activation of naive and memory T cells by interleukin-15

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interleukin-15 (IL-15), a member of the monococyte and other cytokine families, has been shown to have biological activity similar to that of IL-2, including growth stimulation of activated T cells, induction of cytolytic effector cells, and B-cell costimulation for proliferation and Ig production. We report that IL-15 at optimal concentrations rapidly induced memory (CD45RO⁺) CD4⁺ and CD8⁺ T cells and naive (CD45RO⁻) CD8⁺ T cells to express the CD89 activation marker followed by proliferation. By contrast, IL-15 failed to induce naive (CD45RO⁻) CD4⁺ T cells to express CD69 or to proliferate. Similar findings were obtained with IL-2. Unlike the other T-cell subsets, CD4⁺ T cells with a naive phenotype expressed little or no IL-2Rβ chain, a shared component of the IL-2 and IL-15 receptors required for receptor function. A monoclonal antibody to the IL-2Rβ chain, MAbβ1, reduced CD69 expression and proliferation in CD4⁺ memory, CD8⁺ memory, and CD8⁺ naive T cells activated by IL-15. These results confirm the biological similarities of IL-2 and IL-15. They further document that the pool of naive CD4⁺ cells, unlike the pool of memory CD4⁺, memory CD8⁺, and naive CD8⁺ cells, is not regulated directly by the T-cell growth factors IL-2 or IL-15. This is a US government work. There are no restrictions on its use.

Materials and Methods

Reagents and cytokines. Biotinylated monoclonal antibody (MoAb) against CD45RO was obtained from Dako Co (Carpentaria, CA). Streptavidin-Cy-Chrome (Pharmingen, San Diego, CA) and streptavidin-Phycocerythrin (PE) (Becton Dickinson Immunocytometry Systems, San Jose, CA) were used for staining biotin-conjugated antibodies. CD45RO⁺ and CD8⁺ memory, and CD8⁺ naive T cells activated by IL-2 were stained with anti-CD45RO (Leu-23) and fluorochrome isothiocyanate (FITC) conjugated MoAbs against CD3 (Leu-4), CD4 (Leu-3a), and CD8 (Leu-2a), were purchased from Becton Dickinson. MoAbs anti-Tac (anti-human IL-2Ra, mouse IgG2a), 12 MAbβ1 (anti-human IL-2Rβ, mouse IgG2a), 13 and TG144 (anti-human IL-2Rγ, rat IgG2b) 15 were kind gifts from Dr. T.A. Waldmann (National Institute of Health, Bethesda, MD), Dr. M. Tsu (Unichika Hospital, Kyoto, Japan) and Dr. K. Sugamura (Tohoku University, Sendai, Japan), respectively. Isotype matched control (mouse IgG2a and rat IgG2b) were obtained from Pharmingen. PE-conjugated goat antimouse Ig and antirat Ig were purchased from Southern Biotechnology Associates (Birmingham, AL). Human rIL-2 was a gift of Chiron Co (Emeryville, CA), rIL-15 was a gift of Immunex Co (Seattle, WA).

