Effect of Recombinant Human Growth Hormone on Acute and Chronic Human Immunodeficiency Virus Infection In Vitro

By Jeffrey Laurence, Bryn Grimison, and Amnon Gonenne

Growth hormone (somatotropin) is a potent anabolic protein currently being evaluated clinically in cachexia associated with malignancy and human immunodeficiency virus (HIV) disease. Growth hormone can also lead to enhancement of lectin-mediated cellular proliferation, macrophage activation, and cytokine induction, events linked to induction of latent HIV in vitro. We thus explored the ability of recombinant human growth hormone (rhGH) to affect viral replication in acute and chronic HIV infection, and to alter transcription at the HIV-1 long terminal repeat (LTR). A clone of promonocytic cells, chronically infected with HIV-1 and susceptible to viral induction by a variety of cytokines and protein kinase C activators, was unperturbed by rhGH used over broad concentrations (10 to 500 ng/mL) and time intervals. This unresponsiveness paralleled the lack of effect of rhGH on HIV-associated trans-activation in both monocytic and CD4+ T-cell lines. In contrast, rhGH enhanced viral replication in acutely infected peripheral blood mononuclear cells (PBMC) by twofold to 20-fold, albeit having no adverse effect on the antiviral efficacy of zidovudine (AZT). Augmentation of HIV growth correlated with stimulation of cellular DNA synthetic responses and an increase in tumor necrosis factor-α (TNF-α) secretion. These data are discussed in the context of ongoing clinical trials of rhGH in HIV-seropositive individuals with wasting syndromes.

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We had previously defined a model to study the effects of gonadal and steroidal hormones and their antagonists on chronic and acute HIV infection in vitro.13,14 Using these systems, we now report the ability of rhGH to enhance HIV replication and tumor necrosis factor-α (TNF-α) secretion in peripheral blood mononuclear cells (PBMC), in the absence of viral induction from chronically infected cell lines or a direct effect on HIV-1 transcription. The enhanced replication of HIV is consistent with rhGH-mediated stimulation of cell growth and cytokine generation.

MATERIALS AND METHODS

Cells

U1.1A cells were subcloned from U1, a clone of the human monocytic cell line U937, which had been infected with the lymphadenopathy-associated virus (LAV) strain of HIV.15 The cells contain an average of two proviral copies of HIV-1 per cell. H9 is a subclone of HUT-78, a human CD3+, CD4+ lymphoblastoid cell line permissive for replication of HIV and partially resistant to its cytolytic effects. Cells were cultured in RPMI-1640 (Flow Laboratories, McLean, VA) plus 10% ultra-filtered fetal bovine serum, at a concentration of 5 × 10^4 cell/mL. PBMC were isolated from heparinized venous blood of HIV-seronegative donors by ficoll-hypaque density gradient centrifugation.

Reagents

4B-phorbol-12-myristate-13-acetate (PMA) (Sigma Chemical, St Louis, MO) was prepared as a stock solution of 100 µg/mL in

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were collected from wells representing each experimental condi-
tion and 0.1 mL plated per well in triplicate in polystyrene
flat-bottom microwell plates. Cells were pulsed with 0.1 mCi
'H-methyl-thymidine (1.9 Ci/mmol; specific activity: New En-
labs, Chicago, IL).

HIV was measured as previously reported.14 Two plasmids were
infectious doses of viral stock, representing supernatants of H9 T
lymphoblasts infected with the HTLV-IIIB strain of HIV,16 were
added, and the medium was completely changed 18 hours later.
One half of the medium was removed and replaced with fresh
culture medium every 3 or 4 days. Infection was followed by ELISA
assay for p24 core antigen. In select experiments, quantities of
anti-TNF-a sufficient to neutralize 10 to 2,500 U of TNF-a were
prepared as a 5-mmol/L stock solution in distilled water.


