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Homeostasis of Naive and Memory CD4+ T Cells: IL-2 and IL-7 Differentially Regulate the Balance Between Proliferation and Fas-Mediated Apoptosis

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Cytokines play a crucial role in the maintenance of polyclonal naive and memory T cell populations. It has previously been shown that ex vivo, the IL-7 cytokine induces the proliferation of naive recent thymic emigrants (RTE) isolated from umbilical cord blood but not mature adult-derived naive and memory human CD4+ T cells. We find that the combination of IL-2 and IL-7 strongly promotes the proliferation of RTE, whereas adult CD4+ T cells remain relatively unresponsive. Immunological activity is controlled by a balance between proliferation and apoptotic cell death. However, the relative contributions of IL-2 and IL-7 in regulating these processes in the absence of MHC/peptide signals are not known. Following exposure to either IL-2 or IL-7 alone, RTE, as well as mature naive and memory CD4+ T cells, are rendered only minimally sensitive to Fas-mediated cell death. However, in the presence of the two cytokines, Fas engagement results in a high level of caspase-dependent apoptosis in both RTE as well as naive adult CD4+ T cells. In contrast, equivalently treated memory CD4+ T cells are significantly less sensitive to Fas-induced cell death. The increased susceptibility of RTE and naive CD4+ T cells to Fas-induced apoptosis correlates with a significantly higher IL-2/IL-7-induced Fas expression on these T cell subsets than on memory CD4+ T cells. Thus, IL-2 and IL-7 regulate homeostasis by modulating the equilibrium between proliferation and apoptotic cell death in RTE and mature naive and memory T cell subsets. The Journal of Immunology, 2003, 171: 61–68.
through Fas/CD95. Stimulation of CD95 by its ligand results in the formation of a death-inducing signaling complex. Recruitment and activation of caspase 8 in the death-inducing signaling complex induces a proteolytic cascade that leads to apoptosis. Although both CD95 and its ligand (CD95L) are induced in T cells upon engagement of the TCR (15), expression of CD95 is not in itself a measure of the sensitivity of a cell to Fas-mediated apoptosis. Memory peripheral blood T cells are resistant to cell death induced by agonistic α-Fas mAbs despite detectable levels of surface CD95 (16–18). Moreover, while T cells acquire sensitivity to AICD following TCR engagement, the responses of naive and memory T cell pools are not equivalent: memory T cells are more resistant to AICD after Ag restimulation than primary Ag-stimulated naive T cells (19–21).

In this study, we assessed whether the susceptibility of CD4⁺ T cell subsets to Fas-mediated apoptosis is modified by the presence of cytokines and whether this susceptibility correlates with cytokine-mediated survival and/or proliferation. Although the IL-7 cytokine promotes the survival of naive CD4⁺ T cells (22–24), we now show that together with IL-2, it primes naive peripheral blood T cells as well as RTE for Fas-mediated cell death. In contrast, IL-2/IL-7-stimulated memory T cells are significantly more resistant to Fas-induced cell death. The level of Fas-induced cell death does not correlate with proliferation as only RTE divided significantly in response to the combination of these homeostatic γc cytokines. However, in contrast to the previously reported inhibitory effect of IL-2 on IL-15-induced proliferation of CD8⁺ T cells (25, 26), IL-2 potentiated the IL-7-induced proliferation of CD4⁺ T cells. Thus, the IL-2 and IL-7 cytokines distinctly modulate the proliferation of naive and memory T cell subsets and contribute significantly to the differential susceptibility of these lymphocytes to Fas-induced cell death.

