

Alpha-1-antitrypsin inhibits human immunodeficiency virus type 1

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ABSTRACT Several observations suggest the existence of potent endogenous suppressors of human immunodeficiency virus type 1 (HIV-1) production, and inhibitors of serine proteases may participate in this effect. Alpha-1-antitrypsin (AAT) is the most abundant circulating serine protease inhibitor. Physiological AAT concentrations inhibited HIV-1 production in chronically infected U1 monocytic cells, reduced virus replication in freshly infected peripheral blood mononuclear cells, and blocked infection of permissive HeLa cells. In U1 cells, AAT suppressed activation of the HIV-1-inducing transcription factor NF- κ B. Similar results were obtained using CE-2072, a synthetic inhibitor of host serine proteases. HIV-1 did not replicate in blood obtained from healthy volunteers, but marked replication was observed in blood from individuals with hereditary AAT deficiency. These results identify AAT as a candidate circulating HIV-1 inhibitor *in vivo*. Two different mechanisms of AAT-induced HIV-1 inhibition were identified, including reduced HIV-1 infectivity and blockade of HIV-1 production. A novel host-pathogen interaction is suggested, and an alternative strategy to treat HIV-1-related disease may be possible.—Shapiro, L., Pott, G. B., Ralston, A. H. Alpha-1-antitrypsin inhibits human immunodeficiency virus type 1. *FASEB J.* 15, 115–122 (2001)

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THERE ARE NO published reports of human immunodeficiency virus type 1 (HIV-1) propagation from infected and unaltered whole blood. HIV-1 production in infected blood has been demonstrated only after dilution with tissue culture medium and the addition of stimulated lymphocytes or lymphocytic cells (1, 2). These manipulations facilitate virus production and suggest the presence of circulating inhibitors. Investigations of HIV-1 production in infected patients have emphasized virus proliferation in lymph nodes, where concentrations of specific serum constituents are reduced (3, 4). Furthermore, the risk of contracting HIV-1 infection after percutaneous exposure to infected blood is ~1 in 250–300 (5, 6); in contrast, the risk of infection for hepatitis B virus after needlestick exposure may be as high as 1 in 8 (7).

HIV-1 infection is rarely contracted by the oral route, and saliva contains HIV-1-suppressive factors (8–13). Salivary secretory leukocyte protease inhibitor (SLPI) has been reported to inhibit HIV-1 *in vitro*, and immunodepletion of native SLPI from saliva partially neutralized the HIV-1-inhibitory activity (14, 15).

Considered together, these observations suggest the existence of potent endogenous inhibitors of HIV-1. Since the role of SLPI *in vivo* is inactivation of host-derived serine proteases, it is also suggested that endogenous serine proteases and their inhibitors may have a role in HIV-1 pathogenesis.

Alpha-1-antitrypsin (AAT) is the most abundant circulating serine protease inhibitor. The normal serum AAT concentration is 1.5–3.5 mg/ml, but peak concentrations as great as fourfold that of normal may occur during inflammation (16–18). It is a 394 amino acid, 52 kDa glycoprotein synthesized in the liver and secreted into the circulation with a half-life of 4–5 days (16). We examined AAT for HIV-1 inhibitory effects.

MATERIALS AND METHODS

Materials

Recombinant human interleukin 18 (IL-18) was processed to the mature form with recombinant IL-1 β -converting enzyme (19). Interleukin-6 was obtained from R&D Systems (Minneapolis, Minn.) and lipopolysaccharide (LPS, *Escherichia coli* serotype 055:B5) was obtained from Sigma (St. Louis, Mo.). Medium for U1 and MAGI-CCR-5 cell cultures consisted of RPMI 1640 medium (Mediatech, Herndon, Va.) containing 2.5 mM L-glutamine, 25 mM HEPES, 100 units/ml penicillin, and 100 μ g/ml streptomycin (Life Technologies, Grand Island, N.Y.) with 10% (for U1 cells) or 7.5% (for MAGI-CCR-5 cells) v/v heat-inactivated fetal bovine serum (Life Technologies). Lymphocyte-tropic HIV-1 strain AO18A was supplied by the National Institutes of Health (NIH) AIDS Research and Reference Reagent Program, National Institute of Allergy and Infectious Diseases (NIAID). The monocytotropic HIV-1 isolate used in the whole blood studies was

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obtained and expanded from a patient participating in a clinical evaluation of antiretroviral drug resistance (20).

