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Cytokine Deprivation of Naive CD8\(^+\) T Cells Promotes Minimal Cell Cycling but Maximal Cytokine Synthesis and Autonomous Proliferation Subsequently: A Mechanism of Self-Regulation\(^1\)

Subash Sad\(^2\)*† and Lakshmi Krishnan*

Naive CD8\(^+\) T cells differentiate into effectors secreting various cytokines that aid their function. IL-2, but not IL-15, promoted this differentiation of naive CD8\(^+\) T cells into effectors. However, the amount of IL-2 present during differentiation had a dichotomous effect on their subsequent function. High concentrations of IL-2 enhanced proliferation and cell cycling initially, but the effectors subsequently failed to produce cytokines and proliferate autonomously, although CD28 expression was maintained. In contrast, suboptimal amounts of IL-2 during priming promoted apoptosis, little proliferation and cell cycling, yet the CD8\(^+\) effectors generated produced high levels of cytokines and proliferated autonomously. Interestingly, the effects of IL-2 on naive CD8\(^+\) T cells were totally opposite those on naive CD4\(^+\) T cells. Although IL-2 impaired cytokine synthesis by CD8\(^+\) T cells, the expression of LFA1 and CD44 as well as Fas-dependent cytotoxicity were enhanced. However, loss of cytokine synthesis was not due to increased cytotoxicity, as inhibition occurred even in the absence of perforin/FasL. Interestingly, CD8\(^+\) effectors secreting reduced amounts of cytokines exhibited enhanced IL-2R\(^{a}\), but reduced IL-2R\(^{b}\), expression. Furthermore, sorted CD8\(^+\) IL-2R\(^{a}\)high cells secreted less cytokines than IL-2R\(^{a}\)low cells. These results suggest that the presence of excessive IL-2 during the activation of naive CD8\(^+\) T cells, while promoting cell cycling initially, may compromise long-term immunity. *The Journal of Immunology, 1999, 163: 2443–2451.*

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Materials and Methods

**Mice**

Female C57BL/6 mice, 6–8 wk old, were obtained from Charles River Canada (St. Constant, Canada). C57BL/6-perforin\(^{-/-}\), C3H/HeJ-Fas ligand\(^{-/-}\) (FasL\(^{-/-}\)), and their controls were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were maintained in the animal facility of the Institute for Biological Sciences (National Research Council, 100 Sussex Drive, Room 4105, Ottawa, Ontario, Canada K1A 0R6. E-mail address: subash.sad@nrc.ca

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T CELLS BY CYTOKINES

Cytokine assays
T cells were generated as described above and were used as effectors after 7–8 days of culture. M12.4.1, L1210, and L1210/Fas target cells (102) were labeled with 35S (100 mCi) in 50 μl of RPMI plus 8% FBS medium for 45 min. Targets were washed, various ratios of effectors and targets were cocultured for 4 h in 96-well round-bottom tissue culture plates, the supernatants were collected, and radioactivity was measured using a gamma counter.

Flow cytometric assay
T cells were incubated on ice (106 cells in 50 μl of RPMI 1640 plus 1% FBS) with anti-mouse CD32/CD16 (FcγRII/III receptor). After 30 min aliquots were washed and incubated separately in 50 μl of RPMI plus 1% FBS with the following Abs: anti-mouse CD4, CD8, LFA-1, IL-2Rα, CD44, NK1.1, and CD28. Incubation lasted for 30 min on ice. Cells stained with biotinylated Abs were subsequently incubated with streptavidin-FITC after thorough washing. Cells were fixed in 1% formaldehyde in PBS and analyzed by flow cytometry.

For cell cycle analysis, cells were stained with FITC-labeled anti-CD8 Ab for 30 min on ice in 50 μl of RPMI plus 1% FBS. Cells were washed and fixed in 70% ethanol overnight. Cells were stained with the propidium iodide dye in the presence of RNase A (100 U/ml) and analyzed by flow cytometry. DNA content histograms gated on CD8+ T cells were obtained using EXPO software (Coulter). The percentage of cells in the G1/M phase of the cell cycle was calculated.

