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Differential Effect of the Immunomodulatory Hormone Somatostatin on Replication of Human Immunodeficiency Virus Type 1 in CD4$^+$ and CD8$^+$ T Lymphocytes

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The long-acting somatostatin analog octreotide (SMS 201-995) possesses immunosuppressive properties and has been successfully used for the management of human immunodeficiency virus (HIV)-associated diarrhea, a condition commonly observed in the absence of known enteric pathogens. Since HIV type 1 (HIV-1) replication can occur in both CD4$^+$ and CD8$^+$ lymphocytes, we hypothesized that this benefit might be due to local effects on HIV-1 replication in these two T-cell subsets. As a model, we studied the effects of two synthetic molecules, SRIH 1-14 and SRIH 1-28, closely related to naturally occurring forms of somatostatin, as well as SMS 201-995 on HIV-1 replication in CD4$^+$ and CD8$^+$ cells derived from peripheral blood mononuclear cells (PBMC). We found that HIV-1 replication was inhibited in CD8$^+$ cells but enhanced in infected CD4$^+$ lymphocytes, as measured by p24 antigen levels in culture fluids. These differential effects were drug concentration dependent. We also observed that somatostatin inhibited the mitogen-induced proliferative responsiveness of both cell types. These effects on both HIV-1 replication and cell proliferation were independent of somatostatin gene expression, since somatostatin mRNAs were not detected in mitogen-stimulated PBMC, as determined by reverse transcription-PCR.

AIDS is commonly associated with chronic diarrhea, with incidence rates among AIDS patients ranging from 30 to 60% in the industrialized countries of North America and Europe to 60 to 90% in developing countries (30). A number of enteric pathogens, including viruses, bacteria, protozoa, and fungi, have been linked to the etiology of human immunodeficiency virus type 1 (HIV-1)-associated diarrhea, but 25% of cases are unexplained (30). In the absence of identifiable microbial agents, HIV-1 itself has been proposed as a possible factor, since the gastrointestinal (GI) tract is the largest lymphoid organ in the body and therefore constitutes a major target for HIV-1. Numerous studies have confirmed the infection of lymphocytes by HIV-1 in the lamina propria of the GI tract (13, 18).

Distinct subsets of CD8$^+$ cells have been shown to be susceptible to infection by HIV-1. Included among these are CD8$^+$ cells which possess natural killer activity (CD16$^+$) but are negative for expression of the T-cell receptor (45). In contrast, CD8$^+$ T cells that are T-cell receptor positive (either αβ or γδ) are relatively resistant to HIV replication (10, 11, 26).

The large intestine contains almost equal numbers of CD4$^+$ and CD8$^+$ cells, while CD8$^+$ cells make up about 70% of intraepithelial lymphocytes (IEL) of the small intestine (7). Some doubly positive CD4$^+$ CD8$^+$ cells have also been identified among the IEL population of both the small and large intestine (7). Peripheral and thymic CD8$^+$ lymphocytes from normal donors can be infected by HIV-1 in tissue culture through either transmission from CD4$^+$ lymphocytes by cell-to-cell contact (10, 26, 33), infection by cell-free virions (11, 39, 45), or infection of doubly positive CD4$^+$ CD8$^+$ cells (23, 38), which may sometimes be abnormally expanded in HIV-1-infected subjects (32). Although certain studies failed to document infection of CD8$^+$ cells sorted from the blood of HIV-1-seropositive subjects, other workers have reported that in vivo, infection of CD8$^+$ cells by HIV-1 can occur in both humans (8, 46) and SCID-hu mice (2, 44).

Somatostatin (somatotropin release-inhibiting hormone [SRIH]) is an immunosuppressive peptide hormone found widely throughout the body. Exposure to SRIH can lead to inhibition of secretion of immunoglobulins (Igs) by B lymphocytes (21), gamma interferon (IFN-γ) (5), and colony-stimulating activity (19) by mononuclear leukocytes, inflammatory mediators such as histamine and leukotriene D_4 by basophils (15), and superoxide anions by monocytes (28). SRIH can also inhibit the growth of mononuclear leukocytes (29). Specific SRIH receptors have been identified at the surface of CD4$^+$ (5, 20, 42, 43) and CD8$^+$ (20, 43) T lymphocytes, B cells (42, 43), monocytes (4), and mast cells (31).