U1 cells

U1 cells were obtained from the NIH AIDS Research and Reference Reagent Program, NIAID (21). All final cell concentrations were $1 \times 10^6 \text{ ml}^{-1}$ in a 0.5 ml volume in 24-well polystyrene tissue culture plates (Falcon, Lincoln Park, N.J.). Cells were incubated without or with AAT or CE-2072 for 1.0 h (37°C , 5% CO_2), followed by the addition of stimulus. After 48 h of culture, Triton-X-100 (1% v/v final concentration, Sigma) was added to each culture and the cultures were frozen at -70°C until p24 antigen measurement by enzyme-linked immunoassay (ELISA) (National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Md.). Quantification of p24 using the ELISA was not affected by the presence of AAT.

Peripheral blood mononuclear cells (PBMC)

PBMC isolated from three healthy donors were incubated for 2 days prior to HIV-1 infection in R3 medium [RPMI 1640 medium, 20% fetal bovine serum, penicillin/streptomycin, and 5% (v/v) IL-2 (Hemagen, Waltham, Mass.)], which was supplemented with an additional 5% (v/v) IL-2 and 3.3 $\mu\text{g/ml}$ phytohemagglutinin (Sigma) (22). After the 2 day incubation, peripheral blood mononuclear cell suspensions from each donor were aliquoted equally into two 50 ml polypropylene tubes (Falcon); the cells were concentrated by centrifugation (400 *g*) and the supernatants decanted. One of the two 50 ml tubes received AAT vehicle and the second tube received 3.0 mg/ml AAT, each in a final volume of 500 μl . After 30 min of incubation (37°C , 5% CO_2), 250 tissue culture infective doses (TCID_{50}) of HIV-1 strain A018A per 1×10^6 PBMC were added to each tube (23). The tubes were then incubated for 3 h to allow infection of the cells. The infected PBMC in each of the two tubes were washed in R3 medium, and the cells were pelleted (400 *g*) and resuspended at $2 \times 10^6 \text{ ml}^{-1}$ in fresh R3 medium. A single 250 μl aliquot of infected PBMC suspension was pipetted from each of the two 50 ml tubes and placed into separate polypropylene tubes (Falcon) with 250 μl of R3 and 1% (v/v, final concentration) Triton-X-100. These aliquots were frozen (-70°C) until p24 assay and designated time 0 ($T=0$). Two hundred-fifty microliters of infected PBMC suspension from each of the two 50 ml tubes were then aliquoted in parallel into separate 24-well polystyrene tissue culture plates (Falcon). The cultures received an additional 250 μl of R3 medium alone (spontaneous cultures) or 250 μl of R3 medium containing AAT sufficient to produce the final AAT concentrations. Cultures were incubated (37°C , 5% CO_2) for 4 days, after which Triton-X-100 (1% v/v) was added and the cultures frozen (-70°C) until p24 assay.

MAGI-CCR-5 cell infection

MAGI (multinuclear activation of a galactosidase indicator)-CCR-5 cells (NIH AIDS Research and Reference Reagent Program, NIAID) were aliquoted into 24-well polystyrene plates (Falcon) at 4×10^4 per well in a 1.0 ml volume (24). After 24 h of incubation (37°C , 5% CO_2), all medium was removed from each well and 200 μl fresh medium was added without or with AAT at the final concentration. AAT diluent was added to a separate culture at the largest concentration used and served as a vehicle control. All cultures were then incubated for 1.0 h. One hundred-thirty TCID_{50} of A018A strain of HIV-1 and 20 $\mu\text{g/ml}$ DEAE dextran in 100 μl

medium were then added to the cell-containing wells. To evaluate background reporter activation, a separate cell-containing well received DEAE dextran in medium without virus. After a 2 h incubation, medium was added to each culture to adjust the final volume to 500 μl , and the cultures were incubated for 48 h. Medium was then aspirated, the cells were fixed, and a β -galactosidase staining solution was added as described (24). After 50 min of incubation, a blinded count of pigmented (reporter-activated) cells under a $100\times$ microscope was conducted.