The number of apoptotic cells was enumerated using the procedure described by Telford et al. (21). Cells were processed and acquired on the flow cytometer as described above for cell cycle analysis. The apoptotic fraction was measured by gating on CD8+ T cells and by the subdiploid population staining in the region below the large G1/G0 peak.

Results
IL-2, but not IL-15, is required for the differentiation of naive CD8+ T cells into effectors
CD8+ CD44low T cells (1 × 105/well; >99% pure) were stimulated with irradiated J774A.1 cells (1 × 106/well). In this differentiation culture the generation of effector CD8+ T cells can be appreciated between days 4–5, depending on the presence of cytokines in the culture. In the absence of exogenous IL-2 no detectable T cell proliferation occurred (Fig. 1a). This may be attributable to naive CD8+ T cells (as opposed to naive CD4+ T cells) producing minute amounts of cytokines (S. Sad, unpublished observations), not sufficient to drive their differentiation. Addition of increasing amounts of exogenous IL-2 resulted in a corresponding increase in CD8+ T cell proliferation in a dose-dependent manner. However, IL-15, even at 10 ng/ml, failed to induce significant proliferation.

We also compared the proliferative responses of effectors CD8+ T cells to IL-2 and IL-15. Effector CD8+ T cells (H-2b) were generated after stimulation with allogeneic J774A.1 cells (H-2b) in the presence of 1 ng/ml IL-2. On day 7, effectors generated were washed and restimulated with irradiated J774A.1 cells in the absence or the presence of increasing amounts of IL-2 or IL-15. Proliferation was assessed at 72 h by measuring the incorporation of tritiated thymidine. Fig. 1b indicates that at lower cytokine concentrations effector CD8+ T cells proliferate more strongly in response to IL-15 than to IL-2. At a cytokine concentration of 1 ng/ml, IL-2 induced 3,171 cpm, whereas IL-15 induced 24,439 cpm. However, we observed consistently that CD8+ T cell proliferation in response to IL-15 saturated around a concentration of 1 ng/ml, whereas IL-2 continued to induce proliferation even at higher cytokine levels. These results indicate that naive and effector CD8+ T cells respond differentially to IL-2 and IL-15.
using the antiapoptotic cytokine, IL-15. Addition of IL-15 to concentrations of 1 and 10 ng/ml. These results were further corroborated with the number of apoptotic cells declining at IL-2 concentrations. On the other hand, Fig. 2 clearly indicates that addition of increasing concentrations of IL-2 promote apoptosis, as opposed to cell cycling, in the primary culture. On the other hand, CD8 T cells produced maximal amounts of cytokine synthesis, by CD8 T cells. C57BL/6 CD44low CD8 T cells (H-2b; 1 × 10⁵/well) were stimulated with irradiated allogeneic M12.4.1 cells (H-2d; 1 × 10⁵/well) in the presence of IL-12, anti-IL-4, and various concentrations of IL-2 and IL-15. a, Proliferation was measured on day 5 by liquid scintillation. A parallel culture of effector cells that was derived in the presence of IL-12, anti-IL-4, and 1 ng/ml IL-2 was continued until day 7. The effector cells generated were then washed and restimulated (1 × 10⁶/well) with irradiated allogeneic J774A.1 cells (5 × 10⁵/well) in the absence or the presence of increasing concentrations of IL-2 or IL-15. b, Proliferation was measured at 72 h poststimulation by liquid scintillation. The mean ± SD of triplicate cultures are shown.

