HIV-1 Antiviral Activity of Recombinant Natural Killer Cell Enhancing Factors, NKEF-A and NKEF-B, Members of the Peroxiredoxin Family*

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CD8+ T-cells are a major source for the production of non-cytolytic factors that inhibit HIV-1 replication. In order to characterize further these factors, we analyzed gene expression profiles of activated CD8+ T-cells using a human cDNA expression array containing 588 human cDNAs. mRNA for the chemokine I-309 (CCL1), the cytokines granulocyte-macrophage colony-stimulating factor and interleukin-13, and natural killer cell enhancing factors (NKEF) -A and -B were up-regulated in bulk CD8+ T-cells from HIV-1 seropositive individuals compared with seronegative individuals. Recombinant NKEF-A and NKEF-B inhibited HIV-1 replication when exogenously added to acutely infected T-cells at an ID50 (dose inhibiting HIV-1 replication by 50%) of ~130 nM (3 μg/ml). Additionally, inhibition against dual-tropic simian immunodeficiency virus and dual-tropic simian-human immunodeficiency virus was found. T-cells transfected with NKEF-A or NKEF-B cDNA were able to inhibit 80–98% HIV-1 replication in vitro. Elevated plasma levels of both NKEF-A and NKEF-B proteins were detected in 23% of HIV-infected non-treated individuals but not in persons treated with highly active antiretroviral therapy or uninfected persons. These results indicate that the peroxiredoxin family members NKEF-A and NKEF-B are up-regulated in activated CD8+ T-cells in HIV infection, and suggest that these antioxidant proteins contribute to the antiviral activity of CD8+ T-cells.

CD8+ T-cells inhibit HIV-1 replication by both cytolytic and non-cytolytic mechanisms (1). The importance of cell-mediated cytotoxic immunity for the partial control of human immunodeficiency virus type 1 (HIV-1) replication in infected individuals is now widely recognized (2–10). The direct killing of virus-infected cells by antigen-specific cytotoxic T-lymphocytes is considered to be the dominant mechanism of virus suppression. Nevertheless, chemokines (MIP-1α, MIP-1β, and regulated on activation normal T-cell expressed and secreted (RANTES)) produced by CD8+ T-cells have been shown to inhibit HIV-1 replication in vitro (11, 12) at the level of viral entry (13, 14) and may play a critical role in vivo as an antiviral host defense (15, 16). CD8+ T-lymphocytes can suppress human immunodeficiency virus type 1 (HIV-1) replication in vitro by secreting a soluble factor(s) that differs from the chemokines in the mechanism of inhibition. These factors remain undefined (17–26) and have been termed CD8+ T-lymphocyte antiviral factors (CAF). Although the cytotoxic T-lymphocytes response is major histocompatibility complex class I-restricted, this restriction does not apply to inhibition of HIV-1 replication by CAF (17, 25). Moreover, the production of CAF action appears to be the property of stimulated CD8+ T-cells and does not require HIV infection (19, 20, 26, 27). One site of CAF action is the inhibition of HIV-1 RNA transcription, particularly at the long terminal repeat (LTR) that is assumed to function through down-regulation of the NF-κB pathway (19, 21–23). It seems probable that the antiviral action of CAF is achieved by more than one cytokine or chemokine secreted by CD8+ T-cells, perhaps acting in concert (28, 29).

Recently we showed that CAF consists at least two components (26), a heparin-binding >50-kDa molecule, which we identified as a modified form of antithrombin (30), and a smaller molecule, possibly the α-defensins (31). In this study, we used a gene array to perform a more comprehensive analysis of gene expression by CD8+ T-cells from HIV-infected persons. We demonstrate here that the peroxiredoxin family proteins NKEF-A and NKEF-B are up-regulated by CD8+ T-cells following activation. These proteins exogenously added or intracellularly expressed have anti-HIV activity and have been described to increase natural killer cell activity (32–35), increase cell resistance to oxidative stress (36, 37), and regulate transcription activator protein (AP-1) (see Ref. 38 and reviewed in Ref. 39). We show that recombinant NKEF-A and NKEF-B protein exogenously added to HIV-1 cultures can inhibit HIV-1 replication and that T-cells transfected with NKEF-A or NKEF-B cDNA were resistant to HIV-1 infection.