Materials and Methods

T cell isolations and culture conditions

Adult peripheral blood (APB), obtained from healthy adult donors after informed consent, as well as umbilical cord blood (UCB), obtained immediately after delivery of full-term infants (Montpellier, France), was collected in heparinized tubes. CD4⁺ T cells were purified by negative selection using tetrameric complexes in which one Ab recognizes a surface Ag on B cells, monocytes, NK cells, or CD8⁺ cells and the other recognizes glycoporphin A on the surface of B cells. Cells were then pelleted upon Ficoll-Hypaque (Sigma-Aldrich, St. Louis, MO) separation and nonbound CD4⁺ T cells were recuperated. To separate naive and memory T cell subsets, CD4⁺ lymphocytes were incubated with CD45RO (DAKO, Trappes, France) or CD45RA (Immunotech, Marseille, France) mAbs, respectively, in combination with HLA-DR and CD69 mAbs (clone TP1.55.3 and 7C11; Immunotech) Ab for 16 h at 37°C. Protein lysates were blocked for 1 h at room temperature in TBS (150 mM NaCl, 1% Nonidet P-40, 1 mM sodium orthovanadate, 10 mM sodium fluoride, 10 mM glycerophosphate, 20 mM sodium pyrophosphate, 7.5 μg/ml aprotinin, and 200 μM PMSF). Cells that were incubated previously that RTE were not stimulated with either polyclonal Ab recognizing Tyr phosphorylated STAT 5 (1/10 000 dilution; Cell Signaling Technology, Beverly, MA) or an extracellular signal-regulated kinase (Erk-2) mAb (1/2000 dilution; BD Transduction Laboratories, Lexington, KY) for 1 h at room temperature. Blots were then incubated with goat anti-rabbit or anti-mouse secondary Ab linked to peroxidase (Amersham, Arlington Heights, IL), respectively, and immunoreactive proteins were visualized using the ECL detection system (Amersham).

Flow cytometry for surface markers

To detect expression of CD69 and CD95, cells were incubated for 20 min on ice with the appropriate PE-conjugated mAb (clones TP1.55.3 and 7C11, respectively; Immunotech), at the indicated time points. Background fluorescence was measured using an Ig isotype control Ab. Cells were washed with PBS (Bio Media, Boussens, France), analyzed on a FACS-Calibur (BD Biosciences) and data analysis was performed using CellQuest software (BD Biosciences).

Results

Proliferation of RTE and mature adult CD4⁺ T cells in response to γc cytokines

The naive T cells present in UCB have previously been defined as RTEs on the basis of TCR excision circle levels (12) whereas naive CD4⁺ T cells present in the peripheral circulation of adults often emigrated from the thymus years earlier. We and others have previously shown that RTE from UCB, but not adult CD4⁺ lymphocytes, proliferate in response to the IL-7 cytokine, despite high receptor expression (IL-7Rα and γc chains) on all subsets (12, 13). The range of biological activities induced by a second γc cytokine, IL-2, on RTE is not known, but its effects on mature CD4⁺ T lymphocytes are pleiotropic with responses including proliferation, protection from apoptosis, increased susceptibility to AICD, and limited T cell expansion (26–29). Using the CFSE dilution method to precisely monitor cell division, we first compared the proliferative responses of RTE as well as mature naive (TNg) and memory (Tcm) CD4⁺ T cells from adult peripheral blood (APB) to IL-2, IL-7, and the combination of these two γc cytokines. IL-2 alone did not stimulate cell division of either TNg or Tcm whereas 30% of RTE divided at least once under these conditions (1). As previously reported (12, 13) IL-7 stimulated division of RTE but only minimal proliferation was observed in APB CD4⁺ lymphocytes.

Western blot analysis

Cells were collected at the indicated time points and immediately lysed in ice-cold lysis buffer (20 mM Tris-HCl, pH 7.5, 5 mM EDTA, 150 mM NaCl, 1% Nonidet P-40, 1 mM sodium orthovanadate, 10 mM sodium fluoride, 10 mM glycerophosphate, 20 mM sodium pyrophosphate, 7.5 μg/ml aprotinin, and 200 μM PMSF). Cells that were stimulated immediately after isolation were incubated in the presence of either IL-2 (10³ U/10⁶ cells), IL-7 (100 ng/10⁶ cells) or both cytokines (10³ U/10⁶ cells and 100 ng/10⁶ cells, respectively) for 30–60 min at 37°C. Protein lysates were resolved on SDS-PAGE gels and transferred electrophoretically to Protran nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). Membranes were blocked for 1 h at room temperature in TBS (150 mM NaCl, 20 mM Tris, pH 7.5, 0.05% Tween 20) containing 5% milk and with either a polyclonal Ab recognizing Tyr⁶⁹⁴ phosphorylated protein kinase B (Akt) (1/2 000 dilution; BD Transduction Laboratories, Lexington, KY) or an extracellular signal-regulated kinase (Erk-2) mAb (1/2 000 dilution; BD Transduction Laboratories, Lexington, KY). Membranes were incubated with goat anti-rabbit or anti-mouse secondary Ab linked to peroxidase (Amersham, Arlington Heights, IL), respectively, and immunoreactive proteins were visualized using the ECL detection system (Amersham).