Cell preparations and cultures. PBMC were isolated from normal PB by density gradient centrifugation. Culture medium consisted of RPMI 1640 (Life Technologies, Inc, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (Intergen, Purchase, NY) 2 mmol/L-glutamine (Life Technologies) and 5 μg/mL gentamicin (Sigma Chemical Co, St Louis, MO). Experiments to assess CD69 expression were performed with PBMC incubated for 12 to 16 hours in culture medium (2 x 10⁶ cells/well, final volume 1 mL) in 24-well cell culture plates (Costar, Cambridge, MA) with or without rIL-2 or rIL-15 at various concentrations. Proliferative
responses to cytokines were evaluated using highly purified T-cell subpopulations. T cells were separated from PBMC by rosetting with 2-aminooethylisothiouronium bromide (Sigma)-treated SRBC, followed by density gradient centrifugation. E-rosetting T cells were then stained with a biotinylated MoAb against CD45RO, followed by incubation with PE-conjugated streptavidin, and further staining with a FITC conjugated MoAb against CD4 or CD8. CD45RO<sup>-</sup> (naive) and CD45RO<sup>+</sup> (memory) T cells were isolated using a FACStar plus electronic cell sorter (Becton Dickinson). The selected naive and memory T-cell subpopulations were more than 90% and 95% pure, respectively. In each cell separation, the purified cells were seeded at 2.5 X 10<sup>5</sup> cells/well in 96-well round bottom plates (Costar) in a final volume of 200 µL of culture medium, and cultured for 5 days. IL-2 and IL-15 were added to the cultures at various concentrations. DNA synthesis was determined by [³H] thymidine incorporation (0.5 µCi/well, 6.7 Ci/mmol; New England Nuclear, Boston, MA) during the last 16 hours of culture.

Three-color immunofluorescence analysis. Three-color immunofluorescence was performed to evaluate CD69 expression on T-cell subsets activated with IL-2 and IL-15. The cells were first treated with biotin-conjugated MoAb against CD45RO, followed by incubation with PE-conjugated streptavidin-Cy-Chrome. The cells were further stained with a PE-conjugated MoAb against CD69 and FITC-conjugated MoAbs against CD4 or CD8. The cells were then analyzed using a FACScan flow cytometer equipped with a Cell Quest data analysis program (Becton Dickinson). Three-color immunofluorescence was also used to assess expression of the IL-2R subunits on T-cell subpopulations. PBMC were first treated with anti-Tac (anti-IL-2Ra), Mikβ1 (anti-IL-2Rβ), TUGβ4 (anti-IL-2Rγ), or isotype matched control (mouse IgG2a or rat IgG2b) MoAbs, and then stained with PE-conjugated goat antimouse or antirat Ig antisera. After incubation with 10% mouse serum and 10% rat serum to block sites not occupied by secondary antibodies, the cells were stained with biotin-conjugated MoAbs against CD45RO<sup>-</sup>, followed by staining with streptavidin-Cy-Chrome. The cells were further stained for identification of CD4 or CD8<sup>-</sup> T cells with the corresponding MoAb labeled with FITC. The stained cells were analyzed using a FACScan flow cytometer.

RESULTS

Activated T cells are known to proliferate in response to IL-15 and to IL-2. To examine whether phenotypically naive T cells can respond to IL-15, PBMC were cultured for 12 hours in medium alone or in medium supplemented with either IL-15 (100 ng/mL) or IL-2 (1,000 U/mL). Naive (CD45RO<sup>-</sup>) and primed/memory (CD45RO<sup>+</sup>) T cells obtained before or after incubation were examined for surface expression of the early activation marker CD69 by three-color FACS analysis. We selected a 12- to 16-hour time point because preliminary determinations (not shown) indicated peak CD69 expression on T-cell subsets at this time point, before the occurrence of cell proliferation. As shown in a representative experiment (Fig 1), after 12 hours exposure to IL-15, a proportion of CD8<sup>+</sup> cells with either a memory/primed (11.9%) or naive (11.5%) phenotype exhibited CD69 surface expression. Because no CD69 expression was measured on these T cells before culture or in control cultures, these findings are indicative of IL-15-induced activation. A proportion (8.7%) of memory CD4<sup>+</sup> cells also exhibited CD69 surface expression after 12 hours exposure to IL-15, but not before culture or after 12 hours incubation in medium. By contrast, little or no CD69 expression was noted among CD45RO<sup>-</sup> (naive) CD4<sup>+</sup> cells. We considered the possibility that IL-15 might have induced a switch of naive (CD45RO<sup>-</sup>) CD4<sup>+</sup> cells into the memory (CD45 RO<sup>+</sup>) pool. However, the percent of cells with either a memory or naive phenotype did not change substantially during incubation. When IL-2 was used as a stimulus, CD69 expression on T-cell subsets was comparable to that observed with IL-15 stimulation (Fig 1). Some donor variation was observed, but in 5 experiments, the mean proportion of CD4<sup>+</sup>, phenotypically naive, T cells that scored positive for CD69 after 12 hours culture with IL-15 was 0.6%, and with IL-2 was 0.5% (Table 1). Thus, memory CD4<sup>+</sup>, memory CD8<sup>+</sup> and naive CD8<sup>+</sup> cells had evidence of activation by IL-15 which was similar in magnitude to that induced by IL-2. However, by the same test method, phenotypically naive CD4<sup>+</sup> cells had little or no evidence of activation by either IL-2 or IL-15.

