Human Immunodeficiency Virus Type 1 (HIV-1) Infection of Herpesvirus Saimiri-Immortalized Human CD4-Positive T Lymphoblastoid Cells: Evidence of Enhanced HIV-1 Replication and Cytopathic Effects Caused by Endogenous Interferon-γ

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Herpesvirus saimiri (HVS) is a nonhuman primate gamma herpesvirus which can immortalize human T lymphocytes similar to Epstein-Barr virus immortalization of B cells. The HVS-immortalized T cell lines can be cloned and they remain functional, including susceptibility of CD4 expressing T cells to infection with human immunodeficiency virus type 1 (HIV-1). In this report, we have used five such HVS-transformed CD4-positive T cell clones to reevaluate the role of endogenous interferon gamma (IFN-γ) in HIV-1 replication in T cells. All five clones had similar phenotypes; and four clones constitutively produced IFN-γ and one clone did not. All five clones could be efficiently infected with HIV-1. HIV-1 infection of the IFN-γ-positive cells also upregulated IFN-γ mRNA production and IFN-γ secretion but not production of IL-2 or IL-4. In contrast, infection of IFN-γ-negative cells did not induce IFN-γ, IL-2, or IL-4. Exposure to anti-IFN-γ antibodies after HIV-1 infection significantly reduced virus production and inhibited virus-induced death of IFN-γ-positive cells but had no effect on IFN-γ-negative cells. We conclude that in CD4-positive T lymphocytes immortalized by HVS endogenous IFN-γ does not inhibit HIV-1 but enhances HIV-1 replication and cytolysis. The potential augmenting effects of IFN-γ on HIV-1 replication in CD4-positive T cells recommend caution in a therapeutic use of this cytokine in AIDS.

INTRODUCTION

The ability of Epstein-Barr virus (EBV) to immortalize efficiently human B lymphocytes in vitro has been very useful in studies on B cell biology, cell transforming function of EBV, and lymphomagenesis (reviewed by Kieff and Liebowitz, 1990). Until recently, no similarly efficient approach existed for the immortalization of human T lymphocytes. In 1992, Biesinger et al. (1992) demonstrated that certain strains of HVS, which, like EBV, is a member of gamma Herpesviruses and which causes T cell lymphomas in New World monkeys (Roizman et al. 1990), can infect and transform human T lymphocytes in vitro. Like EBV-positive B-lymphoblastoid cell lines, HVS-immortalized T lymphoblastoid cell lines (T-LCLs) carry latent virus and grow for extended periods of time without antigenic or mitogenic stimulation (Biesinger et al., 1992; Weber et al., 1993). T-LCLs also maintain important T lymphocyte functions, including functional receptors for antigen, signal transduction through TCR, CD3, CD4, or IL-2 receptors (Broker et al., 1993; Del Prete et al., 1994; Mitrucker et al., 1993; Saha et al., 1996; Weber et al., 1993), and B cell help (Del Prete et al., 1994; Saha et al., 1996). In contrast to the majority of the CD4-positive human T cell lines of tumor origin, CD4-bearing T-LCLs are susceptible to highly productive infection with both laboratory and primary strains of HIV-1 and HIV-2 (Nick et al., 1993). Thus, HVS-immortalized T-LCLs mimic many respects normal T lymphocytes and they can be used for studies of T cell biology and function in long-living clonal T cells. In the present work, we investigated whether such T cell clones can be used to resolve the controversial role of IFN-γ in HIV-1 infection in T cells.