The long-acting somatostatin analog octreotide has been reported to be useful in the management of HIV-associated diarrhea (25, 34). Given the putative involvement of HIV-1 in GI abnormalities (30), we hypothesized that the benefit of octreotide might result from an SRIH-mediated inhibition of HIV-1 replication in CD4$^+$ and CD8$^+$ lymphocytes of the GI tract. To test this notion, we studied the effect of octreotide on CD4$^+$ and CD8$^+$ cells in tissue culture and found that such an effect could be demonstrated for CD8$^+$ cells only. However, SRIH inhibited the mitogen-induced proliferative responsiveness of both cell types.

We were unable to detect SRIH transcripts in phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC), suggesting that somatostatin, in this system, does not act through an autocrine pathway.

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MATERIALS AND METHODS

Cell lines. The TT thyroid medullary carcinoma and U-373MG astrocytoma cell lines were obtained from the American Type Culture Collection (Rockville, Md.) and grown in McCoy's 5A medium (ICN Biomedicals, Inc., Costa Mesa, Calif.) supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 250 U of penicillin per ml, and 250 μg of streptomycin per ml.

Virus production. The HIV-III_B strain of HIV-1 (kindly provided by R. C. Gallo) was grown in the U-937 promonocytic cell line. Viral particles from cell-free culture fluids were pelleted at 25,000 × *g* for 1 h, resuspended at 1/100 the original volume in TNE buffer (20 mM Tris [pH 7.5], 100 mM NaCl, 1 mM EDTA), and frozen at −70°C in small aliquots. The 50% tissue culture infectious dose ($TCID_{50}$) was determined on MT-4 cells by means of an indirect immunofluorescence assay for viral p24 antigen on fixed cells, with mouse monoclonal antibodies (MAbs) against p24, obtained through the National Institutes of Health AIDS Research and Reference Reagent Program. Levels of p24 antigen in culture fluids were determined by a commercially available enzyme-linked immunosorption assay (ELISA; Abbott Laboratories, North Chicago, Ill.).

Infection and purification of T-lymphocyte subsets. Peripheral blood was collected by venipuncture from healthy, HIV-1-seronegative donors; PBMC were purified by centrifugation on a Ficoll-Paque gradient (Pharmacia, Uppsala, Sweden). PBMC were stimulated for 3 days with 10 μg of PHA (PHA-P; Difco, Detroit, Mich.) in RPMI 1640 medium (Gibco Corp., Toronto, Canada) supplemented as described above for cell lines and also containing 10 U of interleukin-2 (Boehringer-Mannheim, Montreal, Canada) per ml. Monocytes and macrophages were removed from PBMC by adherence as described before (26), prior to infection of PBMC by HIV-1. The absence of monocytes and macrophages as well as B lymphocytes was confirmed by the absence of reactivity with the anti-CD14 (Mo2; Coulter Immunology, Sarasota, Fla.) and OKB7 (Ortho Pharmaceuticals, Toronto, Canada) MAbs, respectively, as determined by flow cytometric analysis (26).

Infection of PBMC was performed at a multiplicity of infection of 0.02 $TCID_{50}$ per cell in 500 μl at 37°C for 3 h. Cells were washed twice to remove unadsorbed viral particles and incubated as described below.

Distinct protocols were used for infection of $CD4^+$ and $CD8^+$ cells because the former cannot be efficiently purified after infection by positive selection because of downregulation of cell surface CD4 (37), and because $CD8^+$ cells are more susceptible to HIV-1 infection after coculture with infected $CD4^+$ cells (10, 26, 33). Therefore, $CD4^+$ cells were first purified from uninfected PBMC and then infected with HIV-1. In contrast, $CD8^+$ cells were purified from infected PBMC as explained below, 8 days after viral inoculation. The successful use of this protocol in our laboratory has been described previously (26).

In both cases, immunomagnetic particles were used (26). Briefly, stimulated PBMC (10^7 cells) were labeled with 0.3 ml of either an anti-CD4 (Leu3a) or anti-CD8 (Leu2a) MAb (Becton Dickinson, Mountain View, Calif.) for 30 min on ice. Cells were washed twice in phosphate-buffered saline (PBS) and then incubated at 37°C for 1 h in 0.5 ml with 2.5×10^8 magnetic particles coated with goat anti-mouse IgG, specific for the Fc (Advanced Magnetics Inc., Cambridge, Mass.). Thereafter, the particles were sorted by means of a magnetic field; immunomagnetic particles were dissociated from cells by incubation at 37°C for 48 h.

