Processing and Release of IL-16 from CD4⁺ But Not CD8⁺ T Cells Is Activation Dependent

David M. H. Wu, Yujun Zhang, Nereida A. Parada, Hardy Kornfeld, John Nicoll, David M. Center and William W. Cruikshank

_J Immunol_ 1999; 162:1287-1293; ; http://www.jimmunol.org/content/162/3/1287

---

**References**  This article cites 25 articles, 15 of which you can access for free at: http://www.jimmunol.org/content/162/3/1287.full#ref-list-1

**Subscription**  Information about subscribing to _The Journal of Immunology_ is online at: http://jimmunol.org/subscription

**Permissions**  Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**  Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Processing and Release of IL-16 from CD4\(^+\) But Not CD8\(^+\) T Cells Is Activation Dependent\(^1\)

David M. H. Wu, Yujun Zhang, Nereida A. Parada, Hardy Kornfeld, John Nicoll, David M. Center, and William W. Cruikshank\(^2\)

IL-16 is synthesized as a precursor molecule of 68 kDa (pro-IL-16) that is processed by caspase-3, a member of the IL-1 converting enzyme (ICE) family. This cleavage results in a 13-kDa carboxy terminal peptide, which constitutes the bioactive secreted form of IL-16. We have previously reported constitutive IL-16 mRNA expression and pro-IL-16 protein in CD4\(^+\) and CD8\(^+\) T cells. Although bioactive IL-16 protein is present in unstimulated CD8\(^+\) T cells, there is no bioactive IL-16 present in CD4\(^+\) T cells. Among these lines, unstimulated CD8\(^+\) T cells contain active caspase-3. In the current studies we investigated the regulation of IL-16 protein and mRNA expression in CD4\(^+\) T cells and determined the kinetics of secretion following stimulation of the TCR. CD4\(^+\) T cells release IL-16 protein following antigenic stimulation, and this release is accelerated in time by costimulation via CD82. However, CD3/CD28 costimulation did not alter IL-16 mRNA appearance or stability in either CD4\(^+\) or CD8\(^+\) T cells. The secretion of bioactive IL-16 from CD4\(^+\) T cells correlated with the appearance of cleavage of pro-caspase-3 into its 20-kDa active form. Thus, resting CD8\(^+\) T cells contain active caspase-3 that is capable of cleaving pro-IL-16, whereas CD4\(^+\) T cells require activation for the appearance of active caspase-3. The mechanism of release or secretion of bioactive IL-16 is currently unknown, but does not correlate with cellular apoptosis.


In CD8\(^+\) T cells, pro-IL-16 is cleaved by caspase-3 (1, 2), resulting in the constitutive presence of stored bioactive 13-kDa IL-16 (3–6), as evidenced by the presence of IL-16-specific bioactivity detected in cell lysates of unstimulated CD8\(^+\) T cells (7). A noncytotoxic, noncytolytic release of IL-16 from CD8\(^+\) T cells occurs within 4 h after stimulation with either histamine or serotonin (7, 8). The secretion of IL-16 from CD8\(^+\) T cells following stimulation with vasoactive amines is not affected by inhibitors of protein synthesis. Furthermore, Northern blot analyses indicate that neither histamine nor serotonin induces changes in the level of IL-16 mRNA (7). In those studies that defined the role of CD8\(^+\) T cells as a source of IL-16, we found that IL-16 mRNA was constitutively expressed not only in CD8\(^+\) cells but in CD4\(^+\) cells as well (7). However, in contrast to CD8\(^+\) T cells, vasoactive amine stimulation of CD4\(^+\) T cells did not result in IL-16 release; further, CD4\(^+\) T cell lysates did not contain bioactive IL-16 protein (7). We have subsequently demonstrated that unstimulated CD4\(^+\) T cells contain pro-IL-16 in quantities similar to that found in CD8\(^+\) T cells (9). Although a number of cell types have recently been shown to produce bioactive IL-16, including epithelial cells (10), eosinophils (11), and mast cells (12), CD4\(^+\) T lymphocytes have not been identified as a source of bioactive IL-16. The synthesis and secretion of IL-16 by CD4\(^+\) T cells could represent a positive amplification mechanism for further recruitment and activation of CD4\(^+\) T cells.