Cytokines play important but complex roles in AIDS pathogenesis (Poli and Fauci, 1993; Barker et al., 1995; Clerici and Shearer, 1994). Progression to AIDS appears to correlate with a shift from Th1 to Th2 cytokines (e.g., there is a decrease in IFN-γ), but the significance of these changes is not clear (Clerici and Shearer, 1994). Cytokines like TNFα induce HIV-1 expression in vitro (Poli et al., 1990; Tadmori et al., 1991), while IFNα2 inhibits virus production (Brinchman et al., 1991). The Th1 cytokine IFN-γ has dichotomous effects on HIV-1 replication in macrophages and T cells, the two main target cells for HIV-1 infection (Poli and Fauci, 1993). While IFN-γ protects primary macrophages and mononuclear cell lines against HIV-1 infection (Fan et al., 1994; Dhawan et al., 1994; Hammer et al., 1986), its effect on HIV-1 infection of T cells varies from enhancement (Kinter et al., 1995; Vyakaram et al., 1990) to inhibition (Wong et al., 1988),...
with several studies showing little or no effect (Yamamoto et al., 1986; Hartshorn et al., 1987). The inconsistent effects of IFN-γ on HIV-1 replication in T cells can be attributed to the experimental systems used, which in most cases included unfractonated PBMC rather than isolated CD4-positive T lymphocytes. Other, more extensively studied cytokines such as TNF-α regulate HIV-1 expression through both autocrine and paracrine pathways (Poli et al., 1990; Tadmori et al., 1991; Kinter et al., 1995), but the function of endogenous IFN-γ in HIV-1 replication in T cells is unknown. In this work, we have employed HVS-immortalized T-LCLs as a means to address these problems. First, we were able to clone T-LCLs to ensure the uniformity of T cell populations studied. Second, to determine the role of endogenous IFN-γ in HIV-1 replication, we have examined HIV-1 infection in phenotypically similar T cell clones which differed in their expression of IFN-γ. We report that in this system of long-lived CD4-positive T cell clones, endogenous IFN-γ augments HIV-1 replication and facilitates cytolsis of infected cells.

MATERIALS AND METHODS

Cells and viruses

Establishment of HVS-immortalized CD4+ T cell clones has been described (Saha et al., 1996). Briefly, PBL were isolated from freshly drawn blood from HIV-1-negative donors on Ficoll–Hypaque gradient (Sigma Chemicals, St. Louis, MO) and adherent cells were separated by incubation on plastic tissue culture plates for 2 hr. Nonadherent cells were resuspended at 1 × 10⁶ cells/ml in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, penicillin (100 units/ml), and streptomycin (100 μg/ml) and infected with HVS at a multiplicity of infection of 0.1. Infected cells were cultured in bulk for 2–3 weeks until immortalization was evident and then cloned at 0.5–1.0 cells/well in 96-well culture plates with irradiated allogeneic PBL (100,000/well). The two CD4+ T cell clones, CHCD4 and KRC4D, that have been studied most extensively in this report were derived from PBL of two different donors and were maintained in the absence of exogenous IL-2 for over 1 year. Other IFN-γ-producing CD4+ T cell clones used in this study are MC2-54, MC2-11, MC3-12, and DECD4; they were similarly developed from two other normal donors and maintained in IL-2 (20 units/ml) containing medium. Consistent with other reports (Biesinger et al., 1992; Del Prete et al., 1994), the transformed cells carried HVS genomes as determined by polymerase chain reaction (PCR) amplification of HVS DNA (not shown) and did not produce HVS as determined in a coculture assay (Berend et al., 1993). HVS, group C, strain 488-77, was kindly provided by R. C. Desrosiers (Harvard Medical School, Boston, MA). The virus was propagated in owl monkey kidney cells and titered by plaque assay in these cells as described (Berend et al., 1993). HIV-1 strain HTLV-IIIb was kindly donated by R. C. Gallo and was propagated and titered by the reverse transcriptase (RT) activity in H9 cells as described (Popovic et al., 1984).

HIV-1 infection

Cells were infected by resuspending 2 × 10⁶ cells/ml in 1 ml of cell-free supernatant from H9/HTLV-IIIb cells containing 5 × 10⁵ cpm of RT activity. The cells were incubated for 2 hr at 37°, thoroughly washed, and resuspended in fresh medium at 2 × 10⁶ cells/ml. Control cells were mock-infected with a filtered, cell-free supernatant from an uninfected H9 cell culture. Anti-cytokine or control (nonimmune) antisera were added at 5 μg/ml as indicated. Viable cells were counted by trypan blue exclusion and supernatants were collected at designated days postinfection (dpi) and stored at −70° for analysis of HIV-1 and cytokine production.

RT and p24 assays

RT assays were performed in triplicates as previously described (Willey et al., 1988). In brief, 5-μl samples were added to 25 μl of a mixture containing poly(C), oligo(dG) (Pharmacia Biotech Inc., Piscataway, NJ), MgCl₂, DTT, NP-40, NaCl, H₂O, and 32P-labeled deoxyguanidine triphosphate (Amersham Corp., Arlington Heights, IL). The mixture was incubated for 2 hr at 37° and 6-μl samples were blotted onto DE81 paper, air dried, washed three times in 2× SSC, and at times in 95% ethanol. The filters were dried under a heat lamp and counted in a Beckman LS scintillation counter.

