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We previously characterized several tumor-specific T cell clones from PBL and tumor-infiltrating lymphocytes of a lung cancer patient with identical TCR rearrangements and similar lytic potential, but with different antitumor response. A role of the TCR inhibitory molecule CD5 to impair reactivity of peripheral T cells against the tumor was found to be involved in this process. In this report, we demonstrate that CD5 also controls the susceptibility of specific T cells to activation-induced cell death (AICD) triggered by the tumor. Using a panel of tumor-infiltrating lymphocytes and PBL-derived clones expressing different levels of CD5, our results indicate that T lymphocyte AICD in response to the cognate tumor is inversely proportional to the surface expression level of CD5. They also suggest a direct involvement of CD5 in this process, as revealed by an increase in tumor-mediated T lymphocyte AICD following neutralization of the molecule with specific mAb. Mechanistically, our data indicate that down-regulation of FasL expression and subsequent inhibition of caspase-8 activation are involved in CD5-induced T cell survival. These results provide evidence for a role of CD5 in the fate of peripheral tumor-specific T cells and further suggest its contribution to regulate the extension of CTL response against tumor. The Journal of Immunology, 2007, 178: 6821–6827.

H uman CD5 is a membrane glycoprotein constitutively expressed on thymocytes and mature T cells (1). It belongs to the conserved family of receptors containing extracellular domains of the scavenger receptor cysteine-rich type (2) and is associated both physically and functionally with the TCR/CD3 complex. Accumulating evidence indicates that T lymphocyte activation and selection are sensitive to variations in the expression level of the cell surface CD5. Indeed, based on data from CD5-deficient mice, it has been reported that CD5 exerts a negative effect on TCR signaling of immature T cells (3). CD5 also inhibits peripheral blood T cell signaling and CD5+ T cells show an enhanced response upon TCR triggering (3, 4). More recently, it has been documented that CD5 is recruited and tightly colocalized with CD3 at the immunological synapse so as to inhibit TCR signaling in T cells interacting with APCs, without influencing conjugate formation (5). Furthermore, it has been described that CD5-mediated inhibition of TCR signaling does not require a CD5 extracellular domain, but only requires its cytoplasmic domain (6) in which a pseudo-ITAM motif is likely to play a role (5).

It is well-established that a properly functioning immune system is dependent on programmed cell death at virtually every stage of lymphocyte development and activity. In this regard, activation-induced cell death (AICD), an apoptotic pathway triggered by death receptors following cell hyperactivation, plays a crucial role in the control of immune responses (7). Indeed, failure of AICD can lead to immunopathological diseases such as malignancy and autoimmunity (8). With regard to T lymphocytes, it has been reported that AICD controls the expansion of activated T cells during Ag engagement and induces T cell tolerance (9, 10). In a previous report, we characterized two tumor-specific T cell clones, isolated either from PBL or tumor-infiltrating lymphocytes (TIL) of a lung cancer patient, expressing a unique TCRα/TCRβ and displaying similar lytic potential (11). These clones were found to mediate an antitumor responsiveness, which was inversely proportional to the expression level of the TCR inhibitory molecule CD5. In the present study, we investigated the role of CD5 in T cell survival following stimulation with autologous tumor cells. Our results indicate that CD5 promotes T cell survival by preventing overactivation leading to AICD and point to a role for CD5 in protecting T lymphocytes from TCR-mediated cell death by regulating FasL expression and caspase-8 activation.

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4 Abbreviations used in this paper: AICD, activation-induced cell death; TIL, tumor-infiltrating lymphocyte; PL, propidium iodide; DISC, death-inducing signaling complex; c-FLIP, cellular FLIP.

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Materials and Methods

Derivation and culture of tumor cell lines and T cell clones

The IGR-Heu lung carcinoma cell line was derived and maintained in culture as described (12). Heu171 and H32-22 T cell clones were derived from autologous TIL and PBL, respectively (11). Tag Jurkat cells (JTag), a derivative of the human T cell leukemia Jurkat stably transfected with the SV40 large T Ag, were grown in RPMI 1640 medium (Seroned; Biochrom) supplemented with 10% FCS, antibiotics (50 U/ml penicillin, 50 μg/ml streptomycin), 2 mM l-glutamine, and 1 mM sodium pyruvate.