In the current studies, we investigated the synthesis and release of IL-16 by CD4\(^+\) T lymphocytes after stimulation with specific Ag and anti-CD3 Ab and assessed the effect of costimulation of anti-CD3 with an activating anti-CD28 Ab. We observed that CD3 stimulation of peripheral blood CD4\(^+\) T cells results in the secretion of bioactive IL-16 without detectable changes in the level of expression of IL-16 mRNA. Although costimulation through CD28 results in a more rapid secretion, with increased levels of bioactive IL-16 in cell supernatants, we could not detect any change in IL-16 mRNA levels, rate of transcription, or stability. Furthermore, we could not detect any changes in the level of total pro-IL-16 protein. Rather, we found that, unlike CD8\(^+\) T cells, which contain active caspase-3 (1), CD4\(^+\) T cells require activation to induce the cleavage of pro-caspase-3 into its 20-kDa enzymatically active form. There is a temporal relationship between the appearance of active caspase-3 and bioactive IL-16 in anti-CD3-stimulated CD4\(^+\) T cells; in a similar fashion, CD28 costimulation augments the coincident appearance of both active caspase-3 and bioactive IL-16. These studies indicate that CD4\(^+\) and CD8\(^+\) T cells have distinct mechanisms and kinetics of caspase-dependent processing of IL-16. Whereas CD8\(^+\) T cells do not require antigenic activation for the release of IL-16, the cleavage of pro-IL-16 and the release of bioactive IL-16 by CD4\(^+\) T cells do require antigenic stimulation.

Materials and Methods

Reagents

Cycloheximide (CHX)\(^3\) was obtained from Sigma (St. Louis, MO). Actinomycin D was obtained from Life Technologies (Grand Island, NY). Caspase-1 and -3 inhibitors were obtained from Bachem (King of Prussia, PA).

Antibodies

An affinity-purified polyclonal rabbit anti-rIL-16 was prepared from rIL-16-immunized rabbit sera as described previously (3, 6). Mouse monoclonal anti-human CD4 Abs, mouse anti-human CD8 Abs, FITC-conjugated anti-CD4 or anti CD8 mAbs, and phycoerythrin-conjugated anti-CD3 were
CD28 Ab (1 μm) was incubated at 37°C in a 5% CO₂ atmosphere for 2 h. Test samples, which did not recognize IL-16 by Western blot analysis (9) and had no effect on moattractant chemokines tested thus far (14–16). Preimmune rabbit serum Ab with the chemotactic activity induced by any of the lymphocyte chemokine rhuIL-16 is completely neutralized. There is no cross-neutralization by this antibody.

Migration was quantified by counting the total number of cells migrating through the filters completed cell populations contained <1% of CD4⁺/CD3⁺ lymphocytes and that CD8-depleted cell populations contained <1% of CD8⁺/CD3⁺ cells.

Chemotaxis assay
The presence of IL-16 in cell-free supernatants was assessed by quantification of human NWNA-T lymphoid migration using a modified Boyden chemotaxis chamber technique (4). A total of 52 μl of NWNA-T suspension (10 × 10⁶ cells/ml) was placed in the upper compartments of the 48-well microchemotaxis chambers separated from 32 μl of test samples by 8-μm micropore nitrocellulose filters (Neuroprobe, Cabin John, MD) and was incubated at 37°C in a 5% CO₂ atmosphere for 2 h. Test samples included cell-free supernatants diluted at various concentrations. The filters were fixed with hematoxylin, dehydrated, and mounted on glass slides. Cell migration was quantified by counting the total number of cells migrating beyond 50 μm into the filter by light microscopy, with baseline migration under control buffer conditions of 10–15 cells at the same time that the cells were added to the culture wells containing immobilized anti-CD3 and anti-CD8 Abs. CD4⁺ T cells were incubated in the presence or absence of 100 μM of the tetrapeptide inhibitors Ac-DEVD-CHO or Ac-YVAD-CHO at 37°C in 5% CO₂ for ≤24 h. The culture medium was collected at 6 and 24 h and assessed for IL-16 bioactivity by chemotaxis.

Western blot analysis
Purified CD4 or CD8 cells were prepared by negative selection as described above. Cells under control, anti-CD3, or CD28 costimulatory conditions were washed once with PBS, released by scraping, and harvested by centrifugation. Cells were immediately lysed by sonication in a buffer containing PBS (pH 7.5), 1 mM PMSF, 10 μg/ml aprotonin, and 10 μg/ml leupeptin and subjected to electrophoresis through a 15% SDS polyacrylamide gel. Proteins were electrophotorectively transferred to a nitrocellulose membrane and probed with goat-polyclonal anti-caspase-3 against the P20 subunit (Santa Cruz Biotechnology, Santa Cruz, CA). The secondary Ab, which was an anti-goat Ig labeled with horseradish peroxidase (Santa Cruz Biotechnology), was used at a dilution of 1/5000, and the signal was detected by chemiluminescence (Pierce, Rockford, IL).