Proliferation of $CD4^+$ and $CD8^+$ T lymphocytes. Uninfected, enriched populations of both $CD4^+$ and $CD8^+$ lymphocytes were stimulated with PHA (10 μg/ml) for 3 days prior to assessment of proliferative responsiveness. Cells of each phenotype were plated (10^5 cells per well) in triplicate in a 96-well plate in the absence or presence of different SRIH molecules (SRIH 1-14, SRIH 1-28, and SMS 201-995) at concentrations ranging between 10^{-6} and 10^{-14} M. After 48 h, cultures were pulsed with 1 μCi of [^{3}H] thymidine (New England Nuclear, Boston, Mass.) for an additional 22 h in the presence of fresh medium and drug. Cells were harvested on filters with a Titertek cell harvester (Skatron Inc., Sterling, Va.). Filters were dried and resuspended in scintillation liquid (Betamax; ICN Biomedicals, Irvine, Calif.), and radioactivity was counted in a liquid scintillation counter.

RT-PCR. Total RNA (1 μg) extracted from each cell type was heated at 85°C for 3 min to denature secondary structure, cooled to room temperature, and put on ice. Then, for each RNA sample, the following reagents were added to synthesize complementary DNA by reverse transcription (RT): 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 2 mM dithiothreitol, 0.5 mM each of the four 2′-deoxynucleoside 5′-triphosphates (dNTPs; Pharmacia, Baie d'Urfé, Canada), 0.1 mg of bovine serum albumin per ml, 7,250 U of RNA Guard RNase inhibitor (Pharmacia), and 50 U of Moloney murine leukemia virus reverse transcriptase (MoMLV-RT; Gibco BRL, Burlington, Canada) in a total volume of 25 μl. These reagents were incubated for 1 h at 42°C, heated at 95°C for 5 min to denature the MoMLV-RT, and immediately cooled on ice.

PCRs were carried out by adding 25 μl of the RT reaction mix to 25 μl of a mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (wt/vol) gelatin, 200 μM each of the four dNTPs, 3 U of *Taq* DNA polymerase (Bethesda Research Laboratories), 30 ng of unlabeled antisense primer, and 20 ng of radiolabeled sense primer. The reaction mixture (50 μl) was overlaid with 2 drops of mineral oil to avoid evaporation. Each sample was subjected to 30 cycles of denaturation for 1 min at 95°C, annealing at 61°C for 1 min, and polymerization at 72°C for 2 min with a DNA thermal cycler (Perkin Elmer Cetus, Montreal, Canada). Amplified products were analyzed by electrophoresis on nondenaturing 8% polyacrylamide gels, which were subsequently dried for 1 h and visualized by direct autoradiography. The sizes of PCR products were determined by reference to radiolabeled fragments obtained by digestion of plasmid pAT153 with *Hae*III enzyme (Pharmacia).

Primers. We used a primer pair established from the nucleotide sequence of the SRIH gene (41) for amplification of related cDNA. We took advantage of the presence of an intron of 877 bp in the SRIH gene to design a primer pair that could specifically amplify SRIH cDNA. The sense primer (SMS_{187}: 5′-GAC CCC AGA CTC CGT CAG TTT-3′) and antisense primer (SMS_{1204}: 5′-GGA CAG ATC TTC AGG TTC CAG-3′) are located upstream and downstream of the intron, respectively. The PCR conditions used did not permit amplification of a 1,018-bp sequence which represents the sequence encompassed by both primers on the unspliced SRIH DNA; however, spliced SRIH cDNA yielded a PCR product of 141 bp.

Amplification of the human elongation factor-1α (EF-1α) gene, which promotes cytosolic translation in most eukaryotic cells, was used as an internal control in our experiments. The sense (LMS-1: 5′-CTG ATC TAT AAA TGC GGT GGC-3′) and antisense (LMAS-2: 5′-CCA CAA GGA GAT ATC AAT GGT-3′) primers, located in exons 2 and 3, respectively, were designed from the nucleotide sequence of the human EF-1α gene reported previously (47). The coordinate use of the LMS-1 and LMAS-2 primers generated a PCR product of 166 bp.