EXPERIMENTAL PROCEDURES

Subjects—Plasma was obtained from 13 long term nonprogressors. Control plasma samples were obtained from 13 HIV-1 seronegative, healthy donors. Additional plasma samples from 6 asymptomatic, 5
symptomatic, and 2 AIDS patients, all under highly active antiviral therapy treatment for progressive disease, were investigated. Bulk CD8+ T-cells were purified, expanded, and stimulated as described previously (26) by anti-CD3 cross-linking from peripheral blood mononuclear cells, which were obtained from six HIV-1-infected long term non-progressors (17393, 15188, CT5-02, NEW, RK2000, and CX471) (26) and from seven HIV-1 seronegative individuals. Bulk CD8+ T-cells for each individual were treated separately. Long term non-progression was defined as being infected for more than 10 years, having plasma HIV-1 loads ≤ 400 RNA copies per ml, and CD4+ T-cell counts > 500 per μl in the absence of therapy.

**Assay for Inhibition of HIV-1 Replication**—For the inhibition tests, HIV-1_105, a T-cell tropic strain of HIV-1 (ATCC CRL-8543) was used. CD8+ T-cells were acutely infected with HIV-1_105 at a multiplicity of infection of 10−2 TCID50/ml and resuspended in RPMI 1640 (Sigma) supplemented with 20% (v/v) heat-inactivated fetal calf serum (Sigma; R20 medium) (26). The cells were then plated in 2 ml of R20 medium at 5 × 105 cells/ml in a 24-well plate. 9H cell supernatant (1 ml) was removed every 3 days and replaced with 1 ml of fresh R20 medium supplemented with recombinant NKEF-A or NKEF-B protein. After 9 days the concentration of p24-antigen was measured using an HIV-1 p24 ELISA kit (PerkinElmer Life Sciences). For the dual-tropic SIV-239 or dual-tropic SHIV, studies, the human T-cell line 174xCEM was infected with a multiplicity of infection of 10−2 TCID50/ml and resuspended in R20. The cells were then plated as described above. At 9 days the concentration of SIV p27-antigen was measured by ELISA (Coultier, Miami, FL). The medium controls demonstrated p24 or p27 antigen levels in excess of 100 ng/ml at day 9 and were used to calculate percent virus inhibition.

**Transfection with the NKEF-A and NKEF-B Gene**—Jurkat cells were used for inhibition experiments following transfection with NKEF-A and NKEF-B. NKEF-A- and NKEF-B-expressing vectors (31) were digested with BamHI and XhoI (New England Biolabs, Beverly, MA). The digest was treated with T4 polymerase (Invitrogen) for blunt-end ligation according to the manufacturer's instructions. The NKEF-A and NKEF-B fragments were then inserted into the Smal cloning site of the pRSRE2-EGFP expression vector (Clontech, Palo Alto, CA) and cultured in Escherichia coli. Plasmid DNA were isolated, and the correct inserts was confirmed by DNA sequencing. Jurkat cells were then transfected with a DNA/liposome mixture (FuGENE, Roche Diagnostics) and selected using G418 (Sigma) pressure (1.5 mg/ml). Stable transfected cells were then used in the above described inhibition test using 1.5 mg/ml G418. For days 1–9 the concentration of p24 antigen was measured using an HIV-1 p24 ELISA kit (PerkinElmer Life Sciences). The percentage of inhibition was calculated against a control with the empty pRSRE2-EGFP vector.