Staining for apoptosis
T cells harvested from the cultures were washed twice in PBS before staining with propidium iodide (1 μg/ml final concentration) for 5 min at room temperature. Apoptosis was determined using fluorescence microscopy and was quantitated by counting the percentage of stained nuclei.

Results
Effects of anti-CD3 Ab and Ag stimulation on generation of IL-16 by CD4⁺ and CD8⁺ T cells
The current studies were undertaken to determine under what circumstances CD4⁺ T cells could process pro-IL-16 and secrete bioactive IL-16. First, we investigated the effects of immobilized anti-CD3 Ab and Ag stimulation on the secretion of IL-16 in human T lymphocytes incubated as a mixed T cell population. Monocytes represented 5% of the total cell numbers, which was sufficient to facilitate T cell activation. The cells were stimulated with either anti-CD3 Ab for ≤24 h. Cells isolated from normal individuals were stimulated with anti-CD3 Ab (anti-ε, 1 μg/ml). Because pro-IL-16 is recognized by current ELISAs but has no chemotactic activity (1, 9), we identified the presence of bioactive IL-16 by chemotaxis of human T cells in the presence or absence of IL-16 neutralizing Ab. As shown in Fig. 1, anti-CD3 induced a significant increase in the overall T cell chemotactic activity detected in the supernatants of mixed T cell cultures by 6 h, which increased through 12, 18, and 24 h. The contribution of IL-16 to the overall chemotactic activity was determined by coincubating the supernatants with neutralizing anti-IL-16 Ab for 30 min before assessing cell motility. The IL-16 neutralizing Ab studies indicate that IL-16 contributed the majority of the chemotactoactivity.
activity that was present at the 6-h timepoint for anti-CD3 stimulation. IL-16 contributed approximately one-half of the total chemotactic activity for anti-CD3 (Fig. 1). Although the total detectable chemotactic activity increased from 6 to 24 h, the greatest contribution of IL-16, calculated as a percentage, was seen in the earlier timepoints.

To determine the time course and subset specificity for IL-16 generation following CD3 stimulation, mixed T lymphocytes were then fractionated into CD4+ or CD8+ T cells by negative selection. Subset purity was determined to be 96% CD4+ cells and 95% CD8+ cells, with both populations containing 4–5% monocytes as indicated by FACS analysis (data not shown). To determine the kinetics of IL-16 release, CD4+ or CD8+ cell cultures were stimulated with 1 μg/ml immobilized anti-CD3 Ab. After 6 h and then again after 24 h, culture supernatants were removed and cells were recultured on anti-CD3-coated plates in fresh media. As shown in Fig. 2, anti-CD3 stimulated the release of chemotactic activity at the 6-h timepoint from both cell populations. Neutralizing anti-IL-16 Ab had no effect on total chemotactic activity associated with either unstimulated CD8+ T cells or CD4+ T cells treated with CHX, indicating that either the synthesis or secretion of all lymphocyte chemotactic activities required new protein synthesis (Fig. 3). Similarly, at 24 h, all of the anti-CD3 induced chemotactic activity was inhibited by CHX treatment. These findings are consistent with our previous reports of a lack of preformed chemotactic activity contained in CD4+ T cell lysates.

In contrast to CD4+ cells, treatment with CHX did not reduce the chemotactic activity in CD8+ cell supernatants at 6 h (Fig. 3). The addition of anti-IL-16 Ab to the chemotaxis assay resulted in the loss of all chemotactic activity. All of the chemotactic activity associated with either unstimulated CD8+ cells or cells stimulated with anti-CD3 for ≤6 h is attributable to IL-16, which is consistent with the presence of preformed, bioactive, IL-16. CHX pretreatment did reduce the chemotactic activity released by stimulated CD8+ T cells at 24 h by ~50%; however, all of the CHX-insensitive chemotactic activity was inhibited by anti-IL-16 Abs. The stimulation of CD8+ T cells induces the release of preformed IL-16 by 6 and 24 h, whereas the additional chemotactic activity produced by CD8+ T cells over 24 h is not IL-16 and requires new protein synthesis. Chemokines are a likely source (14, 15) for the residual chemotactic activity. In that regard, we identified RANTES and macrophage inflammatory protein (MIP)-1β as the other two chemotactic factors by neutralizing Ab studies. No MIP-1α bioactivity was detected. A combination of anti-RANTES, anti-IL-16, and anti-MIP-1β Abs completely neutralized all chemotactic activity (data not shown).
Costimulation with CD28 increases IL-16 protein production