RESULTS

Effect of somatostatin on HIV-1 replication. Both the $CD4^+$ and $CD8^+$ positively sorted populations were greater than 96% pure, as determined by two-color cytofluorometry. The kinetics of viral replication were different in $CD4^+$ and $CD8^+$ lymphocytes. In the absence of drugs, we found that p24 levels were highest after 8 to 11 days in $CD4^+$ cells and 24 to 27 days in $CD8^+$ cells (Fig. 1). Moreover, $CD4^+$ cells produced two to three times more p24 then did $CD8^+$ cells. Peripheral $CD4^+$ cells were susceptible to cytopathic effects induced by HIV-1 replication, while $CD8^+$ cells were resistant, in accordance with previous observations (11, 26). After 31 days, the viability of infected $CD8^+$ cultures was largely intact, while infected $CD4^+$ populations had died, as demonstrated by trypan blue dye retention. The viability of infected $CD8^+$ cells was maintained throughout the culture period.

Two synthetic molecules, SRIH 1-14 and SRIH 1-28, as well as the SRIH-related octreotide (SMS 201-995) were tested over concentrations ranging between 10^{-6} M and 10^{-14} M for their effect on HIV replication, as measured by generation of viral p24 antigen. Figure 1 depicts representative results obtained with cells from a single donor studied in three replicate samples. We found that SRIH 1-14, SRIH 1-28, and octreotide at concentrations between 10^{-6} and 10^{-14} M each caused a consistent stimulation (i.e., >43%) of p24 levels in $CD4^+$ lymphocytes (Fig. 1A, C, and E). In contrast, p24 production was inhibited by up to 67% in $CD8^+$ cells treated with the same compounds (Fig. 1B, D, and F). Each experiment was performed three times, with cells from three different donors and three replicate samples, and yielded similar results each time. In no instance were pooled donor samples used in these experiments.

The dose dependence of these activation and inhibitory effects is illustrated in Fig. 2, which presents average results obtained from a total of five patients evaluated. We found significant differences between $CD4^+$ and $CD8^+$ cultures for each SRIH analog tested (paired *t* test, $P < 0.01$ in each case). Inhibition of viral replication in $CD8^+$ cultures was not due to drug-mediated cytotoxic effects, since no loss of cell viability occurred, as determined by trypan blue dye exclusion, at the concentrations tested (not shown).

Effect of somatostatin on proliferative responsiveness of $CD4^+$ and $CD8^+$ cells. SRIH-mediated inhibition of PHA-driven proliferation of both $CD4^+$ and $CD8^+$ cells, in comparison with proliferation in untreated cultures, was generally