IL-2-induced proliferation of CD8 T cells impairs their subsequent cytokine synthesis ability

As experiments in Fig. 1 clearly suggested that IL-2 plays a pivotal role in the differentiation of naive CD8 T cells, we evaluated the optimal amounts of IL-2 required. C57BL/6 CD44low CD8 T cells were stimulated with irradiated allogeneic M12.4.1 cells (H-2d) as described in Fig. 1. On day 6 effector cells were harvested, washed, and analyzed directly for cell cycle and the number of apoptotic cells. Fig. 2a clearly indicates that addition of increasing concentrations of IL-2 enhanced the frequency of cells going into the G2/M phase of the cell cycle. On the other hand, Fig. 2b shows that low IL-2 concentrations (0.1 ng/ml) induced maximal apoptosis, with the number of apoptotic cells declining at IL-2 concentrations of 1 and 10 ng/ml. These results were further corroborated using the antiapoptotic cytokine, IL-15. Addition of IL-15 to CD8 T cell cultures differentiating in the presence of 0.1 ng/ml IL-2 increased T cell yield by about 5-fold. However, IL-15 had no effect on T cell yield when 1 or 10 ng/ml IL-2 was used during the differentiation of CD8 T cells (data not shown). Thus, the cell cycle and apoptosis data taken together indicate that low amounts of IL-2 promote apoptosis, as opposed to cell cycling, in the primary culture.

We then addressed the question of whether the IL-2-induced increase in proliferation also manifests in increased cytokine synthesis by the effectors generated. C57BL/6 CD44low CD8 T cells were stimulated with irradiated allogeneic M12.4.1 cells (H-2d) as described in Fig. 1. After 7 days the numbers of effector cells generated (>99% pure) were evaluated using a hemocytometer. Fig. 3a shows that the presence of increasing amounts of IL-2 enhanced the development of effectors. To evaluate the effects on cytokine synthesis, we washed the effectors, removed dead cells on Lympholyte M, and restimulated them (1 × 10⁵/well) with Con A (5 ng/ml) for 18 h. Cytokine synthesis by CD8 T cells in the secondary culture inversely correlated with their proliferation in the primary culture. CD8 T cells produced maximal amounts of IL-2 in the secondary culture when they were cultured with very low levels of IL-2 in the primary culture. On the other hand, CD8 effectors generated in the presence of high IL-2 concentrations in the primary culture produced low cytokine levels subsequently (Fig. 3b). Addition of IL-15 (10 ng/ml) to these cultures did not restore their cytokine synthesis defect (data not shown).

IL-2 enhances both the proliferation as well as the subsequent cytokine synthesis of CD4 T cells

We then addressed whether naive (CD44low) CD4 T cells were also susceptible to the inhibitory effects of IL-2. C57BL/6 CD44low CD4 T cells were stimulated with allogeneic M12.4.1 cells (H-2d) in the presence of anti-IL-4, IL-12 and various amounts of IL-2. After 7 days, the number of effectors generated was counted (Fig. 4a), and they were restimulated with either Con A (Fig. 4b) or M12.4.1 cells (Fig. 4c) to determine cytokine synthesis. Interestingly, IL-2 stimulated the proliferation of effector CD4 Th1 cells (Fig. 4a) and also enhanced their subsequent ability to produce cytokines (Fig. 4, b and c). This, IL-2 enhanced the proliferation of both CD4 and CD8 effector cells, but it had contrasting effects on their subsequent ability to produce cytokines. Although IL-2 enhanced cytokine synthesis by CD4 effectors, it inhibited cytokine synthesis by CD8 effectors.
Differential restimulatory conditions reveal strong inhibition of CD8$^+$ T cell cytokine synthesis by IL-2

We wanted to establish the extent of inhibition induced by IL-2. To this end, we addressed the possibility that restimulation of CD8$^+$ effectors with potent APC/reagents might restore their cytokine synthesis defect. Allo-specific (H-2$^b$ anti-H-2$^d$) effector CD8$^+$ T cells were generated in the presence of various concentrations of IL-2. After their differentiation into effectors, CD8$^+$ T cells were washed and restimulated (1 $\times$ 10$^5$ cells/well) variably as described in the figure legends. Compared with Con A (Fig. 3b), or activated B cell lymphoma (M12.4.1; data not shown), restimulation of CD8$^+$ effectors with an activated allo-macrophage cell line (J774A.1) resulted in quantitatively higher cytokine levels. Although this may be attributable to the potency of J774A.1 cells as APCs (10, 22), even under these potent restimulatory conditions, the pattern of IL-2-induced inhibition of cytokine synthesis continued (Fig. 5a). Similarly, restimulation with plate-bound anti-CD3 Abs (Fig. 5b) or with PMA plus Ca$^{2+}$ ionophore (A23187; Fig. 5c) did not restore the IL-2-induced loss of cytokine synthesis. Even the use of high amounts of Ca$^{2+}$ ionophore (10 $\mu$g/ml) resulted in only partial restoration of cytokine synthesis (data not shown).