Abs and immunofluorescence analyses

Anti-human CD5 (O490D) mAb was produced as previously described (13). Anti-human TRAIL-R1 (M271) and TRAIL-R2 (M413) were provided by M. Kubin (Amgen, Seattle, WA). Anti-Fas (UB2), neutralizing anti-Fas (ZB4), anti-FasL (4H9), anti-CD3 (UCHT1), anti-CD11a (LFA-1; 25.3.1), PE- and FITC-conjugated goat anti-mouse, PE-conjugated streptavidin, hamster isotopic control and mouse isotopic control Ab were purchased from Beckman Coulter. Biotin-conjugated goat anti-hamster mAb was provided by Jackson ImmunoResearch Laboratories.

Phenotypic analyses were performed by indirect immunofluorescence using a FACS Calibur flow cytometer as described (11). For FasL expression, cells were preincubated or not with anti-CD5 for 2 h, stimulated with autologous tumor cells at indicated time points, and then labeled with 4H9 mAb (5 μg/ml) or control hamster Ab followed by biotinylated goat anti-hamster IgG and then PE-labeled streptavidin. Cells were analyzed as previously described, after fixation with 1% formaldehyde (14). In coculture experiments, cells were fixed before incubation with primary Ab and FITC-labeled anti-human CD3 mAb was included to identify CTL.

AICD and apoptosis assay

For AICD induction, T cell clones were stimulated with autologous tumor cells or anti-human CD3 mAb (UCHT1; 100 ng/ml). Briefly, tumor cells were seeded (10^5 cells/well) on round-bottom 96-well plates 24 h before the experiments. T cells, preincubated or not for 2 h with anti-CD5 (100 μl of ascitic fluid diluted at saturating concentration predetermined by immunofluorescence analysis), anti-LFA-1 or isotropic control (NKTa) mAb were then added at 1:1 ratio and incubated for 6 h. For AICD inhibition, CTL were preincubated 2 h with agonistic anti-Fas mAb (ZB4; 125 ng/ml), then stimulated with IGR-Heu as described above. Apoptosis was measured by flow cytometry using an Annexin V^FITC apoptosis detection kit (BD Biosciences). Briefly, cells were washed and incubated for 15 min in 100 μl of annexin V-labeling buffer with 10 μl of propidium iodide (PI). Samples were analyzed within 1 h using a FACS Calibur flow cytometer. Statistical analyses were performed using Student’s t test.

Real-time quantitative RT-PCR analysis

For analysis of FasL, induction, T cells were stimulated with tumor cells at a 1:1 ratio during 0.2, and 4 h. Total RNA was extracted from 2 × 10^5 T cells using a modified guanidine isothiocyanate phenol/chloroform method (TRIzol reagent; Invitrogen Life Technologies). Real-time quantitative RT-PCR analysis of the FasL gene was performed by TaqMan (Applied Biosystems). PCR primers and probe for the FasL gene were designed by Applied Biosystems and used as the manufacturer’s recommendations. The amount of RNA sample was normalized by the amplification of an endogenous control (18S). Quantification of FasL transcript was determined using the standard curve method based on the use of human FasL cloned in pGEM-T Easy vector (provided by F. Rieux-Laucat, Hoˆpital Necker, Paris, France) as previously described (15).

Cytotoxicity and caspase-8 activity assays

The cytotoxic activity of the T cell clones was measured by a conventional 4-h 51Cr-release assay at indicated E:T ratios. B, Cytotoxic activity of Heu171 and H32-22 T cell clones toward the autologous EBV-transformed B cell line. Autologous HLA-A2^+ lymphoblastoid cells were incubated in medium or in the presence of mutated α-actinin-4 peptide (0.1–100 nM) before addition of Heu171 or H32-22 clones at 30:1 E:T ratio. Data shown are representative of three independent experiments. C, Flow cytometry analysis of Heu171 and H32-22 T cell clones. Cells were stained with CD5-specific O490D mAb (gray) or with an isotype-matched control (white). Percentages of positive cells are indicated. Numbers in parentheses correspond to mean fluorescence intensity. Data shown are representative of five independent experiments.

