IL-15 enhances the function and inhibits CD95/Fas-induced apoptosis of human CD4+ and CD8+ effector-memory T cells

Yvonne M. Mueller1, Vera Makar1, Paul M. Bojczuk1, James Witek2 and Peter D. Katsikis1

Departments of 1Microbiology and Immunology, and 2Medicine, Drexel University College of Medicine, Drexel University, 2900 Queen Lane, Philadelphia, PA 19129, USA

Keywords: apoptosis, CD69, HIV, IFN-γ, lymphocyte activation

Abstract

Although the effect of IL-15 has been described on murine cells in vitro and in vivo, its effect on human memory CD8+ T cells is not well characterized. We show here that IL-15 preferentially enhances the activation and effector function of human effector-memory CD45RA ±CD62L± and CD45RA+CD62L± CD4+ and CD8+ T cells in both healthy and HIV-infected individuals. We find that IL-15 increases 2- to 5-fold both the activation and secretion of the effector cytokines IFN-γ and tumor necrosis factor (TNF)-α by anti-CD3-stimulated purified CD4+ and CD8+ T cells and peripheral blood mononuclear cells from healthy and HIV-infected individuals. Furthermore, IL-15 potently inhibits CD95/Fas-induced apoptosis of the effector-memory CD4+ and CD8+ T cells from HIV-infected individuals. These findings suggest that in addition to being a growth and survival factor for memory CD8+ T cells, IL-15 is also a potent activator of human effector-memory CD8+ T cells both in healthy and in HIV-infected individuals.

Introduction

IL-15 is a pluripotent cytokine that is secreted by antigen-presenting cells such as monocytes/macrophages (1,2) and dendritic cells (3,4), but also a variety of non-lymphoid tissue (5). In vitro studies have demonstrated that IL-15 can maintain murine memory CD8+ T cell, but not CD4+ T cell, numbers (6), and that IL-15 selectively expands antigen-experienced and more differentiated CD28−CD8+ T cells (7), suggesting that IL-15 preferentially affects memory CD8+ T cells. Overexpression of IL-15 in transgenic mice results in increased numbers of memory CD8+ T cells, NK and γδ T cells (8). The critical role of IL-15 in the generation or survival of memory CD8+ T cells was shown in IL-15Rα−/− and IL-15−/− mice, where greatly reduced numbers of memory CD8+ T cells, but not memory CD4+ T cells, were found (9,10). Furthermore, IL-15 treatment of wild-type (6,11) and IL-15−/− mice (9) greatly expands memory CD44hi CD8+ T cells and not memory CD4+ T cells.

Despite this intriguing data on the effect of IL-15 on mouse memory CD8+ T cells, there is a paucity of data as to the effect of IL-15 on human memory CD8+ T cells. The fact that the IL-2Rβ chain is selectively high on mouse memory CD8+ T cells, whereas it is expressed on both human naive and memory CD8+ T cells and memory CD4+ T cells suggests that the effect of IL-15 on human T cells may be very different (6,12). Since the IL-15Rα chain is up-regulated on activated human CD8+ T cells (13) and IL-15 can inhibit T cell apoptosis (8,14), IL-15 may act as a survival factor for human memory CD8+ T cells. Furthermore, IL-15 enhances the proliferation of peripheral blood mononuclear cells (PBMC) to mitogen, recall antigens and HIV-specific antigens (15,16). IL-15 was reported to be more potent than IL-2 in activating and stimulating the proliferation of naive and memory CD8+ T cells as well as memory CD4+ T cells in PBMC from HIV-infected individuals (17). In this later study, however, CD45RA and CD45RO expression was used to distinguish naive CD45RA+ and memory CD45RO+ cells. Given that there are CD45RA+ memory CD8+ T cells in peripheral blood, the effect of IL-15 on naive CD8+ T cells is questionable. T cells recently were classified into naive and memory/effector populations using CD45RA and lymph node homing receptors such as CD62L or CCR7. Naive T cells are described as CD45RA+CD62L+CCR7+ (18–20), while the CD8+ T cell memory population was subdivided into a central memory T cell subset (CD45RA−CD62L+CCR7+) and two effector-memory subpopulations (CD45RA−CD62L−CCR7− and CD45RA+CD62L−).
CCR7). Using this classification instead of CD45RA/CD45RO, one should gain more precise information about the effect of IL-15 on naive and memory CD4+ and CD8+ T cells, and whether central memory and effector-memory T cells are differentially affected by IL-15.

