Culturing of HIV-1-specific cytotoxic T lymphocytes with interleukin-7 and interleukin-15

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Abstract

The ability to study HIV-1-specific cytotoxic T cell (CTL) clones in models in vitro or to expand them for immunotherapeutic use is limited by the technical difficulty of propagating these cells. The factors that determine the survival and proliferation of the cells are incompletely understood and could include cytokines provided from feeder cells or serum. We therefore investigated the effects of adding two cytokines reported to have effects on T cell proliferation and function, interleukin (IL)-7 and IL-15. Four HIV-1-specific clones derived from infected persons were cultured under standard conditions with IL-2 compared to IL-7 or IL-15 alone or in combination with IL-2. Proliferation and survival, as reflected by cell numbers after stimulation, were poorly supported by IL-7 or IL-15 alone, and these cytokines appeared to provide no additional benefit when added to IL-2. Similarly, these cytokines alone did not support the functional status of these cells as measured by chromium release assays with peptide-pulsed target cells. Addition of IL-7 or IL-15 to IL-2 did not augment function of the cells. These data suggest that supplementing CTL cultures with these cytokines does not provide improvement of cell growth or function.

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Introduction

CD8⁺ HIV-1-specific cytotoxic T cells (CTLs) are believed to play an important protective role in immunopathogenesis (reviewed in (Yang and Walker, 1997)) and therefore have been a focus in vaccine design and immunotherapeutics. Recent CD8-depletion experiments in macaques have underscored the effector function these cells play in suppressing viral replication (Jin et al., 1999; Matano et al., 1998; Schmitz et al., 1999), although early attempts at ex vivo expansion of autologous HIV-1-specific CTL and reinfection into infected persons have met with limited success (Brodie et al., 1999, 2000; Koenig et al., 1995; Lieberman et al., 1997; McKinney et al., 1999).

We (Yang et al., 1996, 1997a, 1997b, 2002) and others (Baalen et al., 2002; Buseyne et al., 1996; Schutten et al., 2001; Shankar et al., 1998, 1999; Van Baalen et al., 1998) have therefore been interested in evaluating the determinants of CTL antiviral function in controlled systems with acutely HIV-1-infected target cells. To manipulate and study factors such as CTL epitope specificity, lytic function, cytokine production, viral sequence and accessory gene function, and infection kinetics, highly defined in vitro models utilizing CTL clones are crucial. Such issues are difficult or impossible to study in vivo, where correlative data, at best, are obtainable.

A significant technical barrier to such work, however, is the difficulty of propagating CTL clones. Standard methodology involves providing a T cell receptor signal in the presence of feeder mononuclear cells and interleukin (IL)-2 in medium with serum (Walker et al., 1989), and the factors that determine the survival and proliferation of the cells are incompletely understood. Working with primary CTL clones is a challenging technical barrier as these cells tend to exist in a terminally differentiated state in vivo probably...
due to continuous turnover in the face of persisting HIV-1 infection (recently reviewed in Appay and Rowland-Jones, 2002; Van Baarle et al., 2002)) and survive poorly once isolated in vitro.

We therefore evaluated the ability of two cytokines known to have effects on T cell development, survival, and function: interleukin IL-7 and IL-15. Because the factors provided by feeder cells and serum influencing the ability of CTL to proliferate are poorly defined, these cytokines were tested as potential candidates for increasing the efficiency of CTL propagation.

Results

Neither IL-7 nor IL-15 appears to augment proliferation of HIV-1-specific CTL clones in culture

Four HIV-1-specific CTL clones were restimulated using anti-CD3 antibody, irradiated allogeneic feeder PBMC, and various cytokines (Fig. 1). A standard condition, utilizing 50 U/ml of IL-2, served as a comparison for tests of IL-7 and IL-15. Either IL-7 or IL-15 alone at “low” (0.3 ng/ml ≥ 600 U/ml) or “high” concentrations (1 ng/ml ≥ 2000 U/ml) did not support growth as well as IL-2, yielding cell numbers of about 40–50% by comparison. When these concentrations of IL-7 and IL-15 were added to IL-2, cell numbers after expansion were not significantly changed compared to IL-2 alone, ranging from about 100% to 120% by comparison. These data indicated that neither IL-7 nor IL-15 significantly boosted the proliferation or survival of the CTL clones, over that of IL-2 alone, whether given alone or in conjunction with IL-2.