CFSE labeling

Freshly isolated T cell subsets were washed and resuspended in PBS at a concentration of 2.5 × 10⁶ cells/ml for labeling with the fluorochrome CFSE (Molecular Probes, Eugene, OR). Cells were incubated with CFSE at a final concentration of 2.5 μM for 3 min at room temperature. Labeling was terminated by the addition of FCS (30% of total volume), cells were washed twice, and then cultured as indicated. Division was analyzed on a FACS Calibur on the FL-1 wavelength, at the indicated time points.

Anti-Fas Ab-induced apoptosis

To monitor CD95-induced apoptosis, T cell subsets were left untreated (control), or incubated with 100 ng/ml of an agonistic CD95 mAb (clone 7C11; Immunotech) Ab for 16 h at 37°C. To investigate whether Fas-induced apoptosis requires caspase activation, cells were either left untreated or pretreated with the broad spectrum caspase inhibitor Z-VAD-fmk (50 μM; Sigma-Aldrich) for 30 min and then incubated with the agonistic CD95 Ab for 16 h. Cells (5 × 10⁴) were harvested and incubated with annexin FITC solution (200 ng/ml) and propidium iodide (10 μg/ml; Annexin VFluos kit; Immunotech) for 10 min on ice. After washes, apoptotic cells were quantified by FACS analysis, counting annexin V-positive cells.
Nevertheless, significantly more RTE underwent three or more divisions in the presence of both γc cytokines as compared to IL-7 alone (55 vs 20% in a representative experiment; Fig. 1). Proliferation of APB CD4⁺ lymphocytes was also increased but to a much lower level, with only 15–25% of cells having undergone one or more divisions. Notably, in all cases where proliferation was observed, the kinetics of γc cytokine-induced division were much slower than that observed following TCR stimulation with a first division noted after 72–96 h as compared to 30–48 h in Ag-stimulated cells (data not shown). Moreover, the effects of IL-2 and IL-7 are additive as supplementing higher concentrations of either of these cytokines alone did not modify proliferation profiles in any of the T cell subsets (data not shown).

Expression of Fas on γc cytokine-stimulated CD4⁺ lymphocyte subsets

Immunological responsiveness is controlled by a balance between proliferation and cytokine secretion on the one hand, and apoptotic cell death, on the other. However, the vast majority of studies performed to date have monitored apoptotic cell death in the context of TCR stimulation. As the contribution of the Fas-mediated cell death pathway plays an important role in controlling peripheral T cell immune responses, it was important to analyze the effects of γc cytokines on the susceptibility of CD4⁺ T lymphocyte subsets to Fas-induced apoptosis in the absence of MHC/peptide signals.

We first monitored the levels of Fas on freshly isolated CD4⁺ lymphocyte subsets by flow cytometry. As previously reported (30–32), Fas was not detected on RTE (Fig. 2A). Moreover, Fas was not detected on T_N although it was present at low levels on a significant percentage of T_M. Expression of Fas was not in itself an intrinsic marker of T cell activation, as expression of an unrelated activation marker, CD69, was similar in all three CD4⁺ T lymphocyte subsets (Fig. 2A).

It has previously been shown that Fas is up-regulated on T cells following Ag stimulation (30, 33, 34) but its expression on γc cytokine-exposed T cell subsets is not known. Exposure to either IL-2 or IL-7 alone resulted in a small increase in Fas expression on both RTE and T_N (Fig. 2B). Notably though, ex vivo culture in the absence of cytokines also resulted in a small augmentation of Fas levels on these T cells, likely due to the presence of FCS in the media (compare Fig. 2, A and B, untreated cells). Nevertheless, concomitant exposure to both γc cytokines resulted in the induction of significant levels of Fas on these cells, with ~80% of RTE and T_N expressing this molecule in a representative experiment (Fig. 2B). This increase in Fas expression was not associated with activation per se as CD69 levels were only marginally augmented following cytokine stimulation (Fig. 2B). Although basal Fas levels were higher on T_M than on the other CD4⁺ lymphocyte subsets, Fas expression on these former cells was not augmented by either IL-2, IL-7, or the combination of IL-2 and IL-7 (Fig. 2B). Moreover, CD69 levels remained unchanged on T_M in the presence of γc cytokines. It should be noted though that this phenomenon was specific to γc cytokine stimulation as both Fas and CD69 levels were significantly augmented under conditions of Ag stimulation (data not shown). Thus, the responsiveness of CD4⁺ lymphocytes to treatment with the IL-2 and IL-7 cytokines, as measured by Fas induction, differs significantly between RTE and naive T cell subsets, on the one hand, and memory T cell subsets, on the other.

