-2b mice, CD5 deficiency enhances TCR signaling in response to thymic I-Ak ligands. The resultant increase in the TCR signal is then sufficient to increase the probability that a CD4+CD8+ thymocyte will be deleted, but is insufficient to select efficiently against high levels of the TCR clonotype. A similar split phenotype of effective positive selection in the context of reduced thymic cellularity occurs in other TCR transgenic mice, such as those expressing the 2C receptor on an H-2b background (30, 31). CD5 deficiency has no obvious effect on the representation of CD4+CD8− cells in nontransgenic thymocytes (16), suggesting that it is not normally involved in regulating the expansion of clones after they have received the pre-TCR signal. Thus, the reduction in CD4+CD8− cells observed in CD5−DO11.10 TCR transgenic mice is most likely the result of MHC-dependent negative selection.

As an additional demonstration of the developmental significance of CD5, we showed that loss of CD5 could partially suppress the block of development of the CD4 lineage in CD4− mice. Rescue of CD4 lineage cells was evident in both the thymus and the periphery, although, interestingly, the effect was of greater apparent magnitude in the thymus (~3-fold vs 2-fold). Signaling thresholds for survival in the thymus may well be distinct and regulated differently from those in the periphery. Therefore, it seems feasible that the gain in signaling responsiveness caused by loss of CD5 could facilitate thymic development and peripheral survival/expansion to varying degrees. Alternatively, it is possible that CD4 lineage cells do not exit the thymus efficiently in the CD4− mice and perhaps accumulate to some extent in the medulla. Our BrdU labeling studies indicate that the development of CD4 lineage cells is enhanced by the loss of CD5 in CD4− mice, but they do not address the potential for accumulation in the medulla. Nonetheless, although we have not yet explored the basis for the difference in representation of CD4 lineage cells in the thymus vs the periphery, it is apparent that the absence of CD5 affords a substantial cellular and functional rescue of the CD4 lineage. In this respect, the findings are consistent with the proposed negative regulatory function for CD5.

Perhaps the most direct demonstration of CD5-mediated negative regulation came from the T cell hybridoma studies (Fig. 4), in which the expression of CD5 suppressed TCR-dependent responses. Negative regulation in this setting did not occur in the absence of the CD5 cytoplasmic tail, suggesting that the effect

FIGURE 4. Impact of CD5 expression on the response of mature T cells to TCR stimulation. A, The graph shows Ag-specific proliferation ([3H]thymidine incorporation) of purified H-2d DO11.10 CD4+ T cells from control (○) or CD5− (●) mice. T cells were activated in vitro with syngeneic T cell-depleted splenocytes that had been pulsed with the OVA 323–339 peptide as described in Materials and Methods. Results are from one representative experiment of four performed. Enhanced proliferation of Tg+ CD4 cells was observed in six of seven CD5− mice tested. 2A4.3 (B) and 171.1 (C) T cell hybridomas were activated with anti-CD3 mAb or HEL peptide, respectively. The graphs show IL-2 responses 24 h after stimulation of parental hybridomas (○) and hybridomas expressing wild-type CD5 (●), tailless CD5 (▲), or GFP (■). Results are from one representative experiment of three (2A4.3) and six (171.1) performed.
CD5 regulates CD4 lineage development and responses

Depended on intracellular interactions mediated by CD5. The nature of these interactions is currently a matter of speculation. CD5 may associate with several cytoplasmic proteins that have been implicated in various forms of negative regulation. One of these is SHP-1 (Src homology 2 domain-bearing protein tyrosine phosphatase-1) (32), a protein tyrosine phosphatase that negatively regulates signaling from a variety of hemopoietic receptors, including NK inhibitory receptors and the B cell receptor (33–36). Dominant negative forms of SHP-1 impair TCR signaling (37), and thymocytes from SHP-1-defective Me6 mice are hyperproliferative (32). Recent data suggest that the tyrosine residue at position 378 of CD5 may be important for association with SHP-1 and may be required for negative regulatory activity (38). Our data appear somewhat difficult to reconcile with this conclusion, because tyrosine 378 is retained in a tailless CD5 mutant that does not negatively regulate TCR responses in the hybridomas we have tested. Although we can coprecipitate SHP-1 with wild-type CD5 from lysates of pervanadate-stimulated T cell hybridomas, we do not detect SHP-1 associating with the tailless mutant (unpublished observations). Additional experiments are required to explore further the potential significance of SHP-1 for CD5-mediated negative regulation.