Electrophoretic mobility shift assay (EMSA)

Ten million U1 cells at $5 \times 10^6 \text{ ml}^{-1}$ final concentration were suspended in medium alone as a control and in medium without or with 5.0 mg/ml AAT or 30 μM CE-2072. Cells were incubated for 30 min (37°C , 5% CO_2), after which IL-18 was added (40 ng/ml) to each culture except the control. After 45 min of incubation, nuclear protein extracts were obtained from each culture as described previously (25). A double-stranded oligonucleotide probe containing an NF- κB binding region (5' AGT TGA GGG GAC TTT CCC AGG C 3'; Promega, Madison, Wis.) was end-labeled with [γ - ^{32}P] ATP (New England Nuclear, Boston, Mass.) following the manufacturer's protocol (Life Technologies). The labeled probe was purified using a NuTrap column (Stratagene, La Jolla, Calif.) and activity was determined using a scintillation counter (Beckman LS 6500). Two hundred thousand counts per minute of probe were combined with 3.0 μg nuclear protein extract from each culture condition and 0.03 μg of poly-dI/dC in separate polypropylene tubes. EMSA buffer (50 mM NaCl, 0.5 mM EDTA, 10 mM Tris-HCl pH 7.5, 1.0 mM MgCl_2 , 4% glycerol, and 0.5 mM DTT) was added to each tube to produce a final volume of 25 μl .

Supershift and competition experiments were also performed. For each culture condition, two parallel duplicate tubes contained either anti-p65 antibody (5.0 μl , Santa Cruz Biotechnology, Santa Cruz, Calif.) or nonlabeled probe (100-fold molar excess). After 30 min of incubation at room temperature, 20 μl of material was pipetted from each tube and loaded into a 5% acrylamide, $0.5\times$ Tris-borate-EDTA, 2.5% glycerol gel and run at 10 V/cm. The gel was removed after the free probe had run three-fourths of its length. The gel was then dried onto Whatman paper and exposed to X-ray film overnight at -70°C .

Whole blood HIV-1 infection

Blood was obtained by venipuncture from each participant and aspirated into a prepared glass vacuum tube containing premeasured buffered sodium citrate anticoagulant (final concentration 10.5 mM, Becton Dickinson, Franklin Lakes, N.J.). One milliliter of blood was pipetted into a sterile capped polypropylene tube (Falcon). Two thousand TCID_{50} of a single-passage HIV-1 clinical isolate that was non-syncytium inducing (monocyte-tropic) and titered by standard protocol was added to the blood (20, 26, 27). A 180 μl time 0 ($T=0$) aliquot was removed prior to incubation, and Triton-X-100 added (1% v/v final concentration) and frozen at -70°C . The remaining infected blood was then cultured for 4 days (37°C , 5% CO_2) with the polypropylene cap loosely applied. A 450 μl sample was collected after culture, Triton-X-100 was added (1% v/v), and the sample was frozen at -70°C until p24 assay.

Statistical analysis

Replicate experiments were independent, and summary results are presented as means \pm SE. Differences were consid-

ered significant for $P < 0.05$. Group means were compared by repeated measures analysis of variance (ANOVA) using Fisher's least significant difference. For U1 cell and PBMC experiments, percent reduction/inhibition for each sample was calculated by subtracting the percent p24 from 100%. Percent p24 in each sample was derived from the equations:

percent p24 in U1 cell sample

$$= \left[\frac{\text{sample p24 concentration} - \text{control p24 concentration}}{\text{stimulus alone p24 concentration} - \text{control p24 concentration}} \right] \times 100\%$$

percent p24 in PBMC sample

$$= \left[\frac{\text{sample p24 concentration} - (\text{T} = 0 \text{ p24 concentration})}{\text{spontaneous p24 concentration} - (\text{T} = 0 \text{ p24 concentration})} \right] \times 100\%$$

For MAGI-CCR-5 results, percent reduction for each sample was calculated by subtracting the percent reporter-activated counts from 100%. Percent of reporter-activated counts in each sample was derived from the equation:

percent reporter-activated counts in MAGI-CCR-5 cell sample

$$= \left[\frac{(\text{counts in sample}) - (\text{counts in the absence of HIV-1})}{(\text{counts in HIV-1 alone culture}) - (\text{counts in the absence of HIV-1})} \right] \times 100\%$$