HIV-1 core antigen p24 was measured using HIV Ag kit (Coulter, Hialeah, FL) according to the manufacturer’s instructions.

Analysis of mRNA

Cellular RNA was isolated using a modified SDS/phe- nom extraction method as described (Liu et al., 1994). Control or HIV-1-infected cells 3 days after infection were washed and lysed in SDS/EDTA/Proteinase K solution. RNA was extracted by phenol followed by phenol/chloro- form/isoamylalcohol, precipitated by ethanol, and dissolved in DEPC-treated water. cDNA was synthesized by incubating 2 μg of oligo dT-primed RNA, MuLV RT, and 1 mM dNTP (Perkin–Elmer–Cetus, Norwalk, CT) in a final volume of 40 μl at 42° for 15 min followed by 5 min at 99° and 5 min at 4°. The cDNA served as a template for amplification of human IL-2, IL-4, IFN-γ, or HIV-1 env-specific sequences by PCR using commercially available primers (Clontech, Palo Alto, CA) and according to manufacturer’s instructions. The amplifications yielded DNA fragments of 305, 344, and 427 base pairs (bp) for IL-2, IL-4, and IFN-γ, respectively. Each RNA sample was subjected to the reverse transcription step with or without RT to control for cellular DNA contamination. Plasmids containing cytokine genes were used as positive controls.


Cytokine assays

Culture supernatants from uninfected or HIV-1-infected cells were collected at 3 days after infection and were stored in aliquots at −70°C. Levels of IL-4 and IFN-γ were measured by ELISA using commercial kits for human IL-4 (Genzyme Framingham, MA) and IFN-γ (BioSource International, Camarillo, CA) according to the manufacturers’ directions. Levels of IL-2 were measured using IL-2 responsive CTLL cell line as previously described (Mosmann et al., 1986).

Flow cytometry (FACS) analyses

FACS analyses of T cells were performed using cell suspensions as previously described (Saha and Wong, 1992) using either FITC- or PE-labeled monoclonal antibodies to CD4 and CD8 (Sigma, St. Louis, MI); TCR-v/β, CD20, CD25 (Tac) (Becton-Dickinson, San Jose, CA); and CD3 and HLA-DR (Coulter Corp., Hialeah, FL). TCR-v/β gene rearrangements were identified using a panel of anti-v/β antibodies, including V/β2 and V/β3 (Amac Co, MA) and V/β5a, 5b, 5c, 6a, 8a, 12a, 13, and 17 (T Cell Sciences, Cambridge, MA).

RESULTS

Characterization of HVS-immortalized T cell clones

The HVS-immortalized cells were characterized by surface marker analysis at various times after cloning and the overall phenotypes of two representative clones, CHCD4 and KRCD4, are summarized in Table 1. Both clones expressed CD4 and other surface markers typical for activated T lymphocytes, but not CD8 or CD20 (B cell marker). Notably, CHCD4 but not KRCD4 expressed the T cell receptor V/β2 chain, indicating that HVS does not selectively immortalize T cells carrying a specific rearranged TCR-V/β chain, consistent with our previous data (Saha et al., 1996). Previous studies indicated that HVS-immortalized human CD4-positive T-LCLs expressed predominantly IFN-γ, a Th1 cytokine, but not IL-4, a Th2 cytokine (DeCarli et al., 1993; Saha et al., 1996). We tested CHCD4 and KRCD4 cells for the expression of IL-2, IL-4, and IFN-γ at the protein and mRNA levels. Significant levels of IFN-γ were detected in the supernatants of CHCD4 but not KRCD4 cells (Table 2). High level production of IFN-γ was also detected in MC2-5.4, MC2-11, MC3-1.2, and DEC4 clones used in this study and in 20 other CD4+ T cell clones established in our laboratory (not shown). Thus, KRCD4 is the only HVS-transformed CD4+ T cell clone described thus far which exhibits IFN-γ-negative phenotype. Stimulation of CHCD4 cells (TCR-V/β2) with TSST (a V/β2-specific superantigen) resulted in increased IFN-γ levels, indicating that the sig-

TABLE 1 Phenotypes of CHCD4 and KRCD4 Cells

<table>
<thead>
<tr>
<th>Surface molecule</th>
<th>CHCD4</th>
<th>KRCD4</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD8</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>TCRv/β</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD20</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>CD25</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD69</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TCR-V/β2</td>
<td>+</td>
<td>UD b</td>
</tr>
</tbody>
</table>

a Data from FACS analyses at various times after cell cloning. b UD, undetectable with the panel of anti-V/β antibodies used.