In additional experiments, we examined IL-2- and IL-15-induced CD69 expression on T-cell subsets as a function of cytokine dose. PBMC, cultured for 16 hours in medium alone or in medium supplemented with either IL-15 (0.41 to 100 ng/mL) or IL-2 (4.1 to 1,000 U/mL) were examined by three-color FACS analysis at the end of culture (Fig 2A and B). At the highest IL-15 (100 ng/mL) and IL-2 (1,000 U/mL) doses tested, a proportion of CD8<sup>+</sup> memory, CD8<sup>+</sup> naive, and CD4<sup>+</sup> memory T cells expressed the CD69 activation marker, and both the magnitude and the hierarchy of T-cell subset responses to the cytokines were similar. At suboptimal IL-2 concentrations, particularly at 111 U/mL, the percent cells expressing CD69 was higher in CD4<sup>+</sup> memory T cells than among the other IL-2 responsive T-cell subsets. This finding suggested that high affinity IL-2 receptors might be expressed more frequently in CD4<sup>+</sup> memory
T cells than in other T-cell subsets. In contrast, at suboptimal IL-15 concentrations (0.41 to 11 ng/mL), CD69 expressing cells were not more frequent in the memory CD4+ T-cell subset than in the other IL-15 responsive subsets. This difference in T-cell responses induced by suboptimal concentrations of IL-2 and IL-15 raised the possibility that high affinity IL-2 and IL-15 receptors might be distributed differently on T cells.

To assess further T-cell subsets susceptibility to activation by IL-15, proliferative responses were measured. Subsets of CD4+ CD45RO- (memory CD4+), CD4+ CD45RO+ (naive CD4+), CD8+ CD45RO+ (memory CD8+), and CD8+ CD45RO- (naive CD8+) cells obtained by electronic cell sorting were cultured for 5 days in the presence of either IL-15 (1.2 to 100 ng/mL) or IL-2 (12 to 1,000 U/mL). As shown in Fig 2, highly purified memory CD8+ memory CD4+, and naive CD8+ cells proliferated in response to IL-15 and IL-2, particularly at the higher cytokine doses. By contrast, little or no proliferation was measured in the CD4+ naive T-cell subset stimulated with either IL-2 or IL-15. At the highest cytokine dose, the magnitude of responses to IL-15 and IL-2 were comparable, and the hierarchy of proliferative responses to IL-15 and IL-2 was similar among the T-cell subsets. At suboptimal doses of IL-2, particularly at 37 U/mL, CD4+ memory T cells proliferated somewhat better than the other IL-2 responsive subsets. However, at suboptimal doses of IL-15 (1.2 to 11 ng/mL) CD4+ memory T cells did not proliferate better than the other IL-15 responsive subsets. These findings are consistent with the results of CD69 expression induced by IL-2 or IL-15, and confirm that memory CD8+, memory CD4+ and naive CD8+ cells are responsive to IL-15 and IL-2, but naive CD4+ cells are not. In addition, these results suggest the possibility that high affinity IL-2 receptors are distributed differently in T-cell subsets than high affinity IL-15 receptors.