RESULTS

AAT inhibits HIV-1 production in stimulated U1 cells

AAT was purified from Cohn fraction IV-1 from the plasma of healthy volunteers. Successive anion and cation exchange chromatography produced fully active AAT as assessed by porcine elastase inhibition assay (28) and array nephelometric antigen analysis (29). The AAT was > 94% pure by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and high-performance liquid chromatography assessment (data not shown). The capacity of purified AAT to affect HIV-1 production was examined in the chronically infected U1 cell line, which contains inducible HIV-1 incorporated as a latent provirus (21). Virus production was determined in the U1 cells after 48 h of culture by quantification of HIV-1-specific p24 antigen. As shown in **Fig. 1A**, p24 production increased 150-fold to 6235 ± 1775 pg/ml after stimulation with 10 ng/ml IL-18 compared to control cultures containing cells in medium alone (Ctl) (25). The presence of AAT reduced IL-18-induced p24 dose-dependently, with 1 and 3 mg/ml AAT reducing p24 by 65 and 98%, respectively. As an alternative stimulus, we used the HIV-1-inducing cytokine IL-6 (30). One hundred nanograms per milliliter IL-6 increased HIV-1 production 3.6-fold compared to control (**Fig. 1B**), to 4337 ± 2006 pg/ml. Three and 5.0 mg/ml AAT inhibited IL-6-induced p24 by 80 and 100%. To exclude the possibility that AAT

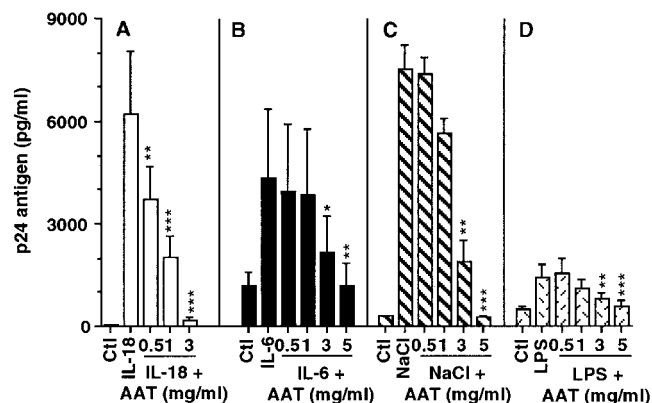


Figure 1. AAT inhibits p24 antigen production in stimulated U1 cells. U1 cells were incubated for 48 h with medium alone as a control (Ctl), with each stimulus alone, or with AAT added 1.0 h prior to each stimulus. Final AAT concentrations are indicated on the horizontal axis. Stimuli shown are A) IL-18 at 10 ng/ml in three separate experiments, B) IL-6 at 100 ng/ml in four separate experiments, C) a 60 mM increase in NaCl in three separate experiments, and D) LPS at 500 ng/ml in five separate experiments. p24 antigen production is indicated on the vertical axis as the mean \pm SE. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ compared to each stimulus alone.

bound and sequestered IL-18 or IL-6, we stimulated HIV-1 production using NaCl-induced hyperosmolarity as described previously (31). Hyperosmolarity induced by the addition of 60 mM NaCl to the cultures increased p24 concentration by 26-fold to 7511 ± 707 (Fig. 1C), which was inhibited by 76 and 100% using 3.0 and 5.0 mg/ml AAT, respectively.

Secondary infections occurring in HIV-1-infected individuals often result in a transient increase in circulating virus (32, 33). Lipopolysaccharide is a surface component of gram-negative bacteria with proinflammatory properties. Therefore, LPS may participate in infection-associated increase in HIV-1 production. U1 cell cultures stimulated with 500 ng/ml LPS contained 1427 ± 391 pg/ml p24, a 3.0-fold increase compared to control, as shown in **Fig. 1D**. Three and 5.0 mg/ml AAT inhibited LPS-induced p24 by 68 and 89%.

Neither a toxic nor antiproliferative effect of 5.0 mg/ml AAT was detected in three separate experiments using trypan blue exclusion, cell counts, and metabolically induced formazan production (CellTiter 96, Aqueous One solution Cell Proliferation Assay; Promega). The AAT vehicle (0.15 M NaCl, 0.02 M NaPO_4 , pH 7.05) added to cultures at the largest volume used did not affect IL-18-induced p24 production (three separate experiments).

We considered the possibility that a substance copurified with the AAT used in these studies was responsible for the inhibitory effects obtained. Therefore, we used CE-2072, a 700 Da peptide-based synthetic inhibitor of the serine proteases neutrophil elastase and proteinase-3 (34). As shown in **Fig. 2**, CE-2072 inhibited HIV-1 production in U1 cells stimulated with 10 ng/ml IL-18 (**Fig. 2A**) or with 60 mM NaCl (**Fig. 2B**). The CE-2072-induced inhibitory effect was dose dependent.