IL-2 enhances the Fas-dependent cytotoxic activity of CD8$^+$ T cells

The experiments described above indicated that the cytokine synthesis function of CD8$^+$ T cells is lost due to exposure of cells to high concentrations of IL-2. We then determined the effects on the cytotoxic function of CD8$^+$ T cells. Effector CD8$^+$ T cells (H-2$^b$ anti-H-2$^d$) were washed and their cytolytic activity was measured by incubation with radiolabeled target cells in various proportions for 4 h. CD8$^+$ T cells from normal mice (H-2$^d$) showed a strong cytotoxic activity toward their specific target cells, M12.4.1 (Fig. 6a). The absence of such cytotoxic activity in perforin-deficient CD8$^+$ T cells (H-2$^d$) indicates that M12.4.1 target cells are killed by these CD8$^+$ T cells mainly by the perforin-dependent mechanism. The presence of increasing amounts of IL-2 in the primary culture of naive CD8$^+$ T cells increased their subsequent ability to kill targets by the Fas-dependent mechanism (Fig. 6b). Thus, IL-2 has
distinctly opposite effects on the two CD8+ T cell functions: enhancement of Fas-dependant cytotoxicity but impairment of cytokine synthesis.

**IL-2 induces inhibition of cytokine synthesis even in the absence of perforin- or Fas-dependent cytotoxicity**

We have previously demonstrated that killing of APC by CD8+ T cells results in their reduced cytokine synthesis (13). Thus, IL-2-induced inhibition of cytokine synthesis of CD8+ T cells may be attributable to their increased cytolytic function. To address this question, we stimulated naive CD8+ T cells (normal, perforin-deficient, or FasL-deficient) with allo-APC and increasing concentrations of IL-2. Effector CD8+ T cells were tested for their perforin-dependent (a) or Fas-dependent (b) cytotoxicity 7 days after initiation of culture. To measure perforin-dependent killing, normal or perforin-deficient effector CD8+ T cells were incubated with 51Cr-labeled allogeneic M12.4.1 (H-2d) cells. Fas-dependent killing was measured by testing the cytotoxicity of perforin-deficient effector CD8+ T cells on targets expressing low levels (L1210, H-2d) or high levels (L1210Fas, H-2d) of Fas. Cytotoxicity was tested in a 4-h chromium release assay. The mean ± SD of triplicate cultures are shown.

**FIGURE 7.** Both normal as well as perforin-deficient CD8+ T cells are sensitive to the inhibitory effects of IL-2. Effector CD8+ T cells (H-2b anti-H-2d) from normal or perforin-deficient mice were generated in the presence of increasing concentrations of IL-2. On day 7, cells were washed and restimulated (1 x 10^6 cells/well) with various numbers of irradiated M12.4.1 cells (H-2d). Supernatants were collected after 18 h, and cytokines were tested by ELISA. The mean ± SD of triplicate cultures are shown.

**FIGURE 8.** IL-2 inhibits cytokine synthesis in normal as well as FasL-deficient CD8+ T cells. Effector CD8+ T cells (H-2b anti-H-2d) from normal or FasL-deficient mice were generated in the presence of increasing concentrations of IL-2. To measure cytokine production, cells were harvested on day 7, washed, and restimulated (1 x 10^6 cells/well) with various numbers of irradiated M12.4.1 cells (H-2d). Supernatants were collected after 18 h, and cytokines were tested by ELISA. The mean ± SD of triplicate cultures are shown.
overall produced more cytokines than normal control cells. This was particularly evident in FasL-deficient CD8$^+$ T cells differentiated in high IL-2 amounts, consistent with the cytotoxicity data presented in Fig. 6b. However, both normal and FasL-deficient CD8$^+$ T cells showed similar sensitivity to IL-2-induced inhibition of cytokine synthesis, indicating that the IL-2-induced increase in Fas-dependent killing was not the reason for loss of cytokine synthesis.