The failure of naive CD4+ T cells to either express the CD69 activation marker or to proliferate in response to IL-2 or IL-15 could be because of the absence of appropriate cytokine receptors. In addition, the enhanced responsiveness of CD4+ memory T cells to low dose IL-2 but not IL-15 could be because of differences in receptor chain distribution. The IL-2- and IL-15 receptors share the IL-2R beta and gamma chains. In contrast, the IL-2R alpha chain is a component of the high affinity IL-2 but not IL-15 receptor complex.

Using MoAbs that specifically recognize the IL-2R alpha (anti-Tac), IL-2R beta (Mikbeta) and IL-2R gamma (TUGh4), we looked for IL-2R chain expression in phenotypically naive and memory T-cell subsets. As shown in a representative experiment (Fig 3), the IL-2R gamma chain was expressed at low levels (the mean fluorescence intensity [MFI] change from background ranged between 1.2 and 3.3) in all T-cell subsets, including CD4+ naive, CD4+ memory, CD8+ naive and CD8+ memory cells. The IL-2R beta chain was detected in CD8+ memory (MFI change 3.0), in CD8+ naive (MFI change 2.3) and in CD4+ memory T cells (MFI change 2.6). However, the IL-2R alpha chain was detected at low levels, if at all, (MFI change 0.6) on CD4+ naive T cells. Although not part of the IL-15 receptor complex, the IL-2R alpha chain was expressed in memory CD4+ (MFI change 17.2), memory CD8+ (MFI change 2.5) and naive CD8+ (MFI change 5.4), but was absent or expressed at very low levels on naive CD8+ cells (MFI change 0.2). These findings suggest that the failure of CD4+ naive T cells to proliferate in response to IL-15 or IL-2 might be due to insufficient expression of the IL-2R beta chain. They also suggest that the enhanced responsiveness of CD4+ memory T cells to low-dose IL-2, but not IL-15, might be attributed to their higher level expression of the IL-2R alpha chain compared to the other T-cell subsets.

To assess further the importance of IL-2R beta chain expression to IL-15--induced T-cell responses, T-cell stimulation

| Table 1. CD69 Expression on Naive and Memory T-Cell Subsets |  |
| --- | --- | --- | --- | --- | --- |
| Cell Subset | No Culture | 12 h Medium | 12 h IL-2 | 12 h IL-15 |
| CD4+ CD45RO- | 0.2 (0.1) | 0.4 (0.1) | 9.4 (1.7) | 9.1 (2.5) |
| CD4+ CD45RO+ | 0 | 0.1 (0.1) | 0.5 (0.3) | 8.6 (0.3) |
| CD8+ CD45RO+ | 0.9 (0.3) | 1.5 (0.3) | 12.7 (1.0) | 13.5 (2.5) |
| CD4+ CD45RO- | 0.3 (0.1) | 0.9 (0.5) | 11.7 (5.1) | 12.5 (5.8) |

* The experiment was performed as described in the legend to Fig 1. Values represent the mean (±SEM) of 5 experiments.