![FIGURE 1. IL-2 and IL-7 differentially induce the proliferation of umbilical cord and adult CD4⁺ T cell subsets. Naive (T_N) and memory (T_M) CD4⁺ T lymphocytes from APB, and CD4⁺ RTE from UCB, were purified by negative selection. Cells were labeled with CFSE and cultured in the presence of either IL-2 (100 U/ml), IL-7 (10 ng/ml), or a combination of the two γc cytokines (IL-2/IL-7) for 8 days and division was assessed by flow cytometry. The numbers shown above the peaks indicate the number of cell divisions. One representative experiment of four is shown.](http://www.jimmunol.org/)

![FIGURE 2. IL-2 and IL-7 synergistically induce Fas expression on naive CD4⁺ T cell subsets. A, Freshly isolated naive (T_N) and memory (T_M) CD4⁺ T lymphocytes isolated from APB (T_N, red line; T_M, blue line) and RTE from UCB (green line) were stained with Abs to Fas (CD95) and CD69. B, Following 6 days of culture in the absence (red) or presence of IL-2 (yellow), IL-7 (blue), or IL-2 with IL-7 (green), Fas and CD69 expression were monitored again. Black lines show staining with isotype-matched controls. One representative experiment of five is shown.](http://www.jimmunol.org/)
Susceptibility of CD4+ lymphocyte subsets to Fas-mediated apoptosis

Expression of Fas on T lymphocytes is not in itself a measure of the susceptibility of these cells to Fas-mediated apoptosis (30, 35). The differential susceptibility of activated CD4+ lymphocytes to Fas-mediated apoptosis is not solely dependent on cell surface CD95 levels, (36) but rather may correlate with actin polarization (35) as well as other changes at the cell surface and in the internal cell environment. Therefore, it was important to directly determine whether these γc cytokines sensitize CD4+ lymphocytes to Fas-engagement and whether cell death correlates with the induction of cell surface CD95 expression. CD4+ T cell populations were cultured ex vivo during a short period, 5–7 days, in the absence or presence of these γc cytokines. Sensitivity to Fas-mediated induction of apoptosis was then monitored following addition of the agonistic anti-CD95 Ab, 7C11.

As previously reported, a significantly higher proportion of RTE, as compared to T N or T M, died when γc cytokines were not added to the media (22, 24). However, under these conditions, no further apoptosis was induced in any of the subsets upon Fas engagement. The addition of IL-7, but not IL-2, significantly inhibited the level of spontaneous cell death observed in the ex vivo-maintained RTE. In the presence of either cytokine alone, there was only a minimal level of Fas-induced cell death within T N and T M populations (Fig. 3A). A higher level of cell death was observed in Fas-engaged RTE pretreated with IL-7 (10–40%), but these levels were consistently lower than those detected following Ag activation (55–90%) (Fig. 3A and data not shown). However, upon pretreatment with a combination of the IL-2 and IL-7 cytokines, Fas engagement resulted in significant levels of cell death in both T N and RTE (45–90%), equivalent to that observed in Ag-activated cells (Fig. 3, A and B and data not shown). In contrast, IL-2/IL-7-treated memory T cells were much less susceptible to Fas-mediated cell death, with apoptosis ranging from 20% to a maximum of 45% (Fig. 3, A and B and data not shown). It should be noted that a rather broad range of apoptosis was observed in independent experiments, likely due to variability between T lymphocytes isolated from different donors. Nevertheless, the differences in Fas-mediated apoptosis detected in each culture condition remained constant. Thus, the combination of IL-2 and IL-7 cytokines primed naive T cells as well as RTE for Fas-mediated apoptosis whereas memory cells were relatively refractory to this process.