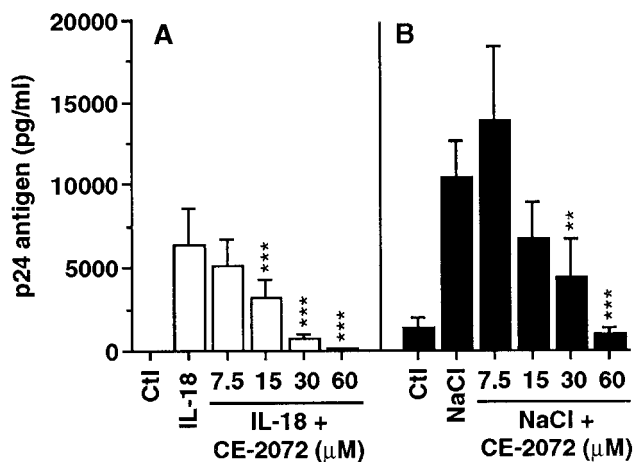


Figure 2. CE-2072 inhibits p24 antigen production in stimulated U1 cells. U1 cells were cultured as described for Fig. 1, except that CE-2072 was added 1.0 h prior to the addition of each stimulus. Shown are cultures stimulated with A) IL-18 at 10 ng/ml in three separate experiments and B) a 60 mM increase in NaCl in three separate experiments. Data are presented as the mean \pm SE. ** $P < 0.01$ and *** $P < 0.001$ compared to each stimulus alone.

CE-2072 (60 μ M) inhibited p24 production in response to IL-18 or NaCl with reductions of 99 and 100%, respectively. Neither a toxic nor antiproliferative effect of 60 μ M CE-2072 was detected in three separate experiments using trypan blue exclusion, cell counts, and metabolically induced formazan production (Promega). The CE-2072 vehicle (DMSO) added to cultures at the largest volume used did not affect IL-18-induced p24 production in three separate experiments.

AAT inhibits HIV-1 production in infected PBMC

To ascertain whether AAT inhibited HIV-1 production in primary cells, PBMC isolated from healthy donors were infected with HIV-1. For each donor, infection was conducted in the absence or presence of 3.0 mg/ml AAT. After infection, the cells were washed in R3 medium to remove free viruses. Washing also removed free AAT from cells infected in the presence of this molecule. **Figure 3A** shows PBMC infected in the absence of AAT. p24 concentration increased after 4 days of culture, from 180 ± 63 pg/ml at time 0 (T=0, cell-associated virus measured after infection but prior to culture) to 7781 ± 1650 pg/ml after 4 days (spontaneous, Spont). Alpha-1-antitrypsin at 4.0 and 5.0 mg/ml reduced 4 day p24 production by 33 and 53%, respectively. **Figure 3B** shows the results in PBMC from the same donors as in Fig. 3A, except that cells were infected with HIV-1 in the presence of AAT. p24 increased after 4 days, from 107 ± 52 pg/ml at time 0 to 8478 ± 629 pg/ml in 4 day spontaneous cultures. AAT at 2.0, 3.0, 4.0, and 5.0 mg/ml reduced p24 by 71, 62, 68, and 69%, respectively.

The presence of AAT at the time of infection (Fig. 3B) enhanced AAT-induced inhibition of spontaneous p24 production in the 4 day cultures. Enhanced inhi-

bition manifested as greater maximum p24 reduction (71% in Fig. 3B) vs. 53% in Fig. 3A) and as p24 reduction at lower AAT concentrations. The difference in enhanced suppression was statistically significant for AAT concentrations 2.0, 3.0 and 4.0 mg/ml ($P < 0.05$ for each comparison by ANOVA).

The presence of 5 mg/ml AAT in 4 day PBMC cultures did not affect proliferation compared to 4 day spontaneous cultures, as assessed using metabolically induced formazan production (Promega, three separate experiments). The AAT vehicle did not affect 4 day p24 production when added at the largest volume used in three separate experiments.

AAT inhibits HIV-1 infection

We examined the effect of AAT on early events during HIV-1 infection using MAGI-CCR-5 cells. When exposed to HIV-1, entry into these cells and production of viral Tat protein activate an intracellular *LacZ* reporter (24). Tat-induced reporter activation initiates production of β -galactosidase, which produces pigment after addition of a substrate. In the absence of virus (Fig. 4, first bar from left), a mean 2.3 ± 1 reporter-activated cells was obtained, representing background activation. Addition of HIV-1 increased the number of reporter-activated cells by 31-fold to 72 ± 13 (Fig. 4, second bar from left). Addition of virus in the presence of 3.0, 4.0, and 5.0 mg/ml AAT reduced the mean number of reporter-activated cells by 41, 66, and 76%, respectively. Alpha-1-antitrypsin vehicle at the largest volume used did not alter HIV-1-induced reporter activation compared to cells infected with HIV-1 alone (Fig. 4, third bar from left).