Antibodies

Rabbit antiserum to IFN-γ, IL-2, and IL-4, and nonimmune rabbit serum used as control, were obtained from Genzyme, (Framingham, MA). Sera were heat inactivated at 56°C for 30 min before use. Biological activities of the antibodies were tested on appropriate cells (Saha et al., 1996).

TABLE 2 Expression of IL-2, IL-4, and IFN-γ in CHCD4 and KRCD4 Clones before and after HIV-1 Infection

<table>
<thead>
<tr>
<th>Cells</th>
<th>Treatment</th>
<th>IL-2 (U/ml)</th>
<th>IL-4 (pg/ml)</th>
<th>IFN-γ (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHCD4</td>
<td>None</td>
<td>&lt;0.01</td>
<td>&lt;45</td>
<td>10,464 ± 2,390</td>
</tr>
<tr>
<td>KRCD4</td>
<td>None</td>
<td>&lt;0.01</td>
<td>&lt;45</td>
<td>&lt;15</td>
</tr>
<tr>
<td>CHCD4</td>
<td>HIV-1</td>
<td>&lt;0.01</td>
<td>&lt;45</td>
<td>18,920 ± 3,170</td>
</tr>
<tr>
<td>KRCD4</td>
<td>HIV-1</td>
<td>&lt;0.01</td>
<td>&lt;45</td>
<td>&lt;15</td>
</tr>
</tbody>
</table>

a Cytokines were measured as described under Materials and Methods by using CTTL cell line for IL-2 and ELISA for IL-4 and IFN-γ. The detection limits were 0.01 U/ml for IL-2, 45 pg/ml for IL-4, and 15 pg/ml for IFN-γ. Positive and negative samples were included in all tests. Results are of three separate experiments ±SEM.

b Cells were infected with HIV-1 and supernatants were collected at Day 3 for cytokine assay as described under Materials and Methods.
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FIG. 1. Expression of IFNγ and HIV-1 envelope mRNA in uninfected and HIV-1-infected CHCD4 and KRCD4 cells. Cells were infected by HIV-1 and RNA was isolated after 3 days as described under Materials and Methods. RT-PCR was performed using IFNγ-specific primers. Lanes 7–15 are with β-actin primers and lanes 16–18 are with HIV-1 env-specific primers. Lane 1, mol wt marker; lane 2, uninfected CHCD4; lane 3, HIV-1-infected CHCD4; lane 4, uninfected KRCD4; lane 5, HIV-1-infected KRCD4; lane 6, IFNγ-positive control; lane 7, uninfected CHCD4; lane 8, HIV-1-infected CHCD4; lane 9, uninfected KRCD4; lane 10, HIV-1-infected KRCD4; lane 11, β-actin-positive control; lane 12, uninfected CHCD4 without RT step; lane 13, HIV-1-infected CHCD4 without RT step; lane 14, uninfected KRCD4 without RT step; lane 15, HIV-1-infected KRCD4 without RT step; lane 16, HIV-1-infected CHCD4; lane 17, HIV-1-infected KRCD4; lane 18, HIV-1-positive control.

HIV-1 infection and effect on cytokine expression

HVS-transformed T cells are highly permissive to HIV-1 (Nick et al., 1993). We tested whether the observed differences in IFNγ expression by KRCD4 and CHCD4 cell lines affect their susceptibility to a laboratory strain HTLV-III b (Figs. 1–3). HIV-1 efficiently replicated in both KRCD4 and CHCD4 cells but virus infection was faster and production was higher in CHCD4 cells compared to KRCD4 cells (Fig. 2A). This may be because CHCD4 cells grew faster than KRCD4 cells (Fig. 3). However, as determined by RT-PCR for HIV-1 V3-loop-specific mRNA, both cells expressed viral envelope-encoding mRNA 3 days after infection (Fig. 1, lanes 16 and 17). Exposure to HIV-1 enhanced the proliferative proliferation of CHCD4 and KRCD4 cells early after infection, but to different levels (Fig. 3). Consistent with a previous report (Nick et al., 1993), HIV-1 infection in these cells was cytopathic and most of the cells in both systems died a short time after reaching peak HIV-1 production (Fig. 3).