Fas-mediated apoptosis in γc cytokine-stimulated CD4+ lymphocyte subsets is dependent upon caspase activation

Caspases, a group of cysteine proteases, play a critical role in the Ag-driven regulation of T cell death (reviewed in Ref. 37), and as such, it was important to determine their involvement in the Fas-mediated cell death observed in IL-2/IL-7-stimulated CD4+ T cell subsets. Addition of a pan-caspase inhibitor, Z-VAD-fmk, did not block the low spontaneous level of cell death observed in any of the IL-2/IL-7-stimulated CD4+ subsets (data not shown). However, it almost completely inhibited α-Fas-induced cell death (Fig. 4). Thus, induction of caspases appears to regulate the Fas-mediated cell death observed in the context of IL-2/IL-7 cytokine stimulation.

STAT 5 activation in γc cytokine-stimulated CD4+ T cell subsets

Signaling pathways activated via γc cytokines are transduced via Janus kinases (JAKs) and STATs. Both IL-2 and IL-7 are known to activate JAK1 and JAK3, as well as STAT 5 which translocates to the nucleus where it functions as a transcription factor (reviewed in Ref. 38). Indeed, STAT 5 has been shown to be involved in long-term survival of an IL-2-dependent cell line and promotes the sensitization of IL-2-treated T cells to AICD (27, 28). Nevertheless, the vast majority of studies assessing the activation of STAT 5 and its role in IL-2-mediated apoptosis have been performed in cell lines or in Ag-stimulated T cells. Therefore we studied the IL-2/IL-7-induced activation of STAT 5 in the context of non-antigen stimulated CD4+ T lymphocyte subsets.

STAT 5 phosphorylation was not detected in freshly isolated CD4+ T lymphocytes, irrespective of the subset. Upon short-term activation with IL-2 (30 to 60 min), significantly higher levels of STAT 5 phosphorylation were induced in APB memory T cells than in naive T cells and only minimal levels were observed in RTE from UCB (Fig. 5A). It is interesting to note that in all these
populations, expression of the α-chain of the IL-2R (CD25) was barely detectable (<10%), albeit by conventional FACS analysis (not shown). In contrast to IL-2-mediated phosphorylation, IL-7 induced STAT 5 phosphorylation in RTE as well as in naive and memory APB subsets (Fig. 5A). Following ex vivo culture (6 days) in the presence of γc cytokine(s), the same general trends were observed. There was a significantly higher level of STAT 5 phosphorylation in IL-2-stimulated memory T cells than in either naive cells or RTE (Fig. 5B). Furthermore, IL-7 consistently induced significantly higher levels of STAT 5 phosphorylation in naive and RTE subsets as compared with memory cells, despite expression of IL-7Rα and γc chains on all subsets (13). Nevertheless, as compared with IL-7 alone, the combination of IL-2 and IL-7 did not appear to increase STAT5 phosphorylation in RTE or naive T cells, at either an early or “steady state” time point (Fig. 5, A and B). Altogether, these data show that although both IL-2 and IL-7 can transduce a signal via STAT 5 in CD4+ T cells, the STAT 5 signaling cascade is differentially regulated in naive and memory CD4+ T cell subsets.

**Discussion**

Many recent studies point toward the importance of the IL-7 cytokine in postthymic development, especially with regard to its role in the homeostatic maintenance of the recirculating T cell pool. Under conditions of lymphopenia, IL-7 clearly supports homeostatic T cell proliferation (6, 7, 39, 40). This effect has been postulated to be of physiological importance because of the finding that patients with lymphopenia, due either to HIV infection or chemotherapy, have increased serum IL-7 levels (41–44). Although IL-7 appears to play a similar proliferative role in nonlymphopenic mice and monkeys, its precise activity under these latter conditions is less clear. It has been reported that rIL-7 alters the CD4:CD8 ratio in nonlymphopenic mice by promoting a more rapid expansion of CD8+ T cells (11). However, in nonlymphopenic primates, rIL-7 increased both CD4+ and CD8+ T cell numbers to similar extents (9). This discrepancy may simply be due to the distinct effects of IL-7 in the context of peripheral blood and lymph nodes (45, 46). The vast majority of lymphocyte analyses in mice are performed in lymph nodes/spleen while analyses in monkeys and humans are generally performed on peripheral blood samples. Thus, rIL-7 may distinctly modulate T cell fate and expansion in different organs, likely due to their particular cytokine microenvironments.