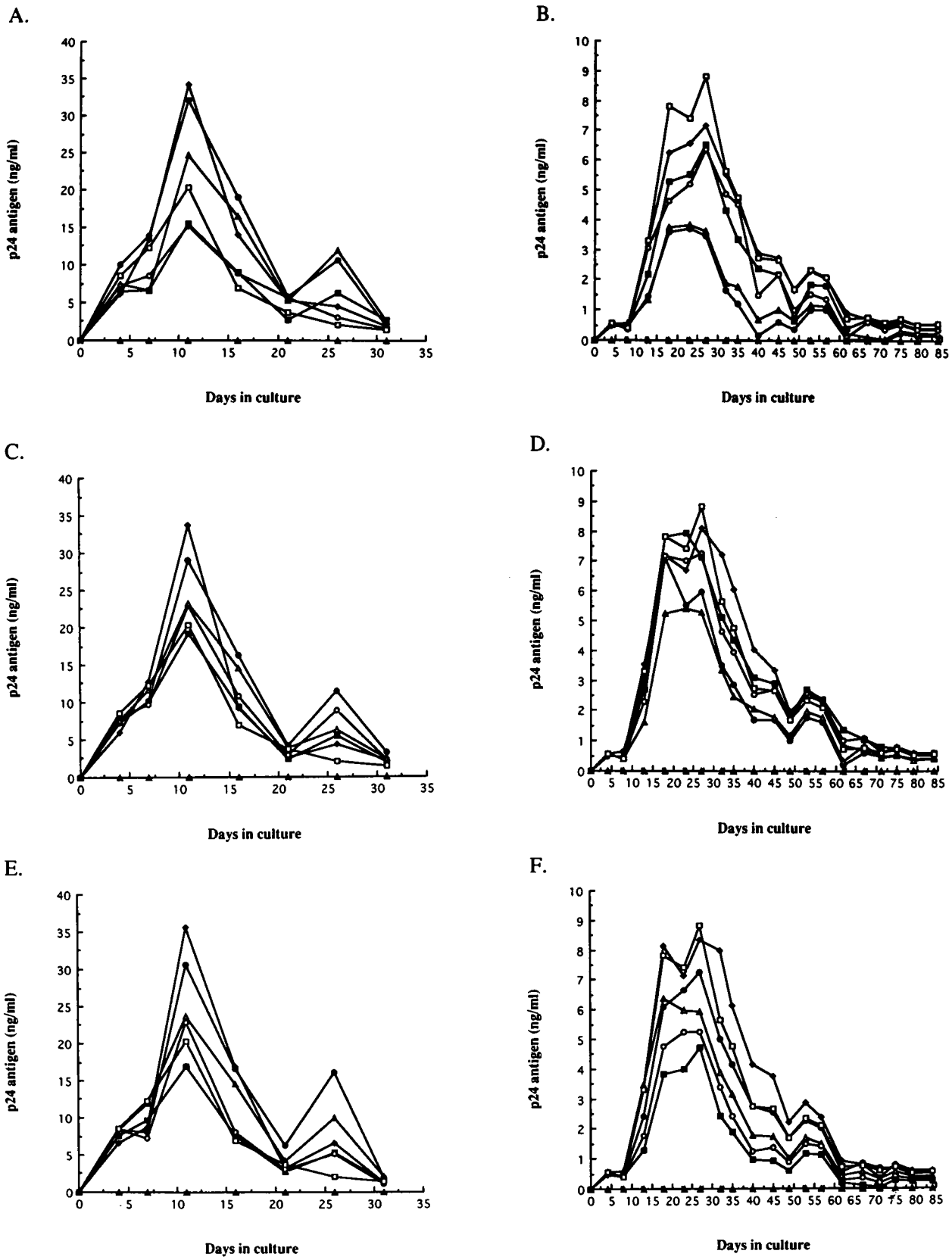

FIG. 1. Effect of somatostatin analogs on HIV-1 replication in peripheral blood T-cell subsets. HIV-1_{IIIB} was grown in cultures enriched for $CD4^+$ cells (A, C, and E) or for $CD8^+$ cells (B, D, and F) in the absence (□) or presence of either SRIH 1-14 (A and B), SRIH 1-28 (C and D), or octreotide (SMS 201-995; E and F) at various concentrations: 10^{-6} M (◆), 10^{-8} M (●), 10^{-10} M (△), 10^{-12} M (○), and 10^{-14} M (■).