CD5^+ Jurkat (JTag) cells were transiently transfected with pEGFP-N1 empty vector or pEGFP-N1-CD5 plasmid (5). Briefly, 10^5 Jurkat were electroporated in a 4-mm cuvette (Eurogenetec) with 10 μg of pEGFP-N1-CD5 or pEGFP-N1 empty plasmid at 900 μF, 300 V (Gene Pulser II; Bio-Rad). After a 24-h incubation at 37°C, fresh medium (RPMI 1640, supplemented with 10% FCS) was added and cells were further cultured for additional 48 h. Expression of CD5 and GFP fluorescence was checked every 24 h. AICD in transfected Jurkat cells was induced at day 3 after 6 h of treatment with PMA (3 μg/ml; Sigma-Aldrich).

Results

T cell CD5 levels inversely correlate with tumor-mediated AICD

Heu171 and H32-22 T cell clones were isolated, respectively, from TIL and PBL of a lung cancer patient (11). Although the two clones expressed a unique TCR and displayed similar lytic potential, only the TIL clone mediated a strong cytotoxic activity toward the autologous IGR-Heu tumor cell line (Fig. 1A). In contrast, both clones were able to lyse the autologous EBV-transformed B cell line pulsed with the antigenic peptide (Fig. 1B). T cell antitumor...
stimulated or not with IGR-Heu tumor cells for 6 h at a 1:1 E:T ratio. Cells were then stained with Annexin V<sub>FITC</sub> and PI. Percent apoptotic cells is shown in the lower right quadrant. Numbers in parentheses correspond to the mean fluorescence intensity. Cells in the upper right quadrant are late apoptotic or necrotic. Data shown are representative of five independent experiments. B, H32-22 (◼) and Heu171 (▲) were incubated with anti-CD3 mAb (UCHT1, 100 ng/ml) or in medium for 6 h. Cells were then stained with Annexin V<sub>FITC</sub> and PI. Percent of apoptotic cells is shown.

FIGURE 2. AICD of T cell clones. A, H32-22 (CD$^{5\text{high}}$) and Heu171 (CD$^{5\text{low}}$) were stimulated or not with IGR-Heu tumor cells for 6 h at a 1:1 E:T ratio. Cells were then stained with Annexin V<sub>FITC</sub> and PI. Percent apoptotic cells is shown in the lower right quadrant. Numbers in parentheses correspond to the mean fluorescence intensity. Cells in the upper right quadrant are late apoptotic or necrotic. Data shown are representative of five independent experiments. B, H32-22 (◼) and Heu171 (▲) were incubated with anti-CD3 mAb (UCHT1, 100 ng/ml) or in medium for 6 h. Cells were then stained with Annexin V<sub>FITC</sub> and PI. Percent of apoptotic cells is shown.

reactivity was inversely proportional to the expression level of TCR inhibitory molecule CD5, which was much higher on the PBL (CD$^{5\text{high}}$) than on the TIL (CD$^{5\text{low}}$) clone surface (Fig. 1C).

It has been widely documented that strong TCR-mediated activation ultimately leads to programmed T cell death (reviewed in Ref. 17). Because CD5 exerts a negative feedback effect upon TCR signaling (3, 4, 11), we asked whether it may contribute to different T cell survival between the various antitumor CTL. We therefore investigated the role of CD5 in regulating T cell AICD in both PBL and TIL clones. For this purpose, we compared T cell survival of H32-22 and Heu171 after activation either with IGR-Heu or with anti-TCR mAb. Apoptotic T cells were detected by double staining with annexin V and PI. As shown in Fig. 2A, annexin V<sup>+</sup>/PI<sup>+</sup> apoptotic cells comprised 43% of Heu171. Interestingly, the same stimulation led to only 22% of apoptotic cells in H32-22. In contrast, stimulation with anti-CD3 mAb (used at 100 ng/ml) induced 85–90% of apoptotic cells in both clones (Fig. 2B), indicating that they displayed similar susceptibility to programmed cell death, and supporting our previous results indicating that CD5-mediated protection is restricted to interactions with autologous target cells (11). These data clearly show that the CD$^{5\text{high}}$ PBL clone survived longer than CD$^{5\text{low}}$ TIL after TCR stimulation with the cognate tumor.