Given the anti-apoptotic effects of IL-15 (6,8–11,21–23), of interest was also the question whether IL-15 can inhibit CD95/Fas-induced apoptosis of T cells from HIV-infected individuals, as we and others have shown previously that T cells from these individuals are prone to undergo apoptosis (24–26), whereas T cells from healthy individuals are resistant (26–28).

The aim of the current study was to examine the effect of IL-15 on the activation and effector function of human naive and memory CD4+ and CD8+ T cells in healthy and HIV-infected individuals. We show here that IL-15 preferentially acts on effector-memory CD4+ and CD8+ T cells in both healthy and HIV-infected individuals, enhancing the activation of effector-memory CD4+ and CD8+ T cells, and their effector function, i.e. IFN-γ production by these cells. Furthermore, IL-15 inhibits CD95/Fas-induced apoptosis of the effector memory CD8+ and to a lesser extent CD4+ T cells from HIV-infected individuals. These data demonstrate that IL-15 increases activation and effector function, and enhances survival of effector-memory CD4+ and CD8+ T cells from healthy and HIV-infected individuals.

**Methods**

** Patients **

Heparinized blood was obtained from 16 HIV-seropositive adult patients and 10 HIV-seronegative healthy individuals following Drexel University Institutional Review Board approval and obtaining informed consent. All HIV-infected individuals were HIV+ since at least 1 year (range 1–18 years), the median CD4 count was 368 cells/μl (range 147–974), the median viral load was 317 RNA copies/ml blood (range 50–61,800), all were asymptomatic and 11 patients were on anti-viral treatment.

** Flow cytometry **

PBMC were isolated from heparinized venous blood of HIV+ and HIV− individuals by Ficoll-Hypaque density centrifugation (Amersham Pharmacia Biotech, Sweden).

The following combinations of mAb were used for the activation studies of memory T cell subpopulations: anti-CD69–FITC/anti-CD62L– phycoerythrin (PE)/anti-CD45RA–PE–Cy5/anti-CD8–APC and anti-CD69–FITC/anti-CD62L–PE/anti-CD45RA–PE–Cy5/anti-CD4–allophycocyanin (APC); and for the apoptosis studies: Annexin V–FITC (kind gift from Dr J. Tait, University of Washington, Seattle, WA)/anti-CD62L–PE/anti-CD45RA–PE–Cy5/anti-CD8–APC and Annexin V–FITC/anti-CD62L–PE/anti-CD45RA–PE–Cy5/anti-CD4–APC (eBioscience, San Diego, CA). One million cells were stained as previously described (26) with the combination of antibodies in HBSS (Cellgro, Herndon, VA), 3% FBS, 0.02% NaN3 for 15 min on ice, washed twice with HBSS, 3% FBS, 0.02% NaN3 and fixed with 1% paraformaldehyde. Annexin V staining was performed in the presence of 2.5 mM CaCl2. Analysis was performed on a FACSCalibur (Becton Dickinson, San Jose, CA) using FlowJo software (TreeStar, San Carlos, CA).

**Activation studies**

Freshly isolated PBMC, purified CD4+ T cells or purified CD8+ T cells from HIV-infected individuals and controls were used in the activation studies. CD4+ T cells and CD8+ T cells were purified from freshly isolated PBMC by negative selection using RosetteSep for CD4+ and CD8+ T cell enrichment (StemCell Technologies, Vancouver, Canada). The purity of CD4+ and CD8+ T cells was >92% by flow cytometry, with the contaminating population being CD3−CD4−CD8−CD45R−.

For the anti-CD3 antibody activation experiments, PBMC and purified CD4+ and CD8+ T cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated FBS, 2 mM L-Glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin sulfate (Cellgro) at 37°C in a 5% CO2 incubator at 1 × 106 cells/ml/well in 24-well plates coated with 0.01 and 0.1 μg/ml monoclonal anti-CD3 antibody (OKT3) in the presence or absence of 5 ng/ml IL-15 (R & D Systems, Minneapolis, MN) as previously described (24). All reagents used were endotoxin free. Fourteen hours after stimulation, supernatants and cells were harvested. Supernatants were assayed for cytokine production while cells were stained for flow cytometry analysis.