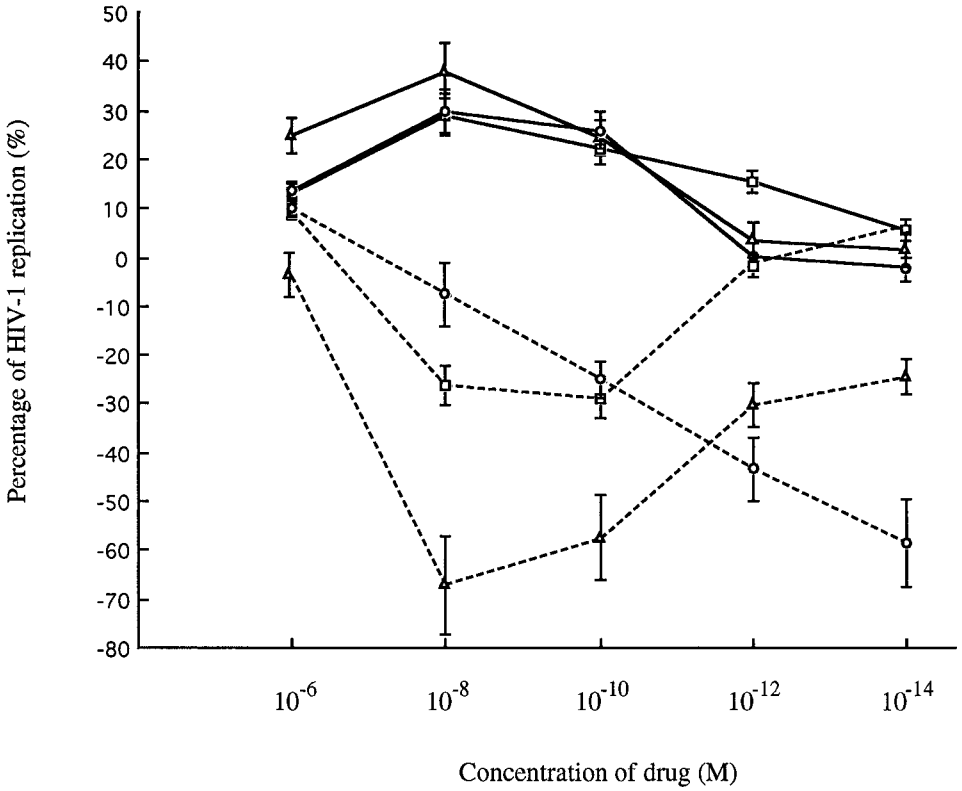

FIG. 2. HIV-1 replication, relative to that in untreated controls, in $CD4^+$ (——) and $CD8^+$ (– – –) cells cultured in the presence of SRIH 1-14 (△), SRIH 1-28 (□), or SMS 201-995 (○). Results are expressed as the mean for cells from five patients ± standard deviations.

modest. Figure 3 depicts the results obtained with cells from a single donor; similar findings were obtained on each of two other occasions. This inhibition appeared to be dose related for all three SRIH molecules studied. With regard to SRIH 1-14 and SRIH 1-28, we found that in most cases, a drug concentration of 10^{-10} M had greater inhibitory effects on both $CD4^+$ and $CD8^+$ cells. This effect was statistically significant (paired t test, $P < 0.01$). By contrast, the more pronounced inhibitory effects with the long-acting somatostatin analog SMS 201-995 were observed at a lower concentration, 10^{-12} M. Higher concentrations (10^{-6} M) of this drug may have had a stimulatory effect (Fig. 3). The issue of proliferative responsiveness relative to receptors and signalling is currently under investigation. Although these studies were performed with the III_B laboratory strain of HIV-1, similar findings were obtained on a more limited basis with a clinical isolate (results not shown).

Somatostatin gene expression in PBMC. We studied the ability of activated PBMC to synthesize SRIH mRNA by means of RT-PCR, with the TT and U-373MG cell lines serving as positive (14) and negative controls, respectively. Figure 4 shows a representative experiment of four performed, showing nonexpression of SRIH transcripts in PHA-stimulated PBMC. This absence of SRIH gene expression cannot be attributed to RNA degradation, since the EF-1α gene was appropriately amplified in each of the RNA samples examined.

DISCUSSION

We found that SRIH caused enhanced HIV-1 replication in $CD4^+$ cells but inhibition in $CD8^+$ lymphocytes. IFN-γ can indirectly up- and downregulate HIV-1 expression in $CD8^+$ and $CD8^-$ subsets, respectively (45). Thus, the inhibition of IFN-γ production by SRIH (5, 27, 48) may conceivably explain its dichotomous effect on HIV-1 replication in $CD4^+$ and $CD8^+$ cells (Fig. 1 and 2). It is not known whether mitogen- or antigen-driven stimulation of either $CD4^+$ or $CD4^-$ cells can affect SRIH receptor expression.

Although some $CD8^+$ T-cell subsets, termed helper cell-independent cytolytic T cells, have the ability to secrete interleukin-2 (IL-2), it is believed that type 1 $CD4^+$ T helper cells are the major source of this factor. Kappa B enhancer elements have been identified in various inducible cellular genes, e.g., those that encode IL-2 and the alpha subunit of the IL-2 receptor, as well as in the long terminal repeat regions of the HIV-1 genome (17). These kappa B enhancer elements compete for binding of inducible transcriptional activators of cellular origin, such as NF-κB. Treatment with SRIH is associated with augmented IL-2 release by T-lymphocyte cell lines (29). Conceivably, this may contribute to the activation of the HIV-1 long terminal repeat in $CD4^+$ cells and increased viral replication (Fig. 2).

Our results on the differential effects of SRIH on HIV-1 replication may also be related to differences in the percentages of cells expressing SRIH receptors at their surface, 53% for $CD8^+$ cells versus 30% for $CD4^+$ lymphocytes (43). In addition, differential patterns of high- and low-affinity SRIH receptor representation on these subsets may play a role in the cell type-dependent effect on viral replication (42), as may differences in the level of SRIH-degrading proteases (9).