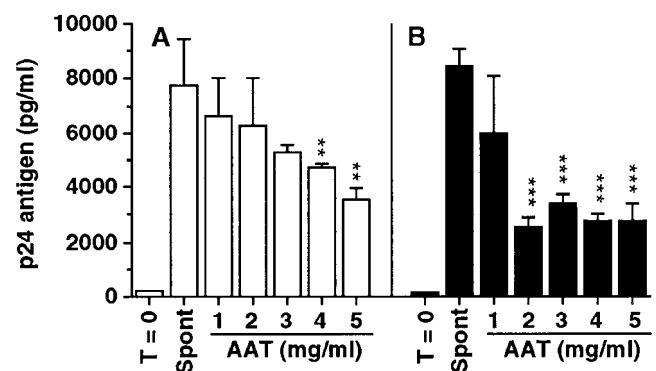


Figure 3. AAT inhibits spontaneous p24 antigen production in PBMC. A) PBMC from three healthy donors were infected with HIV-1. The PBMC were washed and time 0 (T=0) PBMC aliquots were obtained prior to culture. PBMC were cultured for 4 days in medium alone to measure spontaneous (Spont) virus production or PBMC were cultured in the presence of AAT at the concentrations indicated. B) PBMC from the same 3 donors were infected in the presence of 3.0 mg/ml AAT. The cells were washed, T=0 aliquots of cells obtained, and PBMC were cultured for 4 days as described in panel A. Mean \pm SE. p24 production is shown on the vertical axis. ** $P < 0.01$ and *** $P < 0.001$ compared to Spont.

AAT inhibits NF-κB activation in U1 cells

Since AAT is not believed to have intracellular antiprotease activity, the inhibitory effect of AAT in U1 cells (Fig. 1) suggests a requirement for extracellular serine protease function in virus production. Therefore, we investigated the possibility that AAT altered intracellular signaling associated with HIV-1 production. We assessed activation of the transcription factor NF-κB, a well-characterized HIV-1 inducer (35, 36).

Figure 5 shows NF-κB activation in U1 cells as determined by EMSA. Control cultures contained U1 cells in medium alone and did not produce a significant shifted NF-κB binding complex (lane 1). However, a large amount of complex was observed for cells stimulated with 40 ng/ml IL-18 (lane 4), indicating increased NF-κB activation. Compared to 40 ng/ml IL-18 alone, the combination of 5.0 mg/ml AAT and IL-18 (lane 7) resulted in significantly reduced NF-κB activation. CE-2072 (30 μM) also reduced IL-18-activated NF-κB (lane 10).

NF-κB functions as a dimer composed of p50 and p65 components (37). To confirm that bound complexes consisted of NF-κB, we incubated probe/nuclear extract aliquots with an antibody recognizing the NF-κB p65 component. As indicated in Fig. 5 (lanes 5, 8, and 11), supershifted p65-containing complexes were observed in cultures stimulated with IL-18 alone and with IL-18 in the presence of AAT or CE-2072. Lanes 3, 6, 9, and 12 contain probe/nuclear extract incubated with an excess of nonradiolabeled probe. The near absence of NF-κB-shifted complexes demonstrates the specificity of interaction between the probe and NF-κB. Collectively, these studies establish the ability of AAT and CE-2072 to inhibit NF-κB activation.

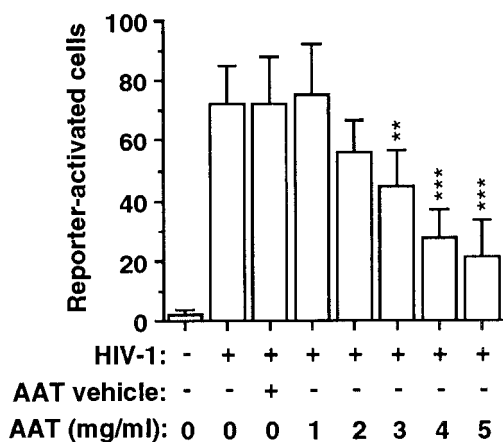


Figure 4. AAT inhibits HIV-1 infection. In 3 separate experiments MAGI-CCR-5 cells were exposed to HIV-1 and reporter-activated cells quantified (mean \pm SE) after 48 h of incubation. Cells were cultured in medium alone (background activation, far left bar), with HIV-1 alone (second bar from left), with virus and vehicle (third bar from left), or with virus and AAT at the concentrations shown. ** $P < 0.01$ and *** $P < 0.001$ compared to cells exposed to HIV-1 alone.