dimethylsulfoxide (DMSO), diluted in RPMI-1640, and used at a
final concentration of 5 or 50 ng/mL. rhGH (Bio-Tropin) was
obtained from Bio-Technology General (New York, NY) and
prepared as a stock solution of 5 mg/mL in distilled water. It
contained less than or equal to 10 endotoxin U/mg, as determined by
the Limulus amoebocyte lysate assay. Zidovudine (3'-azido-3'-
deoxythymidine, AZT) was purchased from Pharmatec (Alachua,
FL), and prepared as a 5-mmol/L stock solution in distilled water.
A rabbit polyclonal anti-human TNF-a antiserum was obtained from
Dr Anthony Cerami (Rockefeller University, New York,
NY). A quantity of 0.002 mL of antiserum is capable of neutralizing
1,000 U of recombinant human TNF-a (B. Sherry, unpublished data).

**RESULTS**

**Effect of rhGH on Chronically Infected Cells and the HIV LTR**

U1.1A cells were exposed to PMA and supernatants
assayed for HIV p24 core antigen at 48 hours. As previously
reported,13,14 a greater than 20-fold increase in p24 antigen
occurred in the presence of PMA (Table 1). U1.1A cells were also exposed to rhGH at physiologic (10 to 50 ng/mL),
pharmacologic (50 to 100 ng/mL), and suprapharmacologic
(250 to 500 ng/mL) concentrations for 24 to 96 hours, and
culture supernatants were analyzed at 24-hour intervals for
p24. No effect was noted on the low basal level of antigen
production by these chronically infected cells at 48 hours
(Table 1), or at any other time point assessed.

Cellular responses to phorbol esters mimic responses
to growth factors, hormones, and immunologic activation. As
the HIV LTR contains a palindromic element shared with
the growth hormone promoter,14 we used a transient cotrans-
fection assay to study possible activation of HIV-LTR-
mediated gene expression in the presence of rhGH. Conver-
sion of radiolabeled chloramphenicol to its acetylated
forms is markedly enhanced by PMA when the HIV-LTR-
CAT and tat plasmids are cotransfected into U937 cells,13,14 the
uninfected parental line of U1.1A. In contrast, rhGH
had no effect on baseline HIV-LTR directed CAT activity,
in the presence or absence of HIV tat (Table 2). An equivalent
lack of effect was demonstrated in H9 CD4+ T

cells, where control levels of chloramphenicol acetylation
remained within 10% of that seen at all levels of rhGH used
(10 to 500 ng/mL).

**Effect of rhGH on HIV Infection of PBMC**

Enhancement of viral activity, as determined by measurement
of p24 antigen in detergent-solubilized supernatants,
was noted in four of five experiments, with a range of viral
enhancement from twofold to 20-fold at doses of 50 and 100
ng/mL. Lower concentrations of rhGH (1, 5, and 10
ng/mL) had no effect (data not shown). No interference
with the antiviral efficacy of AZT was noted when this drug
was included at pharmacologic (0.5 mmol/L), and supraphar-
macologic (5 mmol/L) doses. The kinetics of these re-

<table>
<thead>
<tr>
<th>Sample</th>
<th>PM (ng/mL)</th>
<th>rhGH (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>250</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>500</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>—</td>
</tr>
</tbody>
</table>

Cells were plated at 2 × 10^5/microwell in 0.2 mL culture medium with
the appropriate concentration of inducing agent. Supernatants were
harvested 48 hours later and tested for p24 antigen by an ELISA-based
antigen capture system.
Table 2. Effect of rhGH on HIV-LTR Activity in U937 Cells

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>LTR-CAT</th>
<th>Tat</th>
<th>rhGH (ng/mL)</th>
<th>% Acetylation</th>
<th>Relative Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>0.4</td>
<td>1.0</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>–</td>
<td>10</td>
<td>0.5</td>
<td>1.3</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>–</td>
<td>100</td>
<td>0.4</td>
<td>1.0</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>59.2</td>
<td>148.0</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>10</td>
<td>59.0</td>
<td>147.5</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>50</td>
<td>71.0</td>
<td>177.5</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
<td>100</td>
<td>60.3</td>
<td>150.8</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>250</td>
<td>59.8</td>
<td>149.5</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
<td>500</td>
<td>61.6</td>
<td>154.0</td>
</tr>
</tbody>
</table>