Table 1. The CD5 expression level inversely correlates with T cell clone AICD and caspase-8 activity<sup>a</sup>

<table>
<thead>
<tr>
<th>CD5 Expression (mean fluorescence)</th>
<th>% Apoptotic Cells</th>
<th>% Caspase-8 Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIL</td>
<td></td>
<td></td>
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<tr>
<td>Heu171 CD5&lt;sup&gt;−&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heu171 CD5&lt;sup&gt;+(low)&lt;/sup&gt;</td>
<td>28</td>
<td>51</td>
</tr>
<tr>
<td>PBL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H32-8</td>
<td>88</td>
<td>27</td>
</tr>
<tr>
<td>H32-22</td>
<td>117</td>
<td>19</td>
</tr>
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</table>

<sup>a</sup>Mean fluorescence of CD5 surface expression, autologous tumor cell line-mediated AICD, and caspase-8 activity in CD5<sup>−</sup> and CD5<sup>+(low)</sup> subsets of the Heu171 TIL clone and PBL-derived T cell clones (CD$^{5\text{high}}$). Data shown are representative of four independent experiments. The percentage of apoptotic cells was significantly higher in the CD5<sup>−</sup> fraction than in the CD5<sup>+(low)</sup> fraction of the Heu171 clone (p < 0.07).

CD5 promotes T cell survival

The above experiments suggested that the CD5 molecule is involved in the control of T cell survival. As its distribution on Heu171 was bimodal, we sorted the CD5<sup>−</sup> and CD5<sup>+(low)</sup> subpopulations and investigated T cell survival after 6 h activation with autologous tumor cells. Results in Table I show that the percentage of annexin V<sup>+</sup>/PI<sup>+</sup> apoptotic cells is significantly higher in the Heu171 CD5<sup>−</sup> fraction than in the CD5<sup>+(low)</sup> fraction. These results indicate that within the same clone, the CD5<sup>−</sup> subset was more susceptible to AICD than the CD5<sup>+(low)</sup> subset. Experiments were also performed with H32-22 and one additional PBL CTL clone, H32-8, displaying intermediate levels of CD5 (see Table I). Both PBL-derived clones were much more resistant to apoptosis than the Heu171 CD5<sup>−</sup> subset (Table I). These data indicate that T cell clone sensitivity to AICD was inversely proportional to CD5 expression levels, supporting the hypothesis that CD5 can control T cell survival.

We next performed experiments in which T cell clones were preincubated with a neutralizing anti-CD5 mAb. Data depicted in Fig. 3 show that this treatment led to a prompt increase in H32-22
AICD after stimulation with IGR-Heu tumor cells, reaching a level similar to that observed in Heu171. In contrast, pretreatment with isotypic control mAb or anti-LFA-1 (CD11a, which is highly expressed on the two T cell clones) had no effect (Fig. 3). Note that treatment with anti-CD5 mAb alone did not induce AICD (data not shown). These results further support the notion that CD5 protects T cells from TCR activation-dependent apoptosis triggered after recognition of the specific target.

FIGURE 4. A, Expression of GFP (left panels) and CD5 (right panels) in the transiently transfected Jurkat T cell line. CD5+ Jurkat (JTag) cells were transiently transfected with pEGFP-N1-CD5 (CD5-GFP) or pEGFP-N1 empty vector (GFP) and expression of GFP and CD5 was analyzed at day 3 after electroporation. For CD5 expression, cells were stained with anti-CD5 or an isotypic control mAb. Percent of positive cells is shown. Numbers in parentheses correspond to mean fluorescence intensity. B, AICD in CD5-transfected Jurkat T cell line. AICD was induced in pEGFP-N1 empty vector (GFP) and pEGFP-N1-CD5 (CD5-GFP)-transfected cells by stimulation with PMA (3 μg/ml) for 6 h at day 3 after electroporation. Cells were stained with annexin V-allophycocyanin and PI and analysis was performed on GFP+ cells. Percent of apoptotic cells is shown.