The question of whether T-cell subsets in the gut and peripheral blood have similar functions is relevant to this discussion. Indeed, intestinal IEL differ from their peripheral blood counterparts in that a much higher percentage of $CD8^+$ T cells

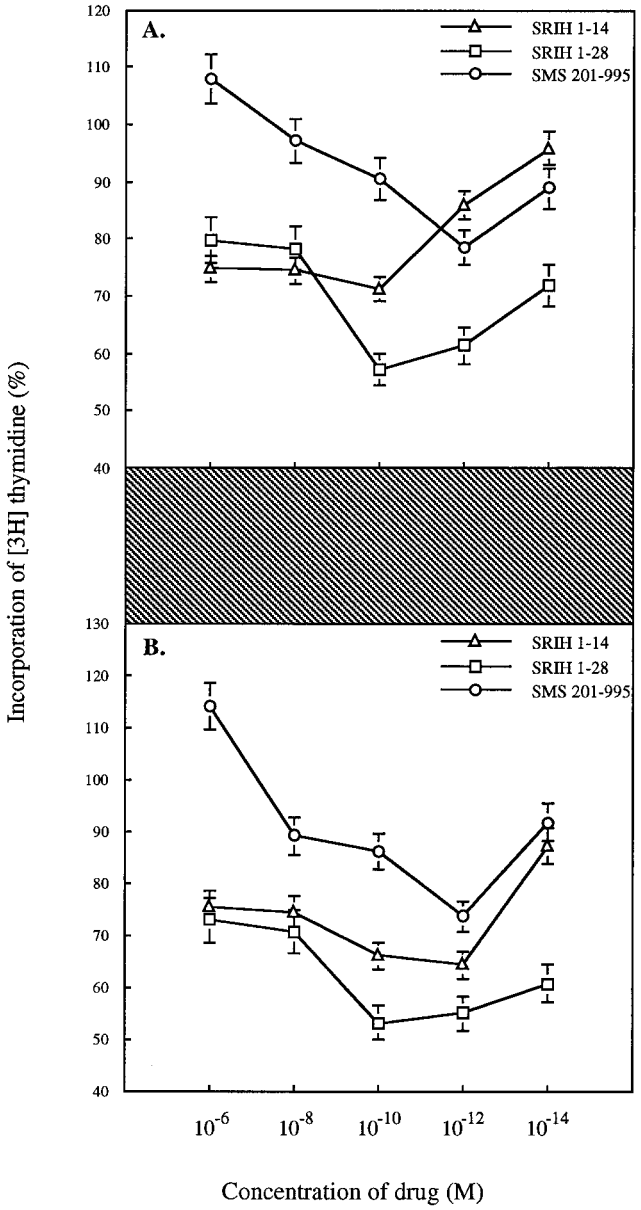

FIG. 3. Proliferative responsiveness of both $CD4^+$ (A) and $CD8^+$ (B) uninfected T lymphocytes to PHA in the presence of SRIH 1-14 (△), SRIH 1-28 (□), or SMS 201-995 (○). Results are expressed as a percentage of [^{3}H]thymidine counts incorporated in the absence of drug. For $CD4^+$ and $CD8^+$ lymphocytes, the actual counts (± standard deviation) incorporated were 25,486 ± 2,058 and 12,520 ± 1,429 cpm, respectively.

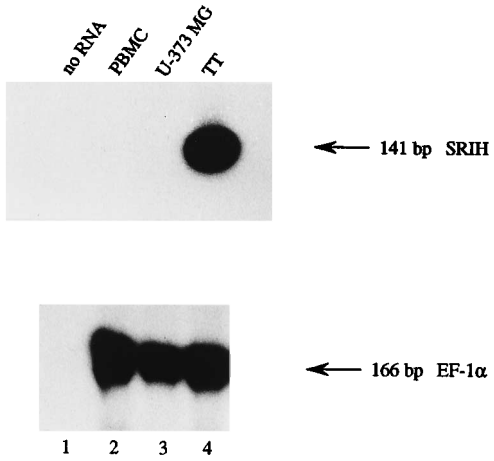

FIG. 4. Failure of PHA-stimulated PBMC to express SRIH mRNA.

and higher levels of cytotoxic activity are present (7). This constituted part of the rationale for studying the effect of SRIH on HIV replication in $CD8^+$ lymphocytes. In contrast, intestinal T lymphocytes of the lamina propria behave similarly to T cells in the circulation (4).

This report is limited through our study of lymphocytes that were derived from PBMC. Conceivably, lymphocytes of intestinal origin might have behaved differently in response to SRIH, although the purity of such populations and the potential difficulty of infecting them in culture might have rendered additional experiments problematic. In addition, we cannot be sure how infection or mitogen treatment might have differentially affected the expression of SRIH receptors on $CD4^+$ and $CD8^+$ intestinal cells.