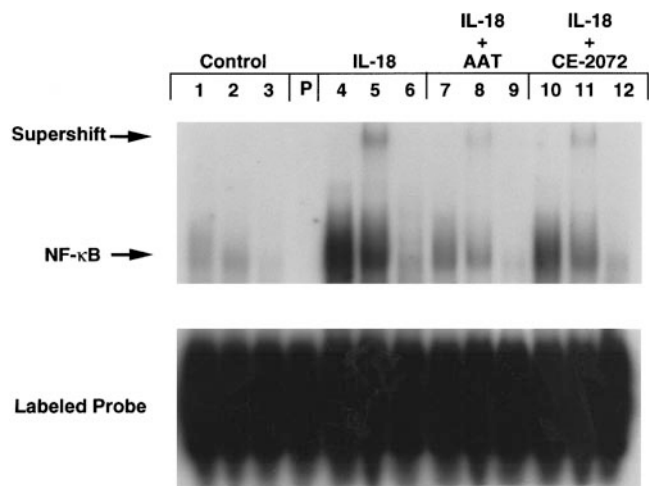


Figure 5. AAT and CE-2072 inhibit activation of NF-κB. U1 cells were cultured in medium alone (control, lanes 1–3), with 40 ng/ml IL-18 (lanes 4–6), with IL-18 in the presence of 5.0 mg/ml AAT (lanes 7–9), or with IL-18 in the presence of 30 μM CE-2072 (lanes 10–12). Activated NF-κB (shifted) binding complexes are indicated, as are supershifted anti-p65-containing complexes. Lanes 1, 4, 7, and 10 contain binary probe/nuclear extract complexes. Lanes 2, 5, 8, and 11 contain ternary probe/nuclear extract/anti-p65 antibody complexes. Lanes 3, 6, 9, and 12 contain binary complexes in the presence of excess unlabeled probe. Lane P contains labeled probe alone. Unbound labeled probe is indicated at the lower portion of the figure.

HIV-1 proliferates selectively in the blood of AAT-deficient individuals

Alpha-1-antitrypsin may function as a natural inhibitor of HIV-1 in the circulation. To test this hypothesis, HIV-1 was added to anticoagulated whole blood obtained from 14 healthy volunteers between 24–40 years of age (10 males and 4 females). Alpha-1-antitrypsin was measured in the serum of these volunteers using a nephelometry assay (AAT Kit, Beckman, Fullerton, Calif.) and concentrations were within the normal range for each individual (data not shown). As shown in Fig. 6A, the addition of clinically derived monocyte-tropic HIV-1 did not result in significant virus production after 4 days of incubation. p24 measured immediately after addition of virus (T=0) was 186 ± 41 pg/ml. After 4 days of incubation the p24 concentration was 338 ± 52 pg/ml, an increase of ~ 0.8 -fold.

We performed similar experiments using blood obtained from individuals with a specific genetic abnormality resulting in deficient amounts of circulating AAT. These subjects exhibited the mutant Z-type variant of AAT, which contains a single point-mutation at amino acid 342 (Glu-Lys), resulting in misfolded protein. Abnormal AAT accumulates within liver cells due to defective secretion, resulting in severely reduced serum concentrations to $< 15\%$ of normal. This mutation affects 70,000–100,000 persons in the United States, who often acquire premature and severe emphysema due to unopposed protease activity in lung tissues (16, 18, 38, 39).