To date, the effects of rIL-7 on mature human T cells have been analyzed exclusively on peripheral blood-derived lymphocytes cultured ex vivo. Under these conditions, the cytokine milieu is more strictly controlled but the effects are clearly biased by the culture conditions as well as the methods used to isolate the selected populations (47, 48). In our study, naive and memory CD4+ T cell populations were isolated by negative selection on the basis of CD45RA and CD45RO expression, respectively. Nevertheless, it should be noted that in vivo some CD45RO cells convert back to CD45RA expression upon entering a quiescent state and thus, CD45RA T lymphocytes from UCB are the only population which is believed to be “truly” naive (49–53). That being said, >95% of the isolated naive CD4+ T cell populations used in these studies, albeit from UCB or from APB, coexpressed CD62L and CD27, two other markers associated with a naive phenotype (data not shown). It remains to be determined whether other factors and subpopulations, such as T regulatory cells, which may differ between UCB and APB, affect the cytokine responsiveness of these naive T cells.

With regard to distinctions between in vitro and in vivo responsiveness to IL-7, it is notable that IL-7 can prime T cells to respond to suboptimal TCR activation (23, 54, 55). In this manner, it can promote homeostatic proliferation in vivo following interaction of the TCR with low affinity ligands that generally do not induce cell cycle entry (56, 57). As the interaction of T cells with a low affinity Ag is significantly less likely to occur in vitro than in vivo, this may explain the more constrained effects of rIL-7 on human T cells cultured ex vivo. However, under the ex vivo conditions used by ourselves and others, rIL-7 does promote a significant division of
human RTE although it does not induce division of naive or memory peripheral CD4+ human T cells (12, 13, 58). In fact, proliferation of mature peripheral blood CD4+ T cells cultured in the presence of IL-7 ex vivo has only been observed following the addition of IL-15, and even then, only memory cells proliferate (58). Thus, responsiveness to a combination of the IL-7 and IL-15 γc cytokines appears to be progressively acquired during T cell differentiation in the periphery.

The roles of γc cytokines in T cell homeostasis are nonredundant (reviewed in Refs. 59 and 60). We now show that in human T cells, IL-2 enhances IL-7-induced proliferation of RTE as well as mature naive and memory CD4+ T cells. Nevertheless, this effect is significantly more pronounced in RTE than in naive or memory populations. In mature T N and T M subsets, the contribution of IL-2 to proliferation is dependent on the presence of IL-7, as IL-2 alone does not induce cell division. This effect may be of important physiological significance as IL-7-stimulated mature T cells appear to be primed to secrete IL-2 (61). With regard to the pleiotropic nature of IL-2, it is interesting to note that this cytokine has recently been shown to limit the expansion of murine CD8+ T cells stimulated with another γc cytokine, IL-15 (25, 26). In contrast, we find that IL-2 enhances IL-7-induced proliferation of human CD4+ T cells. These distinct effects may allow IL-2 to contribute to a differential regulation of CD4+ and CD8+ T cell expansion.

As discussed above, our data and that of many other groups support an important role for IL-7 in homeostatic T cell proliferation. Indeed, treatment of nonlymphopoenic monkeys with a short course of rIL-7 resulted in a significant increase in peripheral T cell numbers (9). Nevertheless, this effect was transient and T cell counts returned to baseline within 10 days after termination of treatment (9). As homeostasis is defined as the maintenance of a fixed number of T cells, this outcome is not unexpected and suggests an important amount of cell death following IL-7 therapy. However, prior to the experiments reported here, studies assessing whether IL-7 modulated T cell susceptibility to apoptotic signals had not been performed. We find that IL-7 alone increased Fas-mediated cell death of RTE, albeit to relatively low levels. In marked contrast, RTE and naive T cells exposed to a combination of IL-2 and IL-7 were extremely susceptible to Fas-induced cell death, to levels equivalent to that observed following TCR activation. Despite equivalent levels of surface Fas, IL-2/IL-7-stimulated memory T cells were significantly more resistant to Fas-induced cell death. Intriguingly, similar differences have been reported for TCR-induced cell death in naive and memory T cell subsets: specifically, Ag receptor-activated memory cells are more resistant to AICD than restimulated naive T cells (19, 20). In the previously studied TCR-stimulated cells as well as in the γc cytokine-stimulated cells studied here, the apoptotic cell death induced in all T cell subsets required activation of caspases. Altogether, these data strongly suggest that the response of CD4+ T cells to Fas engagement is determined by a previous antigenic history, with a selective decrease in vivo conditions of lymphopenia, whereby RTE would die at high levels but their loss would be balanced by a high initial rate of γc cytokine-induced proliferation.