To assess the effect of CD28 costimulation (16–18) on IL-16 expression, we assayed supernatants from CD4+ and CD8+ subsets stimulated with anti-CD3, anti-CD28, or the combination of anti-CD3 and anti-CD28 for 6 and 24 h with and without prior incubation with CHX, as described in Materials and Methods. The T cell supernatants were then assessed in the absence or presence of neutralizing anti-IL-16 Ab as designated in the figure. Migration data are expressed as the mean ± SD for three different experiments. An asterisk (*) denotes supernatants whose chemotactic activity was significantly decreased in the presence of neutralizing anti-IL-16 Ab (p < 0.05).

Regulation of IL-16 mRNA expression

To directly investigate whether anti-CD3 stimulation could modulate IL-16 mRNA, human CD4+ T cells were stimulated with immobilized anti-CD3 for 6 or 24 h before isolation of total RNA and Northern blot analysis. IL-16 mRNA was constitutively expressed and did not appear to increase after 6 or 24 h of anti-CD3 stimulation (Fig. 5). Furthermore, no change in mRNA following anti-CD3 activation could be identified in CD8+ subsets (data not shown). We subsequently examined the effect of costimulation on IL-16 mRNA expression in CD4+ T cells. CD3/CD28 costimulation had no significant effect on the levels of IL-16 mRNA at any
timepoint up to 6 h of stimulation (Fig. 6). Finally, there was no detectable effect of CD3/CD28 costimulation on the stability of IL-16 mRNA (Fig. 7). In these experiments, CD4\(^+\) T cells were incubated in the presence or absence of anti-CD3/anti-CD28 for 24 h, followed by the addition of actinomycin D (10 \(\mu\)g/ml). In both conditions, IL-16 mRNA was detectable at 1 h, decreased at 2 h, and was not detectable by 3 h, indicating no change in mRNA stability. Taken together, these experiments suggest that IL-16 secretion in CD4 cells must be regulated at some critical step after transcription and translation. We hypothesized that this critical step might lie with the pro-IL-16-processing enzyme, caspase-3.

Detection of caspase-3 in activated CD4\(^+\) lymphocyte lysates

We first investigated whether stimulated CD4\(^+\) T lymphocytes contain active caspase-3 and then determined the time course of processing of pro-caspase-3 following anti-CD3 or anti-CD3/anti-CD28 costimulation. In these experiments, purified CD4\(^+\) lymphocytes were stimulated for 6 and 24 h with anti-CD3 or anti-CD3/CD28 costimulation. Cell lysates at these two timepoints were subjected to SDS-PAGE followed by caspase-3 Western blot analysis. As seen in Fig. 8, no active caspase-3 is observed in unstimulated CD4\(^+\) T cells. At 4 h, a timepoint when bioactive IL-16 is not observed in supernatants from anti-CD3-activated CD4\(^+\) T cells, there is only a faint (20-kDa) detectable band correlating to active caspase-3. However, at this timepoint, a 20-kDa band of 10-fold the intensity (lane 3) compared with anti-CD3 stimulated alone (lane 2) is observed with anti-CD3/CD28 co-stimulation. By 24 h, cells stimulated with anti-CD3 alone or co-stimulated with CD3/CD28 all express similar levels of active caspase-3 (Fig. 8). These data suggest that caspase-3 is activated after the stimulation of CD4\(^+\) T cells with anti-CD3 or anti-CD3/CD28 in a time course compatible with the appearance of the bioactive IL-16 detected in the cell supernatants.

In an attempt to corroborate the requirement of caspase activation for the release of bioactive IL-16, CD4\(^+\) T cells were incubated in the presence of peptides Ac-DEVD-CHO and Ac-YVAD-aldehyde, which are inhibitors of caspase-3 and caspase-1 activation, respectively (19–21). CD4\(^+\) T cells were exposed to the inhibitors for 1 h before stimulation with anti-CD3 alone or in combination with anti-CD28. After 24 h, supernatants were assessed for chemoattractant activity attributable to IL-16; cells were assessed for apoptosis by propidium iodide staining to demonstrate that the peptides were effective in an independent assay. Cells treated with Ac-DEVD-CHO were unable to generate detectable bioactive IL-16 in the supernatants after anti-CD3/anti-CD28 stimulation (Fig. 9). Similar effects were observed for stimulation by anti-CD3 alone (data not shown). The involvement of caspase-3 appears to be specific, as the inhibition of caspase-1 by the peptide Ac-YVAD-aldehyde did not alter the magnitude or the time course