**ELISA**

ELISAs for IL-2, IL-4, IL-10, IFN-γ and tumor necrosis factor (TNF)-α were performed using capture and biotinylated detection antibodies and standards purchased from PharMingen (San Diego, CA). Briefly, 96-well Immuno MaxiSorpSurface plates (Nunc, Rochester, NY) were coated with 50 μl of 5 μg/ml of capture antibody in PBS overnight at 4°C, washed 3 times with PBS/0.05% Tween 20 and blocked with 200 μl PBS/0.05% Tween 20/2% BSA for 2 h at room temperature. Plates were then washed 3 times and 50 μl duplicates of culture supernatants or standards were added and incubated overnight at 4°C. After washing 5 times, 50 μl of biotinylated detection antibodies was added at 5 μg/ml for 2 h at room temperature. Following five washes, 100 μl of 1:7000 streptavidin–horseradish peroxidase (Jackson ImmunoResearch, West Grove, PA) was added for 30 min. Following five washes, 100 μl of teramethylbenzidine dihydrochloride microwell peroxidase substrate system (KPL, Gaithersburg, MD) was added for 30 min and color development was stopped with 100 μl 1N H3PO4. Plates were read on a Spectramax plus ELISA reader (Molecular Device, Sunnyvale, CA) at 450 nm. Assay sensitivity was as following: IL-2 14 pg/ml, IL-4 14 pg/ml, IL-10 40 pg/ml, IFN-γ 40 pg/ml and TNF-α 40 pg/ml.

**Apoptosis studies**

Spontaneous apoptosis was determined after 14 h cultivation of PBMC at 1 × 106 cells/ml/well in 24-well plates in the presence or absence of 5 ng/ml IL-15. For CD95/Fas-induced apoptosis, PBMC were cultured in plates coated with 5 μg/ml monoclonal anti-CD95 antibody (IgM, CH11; Immunotech, Brea, CA) in the presence or absence of 5 ng/ml IL-15 or 50 U/ml IL-2 (obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH; performed on a FACSCalibur (Becton Dickinson, San Jose, CA) using FlowJo software (TreeStar, San Carlos, CA).
human rIL-2 from Dr Maurice Gately, Hoffmann-La Roche) for 14 h. Specific apoptosis was calculated using the formula: \[\left(\frac{\% \text{ induced apoptosis} - \% \text{ spontaneous apoptosis}}{100 - \% \text{ spontaneous apoptosis}}\right) \times 100.\]

**Statistical analysis**

Statistical analysis was performed using the Mann-Whitney U-test, Student's t-test, the non-parametric Wilcoxon signed-rank test for paired samples and the Shapiro-Wilk W-test for normality. P values <0.05 were considered significant. The JMP statistical analysis program was used (SAS, Cary, NC).

**Results**

**IL-15 enhances activation of effector-memory CD4+ and CD8+ T cells from healthy individuals**

To determine whether IL-15 can enhance activation of T cell subpopulations, purified CD4+ and CD8+ T cells from healthy individuals were activated with anti-CD3 antibody in the presence and absence of IL-15, and the expression of the early activation marker CD69 was measured on the different naive/memory subpopulations.