**Total RNA Extraction and Northern Blot Analysis**—For total RNA extraction cell pellets of 0 or 4 h, anti-CD3-activated bulk CD8+ T-cells (105) were lysed, 1 ml of RNA STAT60 (Tel-Test B, Friendswood, TX), and the cellular RNA was purified using the RNA STAT60 protocol. To eliminate the DNA contaminant of the RNA the CLEANMAGTM Kit (Gehuniter, Nashville, TN) was used. 10 μg of total cellular RNA was fractionated on a 1.2% agarose, 0.7% formaldehyde gel and hybridized with the inserts confirmed by DNA sequencing. Jurkat cells were then transfected with a DNA/liposome mixture (FuGENE, Roche Diagnostics) and selected using G418 (Sigma) pressure (1.5 mg/ml). Stable transfected cells were then used in the above described inhibition test using 1.5 mg/ml G418. For days 1–9 the concentration of p24 antigen was measured using an HIV-1 p24 ELISA kit (PerkinElmer Life Sciences). The percentage of inhibition was calculated against a control with the empty pRSRE2-EGFP vector.

**RNA Expression Screening with the ATLAS™ Assay**—The ATLAS Array (Clontech) is a nitrocellulose membrane with 588 spotted cDNAs. For the hybridization polyadenylated (polyA+) mRNA was prepared from 100 μg total RNA of 4 h CD3-cross-linked or untreated bulk CD8+ T-cells (105), and bulk CD8+ T-cells of HIV-1 seronegative individuals were also measured separately. The mRNA was suspended in diethyl pyrocarbonate-treated water and separated on poly(A) Quik® RNA Columns (Stratagene, La Jolla, CA) according to the manufacturer's protocol. 1 μg (in 2 μl) of each poly(A)+ mRNA sample was transcribed to radiolabeled cDNA using 1 μl of Moloney murine leukemia virus reverse transcription-strategy (Stratagene) and 0.001 μl [α-32P]dATP (3000 Ci/ mmol, 10 μCi/ml) according to the ATLAS assay protocol and used for hybridization.

The binding of radioactivity to membrane was measured with the Molecular PhosphorImager System GS-363 (Bio-Rad) for an equal amount of time. Signal intensity calculations were performed using the supporting software program Molecular Analyst™ and calibrated against the GAPDH signal.

**Expression in Sf21 Cells and Purification of NKEF-A and NKEF-B from Sf21 Cells**—To produce the NKEF-A and NKEF-B proteins for the above described inhibition test, the NKEF-A and NKEF-B genes were cloned into the baculovirus expression vector pBacPAK9 (Clontech) and overexpressed in Spodoptera frugiperda (SF21 cells; Clontech) as described (33). After transfection SF21 cells were harvested and lysed with insect lysis buffer (Pharmingen) at days 2–4. Afterward recombinant protein was purified as described earlier (35).

**Characterization of Soluble Factors**—For the NKEF-A and NKEF-B ELISAs 2 μg/ml of monoclonal mouse anti-NKEF-A or anti-NKEF-B antibody (Pharmingen) was incubated overnight at 4 °C on protein high-binding EIA/RIA plates (Costar, Cambridge, MA) in coating buffer (0.05 M CO2−/HCO3− buffer, pH 9.6). Plates were washed with PBS buffer (phosphate-buffered saline (PBS), 0.05%/v/v) Triton X-100 (Sigma), pH 7.4) and blocked for 2 h at 37 °C with blocking buffer (PBS, 3%/v/v) goat serum, 3%/w/v bovine serum albumin). Plates were washed with PBS buffer. Standard protein or samples were incubated for 2 h at 37 °C. Plates were washed with PBS buffer. A rabbit polyclonal NKEF-A/NKEF-B detection antibody (32), which recognizes both forms of the NKEFs, was diluted 1:1000 in PBSBS buffer (PBS, 0.1%/w/v) bovine serum albumin, 0.05%/v/v) Triton X-100) and was added at 37 °C for 30 min. After washing with PBS buffer a horseradish peroxidase-coupled anti-rabbit antibody (Vector, Burlingame, CA) was used at 1:5000 dilution at room temperature for 20 min. After washing with PBS buffer the ELISA was developed for 30 min at room temperature with a 1:1 dilution of peroxidase solution B and TMB peroxidase substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD) and stopped with 4 M H2SO4. SDS-PAGE and Western blot was carried out as described previously (26) by polyacrylamide NKEF-A and NKEF-B-specific antibodies (32) at a dilution of 1:10,000. Protein concentration was determined by the BCA method (Pierce).