The HIV-LTR.CAT plasmid (1 μg DNA) was transfected alone or together with a plasmid-containing HIV-1 tat (1 μg DNA). Buffer or rhGH (10 to 500 ng/mL) was present throughout the 48-hour culture period. The percent conversion of ["C"]chloramphenicol to its acetylated forms was determined, and relative CAT activity calculated as ( % acetylation of experimental)/ ( % acetylation of LTR-CAT alone).

Table 3. Effect of rhGH on HIV Infection of PBMC and the Anti-HIV Activity of AZT

<table>
<thead>
<tr>
<th>AZT (μmol/L)</th>
<th>rhGH (ng/mL)</th>
<th>p24 Antigen (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 7</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>26.3</td>
</tr>
<tr>
<td>0.5</td>
<td>—</td>
<td>2.9</td>
</tr>
<tr>
<td>5.0</td>
<td>—</td>
<td>2.5</td>
</tr>
<tr>
<td>—</td>
<td>50</td>
<td>7.8</td>
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<tr>
<td>—</td>
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</tr>
<tr>
<td>—</td>
<td>500</td>
<td>19.9</td>
</tr>
<tr>
<td>0.5</td>
<td>—</td>
<td>2.2</td>
</tr>
<tr>
<td>5.0</td>
<td>—</td>
<td>3.0</td>
</tr>
<tr>
<td>0.5</td>
<td>250</td>
<td>2.9</td>
</tr>
<tr>
<td>5.0</td>
<td>250</td>
<td>2.9</td>
</tr>
<tr>
<td>5.0</td>
<td>500</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Phytohemagglutinin-activated PBMCs were plated at 2 × 10⁶/96-well microplate in 2 mL culture medium containing 32 U/mL IL-2 together with buffer, AZT, and rhGH. One thousand 50% tissue culture infectious doses of HIV-1 were then added, and the medium completely changed 18 hours later. One half of the culture supernatant was removed and replaced with fresh culture medium every 3 or 4 days thereafter. p24 antigen levels were assessed as noted in Table 1.

Table 4. Effect of rhGH on IL-6 and TNF-α Secretion by HIV-Infected PBMCs

<table>
<thead>
<tr>
<th>rhGH (ng/mL)</th>
<th>A. IL-6 (pg/mL)</th>
<th>B. TNF-α (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>—</td>
<td>58 ± 31</td>
<td>17 ± 1</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>100</td>
<td>50</td>
<td>54</td>
</tr>
<tr>
<td>250</td>
<td>—</td>
<td>64</td>
</tr>
</tbody>
</table>

Levels of IL-6 and TNF-α were measured in culture supernatants of PBMC exposed to HIV and buffer or rhGH at or near the peak of viral replication (day 17 [IL-6] or 14 [TNF-α]). ELISA assays for these cytokines had a sensitivity of ≥10 pg/mL.
growth hormone led to our investigation of the effects of this molecule on HIV replication. Growth hormone can stimulate T-cell and monocyte proliferation and cytokine production, factors that promote HIV replication in vitro. High-affinity binding sites for GH have been demonstrated on multiple cell types, including immunocytes, and, as measured in the mouse, all subpopulations of immune cell—helper and cytotoxic T lymphocytes, B cells, monocytes and natural killer cells—can also secrete growth hormone. In addition, sequences related to the estrogen/thyroid hormone receptor element (ERE/TRE) have been identified in the growth hormone promoter, as well as the HIV-1 LTR; they appear to be important for protein complex formation, and may predict regulation by similar cellular factors. We found that rhGH had no direct effect on the HIV LTR, nor did it upregulate virus in latently infected cells. However, it did enhance, to a variable degree, viral replication in acutely infected PBMC.