FIGURE 5. Effect of anti-CD3 stimulation on IL-16 mRNA in CD4\(^+\) T cells. Northern hybridization analysis of total RNA extracted from anti-CD3-stimulated CD4\(^+\) T cells for 0 (lane 1), 6 (lane 2), or 24 (lane 3) h was performed using \(^{32}\)P-labeled cDNA probe specific for IL-16. RNA loading was assessed by hybridization with a \(\beta\)-actin probe. The positions of the 18S and 28S ribosomal RNAs are indicated to the left of the figure.

FIGURE 6. Effect of anti-CD28 costimulation on IL-16 mRNA. Northern hybridization analysis of total RNA extracted from anti-CD3- and anti-CD28-stimulated PBMCs for 0, 1, 2, 4, and 6 h was performed using \(^{32}\)P-labeled cDNA probe specific for IL-16. Equal RNA loading was assessed by hybridization with a \(\beta\)-actin probe. The positions of the 18S and 28S ribosomal RNAs are indicated to the left of the figure.

FIGURE 7. Effect of anti-CD28/anti-CD3 costimulation on IL-16 mRNA stability. CD4\(^+\) T cells were incubated in the presence (lanes 1–4) or absence (lanes 5–8) of anti-CD28 Ab (1 \(\mu\)g/ml) and anti-CD3 Ab (2.5 \(\mu\)g/ml) for \(\leq\) 4 h. Total RNA was extracted at time 0 (lanes 1 and 5), 1 h (lanes 2 and 6), 2 h (lanes 3 and 7), and 3 h (lanes 4 and 8) after the addition of actinomycin D (10 \(\mu\)g/ml). IL-16 mRNA was analyzed by Northern hybridization using a \(^{32}\)P-labeled cDNA probe specific for IL-16. The amounts of RNA loaded in each lane were compared by hybridization with a \(\beta\)-actin probe and ethidium bromide staining. The positions of the 18S and 28S ribosomal RNAs are indicated to the left of the figure.

FIGURE 8. Time course for the activation of caspase-3 in CD3- and CD28-costimulated CD4\(^+\) T cells. CD4\(^+\) T cells were stimulated for 4 or 16 h, at which time total cellular protein was subjected to SDS-PAGE followed by Western blot analysis for the active subunit of caspase-3 (P20). Cells were either left unstimulated (lane 1), stimulated with anti-CD3 for 4 (lane 2) or 16 (lane 4) h, or stimulated with anti-CD3 and anti-CD28 for 4 (lane 3) or 16 (lane 5) h. The presence of caspase-3 was detected using an Ab against its P20 subunit followed by a display of chemiluminescence.
required for expression of bioactive IL-16. It is interesting to note that treatment with the caspase-3 inhibitor did not appear to affect non-IL-16-induced migration for either CD3 alone or for CD3/CD28-costimulated cells, suggesting that IL-16 is the only detectable chemoattractant processed by caspase-3. To confirm the bioactivity of the caspase-1 and -3 inhibitors, their effect on CD3-stimulated apoptosis was assessed at the 24-h timepoint by propidium iodide staining. Based on apoptotic staining, cells treated with the inhibitors demonstrated 23% and 18% apoptotic staining for caspase-1 and -3 inhibitors, respectively, compared with 57% apoptotic staining for untreated cells. This represents a 60.7% and 69.5% decrease in apoptotic cells for the caspase-1 and -3 inhibitors, respectively, compared with untreated cells. Therefore, the data indicate that the caspase-1 inhibitor was functionally active in reducing apoptosis but had no effect on IL-16 release. These findings suggest that the release of bioactive IL-16 is coincident with and dependent upon activation of the enzyme that facilitates its cleavage, caspase-3, and not on transcriptional regulation of the IL-16 mRNA.