In purified CD8+ T cells from healthy individuals, IL-15 significantly enhanced the activation of effector-memory CD8+ T cells as CD69 expression induced by CD3 stimulation was increased in the presence of IL-15 from 17 ± 3.9 to 53 ± 5.0% on the CD45RA-CD62L- effector-memory CD8+ T cells (n = 9, P < 0.005) and from 35 ± 6.7 to 70 ± 5.2% on the CD45RA-CD62L- effector-memory CD8+ T cells (n = 9, P < 0.005) (Fig. 1A and B). IL-15 also increased anti-CD3-induced CD69 expression from 2.1 ± 0.5 to 9.4 ± 2.1% on central memory CD45RA-CD62L+ CD8+ T cells (n = 9, P < 0.005) (Fig. 1A and B). IL-15 had no effect on anti-CD3-induced stimulation of naive CD8+ T cells (n = 9) (Fig. 1A and B). When purified CD4+ T cells were co-cultured with anti-CD3 antibody and IL-15, an increase of CD69-expressing cells was found for the central memory CD45RA-CD62L+ CD4+ T cells (3.8 ± 1.0% after CD3 stimulation alone and 8.4 ± 1.9% after CD3 stimulation in the presence of IL-15 respectively) (n = 10, P < 0.005) (Fig. 1C). A more profound activation-enhancing effect of IL-15 was detected for the effector-memory CD45RA-CD62L- CD4+ T cells, with 15 ± 3.5% CD69+ cells after CD3-stimulation alone and 30 ± 3.4% after CD3 stimulation in the presence of IL-15 (n = 10, P < 0.005) (Fig. 1C). Again, IL-15 did not enhance activation of CD3-stimulated naive CD4+ T cells (n = 10) (Fig. 1C). Similar to previous studies (20), we also could not detect CD45RA-CD62L+ CD4+ T cells in healthy individuals. IL-15 alone in the absence of stimulation through the TCR-CD3 complex had no effect on CD69 expression.
on purified CD4+ and purified CD8+ T cells from healthy individuals (data not shown).

These findings show that IL-15 preferentially enhances the activation of human effector-memory CD4+ and CD8+ T cells, and to a lesser extent that of central memory T cells.

**IL-15 increases activation of effector-memory CD4+ and CD8+ T cells from HIV-infected individuals**

The activation-enhancing effect of IL-15 on memory CD4+ and CD8+ T cells was also observed when CD3-stimulated PBMC from HIV-infected individuals were examined (Fig. 2A–C). IL-15 increased CD69 expression on central memory CD45RA−CD62L+ CD8+ T cells from 3.3 ± 1.2% after anti-CD3 stimulation alone to 27 ± 3.8% after stimulation in the presence of IL-15 (n = 8, P < 0.01) (Fig. 2A and B). IL-15 enhanced activation in both effector-memory CD45RA+CD62L− CD8+ T cells from 12 ± 3.3% for anti-CD3 alone and 63 ± 4.6% for anti-CD3 + IL-15 respectively, n = 8, P < 0.01) and CD45RA+CD62L− CD8+ T cells (19 ± 4.3% for anti-CD3 and 77 ± 4.2% for anti-CD3 + IL-15 respectively, n = 8, P < 0.01) (Fig. 2A and B). Although IL-15 did not increase CD69 expression on CD3-stimulated naive CD8+ T cells from healthy individuals, activation of CD3-stimulated CD45RA−CD62L− naive CD8+ T cells from HIV-infected individuals was enhanced by IL-15 from 2.6 ± 1.0 to 10 ± 2.2% (n = 8, P < 0.01) (Fig. 2A and B). IL-15 alone in the absence of CD3 stimulation had no effect on naive and central memory CD8+ T cells (Fig. 2A and B), but did significantly enhance the CD69 expression on the CD45RA+CD62L− CD4+ T cell effector-memory population from 0.9 ± 0.3 to 13 ± 2.7% (P < 0.01) and on the CD45RA−CD62L− CD4+ T cell effector-memory population from 1.3 ± 0.3 to 16 ± 2.8% (P < 0.01) (Fig. 2A and B). An activation-enhancing effect of IL-15 was also found for memory CD4+ T cells (Fig. 2C). IL-15 increased the expression of CD69 on CD45RA−CD62L− CD4+ T cells from 13 ± 3.6 to 41 ± 6.7% (n = 8, P < 0.01) and on effector-memory CD45RA−CD62L− CD4+ T cells from 20 ± 4.5 to 52 ± 9.2% (n = 8, P < 0.05) (Fig. 2C). No activation-enhancing effect of IL-15 was found for CD3-stimulated naive T cells from healthy individuals.
CD4+ T cells (n = 8) (Fig. 2C). Furthermore, IL-15 in the absence of CD3 stimulation had no effect on the activation of naive or memory CD4+ T cells (Fig. 2C).