In addition to SHP-1, several other signaling molecules may associate with the cytoplasmic tail of CD5. These include the protein kinase casein kinase II (CK2), RasGAP, p116RBD, Tctex, Ca2+/calmodulin-dependent kinase IIβ, and phosphatidylinositol-3-kinase (13, 39–42). In most cases, there is at least the potential for the associated protein to confer a negative regulatory function. For instance, although CK2 has a positive role in regulating the cell cycle (43–45), it may also negatively regulate both MEK1 activation (46) and AP-1 activity (47). RasGAP down-regulates Ras signaling by catalyzing GTP hydrolysis of activated RasGTP (48). Similarly, through a pathway that involves rap1, p116 also down-regulates Ras activity (49) and disruption of the c-erbb gene causes enhanced T cell proliferation (50). Finally, the activation of NF-AT is negatively regulated by phosphatidylinositol-3-kinase activity in T cells (51). The significance of any or all of these associations for negative regulation by CD5 is currently unclear and awaits more selective mutagenesis of the CD5 cytoplasmic tail.

Regardless of the mechanism involved, the immunological significance of negative regulation mediated by CD5 is somewhat obscure. The data presented here and previously show that the selection of T cells occurs differently in the presence or the absence of CD5. The repertoire of TCRs carried by CD5− T cells is therefore likely to be distinct from that of wild-type T cells. Nonetheless, CD5− mice have a full complement of T cells with diverse specificities capable of mediating several types of protective immune responses (16) (C. Peña-Rossi and N. Killeen, our unpublished data). The CD5 molecule is conserved between evolutionarily distant organisms, especially in the cytoplasmic tail (52), suggesting that there is a distinct selective advantage conferred by its function. Given the absence of obvious immunodeficiency in CD5− mice, the nature of this advantage has not yet been established. It seems probable, however, that the change in the TCR repertoire caused by loss of CD5 represents a significant survival risk under certain circumstances. At least two types of approaches could provide further information on the true significance of the CD5 advantage. One is to explore a range of pathogenic challenges in search of ones that would selectively overcome CD5− hosts. A successful immune response to such challenges would then presumably involve either the direct extrathymic involvement of the CD5 molecule or a critical role for cells that do not get selected without CD5. The other is to understand exactly how the TCR repertoire might be compressed, expanded, or shifted without CD5, i.e., to get a better sense of the overall complexity and potential specificity of the CD5− T cell population.

There is accumulating evidence that thymocytes are more sensitive to stimulation through their TCRs than mature T cells (53–56). Such enhanced sensitivity is presumably essential for the efficient elimination of autoreactive thymocytes and for positive selection. The mechanisms that allow for enhanced sensitivity are unclear, but could well involve developmentally regulated changes in the expression of molecules such as CD5. Consistent with this view, double-positive thymocytes express less CD5 than mature single-positive cells. Lower expression of CD5 coupled with the increased expression of positive regulators such as the Syk tyrosine kinase (57) may be essential in establishing a hypersensitive state at the double-positive stage. The complete absence of CD5 in CD5− mice appears to accentuate this state and thereby may bias repertoire selection against cells that make efficacious, but still selectable, engagements with thymic peptide/MHC ligands.

Although the data in this and a previous report (15) reveal a clear role for CD5 in repertoire selection, they also re-emphasize the potential for productive T cell development in its absence. Thus, it is reasonable to conclude that CD5 is largely dispensable for the development of T cells. This, however, does not exclude either the above-mentioned effect on repertoire selection, or the possibility that other molecules share overlapping functions with CD5. An obvious candidate in this last role would be CD6, which is a glycoprotein that is structurally very similar to CD5, at least in its extracellular portion (58, 59). The genes encoding CD5 and CD6 are closely linked in the genome (60, 61) and share a related pattern of expression (62, 63). Thus, it is possible that CD6 may partially compensate for CD5 deficiency in CD5− mice. If so, the true significance of the function that CD5 performs during T cell development and immune responses may only be apparent in mice that lack the expression of both CD5 and CD6.

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