**IL-2 modulates the expression of cell surface molecules in CD8$^+$ T cells**

CD8$^+$ T cells that were generated with allo-APC and increasing amounts of IL-2 in the primary culture were analyzed for the expression of various cell surface markers (Fig. 9). Throughout the culture period, these cells remained pure without any outgrowth of contaminant populations, and the cells did not acquire NK1.1 expression. The expression of CD28 was not altered, suggesting that costimulatory pathways may not be altered as a result of IL-2 exposure. However, IL-2 increased their expression of LFA1 and CD44 (Fig. 9a). To further rule out the possibility that the lack of cytokine synthesis could be due to some other contaminant cells in the culture, we resorted the CD8$^+$ NK1.1$^-$ cells on day 7 and measured their cytokine synthesis after Con A stimulation. After resorting of the cells, the cytokine production pattern remained the same. Cells that were precultured with 0.1 ng/ml of IL-2 synthesized 1.24 ng/ml of IL-2, whereas cells that were precultured with 10 ng/ml of IL-2 synthesized 0.05 ng/ml of IL-2.

The IL-2-induced increase in the expression of LFA1 and CD44 clearly indicated that the cells were in a highly activated state. This was further confirmed by measuring IL-2R$^\alpha$ expression in these cells (Fig. 9b). At low IL-2 concentrations (0.1 ng/ml), only 47.4% CD8$^+$ T cells expressed high levels of IL-2R$^\alpha$. This expression increased substantially to 99.2% at 10 ng/ml IL-2. On the other hand, CD4$^+$ T cells exhibited substantial IL-2R$^\alpha_{high}$ cells (79.1%) even at low IL-2 concentrations (0.1 ng/ml), resulting in a less dramatic enhancement with increasing IL-2 (Fig. 9b). An interesting observation was that IL-2, while enhancing IL-2R$^\alpha$ expression on CD8$^+$ cells in a dose-dependent manner, down-regulated IL-2R$^\beta$ (Fig. 9c).
Thus, IL-2 enhances CD8\(^+\) T cell activation as assessed by the expression of surface molecules and cytolytic activity, yet it inhibits IL-2R\(\alpha\) expression and the subsequent ability to produce cytokines.

**Sorted IL-2R\(\alpha\)\(^{\text{low}}\) cells express more cytokines than IL-2R\(\alpha\)\(^{\text{high}}\) cells**

We determined whether the purified IL-2R\(\alpha\)\(^{\text{low}}\) and IL-2R\(\alpha\)\(^{\text{high}}\) cells differed in their ability to produce cytokines. Allo-Ag-specific effector CD8\(^+\) T cells were derived in the presence of high concentrations (10 ng/ml) of IL-2. Effectors were sorted for IL-2R\(\alpha\)\(^{\text{high}}\) and IL-2R\(\alpha\)\(^{\text{low}}\) expression using flow cytometry and were stimulated with Con A for cytokine synthesis. Fig. 10a shows that on day 7, the effector CD8\(^+\) T cells derived in the presence of high levels of IL-2 were mainly IL-2R\(\alpha\)\(^{\text{high}}\). Fig. 10b indicates that even among the cells derived in the presence of high IL-2 levels, CD8\(^+\) T cells that express low levels of IL-2R \(\alpha\)-chain produce more IL-2 than cells that express high levels of IL-2R \(\alpha\)-chain. This result clearly indicates that the expression of IL-2R\(\alpha\) inversely correlates with the subsequent ability of CD8\(^+\) T cells to produce cytokines.