FIGURE 5. A, Analysis of surface expression of Fas, TRAIL-R1, TRAIL-R2, and TNFR1 on H32-22 and Heu171 clones. Immunofluorescence analysis was performed using anti-TRAIL-R1 (M271), anti-TRAIL-R2 (M413), anti-Fas (UB2; without fill) mAb or isotypic control (black fill). Percentages of positive cells are indicated. Numbers in parentheses correspond to mean fluorescence. B, Role of Fas in T cell AICD. H32-22 and Heu171 were preincubated or not with anti-CD95 (ZB4) mAb for 2 h and then stimulated with autologous tumor cells for 6 h at 1:1 E:T ratio. Cells were stained with Annexin VFITC and PI. Data shown are representative of three independent experiments.
To further strengthen these results, we investigated the role of CD5 in AICD triggered in a CD5 \(^{+}\) Jurkat T cell line (JTag) reconstituted with the molecule and analyzed by FACS. For this aim, cells were transiently transfected with a pEGFP-N1 empty vector used as a control (GFP) or a pEGFP-N1-CD5 vector (CD5-GFP) \(^{5}\) and stimulated with PMA (3 \(\mu\)g/ml) for 6 h to induce AICD. Fig. 4A shows the expression levels of GFP in either GFP or CD5-GFP Jurkat cells 3 days after electroporation (two left panels) and the same cells labeled with the CD5-specific mAb (two right panels). After PMA activation, results obtained on the GFP-gated cells showed that CD5 expression in Jurkat cells induced a significant decrease in annexin V labeling as compared with cells transfected with the empty vector (Fig. 4B). These data further support the finding that CD5 protects T cells from AICD.

CD5 protects T cells from AICD by regulating FasL expression

Initial experiments were performed to determine the mechanism involved in the tumor-mediated T cell clone AICD. For this purpose, we investigated the expression of death receptors of the TNFR superfamily reported to be implicated in mediating TCR-induced apoptosis in activated T cells \(^{18–20}\). Although Heu171 and H32-22 expressed Fas (CD95/Apo-1) at similar levels, they were negative for TNFR1, TRAIL-R1/DR4, and TRAIL-R2/DR5 (Fig. 5A). We therefore examined the role of CD95 in TCR-induced cell death by preincubating target cells with anti-Fas-neutralizing ZB4 mAb. Fig. 5B indicates that ZB4 inhibited tumor cell-triggered AICD in both CD5\(^{high}\) and CD5\(^{low}\) clones. This result suggests that CD5 is involved in the regulation of Fas/FasL pathway, which is known to play an important role in T cell AICD \(^{19,21}\).

Subsequent experiments were conducted to assess the mechanism involved in the control of the Fas/FasL pathway by CD5. For this purpose, we first investigated the expression of Fas and FasL on the surface of H32-22 and Heu171 following stimulation with the autologous tumor. Our results indicate that CD95 is expressed on both clone surface and that this expression is stable following T cell clone stimulation with IGR-Heu (data not shown). With regard to CD95 ligand, it was very weakly expressed on unstimulated clones. However, kinetic studies indicated that it was induced on the Heu171 surface with a peak after 4 h (34% of the cells) of stimulation with the cognate target (Fig. 6A). In contrast, FasL was barely detected on H32-22 stimulated in the same conditions, with a very small increase after 2 h of stimulation (6% of the cells). Importantly, preincubation of H32-22 with anti-CD5 mAb induced roughly a 4-fold increase in FasL expression (22% of cells) after 2 h stimulation with tumor cells (Fig. 6A). This increase was not
observed when the cells were treated with a control mAb. Anti-CD5 mAb used alone also had no effect (data not shown). As it could be expected from the low level of CD5 in Heu171 (11), anti-CD5 mAb treatment had a marginal effect on FasL expression in this cell clone (Fig. 6A). Induction of FasL was also quantitated by real-time RT-PCR analysis after stimulation of TIL and PBL clones with IGR-Heu in the absence or presence of anti-CD5 mAb. As expected, in agreement with membrane expression analysis, the Heu171 clone displayed higher copy numbers of FasL mRNA than the H32-22 clone after 4 h of stimulation with the cognate tumor (Fig. 6B). Anti-CD5 mAb treatment did not induce any increase in FasL mRNA expression (data not shown), suggesting a predominant effect of CD5 blocking on the delivery of the protein at the cell surface. These data emphasize that CD5 regulates FasL expression presumably through inhibition of TCR signaling.