The question of cell purity is important for interpretation of our results. As discussed above, our purified cell populations were devoid of detectable levels of B cells and monocytes/macrophages. Although dendritic cells may have been present in small numbers in the $CD8^+$-enriched fractions (i.e., <0.01%), other data have shown that 3 to 6% of $CD8^+$ cells were productively infected, as shown by two-color flow cytometry analysis for expression of both the CD8 and gp120 antigens (26).

Although the inhibitory effect of SRIH on lymphocyte proliferative responsiveness has been well described, this is the first report to deal with the effect of this hormone on enriched subsets of T cells (Fig. 3). We observed biphasic fluctuations with regard to the effects of SRIH on both HIV-1 replication and cell multiplication. These findings are consistent with previous studies that showed that secretion of IL-2 (29) and IFN-γ (27) by mononuclear cells, of histamine by basophils (15), and of superoxide anions by monocytes (28) was similarly affected by SRIH; so too was proliferation of mononuclear cells (29). The HIV-stimulatory and $CD4^+$ growth inhibition effects of SRIH (Fig. 2 and 3A) are presumably mediated by distinct pathways. The antiproliferative effect of SRIH on both $CD4^+$ and $CD8^+$ lymphocytes could presumably help to prevent the expression of the HIV-1 genome following activation of quiescently infected $CD4^+$ T cells (49) or the effects of tumor necrosis factor alpha of $CD8^+$ origin on chronically infected cells (6). SRIH might also impact on the destruction of uninfected $CD4^+$ lymphocytes by $CD8^+$ cells (16) or the secretion by $CD8^+$ $CD57^+$ lymphocytes of a soluble factor(s) that blocks lymphocyte-mediated cytotoxicity (36).

The pathogenesis of HIV-associated chronic diarrhea is still uncertain. HIV involvement cannot be excluded, even in the presence of known enteric pathogens, since HIV has been detected in the gut tissue of more than 30% of AIDS patients examined (3), with infection occurring in intestinal lymphocytes (13, 18), enterochromaffin (22), and epithelial cells of the GI tract (12). In the last case, HIV entry may occur via a CD4-independent mechanism that employs galactosyl ceramide as an alternative receptor. The beneficial effect of octreotide in patients treated for diarrhea may result from the inhibition of HIV-1 replication in intestinal epithelial cells. Previous studies on SRIH gene expression in thymic and bursal tissue suggested that immature lymphocytes might be a source

of SRIH (1). The lack of expression of SRIH mRNA in developmentally mature lymphocytes (Fig. 4) supports the notion that SRIH is actively synthesized only in immature populations. These results also suggest that the effect of exogenous SRIH on HIV-1 replication in both $CD4^+$ and $CD8^+$ cells may not involve a feedback control loop. Further studies to address the mechanisms involved are in progress and include attempts to interfere with SRIH receptor expression and the ability of antireceptor MAbs to block the SRIH-mediated effects.

Since SRIH gene expression does not apparently occur in mitogen-stimulated PBMC (Fig. 4), the reduction of SRIH immunoreactivity in the gut observed in disease progression to AIDS (40) is unlikely to result from HIV-mediated cytopathic effects in $CD4^+$ intestinal T lymphocytes. In contrast, enterochromaffin cells produce several hormones, including SRIH, and can be infected by HIV-1 (22). Thus, HIV-mediated cytolysis or functional impairment of enterochromaffin cells is a more likely explanation for diminished SRIH immunoreactivity in the duodenum and rectum of HIV-1-seropositive individuals. In addition to a stimulatory effect on HIV-1 replication in $CD4^+$ T cells (Fig. 1 and 2), SRIH and octreotide could boost HIV replication indirectly by inhibiting the proliferation of $CD8^+$ lymphocytes (Fig. 3B), which can limit HIV spread through the soluble factor(s) (24). It will be interesting to determine whether SRIH can exert an effect on either the production or activity of such a factor(s).

The vasoactive intestinal peptide is able to bind to CD4 glycoprotein, suggesting an ability to interfere with infection of $CD4^+$ cells by HIV-1 (35). The clinical use of octreotide is associated with decreased plasma levels of vasoactive intestinal peptide in HIV-infected patients with refractory diarrhea (25). Thus, octreotide therapy could indirectly contribute to increased viral load through this mechanism as well. We are currently studying the effect of SRIH on HIV-infected intestinal epithelial cells (12).

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