**Statistical Analysis**—Fisher's exact test was used to determine significance. Standard error is shown as error bars in the figures.

**RESULTS**

**Differential Gene Expression in CD8+ T-cells of HIV-1 Seronegative and Seropositive Individuals**—CD8+ T-cells are a major source for inhibitory non-cytolytic factors in HIV-1-infected persons. We have shown previously (26) that secretion of soluble antiviral factors is elevated in expanded CD8+ T-cells from HIV-infected persons. In order to assess potential differences in gene expression, which might also be responsible for the antiviral activity, we evaluated mRNA derived from CD8+ T-cells using the ATLAS array, which contained 588 unique genes. Expanded CD8+ T-cell populations of HIV-1 seronegative and the HIV-1 seropositive untreated individuals were evaluated prior to stimulation and 4 h following stimulation with anti-CD3.

Although the ATLAS array includes a wide spectrum of genes, significant differences were limited to the expression of only four genes. These included significant differences (p < 0.001) in mRNA levels for the chemokine I-309, the cytokines GM-CSF and IL-13, and the peroxiredoxin gene NKEF-B (Fig. 1A). The peroxiredoxin data were confirmed by Northern blot analysis using NKEF-A cDNA, a homologue of NKEF-B, which showed differences for this peroxiredoxin gene as well (Fig. 1B). Both proteins have antioxidant function, and a natural killer cell activity was found for the NKEF-A and NKEF-B complex or for recombinant NKEF-A (reviewed in Ref. 39). These mRNA results are consistent with previous studies showing higher secretion of I-309, GM-CSF, and IL-13 in stimulated CD8+ T-cells from seropositive persons (26), and they extend these prior studies by demonstrating the elevation in these peroxiredoxin family mRNAs in CD8+ T-cells from HIV-1 seropositive persons.

**Inhibition of HIV-1 Replication with Recombinant NKEF-A and NKEF-B Protein**—By having demonstrated that GM-CSF, I-309, IL-13, and the NKEFs were preferentially expressed in CD3-activated, HIV-1-infected but untreated individuals, we next evaluated whether some of these gene products might contribute antiviral activity. Although GM-CSF, I-309, and
IL-13 have been shown to influence HIV-1 replication in some in vitro systems (40), we found that these proteins did not inhibit HIV-1<sub>in vitro</sub> replication in acutely infected T-cells when added up to 1 μg/ml, demonstrating that they do not contribute to the observed inhibition (see Ref. 26 and data not shown). In contrast, we found that recombinant NKEF-A and NKEF-B proteins that we expressed in SF21 cells using a baculovirus expression system and purified to homogeneity (Fig. 2A) inhibited HIV-1<sub>in vitro</sub> replication at an ID<sub>50</sub> of 130 nM (3 μg/ml), respectively (Fig. 2B). Additionally, using the NKEF-B protein for the inhibition assay with dual-tropic SHIV and dual-tropic SIV strains, we found nearly complete suppression of these viruses at 3 μg/ml (Fig. 2, C and D). Total protein lysate of uninfected SF21 cells was used as a control and demonstrated no inhibition, excluding contaminating SF21 protein as being responsible for inhibition (data not shown). The observed inhibition did not correlate with a decrease in cell count as measured at log phase of cell growth from day 2 to 6 by trypan blue staining (data not shown). Our data indicate that recombinant NKEFs can inhibit replication of X4 HIV-1, dual-tropic SIV, and dual-tropic SHIV.