**IFN-γ and TNF-α production by T cells from healthy and HIV-infected individuals is enhanced by IL-15**

To determine whether IL-15 enhances the production of effector cytokines, supernatants from PBMC and purified CD4+ and CD8+ T cells stimulated with anti-CD3 antibody in the presence or absence of IL-15 for 14 h were analyzed for IL-2, IL-4, IL-10, IFN-γ, TNF-α, IL-15 enhances IFN-γ production by anti-CD3-stimulated PBMC from 4.6 ± 2.3 ng/ml for anti-CD3 alone to 10.4 ± 2.3 ng/ml for anti-CD3 in the presence of IL-15 (n = 6, P < 0.05) (Table 1). IL-15 also significantly increased anti-CD3-induced IFN-γ production of purified CD4+ T cells and CD8+ T cells. In purified CD4+ T cells, IL-15 increased IFN-γ production by >15-fold from <0.04 ± 0.0 ng/ml for anti-CD3 stimulation alone to 0.63 ± 0.19 ng/ml for anti-CD3 stimulation in the presence of IL-15 (n = 6, P < 0.05) (Table 1). In purified CD8+ T cells, anti-CD3 stimulation induced 0.34 ± 0.25 ng/ml of IFN-γ and this production was increased 6.2-fold by IL-15 to 2.1 ± 0.63 ng/ml (n = 6, P < 0.05) (Table 1). IL-15 also significantly enhanced TNF-α production by T cells. PBMC from healthy individuals stimulated with anti-CD3 produced 7.0 ± 1.3 ng/ml TNF-α and IL-15 increased this to 14.2 ± 3.3 ng/ml (n = 12, P < 0.005) (Table 2). As with healthy individuals, IL-15 did not increase IL-2 secretion of anti-CD3-activated PBMC from HIV-infected individuals.

**IL-15 inhibits CD95/Fas-induced apoptosis in effector-memory CD4+ and CD8+ T cells from HIV-infected individuals**

Since IL-15 predominantly affected the activation of memory T cells and these cells are sensitive to CD95/Fas-induced apoptosis (26), we examined the effect of IL-15 on the CD95/Fas-induced apoptosis of the CD45RA-CD62L- and CD45RA+CD62L+ effector-memory CD4+ and CD8+ T cell subpopulations from HIV-infected individuals.

<p>| Table 2. IL-15 enhances IFN-γ and TNF-α secretion by PBMC from HIV-infected individuals |
|-------------------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Stimulation</th>
<th>IFN-γ (ng/ml)</th>
<th>TNF-α (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.49 ± 0.19</td>
<td>&lt;0.04 ± 0.0</td>
</tr>
<tr>
<td>IL-15</td>
<td>0.44 ± 0.18</td>
<td>&lt;0.04 ± 0.0</td>
</tr>
<tr>
<td>Anti-CD3</td>
<td>1.6 ± 0.52</td>
<td>0.87 ± 0.19</td>
</tr>
<tr>
<td>Anti-CD3 + IL-15</td>
<td>4.8 ± 1.2b</td>
<td>1.6 ± 0.23a</td>
</tr>
</tbody>
</table>

*Mean ± SE, n = 12. P < 0.05, when compared to anti-CD3.
induced apoptosis in 31 ± 3.8% of this subpopulation (n = 14, \( P < 0.005 \)) and this was decreased to 23 ± 4.3% by IL-15 (n = 14, \( P < 0.005 \)) (Fig. 3A and B).

Similar results were found when CD8+ T cell effector-memory subpopulations were analyzed (Fig. 3A and B). In CD45RA*CD62L− CD8+ T cells, 23 ± 5.6% spontaneous apoptosis was observed and this was reduced to 14 ± 3.5% apoptosis by IL-15 (n = 14). In the CD45RA*CD62L+ CD8+ T cells, spontaneous apoptosis was 8.3 ± 1.8% and IL-15 inhibited this to 5.7 ± 11% (n = 14). CD95/Fas stimulation induced apoptosis in 45 ± 4.6% of CD45RA*CD62L− CD8+ T cells (n = 14, \( P < 0.001 \)) and this was potently inhibited by IL-15 to 24 ± 3.4% (n = 14, \( P < 0.001 \)). In CD45RA+CD62L− CD8+ T cells, CD95/Fas induced apoptosis in 15 ± 2.5% of these cells and this was inhibited to 9.0 ± 1.3% by IL-15 (n = 14, \( P < 0.001 \)) (Fig. 3A and B).