Neither IL-7 nor IL-15 appears to augment lytic function of HIV-1-specific CTL clones in culture

The CTLs grown under the above conditions were also compared for their lytic capacity by chromium release assays (Fig. 2). The killing capacity of these clones was suboptimal at baseline in culture with IL-2 alone (about 50% specific lysis). Culturing with IL-7 alone resulted in a relative loss of killing capacity on a per-cell basis (about 40% that of IL-2 alone). There appeared to be a dose-dependent trend for increased cytolytic activity by IL-15 alone in comparison to IL-7, although at the higher dose (1 ng/ml ≥ 2000 U/ml), killing was still about 75% the level of that with IL-2 alone. Combining either IL-7 or IL-15 with IL-2 perhaps slightly increased the killing capacity of the cultured CTL to about 105–110% of that with IL-2 alone, although the increases were not statistically significant. Overall, these data suggested that IL-7 and IL-15 were inferior or of minimal benefit compared to IL-2 alone in preserving or augmenting the cytolytic function of CTL clones.

![Fig. 1. Growth of HIV-1-specific CTL clones cultured with IL-2, IL-7, and IL-15. Four clones were restimulated in parallel in medium containing IL-2 at 50 U/ml, IL-7 at 0.3 ng/ml (low) or 1 ng/ml (high), IL-15 at 0.3 ng/ml (low) or 1 ng/ml (high), or combinations of IL-2 with IL-7 or IL-15. Restimulation was performed with anti-CD3 and irradiated allogeneic feeder PBMC, and cell concentrations were monitored after 1 week. Under the standard condition of IL-2 alone, the clones proliferated to a mean concentration of 5.7 × 10⁶/ml, standard deviation 1.2 × 10⁷. Plotted here are the ratios of cell concentration under the various conditions versus IL-2 alone. Error bars represent one standard deviation. These results were repeated and confirmed using two additional CTL clones with IL-7 and IL-15 (known to be bioactive in a human thymocyte culture system) from other sources (data not shown).](image-url)
Discussion

A major limitation of culturing HIV-1-specific CTL for immunotherapeutic infusions and in vitro functional studies is their expansion. Despite the fact that antiviral CTL responses are easily detectable and fairly high frequency in the majority of infected persons, deriving and maintaining CTL clones are technically demanding. This may be due to exhaustion or abnormal differentiation due to chronic turnover in response to persistent antigen over years of infection as suggested by multiple studies of CTL phenotype in chronically infected persons (Appay and Rowland-Jones, 2002; Van Baarle et al., 2002). We and others have been interested in studying the interaction of defined CTL clones with HIV-1 by in vitro models, and this line of investigation has been limited in a large part by the limited availability of CTL clones, as reflected by the relatively small number of published studies in this area. We therefore evaluated two cytokines reported to have effects on T cell growth and survival to test whether they might enhance the proliferative capacity of HIV-1-specific CTL in vitro.

The cytokine IL-7 likely has a central role in T cell development and survival. Multiple studies suggest that IL-7 is an important factor in the thymus from the development from thymocyte precursors to early T cells (Conlon et al., 1989; Fabbi et al., 1992; Hickman et al., 1990; Kim et al., 1998; Von Freeden-Jeffry et al., 1997). Additional evidence suggests that IL-7 continues to have modulatory effects on mature T cells, contributing to the survival and expansion of naive (Soares et al., 1998) and memory (Welch et al., 1989) T cells, presumably through costimulation in conjunction with T cell receptor signaling (Gringhuis et al., 1997). Two reports have suggested that IL-7 might be useful in stimulating and expanding HIV-1-specific T cells (Ferrari et al., 1995; Kim et al., 1997).

IL-15 has also been reported to have effects on T cell survival and proliferation. This cytokine is believed to share overlapping biologic properties with IL-2, acting through the β and γ chains of the IL-2 receptor (Giri et al., 1994; Tagaya et al., 1996) as well as through a specific IL-15 receptor α chain (Giri et al., 1995), perhaps mediating an anti-apoptotic signal (Bulfone-Paus et al., 1997). Some data have indicated that IL-15 may be useful in expansion of CTL in SIV-infected macaques (Kanai et al., 1996) as well as CD8+ T cells (Agostini et al., 1997) and NK/CD4+ lymphocytes (Chehimi et al., 1997) in HIV-1-infected subjects.

Our data, however, did not indicate that either of these cytokines had marked effects on our ability to grow HIV-1-specific CTL clones in culture. Neither proliferation or survival, as reflected by cell concentrations after stimulation, nor lytic capacity was significantly enhanced by IL-7 or IL-15. Use of either of these cytokines alone instead of IL-2 resulted in lower cell numbers and decreased lytic function. Although both IL-7 and IL-15 alone resulted in similarly less proliferation than IL-2 alone, IL-15 some-
what augmented killing activity in a dose-dependent fashion, perhaps due to its partial functional overlap with IL-2.