Discussion

We have shown previously that mitogen-activated T cells secrete IL-16 (3, 4), and that CD8+ T cells are a major source of this cytokine after 24 h of stimulation (3, 4, 7, 8). Interestingly, there is a pool of preformed, processed bioactive IL-16 in CD8+ T cells that is released within 4–6 h after stimulation with histamine and within 2 h after stimulation with serotonin (7, 8). Although CD4+ T cells contain constitutive IL-16 mRNA and pro-IL-16 protein (9), they contain no stored bioactive IL-16, nor do they release any IL-16 following stimulation with histamine or serotonin (7, 8). The present study demonstrates for the first time that CD4+ T cells are a source of bioactive IL-16 that is secreted following stimulation via the TCR by either specific Ag or anti-CD3 Ab. Although CD8+ T cells secrete IL-16 after anti-CD3 stimulation within 6 h, most of the released IL-16 appears to derive from the preformed bioactive pool, as demonstrated by studies with inhibitors of protein synthesis. However, in CD4+ T cells, the kinetics of secretion of bioactive IL-16 differ from those observed in CD8+ T cells. CD4+ T cells require 24 h to release bioactive IL-16 following anti-CD3 stimulation. This process can be accelerated by costimulation with anti-CD3/anti-CD28, which results in the release of bioactive IL-16 by 6 h. CD3 activation of CD4+ T cells does not result in increases in IL-16 mRNA or in changes in mRNA stability. Rather, we observed that anti-CD3 activation of CD4+ T cells results in the activation of caspase-3 from its proenzyme state, presumably permitting the processing of constitutively expressed pro-IL-16. CD3/CD28 costimulation resulted in a more rapid activation of caspase-3, which correlated with an accelerated secretion of bioactive IL-16. In contrast to the CD8+ T cell, CHX inhibits the appearance of bioactive IL-16 in the supernatants of CD4+ T cells costimulated with anti-CD3/anti-CD28. This finding indicates that de novo protein synthesis is required for the secretion of IL-16. Studies designed to identify the required protein(s) are currently ongoing. Dependence of caspase-3 activation for the processing and release of IL-16 does not appear to be shared by any other detectable chemoattractant, as only IL-16-induced migration was affected.

Of interest, in contrast to CD8+ T cells, IL-16 does not represent a majority of the lymphocyte chemoattractant activity identified in CD4+ T cell supernatants following CD3 or CD3/CD28 stimulation. However, the contribution of IL-16 increases proportionally to ~40% of the overall chemoattractant activity during the first 48 h poststimulation. All other chemoattractant activities did not appear following CHX treatment, suggesting new protein synthesis was required. In that regard, members of the CC chemokine family are likely candidates for this activity.

The requirement of caspase activation for protein processing and secretion has been reported previously for both IL-1β (20) and IL-18 (21). Both of these ILs require activation of caspase-1. The efficiency for IL-1α and -1β processing and release is increased with induced apoptosis but not with necrosis (22). The mechanism for the release of IL-16 has not been determined; however, because IL-16 also lacks a signal peptide, there may be some similarities with IL-1α and -1β. Thus far, our data suggest that apoptosis is not required for the processing and release of IL-16 as the prevention of apoptosis with a caspase-1 inhibitor also did not affect the magnitude or the time course for the release of IL-16. However, we have noted that induction of apoptosis with staurosporin does result in an enhanced release of bioactive IL-16 (Y.Z., unpublished observations). These findings indicate that the processing and release of IL-16 by CD4+ T cells is not contingent upon subsequent cell apoptosis, and further supports the concept that, although activation of caspase-3 enzymatic activity is essential for an apoptotic signal (23, 24), activation of caspase-3 alone is not sufficient for the induction of apoptosis (25).

Based on the kinetics data, one could hypothesize that CD8 cells would represent a rapid release mechanism initiated by the mediators of early inflammation, such as vasoactive amines, and would contribute to the recruitment of CD4+ T cells by the secretion of preformed IL-16. The ability of CD4+ T cells to synthesize and secrete IL-16 may serve to function as a positive feedback mechanism for further cell recruitment and activation. A similar situation may occur with another proinflammatory cell, the eosinophil, which has been shown to generate and release IL-16 protein after activation (11).

In summary, we have shown that CD4+ T cells are a source of IL-16 following TCR activation or CD28 costimulatory conditions. The active form of caspase-3, an important mechanism for further processing of pro-IL-16 and the secretion of bioactive IL-16, can be identified in activated CD4+ T cells in a time course...
that is similar to that seen for IL-16 release. However, an apparent separation does exist between the release of IL-16 following caspase-3 activation, pro-IL-16 processing, and the induction of apoptosis. In addition, our data demonstrate that the mechanisms and requirements for the synthesis and release of IL-16 from CD4+ and CD8+ T cells are entirely different. Taken together, these studies suggest that Ag-activated CD4+ T cells could be a source of IL-16, which might amplify the accumulation of unsensitized CD4+ T cells at sites of Ag-induced inflammation.

References