Calculation of the treatment-specific apoptosis revealed that IL-15 inhibited CD95/Fas-induced apoptosis by 59 and 24% for CD8+ and CD4+ CD45RA*CD62L− effector-memory subpopulation respectively (Fig. 3C). CD95/Fas-induced apoptosis of CD45RA*CD62L− CD8+ T cells was also significantly reduced by 49% in the presence of IL-15, whereas no such effect was seen in CD45RA*CD62L− CD4+ T cells (Fig. 3C). In five patients, we compared the ability of IL-2 and IL-15 to inhibit CD95/Fas-induced apoptosis of CD8+ and CD4+ T cells. IL-2 used at 50 U/ml also inhibited CD95/Fas-induced apoptosis of CD8+ T cells, albeit to a lesser extent than IL-15 (39% inhibition by IL-2 versus 52% inhibition by IL-15), and this inhibition was reflected in both effector memory populations. In CD4+ T cells, IL-2 failed to inhibit CD95/Fas-induced apoptosis (5% inhibition) unlike IL-15 which had a modest effect (21% inhibition).

The above findings show that IL-15 can potently inhibit spontaneous and CD95/Fas-induced apoptosis of effector-memory T cells from HIV-infected individuals.

Discussion

In this study, we examined the effect of IL-15 on the effector function of T cells from healthy and HIV-infected individuals. The effect of IL-15 on the apoptosis of effector-memory T cells from HIV-infected individuals was also studied. We show here that IL-15 preferentially acts on effector-memory CD4+ and CD8+ T cells in healthy and HIV-infected individuals. IL-15 enhances the activation of anti-CD3-stimulated effector-memory CD4+ and CD8+ T cells and enhances effector function, i.e. IFN-γ and TNF-α production, by these cells. Furthermore, IL-15 inhibits CD95/Fas-induced apoptosis of CD4+ and CD8+ effector-memory T cells from HIV-infected individuals. In viral infections and in HIV infection, in particular, such enhancement of the function and survival of effector-memory T cells may prove useful in enhancing the anti-viral response. In support of this we have preliminary data indicating that IL-15 inhibits apoptosis of HIV-specific CD8+ T cells (Mueller and Katsikis, unpublished observations).

We used a combination of CD45RA and the lymph node homing receptor CD62L to identify CD45RA*CD62L− naive T cells, CD45RA CD62L+ central memory T cells and the two effector-memory T cell populations (CD45RA*CD62L− and CD45RA+CD62L− respectively), with the later being the terminally differentiated population (18,20,29). With this combination of surface markers, we show here that IL-15 alone in the absence of signaling through the TCR–CD3 complex does not activate naive or memory subpopulations of purified CD4+ T cells and purified CD8+ T cells in healthy individuals. These results expand the idea that IL-15 is not expressed on resting T cells from healthy individuals (13), and suggest that IL-15 is not expressed on resting naive and memory populations of CD4+ and CD8+ T cells in healthy individuals. These findings are in agreement with mouse studies that show that the IL-15 receptor is only expressed in the memory phenotype murine T cells (6), but is strongly up-regulated on murine CD4+ and CD8+ T cells following TCR activation in vitro (6,30). Since low levels of IL-2βγ chains expression are found on all subsets of human T cells with the exception of naive CD45RA+ CD4+ T cells (12), our data suggest that IL-15 expression is required for IL-15 to act on CD4+ and CD8+ T cells in healthy individuals. Our data are not in agreement with results from Kanegane et al. who reported that IL-15 in the absence of TCR stimulation induced CD69 expression and proliferation of human memory CD4+ and CD8+ T cells and naive CD8+ T cells in healthy individuals (12). Of note, however, is that the concentration of IL-15 used in the study by Kanegane et al. was 50 times higher than the one used in our experiments. At IL-15 concentration similar to ours (5 ng/ml), Kanegane et al. also could not induce CD69 expression in the absence of TCR–CD3 stimulation, and the most likely explanation for the above finding is that at high concentration IL-15 is signaling through the IL-2 receptor chains in the absence of IL-15 activation (31,32). However, whether such high concentrations are physiological relevant is unclear.