**Presence of IL-2 in primary culture impairs proliferation in secondary culture**

Subsequent to the differentiation of naive T cells, effector T cells have to proliferate extensively to curtail the proliferation of the pathogens/tumors. Thus, T cells have to continuously produce cytokines to autonomously aid their proliferation and effector functions. We addressed whether the lack of cytokine production in CD8\(^+\) T cells is due to apoptosis of effector cells in the secondary culture. CD8\(^+\) T cells generated in the presence of varying amounts of IL-2 were washed, and dead cells were removed by layering over Lympholyte M. Cells were restimulated with allo-APC for 24 h and tested for the number of apoptotic cells. In the secondary culture, effector cells derived with low (0.1 ng/ml) levels of IL-2 (in the primary culture) had higher numbers of apoptotic cells than effectors generated in higher amounts of IL-2 (1 and 10 ng/ml; Fig. 11b).

To determine whether the effectors derived in high levels of IL-2 maintained their proliferation in response to exogenous cytokines, we measured the proliferation of CD8\(^+\) effectors in response to allo-APC and increasing concentrations of IL-2 or IL-15 in secondary culture (Fig. 11, c and d). CD8\(^+\) effectors generated in high concentrations of IL-2 retained their proliferative ability to exogenous cytokines in vitro, although at a reduced level.

**Discussion**

The production of cytokines (IL-2, IFN-\(\gamma\), TNF, and lymphotoxin) by CD8\(^+\) T cells correlates with their cytotoxic function. For an effective CD8\(^+\) T cell response, in addition to killing infected cells...
CD8\(^+\) T cells must proliferate sufficiently through the duration of infection to overpower the pathogen. IL-2 drives the autonomous proliferation of CD8\(^+\) T cells (23, 24). At the same time, the presence of IL-2 is required to promote T cell differentiation. We, therefore, analyzed the consequences of differential IL-2 levels during the priming of naive CD8\(^+\) T cells.

Previously, it has been shown that mature effector CD4\(^+\) T cell clones lose their ability to produce cytokines upon preculture with high levels of IL-2 (16). This abrogation of cytokine production was associated with decreased inositol phosphate and diacylglycerol production and impaired calcium-dependent signaling (16, 17). Our observations are different from these studies, as we have addressed the effects of IL-2 during the priming of naive CD8\(^+\) T cells. Furthermore, our studies highlight the finding that IL-2 differentially affects naive CD4\(^+\) and CD8\(^+\) T cells; increasing IL-2 inhibits cytokine synthesis by CD8\(^+\) cells, whereas it enhances cytokine production by CD4\(^+\) cells.

Because cytokine production modulates T cell effector functions, we addressed the consequences of impaired cytokine synthesis by CD8\(^+\) T cells. We observed that while IL-2 impairs subsequent cytokine synthesis by CD8\(^+\) T cells, it enhances their cytotoxic activity, particularly by the Fas-dependent pathway (Fig. 6). Other investigators have also reported that IL-2 enhances Fas-dependent cytotoxicity of T cells (25). Furthermore, we observed that enhancement of cytotoxic function of CD8\(^+\) T cells with IL-2 correlates with the increased expression of IL-2R\(\alpha\), CD44, and LFA1, indicating the activated state of these cells (Fig. 9). McKiscic et al. (26) have shown that IL-2 inhibits the cytolytic activity and cytokine synthesis of IL-2–producing T cell clones. However, we (20, 27, 28) and others (12, 29) have shown that exogenous cytokines do not impair the cytotoxic function of CD8\(^+\) T cells. Effector CD8\(^+\) T cells that are treated with IL-4 lose their cytokine synthesis ability, but maintain their short term cytotoxicity (20). Similarly, anergic cells that fail to produce IL-2, remain highly cytolytic (12, 29). Cytokines affect the cytotoxicity of CD8\(^+\) T cells only over the long term, when T cells have to undergo autonomous proliferation to curtail the proliferation of tumor cells (10).

Rapid killing of APC by effector CD8\(^+\) T cells compromises their stimulation and consequent cytokine synthesis (13). As high levels of IL-2 enhanced the cytotoxicity of CD8\(^+\) T cells, we addressed whether the loss of cytokine synthesis was due to their increased cytotoxicity toward APC. However, Fasl-deficient and perforin-deficient CD8\(^+\) T cells were equally susceptible to IL-2-induced loss of cytokine synthesis.