The mechanism of this enhancement is unclear. It is unlikely to be a result of simply an increase in the number of infected PBMC, as peak enhancement of DNA synthetic responses by rhGH was quite modest and occurred at 10 ng/mL (Fig 1), a concentration that had no effect on p24 antigen production or cytokine release. It may relate to cytokine induction. rhGH augmented TNF-α levels, in the absence of an effect on IL-6. HIV itself can also increase TNF-α production, and, via a positive feedback loop, may induce HIV. TNF-α can activate proteins that bind directly to NF-κB enhancer elements found in the HIV-LTR, as well as promoters of genes for cytokines and their receptors, and regulate cellular activation. Serum TNF-α levels also may directly correlate with clinical stage of HIV disease. It appears paradoxical that an anabolic agent would increase TNF-α/cachexin levels, although the secreted concentrations induced in vitro, while sufficient to enhance HIV replication in vitro, do not correlate with weight loss in HIV-seropositive individuals. Recombinant TNF-α is also being evaluated, in conjunction with interferon-γ, for the treatment of AIDS-related complex, with no augmentation of disease or HIV activity.

However, to the limits of antibody neutralization studies, TNF-α does not appear to be primarily responsible for rhGH-mediated augmentation of HIV replication, with a less than 40% decrease in enhancement in the presence of up to 2,500 nU of antisem. Use of antisense oligomers

<table>
<thead>
<tr>
<th>Table 5. Effect of TNF-α Blockade by an Anti-TNF-α Antiserum on rhGH-Mediated Enhancement of HIV Replication</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhGH (100 ng/mL)</td>
</tr>
<tr>
<td>p24</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>–</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>+</td>
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<tr>
<td>+</td>
</tr>
<tr>
<td>+</td>
</tr>
</tbody>
</table>

Viral cultures were established and harvested as noted in Table 3, except that anti-TNF-α was included at culture initiation and with each change of medium. p24 values are in nanograms per milliliter. Δ indicates change in p24 antigen levels over baseline (cultures without rhGH or anti-TNF).
targeted to TNF-α might be another approach to ascertain the involvement of this particular cytokine. However, it should be noted that several other growth factors capable of enhancing HIV replication, including IL-1 and IL-2, also may be affected by rhGH. In addition, a membrane-associated form of IL-6 or these other cytokines may be involved. This issue is obviously far from settled, and probably involves a complex interaction among diverse factors. Finally, the fact that a differential effect on HIV replication was noted using moderate versus high (250 ng/mL) levels of rhGH may also relate to divergent effects on cytokine production and cellular proliferation, as a trend toward decreased T-cell proliferation (Fig 1) and TNF secretion (Table 4) obtained with the highest concentration used.

The relevance of these in vitro studies to clinical trials already underway with rhGH and other growth factors is uncertain. For example, IL-2 is an immune modulator that also markedly upregulates HIV replication in vitro. Its clinical use in AIDS was linked to an increase in severe bacterial and opportunistic infections. In contrast, while human PBMC infected with HIV-1 in the presence of pharmacologic levels of recombinant granulocyte-monocyte colony-stimulating factor (rGM-CSF) resulted in at least 14-fold enhancement of HIV replication, administration of rGM-CSF or G-CSF to AIDS patients, either alone or in combination with AZT, was not linked to an increase in infection rate and had no measurable affect on HIV production in vivo. However, a more recent study did find significant increases in p24 antigenemia among five of six HIV-seropositive individuals treated with GM-CSF alone. These concerns will be of particular importance as clinical trials of rhGH as an immune modulator in HIV disease are contemplated, based on its ability to augment immune cell proliferation, cytokine secretion, and expansion of antigen-specific T lymphocytes. One must also study whether rhGH will have a differential effect on anti-HIV nucleoside analogs ddI and ddC from that seen with AZT, as has been reported with GM-CSF. Our experiments should thus raise a note of caution in the monitoring of HIV-seropositive patients receiving rhGH. Until more complete clinical data are available, it is suggested that rhGH, as other growth factors, be used in conjunction with zidovudine in HIV-seropositive individuals.

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