Fig 2. Dose-responses of T-cell subset activation by IL-2 and IL-15. (A and B) PBMC were examined for CD69 expression by three-color FACS analysis after 16 hours incubation with either IL-15 (0.41 to 100 ng/mL) or IL-2 (4.1 to 1,000 U/mL). The results are expressed as % CD69+ cells in each T-cell subset. The experiment is representative of 4 performed. (C and D) Naive (CD45RO+ and memory (CD45RO-) CD4+ and CD8+CD45RO+ T-cell populations were obtained from E-rosette-purified T cells by electronic cell sorting. Each subset (2.5 x 10^5 cells/well) was cultured for 5 days with either IL-15 (1.2 to 100 ng/mL) or IL-2 (12 to 1,000 U/mL). [3H] thymidine incorporation was measured during the final 16 hours of culture. The experiment is representative of 4 performed. CD4+ naive ( ), CD4+ memory ( ), CD8+ naive ( ), and CD8+ memory ( ) T-cell subsets.
was performed in the presence of Mikβ1 MoAb that recognizes the IL-2R β chain. PBMC were cultured for 16 hours with IL-15 (100 ng/mL) alone or with either 10 μg/mL Mikβ1 or a control antibody. At the end of culture, three-color FACS analysis was employed to detect CD69 surface expression. As shown in a representative experiment (Fig 4A), the anti-IL-2R β chain MoAb Mikβ1, but not a control MoAb, significantly reduced the fraction of CD8+ naive, CD8+ memory, and CD4+ memory T cells expressing CD69 on activation with IL-15. In additional experiments, subsets of CD4+ CD45RO+ (memory CD4+), CD8+ CD45RO− (naive CD8+), and CD8+ CD45RO− (memory CD8+) cells obtained by electronic cell sorting were cultured for 5 days with IL-15 alone (100 ng/mL), with IL-15 (100 ng/mL) plus 10 μg/mL Mikβ1 MoAb, or with IL-15 (100 ng/mL) plus a control MoAb. In a representative experiment (Fig 4B), the anti-IL-2R β chain MoAb Mikβ1, but not a control MoAb, neutralized the IL-15 induced proliferative responses of memory CD4+, naive CD8+, and memory CD8+ cells. These findings further support a correlation between IL-2R β chain expression and T-cell activation by IL-15.

DISCUSSION

In this study, we have shown that phenotypically primed/memory CD4+ and CD8+ T cells as well as phenotypically naive CD8+ T cells respond to IL-15 stimulation with expression of the early activation antigen CD69 followed by proliferation. In contrast, naive CD4+ cells consistently fail to respond to IL-15 by the same criteria. The IL-2R β and γ chains were previously identified as components of the IL-15 receptor that are required for IL-15 binding and signaling.10 The β chain of the IL-2R is also required for IL-2R signaling after dimerization with the IL-2R γ chain.3 Here we found that the IL-2R β and γ chains are expressed on a proportion of the IL-15 and IL-2 responsive T-cell subsets, i.e., memory CD4+, memory CD8+ and naive CD8+ cells, but the IL-2R β chain is expressed at very low levels, if at all, on CD4+ naive T cells. In addition, an antibody directed to the IL-2R β chain reduced both CD69 expression and proliferation by memory and naive CD8+ as well as memory CD4+ T cells stimulated with IL-15. Thus, phenotypically naive CD4+ cells fail to respond to IL-15 or IL-2 presumably because they lack the IL-2R β chain subunit of a functional IL-15 and IL-2 receptor.

Recently, evidence was gained for the existence of an additional component of the IL-15 receptor, identified as the IL-15R α chain.11 Subsequently, a murine IL-15 binding protein that constitutes the predicted α chain component was cloned and characterized.25 The role this α subunit may play in conferring responsiveness to IL-15 is presently unknown. Simian IL-15 was found to bind to human IL-2R β and γ chains in the absence of the IL-15R α subunit.24 However, simian IL-15 was not capable of binding to a complex of murine IL-2R β and γ chains whereas it was capable of binding in the presence of the murine IL-15R α subunit.24 The IL-2R α chain, confers high affinity binding capacity to IL-2, allowing for increased responsiveness to IL-2 at suboptimal cytokine doses.20 The observation made here that CD4+ memory T cells both express the IL-2R α chain and are activated by suboptimal doses of IL-2 in preference to...
the other T-cell subsets is consistent with the notion that the role and/or distribution of the IL-15R α chain may differ from that of the IL-2R α chain. In addition, because T-cell subsets responded comparably to IL-2 and IL-15 at optimal cytokine concentrations, the present findings support the notion that the α subunits of the IL-2 and IL-15 receptors do not play an essential role in conferring T-cell responsiveness to these cytokines.