To test whether HIV-1 infection affected cytokine expression in CHCD4 and KRCD4 cells, total cellular RNA was isolated from infected cells 3 days after infection and the expression and relative levels of mRNA for IL-2, IL-4, and IFNγ were tested by RT-PCR with uninfected cells serving as controls (Fig. 1). The KRCD4 cells remained negative for IFNγ mRNA regardless of productive HIV-1 infection (lane 5). In contrast, the IFNγ mRNA levels were higher in HIV-1-infected CHCD4 cells (lane 3) than in uninfected cells (lane 2). In agreement with IFNγ mRNA analysis, supernatants of HIV-1-infected CHCD4 cells contained increased levels of IFNγ, but no IFNγ was detected in the supernatants of infected and uninfected KRCD4 cells (Table 2). IFNγ was also upregulated after HIV-1 infection of other IFNγ-producing cell lines (data not shown). HIV-1 infection had no effect on the expression of IL-2 or IL-4, which remained at the background levels (Table 2) and no IL-2 or IL-4 mRNA was detected by RT-PCR (not shown). These results indicate that HIV-1 infection enhances the transcription and production of IFNγ in CHCD4 cells; however, the increased levels of the endogenous and secreted cytokine appear to have no inhibitory effect on HIV-1 replication in these cells.

Effects of neutralization of IFNγ

To determine whether endogenous IFNγ plays any role in HIV-1 infection in CHCD4 cells, we tested whether neutralizing antibodies against the cytokine modulate the course of HIV-1 replication in these cells. The IFNγ-negative KRCD4 cells served as controls in these experiments. CHCD4 and KRCD4 cells were infected with HIV-1, cultured in the presence of neutralizing anti-IFNγ or control antibodies, and virus production was determined by monitoring the RT activity in culture supernatants (Fig. 2B). The presence of anti-IFNγ antibodies had no effect on HIV-1 replication in KRCD4 cells. In contrast, addition of these antibodies significantly inhibited virus replication in CHCD4 cells, particularly early after infection (Fig. 2B). We have previously shown that polyclonal anti-IFNγ antibodies could effectively neutralize endogenously produced IFNγ from HVS-transformed T cells (Saha et al., 1996). The anti-IFNγ at the dose used completely neutralized IFNγ present in culture supernatants of uninfected CHCD4 cells; no IFNγ was detected in these cultures during initial 5–6 days, but cytokine levels gradually increased thereafter (data not shown). Addition of fresh anti-IFNγ antibodies to HIV-1-infected CHCD4 cells at 4- to 5-day intervals did not significantly change the pattern of HIV-1 replication obtained upon single anti-IFNγ treatment (data not shown) as other factors are also likely to be involved in HIV-1 infection of these cells. Presence of
FIG. 2. Infection of IFN-\( \gamma \)-producing (CHCD4) and IFN-\( \gamma \)-negative (KRCD4) cells by HIV-1 (A) and effects of anti-IFN-\( \gamma \) antibodies on HIV-1 production (B). Cells were infected with HIV-1 and cultured in the presence or absence of anti-IFN-\( \gamma \) or control antibodies as described under Materials and Methods. Virus production was monitored by supernatant reverse transcriptase (RT) assay at the times indicated.

anti-IFN-\( \gamma \) antibodies also significantly reduced the cytopathic effects of HIV-1 infection in CHCD4 cells but had little or no effect on viability of infected KRCD4 cells (Fig. 3). In spite of the initial reduction in virus production, HIV-1-infected CHCD4 cells cultured in the presence of anti-IFN-\( \gamma \) continued to produce HIV-1 at lower levels (Fig. 2B) and most of the cells eventually died, similar to infected cells cultured in the absence of the antibody (Fig. 3). Treatment of uninfected CHCD4 cells with anti-IFN-\( \gamma \) antibodies did not affect cell viability or growth rate as determined by trypan blue exclusion or \([^{3}H]\)thymidine incorporation methods, respectively (data not shown), in-
caution in using IFNγ for control of HIV-1 infection in patients.