IL-2, IL-4, and IL-15 all stimulate CD8\(^+\) T cell proliferation. These overlapping activities may be attributed to their sharing multiple components of the receptors used for binding and signal transduction. All three cytokines use the same IL-2R\(\gamma\)-chain, but their receptor \(\alpha\)-chain usage is specific for each cytokine. Furthermore, IL-2 and IL-15 share the usage of IL-2R \(\beta\)-chain. Interestingly, addition of IL-15 to naive CD8\(^+\) cells evoked no T cell proliferation or differentiation (Fig. 1). More importantly, IL-15 could not overcome the inhibitory effects of IL-2 (data not shown). This could be partly due to an IL-2–induced reduction in IL-2R\(\beta\) expression by CD8\(^+\) T cells (Fig. 9c) and consequent impairment of IL-15 signal transduction. Furthermore, reduced IL-2R\(\beta\) expression in CD8\(^+\) effectors may compromise their long term proliferation and consequent memory development.

One of the differences in the proliferative response of effector CD8\(^+\) T cells to IL-2 vs IL-15, is the observation that IL-15-induced proliferation saturates around a concentration of 1 ng/ml, whereas IL-2 continues to induce stronger proliferation even beyond 10 ng/ml. It may be that this unabated proliferation induced by IL-2 renders the cells nonresponsive. Moreover, CD8\(^+\) T cells that express higher levels of IL-2R\(\alpha\) produce lesser amounts of IL-2 on subsequent stimulation than cells that express lower levels of IL-2R\(\alpha\) (Fig. 10). However, CD4\(^+\) effectors that are generated with high amounts of IL-2 have enhanced IL-2R\(\alpha\) expression but produce elevated levels of cytokines. Thus, IL-2R\(\alpha\) expression correlates both positively (in the case of CD4\(^+\) T cells) and negatively (in the case of CD8\(^+\) T cells) with subsequent T cell functions.

Although high expression of IL-2R\(\alpha\) may result in increased usage of IL-2 and, consequently, decreased further production, the effector CD8\(^+\) cells also produced lower amounts of IFN-\(\gamma\) (Fig. 5) and TNF (data not shown), suggesting that a different mechanism may be at play. Furthermore, inhibition of cytokine synthesis occurred even when effectors were restimulated with agents (Con A/plate-bound anti-CD3 Abs, in the absence of APC) that induce T cell activation but not proliferation. Additionally, impairment of autonomous proliferation of CD8\(^+\) effectors (Fig. 11) clearly indicates a functional loss of IL-2 synthesis. To further confirm that the inhibition of cytokine synthesis was not due to increased uptake by CD8\(^+\) T cells, we irradiated CD8\(^+\) effectors (2500 rad) and restimulated them with allo-APC for 18 h. Even under these conditions, CD8\(^+\) T cells that were generated in low IL-2 (0.1 ng/ml) produced 1.95 ± 0.32 ng/ml of IL-2, whereas cells generated in high IL-2 (10 ng/ml) produced <0.05 ng/ml of IL-2.

Various investigators have also shown that effector CD4\(^+\) T cell clones when cultured with high levels of IL-2 undergo activation-induced cell death upon subsequent stimulation with anti-CD3 Abs (15, 30). Interestingly, IL-2–dependent activation-induced cell death occurs only in effector, but not in naive, CD4\(^+\) T cells (30). Our results indicate that during the differentiation of naive CD8\(^+\) T cells into effectors, the presence of low amounts of IL-2 results in increased numbers of apoptotic cells both during the primary culture as well as in the secondary culture (after restimulation with APC for 24 h). As cytokine production and apoptosis in the secondary culture were measured within the same time frame (18–24 h), the loss of cytokine synthesis by CD8\(^+\) T cells appears to be due to defects in signal transduction rather than to the cells undergoing rapid death due to activation. It is, however, conceivable that the failure of effectors to proliferate autonomously due to loss of cytokine production may increase the propensity for apoptosis over the long term.