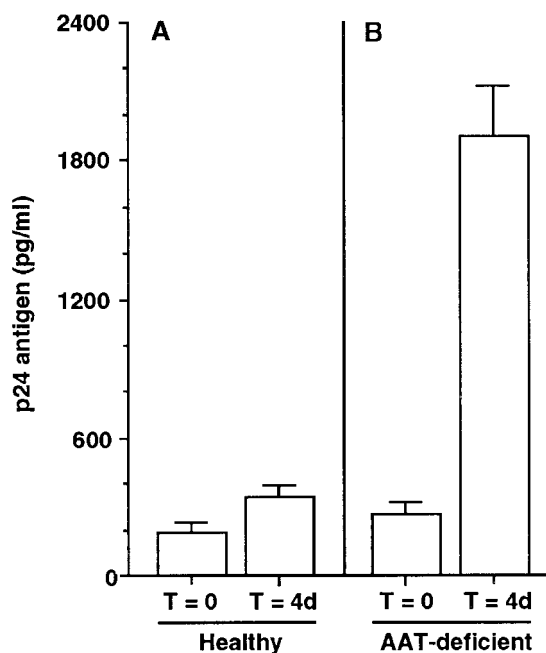


Figure 6. Production of HIV-1 in whole blood from healthy volunteers and in blood from AAT-deficient patients. p24 antigen was measured in blood obtained from *A*) 14 healthy volunteers, and *B*) 5 AAT-deficient patients. Aliquots of blood were collected after the addition of virus (T=0) and after 4 days of incubation (T=4d). Mean \pm se. p24 concentrations are shown for each group.

Blood was collected from five such patients 35–68 years of age (three males and two females) in whom AAT deficiency was established by the exclusive presence of mutant Z-type AAT on isoelectric focusing analysis (40). To confirm reduced circulating AAT levels in these patients, serum AAT was measured using nephelometry, and the level in each patient was below the normal range (data not shown). Four of the patients received cyclic intravenous AAT replacement therapy (41). Blood was obtained from these patients immediately before the next treatment, when AAT levels were at the nadir. Figure 6*B* shows whole blood HIV-1 production in these patients. Aliquots of blood removed immediately after infection (T=0) contained 262 ± 53 pg/ml p24. After 4 days of incubation (T=4d), 1907 ± 221 pg/ml p24 was measured—an increase of ~ 6.3 -fold. These observations support the role of endogenous AAT as an HIV-1-suppressive factor in the circulation of healthy subjects.

DISCUSSION

We propose that AAT is a natural HIV-1 antagonist. The HIV-1-suppressive effect of physiological AAT concentrations was observed in four different biological systems: 1) induction of latent virus from chronically infected U1 cells, 2) viral infection and replication in PBMC, 3) HIV-1 infection of MAGI-CCR-5 cells, and 4) infection and replication in whole blood. Three inde-

pendent measures of HIV-1 production or infection were assessed, including p24 antigen concentration (U1 cells, PBMC, and whole blood), response of a Tat-activated reporter construct (MAGI-CCR-5 cells), and activation of NF- κ B (U1 cells). These results using AAT were supported by the suppressive effect of the synthetic inhibitor of serine proteases CE-2072. The ability of CE-2072 to inhibit HIV-1 diminishes the possibility that a substance copurified with the AAT used in these studies accounted for the observed HIV-1-suppression.

The inhibitory effect of AAT on early infection, as demonstrated in MAGI-CCR-5 cells (Fig. 4), may involve blockade of viral entry into the cell. An interaction between cell surface serine proteases and amino acids within the V3 domain of the gp120 HIV-1 envelope glycoprotein has been described (42–46). This interaction facilitates syncytium formation or infectivity *in vitro* (44, 45, 47). Therefore, AAT may inhibit HIV-1 infectivity (as shown in the MAGI-CCR-5 cell experiments) by disrupting this interaction. A separate mechanism of AAT-induced HIV-1 inhibition was blockade of viral production in latently infected cells, as shown in the U1 cell experiments (Fig. 1). Inhibition of NF- κ B activation (Fig. 5) likely accounted for this effect.

HIV-1 infection and production may be inversely related to amounts of functional AAT, and additional serine protease inhibitors may participate in this effect. Since AAT neutralizes elastase, cathepsin G, trypsin, chymotrypsin, plasmin, thrombin, plasminogen, kallikrein, clotting factor Xa, proteinase-3, and other serine proteases (16, 38, 48), the function of one or more of these proteases is likely required for HIV-1 propagation. However, CE-2072 has known antiprotease activity restricted to neutrophil elastase and proteinase-3, suggesting involvement of these specific proteases in HIV-1 progression (34).

These results may explain the HIV-1-suppressive activity in blood (Fig. 6*A*) and the apparent restriction of virus production to specific tissues. The capacity of AAT to inhibit HIV-1 may also explain, in part, the low risk of infection after percutaneous exposure to infected blood.

All currently approved antiretroviral medications target the virus-specific aspartyl protease or the viral reverse transcriptase (49). Consequently, viral mutation may confer resistance to the inhibitory effect of these drugs. Our results imply endogenous serine protease activity is required for HIV-1 infection and production. Therefore, viral mutation may not confer resistance to targeted inhibition of an endogenous serine protease. Inhibitors of host serine proteases should be considered candidate agents to treat HIV-1-associated disease. **[F]**

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