Phenotypically naive T cells are believed to be the progeny of newly formed T cells released from the thymus.13 They represent 40% to 60% of the circulating CD4+ T cells in normal adult humans.22,23 In cord blood, they represent the vast majority (>90%) of CD4+ T cells.22,23 During infancy and childhood as well as throughout life the frequency of circulating CD4+ CD45RA+ (naive) T cells declines.24 The decline of CD4+ naive T cells has been attributed to the continuous exposure to infectious agents. Release of antigens, mitogens and superantigens by infectious agents induces a switch of naive T cells to memory T cells, and is associated with a change to the memory phenotype (CD45RO+).25-27

The present findings that naive CD4+ T cells, but not naive CD8+ T cells, are neither induced to proliferate or driven to switch to a memory phenotype by IL-2 or IL-15, suggest that the T-cell growth factors IL-2 and IL-15 may not play an important direct role in the control of the naive CD4+ T-cell pool. Exposure to IL-2 or IL-15 is sufficient to activate CD8+, but not CD4+, naive T cells and to recruit them into cell cycle. Our results would suggest that the pool of naive CD4+ T cells is regulated by influx of newly formed T cells from the thymus and departure of cells switched to a memory phenotype after encounter with antigen, rather than through cell proliferation induced by T-cell growth factors. The observation that naive CD4+ T cells, as a whole, have a very slow turnover is consistent with the idea that naive T cells are mostly a resting T-cell population.28,29

Previously, it was noted that the combination of IL-2, IL-6, and TNFα can induce proliferation of a proportion of CD4+ T cells without switch to a memory phenotype.30 It is conceivable that IL-15 in combination with other cytokines, including IL-6 and TNFα, might also activate naive T cells and help maintain the naive CD4+ T-cell repertoire. One might expect to find IL-15 in association with IL-6 and TNFα more frequently than IL-2 because, unlike IL-2 which is produced by activated T cells, IL-15 is produced in monocytes,31 also a source of IL-6 and TNFα. Recently, IL-15 was detected at high concentrations in the synovial fluid of patients with rheumatoid arthritis,32 a site where other proinflammatory cytokines, such as IL-1, TNFα and IL-6 are also abundant.32,33 The T cells found in the synovial fluid of patients with rheumatoid arthritis possess predominantly a memory (CD45RO+) phenotype34 and express surface markers normally associated with activation, including HLA-DR and CD69.35,36 In addition, patients with rheumatoid arthritis generally have decreased numbers of CD4+ naive T cells in the circulation.37,38 These findings are consistent with the present results showing that IL-15 is not an inducer of CD4+ naive T cells.

One of the important issues raised by cytokines with overlapping biological activities, such as IL-2 and IL-15, is the reason for such redundancy. One possibility is simply that production of cytokines with overlapping biological activities is induced by distinct stimuli and performed by different cell types and tissues. We know that IL-2 is produced predominantly by T lymphocytes and that IL-15 is produced by a variety of other tissues, including placenta, skeletal muscle, kidney, and activated monocyte/macrophages.3,38 Although functionally redundant, IL-2 and IL-15 might both be required for effective T-cell activation at different times and/or in different sites. Recently, it was suggested that IL-15 might be essential to the activation of murine γδ T cells during early Salmonella infection, at a time when γδ T cells do not produce IL-2.39 Another possibility is that redundancy of certain biological functions might be coupled with unique functional properties, as a result of the coexistence of shared and unique receptor complexes and signaling pathways. As discussed previously, IL-2 and IL-15 share the IL-2R β and γ chains but not the α chain.10 In addition, endothelial cells were found to selectively bind IL-15 with high affinity, but not IL-2.40 The occurrence of distinct cytokine receptor structures segregating to distinct cell targets could lead to expression of unique combinations of biological activities. Recruitment, homing, and activation of T cells to the endothelium might be a unique set of properties of IL-15.

Although much is presently known about the factors regulating growth and survival of T cells, many other complex functions attributed to these cells are incompletely understood. The present observations that IL-15 and IL-2 can selectively activate memory T cells and naive CD8+ T cells, but not naive CD4+ T cells, points again to the functional similarities of these cytokines with respect to T-cell activation.

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