Previously, in a study examining PBMC from HIV-infected individuals, CD45RO− CD4+ memory T cells, CD45RO+ CD8+ memory T cells and CD45RO− CD8+ naive T cells were shown to be activated by IL-15 alone (17). By using a more detailed phenotypic analysis with the combination of CD45RA and CD62L, we find that in PBMC of HIV-infected individuals only CD45RA CD62L− CD8+ and CD45RA+CD62L− CD8+ effector-memory T cells are directly activated by IL-15 treatment alone. IL-15 alone did not induce the activation of naive and central memory CD8+ T cells or any of the CD4+ T cell subpopulations. Since CD45RO− CD8+ T cells include naive CD45RA+CD62L+ CD8+ T cells, but also the CD45RA+CD62L− effector-memory CD8+ T cell population, the effect of IL-15 on naive CD8+ T cells demonstrated in these earlier studies (17) most likely is due to the response of the effector-memory CD45RA+ T cells in these populations. In agreement with Naora et al. (17), we also demonstrated that IL-15 could directly activate memory CD8+ T cells of HIV-infected individuals. Our observations, however, take these findings one step further by showing that only CD45RA*CD62L− and CD45RA+CD62L− effector-memory and not CD45RA*CD62L+ central memory CD8+ T cells are activated directly by IL-15 in the absence of TCR–CD3 stimulation. In contrast, as noted above, in healthy individuals IL-15 could not activate any CD4+ or CD8+ T cell subpopulations in the absence of TCR–CD3 activation. We believe that the direct effect of IL-15 on enhancing the activation of effector-memory CD8+ T cells of HIV-infected individuals is likely due to an increased activation status of these cells in
Fig. 3. IL-15 inhibits CD95/Fas-induced apoptosis of effector-memory T cells from HIV-infected individuals. (A) Representative flow cytometry showing Annexin V staining on effector-memory CD4+ and CD8+ T cells after stimulation of PBMC with anti-CD95/Fas antibody ± IL-15. (B) Pooled data showing Annexin V binding on effector-memory CD4+ and CD8+ T cells after stimulation of PBMC with anti-CD95/Fas antibody ± IL-15 (n = 14). Bars depict mean ± SE. (C) Percentage of treatment-specific apoptosis shown for anti-CD95/Fas-stimulated CD45RA-CD62L- and CD45RA+CD62L- effector-memory CD4+ and CD8+ T cells (n = 14). Bars depict mean ± SE.
HIV-infected individuals compared to healthy controls. Such increased activation has been previously demonstrated by an increase in CD38 and HLA-DR expression of CD8+ T cells in HIV-infected individuals compared to healthy controls (26,33). Whether this is accompanied by an up-regulation of the IL-15Rα chain has yet to be determined.

We found that IL-15 could also enhance anti-CD3-induced activation of memory CD4+ and CD8+ T cells in healthy individuals and HIV-infected individuals, with the highest activation-enhancing effect being exerted on the CD45RA-CD62L- and CD45RA+CD62L- effector-memory CD4+ and CD8+ T cell subpopulations. This is in agreement with previous studies that have shown that IL-15 expands the antigen experienced and more differentiated CD28- CD8+ T cells (7). This enhanced responsiveness to IL-15 is most likely due to enhanced expression of IL-15Rα following activation as previously shown for mitogen-activated CD4+ and CD8+ T cells (13).

The increase in CD69+ T cells induced by IL-15 could either be due to newly activated T cells or to expansion of these CD69+ T cells or inhibition of activation-induced cell death (AICD). Given that in our cultures cells are incubated with IL-15 overnight, it is unlikely that the increase of CD69+ T cells is due to expansion of these cells. In support of this is the fact that the T cell numbers do not increase in these overnight cultures (data not shown). It is also unlikely that the increase of CD69+ T cells by IL-15 is due to inhibition of AICD as submitogenic doses of anti-CD3 are used, which do not induce AICD in our cultures (data not shown). Furthermore, AICD-inducing mitogenic doses of anti-CD3 we have found that IL-15 cannot inhibit such T cell apoptosis (Mueller and Katsikis, unpublished observations). Therefore, we believe that the increase of CD69+ T cells observed after overnight culture of T cells with low-dose anti-CD3 in the presence of IL-15 is due to increased numbers of newly activated T cells.

Although the effect of IL-15 on T cells concomitantly activated by TCR cross-linking is not well described, reports analyzing the effect of activation through the IL-2R imply a similar mechanism for IL-15 because of the shared IL-2/IL-15Rα chain has yet to be determined.