Secretion of NKEF-A and NKEF-B Proteins by CD8<sup>+</sup> T-cells—By having demonstrated that the NKEFs can inhibit HIV-1 replication, we next tested whether the NKEFs are secreted or released from stimulated CD8<sup>+</sup> T-cells, and whether these proteins contribute to the antiviral activity of these cells (26). Although NKEF-A and NKEF-B proteins were originally described as endogenous proteins (33, 37, 41), thioredoxin (also termed adult T-cell leukemia-derived factor), another protein that serves as the electron donor for most peroxiredoxins (reviewed in Ref. 42), has been shown to be secreted through a novel pathway despite having no signal sequence (43). A similar observation has been confirmed for peroxiredoxin IV (44). We found that both NKEF-A and NKEF-B proteins were secreted after 4 h, regardless of whether the cells were stimulated. The concentrations produced averaged 15–40 ng/ml (Fig. 3) or were at least 10–20 times higher than seen for peroxiredoxin IV (44). We found that both NKEF-A and NKEF-B proteins were secreted after 4 h, regardless of whether the cells were stimulated. The concentrations produced averaged 15–40 ng/ml (Fig. 3) or were at least 10–20 times higher than seen for the chemokines and cytokines at this 4-h time point (26) and were up to ~125 ng/ml at 16 h. The secretion was observed in stimulated CD8<sup>+</sup> T-cells from both infected and uninfected individuals (Fig. 3). Thus, despite higher levels of NKEF mRNA in cells from seropositive persons, supernatants of activated CD8<sup>+</sup> T-cells from both infected and uninfected individuals contained comparable amounts of these proteins, and this might be the result of the missing active protein secretion. This indicates that although NKEFs are able to exert antiviral activity, they are not the elusive CAF, which is produced in greater amounts from CD8<sup>+</sup> T-cells of infected persons (26). Additionally, the concentrations of secreted NKEFs at 4 h (15–40 ng/ml) are at levels below those causing significant inhibition (Fig. 2) at a time when significant inhibition by HIV-1-suppressive factor(s) was observed (26).

Blood Plasma Levels of the NKEF-A and NKEF-B Proteins—By having demonstrated that the NKEFs are secreted,
we next tested blood plasma levels by a specific ELISA to evaluate if plasma concentrations were sufficient to mediate inhibition of HIV-1 replication in vivo. Additionally, we tested if blood plasma levels are dependent on whether individuals are infected or not and treated or untreated. We could not detect a significant difference in NKEF-A or NKEF-B levels in plasma among the uninfected and the long term nonprogressor population. Nevertheless, plasma levels of the NKEFs were elevated (up to 500 ng/ml) in 3 of 13 (23%) HIV-1-infected but untreated persons tested, with levels 2.5–8 times higher than the uninfected or treated HIV patients (Fig. 4). For HIV-1-infected but untreated persons NKEF-A and NKEF-B were found at levels up to 1 μg/ml. At this concentration HIV-1 inhibition was detectable in vitro (Fig. 2B), and an increase of natural killer cell activity in vitro has been noted (35), demonstrating that NKEFs might have an antiviral influence in vivo.

We demonstrated that blood plasma of long term non-progressors have significantly more NKEF-B compared with blood plasma of asymptomatic and symptomatic patients (Fig. 4). Our data indicate that elevated levels of NKEFs are seen in a small percentage of untreated HIV-1-infected individuals and might be the result of a more active CD8+ T-cell response. It was shown earlier that NKEF genes up-regulated in a Th1 response (45) correlate with a more positive disease outcome (46), showing an expansion of the TNF system, probably through an activation of the cytotoxic compartment (47).

**Inhibition of HIV-1 Replication through NKEF-A and NKEF-B Transfection**—Another way to demonstrate inhibitory activity of the NKEFs is to transfect the gene into target cells and determine resistance of these transfected cells to HIV-1. This has been done for peroxiredoxin IV, demonstrating resistance against HIV-1 replication (48). We therefore transfected Jurkat CD4+ T-cells with NKEF-A or NKEF-B expression plasmids, which increased intracellular NKEF levels ~10-fold (Fig. 5A). These NKEF-A- and NKEF-B-transfected T-cells blocked 80–98% HIV-1 replication starting at day 6 post-infection, when compared with the mock-transfected cells (Fig. 5B). Our data indicate that enhanced expression of NKEFs in T-cells might inhibit HIV-1 replication, providing further evidence of the antiviral effect of these compounds.