It is well-established that cytokines transduce signals via multiple protein kinase pathways. As the CD4+ T cell populations studied here demonstrated distinct IL-2/IL-7-mediated biological responses, it was reasonable to assess whether these downstream effects were regulated at the level of proximal signaling intermediates. The Erk mitogen-activated protein kinase pathway has been shown to be stimulated by IL-2 but not by IL-7 (62) and indeed, IL-7 did not induce mitogen-activated protein kinase activation in either RTE or naive/memory T cell subsets (data not shown). In contrast, the JAK/STAT and phosphatidylinositol-3 kinase/Akt pathways play essential roles in the cytokine signaling cascades activated via both IL-2 and IL-7 (38). Akt activation and subsequent Bcl-2 expression appears to be necessary for IL-2-mediated T cell survival whereas STAT 5 is apparently required for both proliferation and sensitivity to AICD (27, 28). We found that although STAT 5 phosphorylation was induced by both IL-2 and IL-7 in all CD4+ T cell subsets, the magnitude of the response differed significantly in these populations. IL-2 induced significantly higher levels of STAT 5 phosphorylation in memory T cells than in naive T cells or RTE, at both early (30–60 min) and late (6 days) time points. In contrast, IL-7-mediated STAT 5 phosphorylation was observed in all CD4+ populations at early time points but was more pronounced in naive T cells and RTE following 6 days of culture. There was no synergy observed when the two cytokines were used in combination. As cytokine-induced proliferation and Fas-mediated apoptosis were most pronounced in distinct populations, IL-7-induced RTE and IL-2/IL-7-induced memory T cells, respectively; our data strongly suggest that STAT 5 phosphorylation per se is not responsible for the diverse downstream biological responses observed in these T cell subsets. Thus, the activation of STAT 5 via different γc cytokines does not result in homogeneous cell environments. Rather, we hypothesize that because of the initial “signatures” of these T cell subsets, they attain distinct “physiological states” following cytokine stimulation, even if the same signaling intermediates are activated. The precise associations between the recruitment of various γc cytokine-induced signaling pathways and the susceptibility of the various T lymphocyte subsets to apoptosis/proliferation remain to be determined.

Much recent evidence indicates that the composition and size of the naive and memory T cell pools are regulated by competition for limiting resources, including cytokines (reviewed in Refs. 59 and 60). In this study, we demonstrate that the IL-2 and IL-7 γc cytokines are important players in the regulation of differential CD4+ T cell subset proliferation in the context of nonlymphoid tissues. Moreover, these γc cytokines differentially modulate the susceptibility of CD4+ T cells to Fas-induced cell death. In this manner, IL-2 and IL-7 can promote homeostatic expansion while limiting the size of the overall T cell pool. The finding that recent thymic emigrants are the most susceptible to the proliferative effects of these γc cytokines (12, 13) may allow for maintenance of optimal diversity, especially under conditions of lymphopenia. Nevertheless, it remains to be determined whether the RTE in neonates respond in the same manner as RTE that develop in adults with T cell depletion. The potential use of recombinant IL-2 and IL-7 molecules as immunorestorative and immunoenhancing agents for these lymphopenic patients, especially patients with HIV infection and those undergoing chemotherapy (reviewed in Refs. 63 and 64), makes it crucial that the wide range of effects of these cytokines be explored. Cytokines that mediate the balance
between proliferation and apoptosis will regulate the size and diversity of RTE, as well as mature naive and memory T cell pools in the peripheral circulation.

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15. References

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