The activation-enhancing effect of IL-15 on effector T cells we observed does not necessarily translate to increased effector function of these cells as it may only result in increased proliferation of effector cells since CD69 expression was shown previously to correlate with proliferative responses (12,39). To determine whether effector function was also increased, we examined the effect of IL-15 on the production of the effector cytokines IFN-γ and TNF-α. Indeed, IL-15 significantly enhanced anti-CD3 antibody-induced IFN-γ and TNF-α secretion by PBMC, purified CD4+ and CD8+ T cells of healthy individuals, and PBMC of HIV-infected individuals. These findings show that IL-15 not only enhances the activation of effector memory T cells, but also preferentially augments effector function by inducing effector cytokines. IL-2 production was not affected by IL-15, suggesting again that naive and central memory T cells were not influenced by IL-15.

IL-15 has been shown previously to enhance the survival of murine CD8+ T cells in vitro and in vivo (6,8–11,22,23,40,41). Although T cells from healthy individuals are not susceptible to CD95/Fas-induced apoptosis after short-term culture (27,28), T cells from HIV-infected individuals are prone to undergo apoptosis after stimulation of the CD95/Fas pathway (24–26). We show here that IL-15 inhibits CD95/ Fas-induced apoptosis in both CD4+ and CD8+ CD45RA-CD62L- effector-memory T cells, and in CD45RA+CD62L- effector-memory T cells. Given that CD4+ and CD8+ T cells play a key role in HIV infection (42–46), enhancing the survival of these two effector T cell populations may prove to be of particular importance in HIV and possibly other viral infections.

As mentioned earlier, IL-15 is produced by a number of cell types including macrophages and dendritic cells, but also by non-lymphoid tissue (1–5). The question arises whether in HIV-infected individuals IL-15 production is affected, as this would potentially influence the function and survival of effector and memory T cells. Unfortunately, very little is known in the literature about IL-15 production in HIV-infected individuals. One study indicated that IL-15 levels are somewhat increased in the serum of HIV-infected individuals (47), whereas another study actually shows that IL-15 production by in vitro stimulated PBMC from HIV-infected individuals is greatly reduced (48). It therefore remains to be determined whether there is a defect of IL-15 production in HIV-infected individuals and whether it correlates with T cell function and survival.

In our studies, as with previous reports (25), IL-2 inhibits CD95/Fas-induced apoptosis in HIV-infected individuals, albeit to a lesser extent compared to IL-15. This is somewhat perplexing given that a number of studies have shown that IL-2 promotes CD95/Fas-induced T cell apoptosis by downregulating FLIP and increasing Fas-associated FADD, and by down-regulating the γc chain (49–51). For this pro-apoptotic effect, however, T cells need to be treated for several days with IL-2. In our studies, cells are treated with IL-2 in overnight apoptosis assays. Given that memory cells exhibit higher IL-2Rβ expression (6,12), and IL-2 induces anti-apoptotic Bcl-2 family members via the IL-2Rβ and γc chains (52), it is not surprising that in our short-term cultures IL-2 exerts predominantly an anti-apoptotic effect. We would like to point out, however, that IL-15 has the added advantage over IL-2 in that it appears not to induce HIV replication (15,48,53–55).

The data presented in this study demonstrate that IL-15 preferentially acts on human effector-memory CD4+ and CD8+ T cells. It can enhance the activation and function of effector-memory CD4+ and CD8+ T cells in both healthy and HIV-infected individuals, and most importantly can inhibit CD95/ Fas-induced apoptosis of these cells in HIV-infected individuals. These findings indicate that IL-15 may prove useful as a strategy to enhance the function of effector cells in disease states where such cells play a protective role.
IL-15 enhances the function of T cells

Acknowledgements
We thank Dr M. Gold and the staff of the Partnership Comprehensive Care Practice of the HIV/AIDS Medicine Division of Drexel University College of Medicine for patient recruitment. This work was supported by National Institutes of Health grants R01 AI46719 and R01 AI52005 to P. D. K.

Abbreviations
AICD activation-induced cell death
APC allophycocyanin
PBMC peripheral blood mononuclear cell
PE phycoerythrin
TNF tumor necrosis factor

References
IL-15 enhances the function of T cells