Use of these cytokines in conjunction with IL-2 did not appear to augment significantly either cell number or function. There are multiple potential explanations. These cytokines could already be provided in feeder cells or culture medium. IL-15 is known to be produced by peripheral blood monocytes (Tagaya et al., 1996), while mature dendritic cells may produce IL-7 (Sorg et al., 1998), although thymic and bone marrow stroma are likely the major sites of IL-7 production (Wiles et al., 1992). Another possibility is that these cells are in a state of activation or differentiation that is not amenable to the action of these cytokines because HIV-1-specific CTLs are believed to be functionally impaired due to chronic turnover or other factor(s). Still another possibility is that the action of these cytokines on CTL is redundant with that of IL-2, particularly in the case of IL-15.

In summary, culturing HIV-1-specific CTL clones with IL-7 or IL-15 was inferior to using IL-2 alone, and the addition of these cytokines to IL-2 did not provide any significant additional advantage. There are several possible explanations why these cytokines did not augment the numbers and function of these cells as reported in other systems, and these possibilities are not mutually exclusive. Our data therefore do not support the use of these cytokines as a means to improve in vitro culture of these cells, although IL-7 and IL-15 may have other activities such as boosting T cell production and memory precursor T cell survival in vivo.

Materials and methods

CTL clones

CTL clones were derived by limiting dilution cloning from PBMC of HIV-1-infected persons and maintained as previously described (Walker et al., 1989). Clone 68A62 (the kind gift of Dr. Bruce D. Walker) recognized the HLA A2-restricted epitope ILKEPVHG (RT aa 309–317) and was isolated by blind limiting dilution cloning from expanded PBMC. Clones 0001/10.7 and 0001/10.18 were independently derived clones recognizing the HLA A2-restricted epitope SLYNTVATL (Gag p17 aa 77–85), each isolated by limiting dilution cloning from a CTL-enriched line produced by immunomagnetic capture of IFN-γ-producing cells (as per manufacturer’s instructions, MACS, Miltenyi). Clone 0010/5.10P was isolated from a CTL-enriched line produced by immunomagnetic capture of IFN-γ-producing cells recognizing an HLA A25-restricted epitope within the peptide sequence FTIPSSINETPGIRY (NIH AIDS Research and Reference Repository catalogue # 5532, RT aa 130–144). Sequence numbers are given relative to HXB2.

Cytokines

Recombinant human IL-2 was obtained from the NIH AIDS Research and Reference Repository. Recombinant human IL-7 and IL-15 were purchased from Biotechdiagnostics, L.L.C. IL-7 and IL-15 were each certified to be >98% pure by SDS-PAGE and HPLC, with a specific activity of >2 x 10⁶ units/mg. For confirmation, the experiments were also repeated with lots of IL-7 from Immunex (Seattle, WA) and IL-15 from Amgen (Thousand Oaks, CA) that have been shown to be bioactive in multiple human thymic culture studies (Gurney et al., 2002; Pedroza-Martins et al., 2002).

CTL restimulation

CTLs that had not been stimulated for at least 7 days were restimulated with the anti-CD3 antibody 12F6 at 0.1 μg/ml, irradiated allogeneic feeder PBMC, and the indicated cytokine(s) in RPMI 1640 (Sigma) supplemented with 10% heat-inactivated fetal calf serum (Hyclone), penicillin-streptomycin, and l-glutamine as previously described (Yang et al., 1996, 1997a). The cells were fed with fresh medium containing the appropriate cytokine(s) after 3 or 4 days, and then counted and tested in chromium release assays after 7 days.

Chromium release assays

Autologous EBV-transformed B cells served as targets for the CTL clones in standard chromium release assays performed as previously described (Yang et al., 1996). Briefly, the target cells were labeled for 1 h with 51Cr with or without the appropriate peptide at 10 μg/ml, followed by washing and plating in a 96-well U-bottom plate at 10⁴ cells/well. The CTL clones were then added at 5 x 10⁴ cells/well in a total volume of 200 μl for a 4-h incubation. Spontaneous and maximal lysis wells utilized medium without CTL and 2.5% Triton x-100 (Sigma), respectively. Supernatants were then harvested to Lumaplates (Packard) and chromium counts were determined by microscintillation counting (Microbeta 1450, Wallac). Percent specific lysis was calculated as: 100 x (experimental release – spontaneous release)/(maximal release – spontaneous release).

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References


earliest T lineage-committed cells depend on IL-7 for Bcl-2 expression and normal cell cycle progression. Immunity 7 (1), 147–154.