The studies reported here have been made possible by the recent development of the efficient method of human T lymphocytes immortalization by HVS (reviewed by Meinl et al., 1995). HVS-immortalized CD4-positive T lymphocytes maintain normal T cell functions, including their susceptibility to efficient HIV-1 infection (Nick et al., 1993). Although the majority of the HVS-immortalized T cell clones described thus far exhibit the Th1 profile, including IFNγ expression (De Carli et al., 1993; Saha et al., 1996), we have identified one T cell clone, KRCD4, which does not express IFNγ (Table 1). Comparison of the KRCD4 cells with the phenotypically similar but IFNγ-producing CD4+ T cell clones permitted us to determine the role of endogenous IFNγ in HIV-1 replication in this model system. Three conclusions can be drawn from these studies:

(i) Contrary to its antiviral function during HIV-1 infection of macrophages (Fan et al., 1994), endogenous IFNγ does not inhibit HIV-1 infection and virus-mediated cytolysis in cloned immortalized T lymphocytes. In fact, HIV-1 replicated faster and to higher levels in IFNγ-positive CHCD4 cells than in IFNγ-negative KRCD4 cells (Fig. 2), indicating that inhibitory effect of the antibody on virus production is not due to antibody effects on target cell physiology. The inhibitory effects of anti-IFNγ antibodies on HIV-1 replication were surprising in that they contradict the postulated role of this cytokine in controlling HIV-1 infection in T cells (Wong et al., 1988). To confirm this phenomenon, we tested the effect of anti-IFNγ antibodies on HIV-1 replication in four other HVS-immortalized T cell clones which constitutively express IFNγ at the levels comparable to that of CHCD4 cells (not shown). As shown in Fig. 4, neutralization of IFNγ significantly inhibited HIV-1 production in a range of 37–56% in all four CD4+ T cell clones. Like CHCD4 cells, although HIV-1 production in these clones was not completely arrested after anti-IFNγ treatments, rate of virus replication (Fig. 4) and virus-induced cytolysis (data not shown) were significantly inhibited. Together, these results demonstrate that not only does endogenous IFNγ fail to inhibit HIV-1 infection in HVS-immortalized CD4+ T cells, but that it can significantly enhance virus replication and its cytopathic effects in these cells.

DISCUSSION

The role of IFNγ in HIV-1 infection of T cells is controversial (Poli and Fauci, 1993). Using phenotypically similar HVS-immortalized T cell clones that differ in their expression of IFNγ, we demonstrate here that endogenous IFNγ does not inhibit HIV-1 infection in T cells. To the contrary, our data suggest that endogenous IFNγ enhances HIV-1 replication and virus-mediated cytolysis in this model. These results recommend caution in using IFNγ for control of HIV-1 infection in patients. 

FIG. 3. Cytopathicity of HIV-1 infection and effect of anti-IFNγ antibodies in IFNγ-producing (CHCD4) and IFNγ-negative (KRCD4) cells. Cells were infected as described in the legend to Fig. 2 and cell viability was determined by trypan blue exclusion at the times indicated.

FIG. 4. Inhibition of HIV-1 production in HVS-immortalized CD4+ T cell clones by anti-IFNγ antibodies. Cells were infected with HIV-1 as described for the experiment described in the legend of Fig. 2. Anti-IFNγ or control antibodies (5 μg/ml) were added to each clone as indicated. Culture supernatants were collected after 7 days and assayed for HIV-p24 production. Percentage inhibition by anti-IFNγ antibodies was calculated in respect to virus production in the presence of control antibodies.
although this difference could also be attributed to the observed variability among HVS-immortalized T cells in HIV-1 replication (Nick et al., 1993). Other HVS-immortalized CD4-positive T cell lines we established from PBL of normal donors (Saha et al., 1996) which constitutively express IFNγ are also susceptible to efficient HIV-1 infection (data not shown). The lack of antiviral activity of IFNγ in T lymphocytes observed here is consistent with the study of Brinchmann et al. (1991) and Kinter et al. (1995) but contradicts the results of Wong et al. (1988), who showed that exogenous IFNγ alone or in combination with TNF-α inhibited HIV-1 production in T cell lines and primary T cells. One explanation of these discrepancies is the use of unfractonated PBMC in the cited studies, a problem which has been alleviated here by employing immortalized T cell clones differing primarily in the expression of secretable IFNγ. These results indicate that the anti-HIV-1 activity of IFNγ is target cell dependent; the cytokine efficiently blocks HIV-1 infection of macrophages but has no such effect in T lymphocytes.