CD5 engagement regulates caspase-8 activation

It is well-established that AICD is initiated through the interaction of FasL with Fas (21, 22). Triggering of the Fas leads to its clustering and the formation of a death-inducing signaling complex (DISC) comprised of the adaptor molecule Fas-associated death domain, procaspase-10, and procaspase-8 (23). Recruitment of procaspase-8 to the DISC leads to autoproteolytic activation of caspase-8 and initiation of the caspase cascade leading to apoptosis (24). We therefore assessed caspase-8 activity in TIL and PBL clones after a 6 h stimulation with the autologous tumor. Consistent with the expression level of FasL, H32-22, and H32-8 exhibited lower caspase-8 activity (17 and 20% of the cells, respectively) than Heu171 CD5<sup>−</sup> (41% of the cells) or CD5<sup>+</sup> (50% of the cells) fractions (Table I). These results further support the conclusion that CD5 protects T cells from AICD through regulated FasL expression and subsequent modulation of caspase-8 activity.

Discussion

We isolated from PBL and TIL of a lung cancer patient several tumor-specific T cell clones recognizing an HLA-A2-restricted epitope encoded by a mutated α-actinin-4 (ACTN4) gene (11, 25). Although two of these clones expressed a unique TCR and displayed similar functional avidity and lytic potential, only the TIL clone lysed autologous tumor cells. We demonstrated that T cell clone antitumor responsiveness inversely correlated with the expression level of the TCR inhibitory molecule CD5. Indeed, tumor-specific circulating T lymphocytes exhibited a much higher level of CD5 than TIL, and failed to kill the cognate target, to secrete cytokines, and to trigger a Ca<sup>2+</sup> response following specific stimulation (11). CD5 is expressed on thymocytes, mature T cells, and a subpopulation of B cells (B1a) (1), and is associated with TCR/CD3 complex and BCR. Because it exerts a negative effect on TCR signaling (3, 4), we investigated its contribution to the control of T cell survival. Our data indicate that CD5 protects T cells from TCR activation-dependent apoptosis triggered following recognition of the specific target. Indeed, tumor-reactive circulating T cells (CD5<sup>high</sup>) were less susceptible to AICD than TIL (CD5<sup>low</sup>) following TCR stimulation with the autologous tumor. Furthermore, our results indicate that preincubation of CD5<sup>high</sup> circulating T cells with neutralizing anti-CD5 mAb led to a prompt increase in tumor-mediated T lymphocyte AICD. It is worth noting that the same treatment had only a weak effect on PBL clone reactivity against tumor cells, as measured by cytotoxic activity and cytokine secretion (data not shown). The molecular mechanism that governs CD5 inhibitory function is not very well-understood and it is yet difficult to explain the overall data obtained. Future studies may allow the determination of the mechanism associated with TCR-signaling inhibition by CD5. It is plausible that CD5-mediated inhibition of TCR signaling does not require the CD5 extracellular domain and that only its cytoplasmic tail is necessary to trigger CD5 inhibitory effect (6). This would imply that triggering of CD5 with its ligand is not required for TCR-signaling inhibition and that blocking of this interaction with neutralizing anti-CD5 mAb leads to increase in PBL clone apoptosis. In agreement with our results, it has been reported that CD5 helps B cells to survive after BCR stimulation, while reducing the BCR-induced Ca<sup>2+</sup> response (26).