The lack of ability to produce cytokines and proliferate autonomously has often been referred to as anergy. Stimulation of T cells with Ag on fixed APCs results in an anergic state, although the cells maintain their short term effector function (11, 12, 29). We have previously shown that IL-4 also induces a similar state in CD8\(^+\) T cells that is characterized by impaired cytokine synthesis, proliferation, and compromised ability to curtail the proliferation of tumor cells (10, 20). IL-10 has also been shown to induce a long term anergy-like state in human CD4\(^+\) T cells (31). CD8\(^+\) T cells proliferate more strongly and produce more cytokines in the absence of IFN-\(\gamma\) (S. Sad, unpublished observations). These results indicate that the induction of an anergy-like state in T cells may be a phenomenon that occurs with high levels of various cytokines.

Cytolytic effector CD8\(^+\) T cells specific for a variety of Ags, including viruses, tumors, and exogenous proteins, persist in the absence of the cognate Ag in vivo and in vitro (32, 33). The nature of stimulus that drives the proliferation of memory T cells in the absence of Ag and the role of IL-2 in the maintenance of memory CD8\(^+\) T cells remain unclear. Although IL-2-deficient mice have normal CTL responses (34), OVA-specific memory CTLs decline strongly in the absence of IL-2, (32), suggesting that cell cycling is important for the maintenance of CD8\(^+\) T cell memory. Our data...
indicate that IL-2 compromises subsequent cytokine synthesis in CD8+ T cells, raising questions about the role of IL-2 in the maintenance of memory cells. It is possible that the production of IL-2 is precisely regulated in vivo so that its level is always maintained at low concentrations, sufficient to drive low level proliferation of cells. Alternatively, IL-2 production by CD8+ T cells may not be important for maintenance of memory CD8+ T cells, as CD4+ T cells can produce copious amounts of IL-2 and are less susceptible to IL-2-induced inhibition of cytokine synthesis. This would mean that CD4+ and CD8+ T cells have to coexist in vivo to sustain memory CD8+ T cells. IL-15 has been shown to be more potent than IL-2 in selectively stimulating the proliferation of memory CD8+, but not CD4+, T cells in vivo (35). Our results also demonstrate that effector, but not naive, CD8+ T cells respond more strongly to IL-15 than to IL-2 (Fig. 1). Thus, it is possible that IL-2 might play a more important role during the initiation of an immune response, whereas at later stages IL-15 might sustain the circulation of memory CD8+ T cells.

Several observations indicate that CD8+ T cells are often dependent on cytokines produced by CD4+ helper cells (36–39). CD8+ T cells may be capable of effectively curtailing an acute phase immune response, as effects of IL-2 are less discernable over memory CD8+ T cells. IL-15 has been shown to be more potent than IL-2 in selectively stimulating the proliferation of memory CD8+, but not CD4+, T cells in vivo (35). Our results also demonstrate that effector, but not naive, CD8+ T cells respond more strongly to IL-15 than to IL-2 (Fig. 1). Thus, it is possible that IL-2 might play a more important role during the initiation of an immune response, whereas at later stages IL-15 might sustain the circulation of memory CD8+ T cells.

As opposed to MHC class II, which is selectively expressed on cells, MHC class I is expressed on almost all cells. Because CD8+ T cells are MHC class I restricted, they can be stimulated more readily, and severe CD8+ T cell responses can cause pathology (40, 41). Thus, multiple mechanisms must maintain the regulation of CD8+ T cell responses. As IL-2 is a potent proliferation-promoting cytokine, it is reasonable that its production needs to be more precisely regulated. Other mechanisms mediated by IL-4−, perforin−, and Fas−dependent killing also help to regulate CD8+ T cell function (13, 20). The lack of production of cytokines may be a self-regulating mechanism for turning off immune responses. Thus, besides the expression of cytokine patterns, the amounts of individual cytokines in the local microenvironment can have totally different temporal effects.

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