(ii) Surprisingly, instead of virus inhibition, endogenous IFNγ-enhanced HIV-1 replication in our clones. Neutralization of IFNγ produced by CHCD4 cells (Fig. 2B) and other CD4-positive clones (Fig. 4) significantly reduced virus production. As expected, anti-IFNγ antibodies had no effect on HIV-1 production in the cytokine-negative KRCD4 cells (Fig. 2B). The mechanism of HIV-1 enhancement by IFNγ is unknown, but it may involve some of the regulatory effects of interferon on the expression of cellular genes (Samuel, 1991) or augmentation of HIV-1 gp41 binding to cell surface, as shown using BJAB cell line (Chen et al., 1993). Since HIV-1 replication invariably results in cytolysis of primary T lymphocytes (Fauci, 1993), our results also indicate that endogenous IFNγ may be responsible for enhanced lysis of CD4-positive T cells in HIV-1-infected individuals simply by increasing HIV-1 production, in addition to the postulated role of this cytokine in programmed cell death (Groux et al., 1992; Liu and Janeway, 1990).

(iii) HIV-1 infection upregulates IFNγ production in T cells which constitutively express the cytokine but not in those which do not express IFNγ (Fig. 1 and Table 2). It is unclear how HIV-1 upregulates IFNγ in most of the HVS-transformed T cells or why no induction was observed in KRCD4 cells. Recent studies have shown that crosslinking of CD4 receptors induces IFNγ and Fas-mediated apoptosis of T cells (Oyaizu et al., 1994) and this mechanism could be defective in KRCD4 cells. On the other hand, it has been shown that HIV-1 gp160 suppresses production of IFNγ in Th0 type CD4-positive T cell lines (Hu et al., 1994) and such a mechanism could be effective in KRCD4 cells but not in other HVS-immortalized T cells. Fan et al. (1993) have demonstrated increased IFNγ and decreased IL-2 expression on a per cell basis in CD4-positive T cells and increased IFNγ has been associated with the falling levels of CD4-positive T cells in vivo. The upregulation of IFNγ production by HIV-1 may contribute to HIV-1 pathogenesis in vivo in several ways, including enhancement of T cell apoptosis (Fauci, 1993, Groux et al., 1992; Clerici et al., 1994), enhanced T cell death through stimulation of HIV-1 replication (Fig. 3), or deleterious effects in cellular gene expression (Balkwill and Burke, 1989; Atwood et al., 1994). IFNγ may also cause harmful effects indirectly, as proposed for brain injury in AIDS through activation of brain macrophages (Gendelman et al., 1994). Although HVS-immortalized T cells do not exactly represent primary T cells and we cannot completely rule out the possibility that other HVS-specific or cellular genes differentially expressed in these cells influenced HIV-1 infection, our results here demonstrate that at least in this system, production of endogenous IFNγ by CD4+ T cells enhances HIV-1 replication.

In conclusion, our results add to the complex picture of the roles of IFNγ and other cytokines in HIV-1 infection. IFNγ augments anti-viral activities of cytotoxic cells (Lolli et al., 1994; Fauci, 1993) and protects monocyte/macrophages against HIV infection (Gendelman et al., 1990). However, IFNγ enhances HIV-1 replication in CD4-positive T cells (Kinter et al., 1995, and this work). The contradictory roles of IFNγ in HIV-1 infection in different cell types may explain the lack of success in one clinical trial of this cytokine in AIDS patients (Agosti et al., 1992). Another study reported rapid exacerbation of Kaposi's sarcoma in an AIDS patient on IFNγ therapy and the authors recommended caution in using IFNγ in HIV-1-infected patients (Albrecht et al., 1994). The system described here will permit detailed studies on the interaction between IFNγ and HIV-1 in T lymphocytes with the aim of uncoupling their reciprocal stimulation of expression.

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HIV-1 INFECTION OF HVS CD4-POSITIVE T CELLS


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