Several evidences indicate that AICD is mediated by members of the TNF superfamily, in particular FasL, and requires activation-induced expression of FasL and Fas, and their subsequent interaction (27). It has been reported that AICD may even occur in a cell autonomous manner, indicating that FasL may interact with Fas on the same cell (22, 28). In the present report, we show that CD5 prevents AICD in T cells through regulation of Fas/FasL pathway. Indeed, our results indicate that CD5<sup>high</sup> T cells express lower FasL mRNA and surface protein than CD5<sup>low</sup> T cells after specific stimulation with autologous tumor cells and that anti-CD5 mAb led to an increase in FasL protein expression on the membrane of the former cells. This increase is likely due to the transport of intracellular FasL molecules to the T cell plasma membrane. Indeed, although transcriptional regulation of FasL is central for the appropriate expression in T cells following activation by target cells (29), increasing evidence indicated that FasL is also critically regulated at posttranscriptional levels. It has been demonstrated that FasL is stored in specialized secretory lysosomes in both T cells and NK cells, and that polarized degranulation controls the delivery of FasL to the cell surface (30, 31). FasL is also concentrated in lipid rafts of primary T cells, and disruption of lipid rafts abrogates FasL apoptosis-inducing activity (32). Our results suggest that CD5 may control FasL expression at both transcriptional and posttranscriptional levels and that it may regulate FasL delivery to the cell surface by controlling TCR-mediated cytotoxic activity. Previous studies demonstrated that cytokines, such as IL-6 and TGF-β1 (33, 34), and the cycle-related molecule, cyclin B1 (35), also inhibit FasL expression and subsequent AICD in T cells. In contrast, other stimuli, including IL-2, IFN-α, and IL-12 (36–38), augment activation-induced T cell death by up-regulation of FasL. These findings support the notion that the regulation of FasL expression on activated T cells is a critical event in the control of AICD and thus of immune responses.

It is well-known that caspases play a crucial role in the signaling of Fas/FasL-mediated cell death. Upon Fas activation and trimerization, several effector molecules are recruited, forming the DISC. Fas-associated death domain, the first protein that binds to Fas, recruits procaspase-8 thereby resulting in activation of caspase-8. Active caspase-8 initiates a cascade of effector caspase activation, including that of caspase-3, which may lead to cleavage of cellular death substrates (39). Our results provide evidence for a role of CD5 in promoting T cell survival through modulation of FasL expression, as a consequence of TCR-signaling inhibition (3, 4), resulting in down-regulation of caspase-8 activation. It has been recently reported that IL-12 inhibits TCR-induced T cell death by regulation of caspase-8 and caspase-3 activation, presumably through the down-regulation of FasL and up-regulation of cellular FLIP (c-FLIP) (38). Similarly, a previous study indicated that KIR2DL1 controls CTL susceptibility to tumor-mediated AICD by a decrease in caspase-8 activity and sustained c-FLIP-L induction (40). Our data, conducted by Western blot analysis using specific mAb, showed that CD5<sup>high</sup> and CD5<sup>low</sup> clones express similar levels of c-FLIP-L protein (data not shown) ruling out its implication in the control of T cell AICD by CD5.

Taken together, our results indicate that CD5 provides survival signals to CD8<sup>+</sup> T cells through regulation of FasL expression and...
capase-8 activation and support a role for CD5 in the control of peripheral CD8+ T cell homeostasis. Along the same line, it has been reported, in TCR-transgenic mice, that the homeostatic behavior of naive CD4+ T cells can be deduced by expression levels of TCR and CD5 (41). One current immunotherapeutic approach in cancer patients is based on adoptive transfer of autologous T lymphocytes (42). The persistence of the adoptively transferred tumor-reactive T cells is crucial for effective antitumor immunotherapy (43). Much effort is now being directed toward developing strategies for prolonging their survival, including that of transduction with the Bcl-2 gene (44). The present study provides evidence for a role of CD5 in the control of tumor-specific T cell survival and suggests its contribution in regulating the extension of the CTL response against tumors.

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Disclosures

The authors have no financial conflict of interest.

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