**DISCUSSION**

CD8+ T-cells are a major source for inhibitory non-cytolytic factors. There are only few studies that compare gene expression between bulk CD8+ T-cells of seropositive and bulk CD8+
T-cells of seronegative individuals. Overall, a Th1 cytokine response is noted as an implication for long term survival (46). Another report shows that the expansion of the TNF system positively correlated with higher expression of TNF-α, interferon-γ, and activation marker secretion of CD8, probably presenting the activation of the cytotoxic compartment (47). Here we report that the NKEF-A and NKEF-B genes are expressed in greater quantity in bulk CD8 T-cells of HIV-1-infected individuals compared with the bulk CD8 T-cells of seronegative individuals (Fig. 1). Furthermore, we show that these genes, normally up-regulated through oxidative stress (49), can be up-regulated by anti-CD3 stimulus in T-cells (Fig. 1). Additionally, we show that these proteins are secreted (Fig. 2). They can inhibit HIV-1, SIV, and SHIV (Fig. 3). They are present in elevated amounts in the plasma of some but not all infected individuals in a way that bleed plasma from long term non-progressors have significantly more NKEF-B than plasma of asymptomatic and symptomatic individuals (Fig. 4). In vitro inhibition tests showed that HIV replication was decreased when the NKEFs proteins were overexpressed in CD4+ T-cells (Fig. 5). It was shown earlier that some peroxiredoxins, e.g. peroxiredoxin IV and its electron donor thioredoxin (42), have antiviral activity against HIV-1, through decreasing free radical concentrations with their antioxidant enzyme function (48, 50, 51) and down-regulating the NF-kB pathway, thereby blocking HIV-1 LTR transcription, a mechanism described for CAF (18, 23). For both NKEF-A and NKEF-B, previously discovered as natural killer cell activity enhancing factors, antioxidant functions were demonstrated (39). Therefore, we hypothesize a similar mechanism for these proteins.

Our data indicate that NKEF-A and NKEF-B are secreted by CD8+ T-cells at concentrations at least 10–20 times higher than found for the chemokines and cytokines 4 h after anti-CD3 stimulation (26). Secretion was observed regardless of whether the cells were stimulated or not or whether they originated from infected or uninfected individuals. These findings exclude the NKEFs from being the elusive CAF for which enhanced secretion is found for CD8+ T-cells of HIV-infected individuals. Furthermore, the concentrations released averaging 15–40 ng/ml at 4 h and ~125 ng/ml at 16 h (Fig. 3) are at levels below those suppressive levels (range, 50–92%) described earlier (26) causing significant inhibition (Fig. 2). CD8+ T-cells having a higher gene expression level, but the same secretion level between infected and uninfected individuals, can be a result of the fact that the NKEFs are not secreted actively (43). Therefore, it is likely that most of the protein might stay in the cytoplasm, and the differences in gene expression may not be high enough to make a significant difference in secretion over the period measured.

Our data indicate that NKEF-A and NKEF-B are elevated in plasma of some but not all HIV-1-infected but untreated individuals. It was already reported that HIV affects antioxidant factors through various mechanisms, and a relationship between thioredoxin levels and the HIV-1 disease progression was already demonstrated (51). Additionally, it was demonstrated that Th1 leukocytes, whose frequency is correlated with a more favorable disease outcome (46), express higher levels of NKEFs (45). Furthermore, elevated levels of thioredoxin correlate with a more favorable disease outcome as long as they were not higher than 30 ng/ml in AIDS patients with CD4+ T-cells counts below 200/μl (52). It was already shown that restoring antioxidant capacity paralleled immunologic and virologic improvements (53–55). The HIV Tat protein was able to suppress antioxidant factor expression (56, 57). Ongoing oxidative stress resulting from HIV infection stimulated Fas-induced CD4+ T-lymphocyte apoptosis and mediated a Tat- and gp160-induced functional impairment of infected T-lymphocytes and enhanced NF-kB-dependent activation of virus transcription (56, 58–60). We found no significant differences between NKEF-A and NKEF-B plasma levels of seronegative and sero-positive treated and combined seropositive untreated individuals (Fig. 4). Nevertheless, some untreated patients (3 of 13) have levels 2.5–8 times elevated than normal or found in seropositive treated individuals. The plasma levels were up to 500–1000 ng/ml for NKEF-A and NKEF-B when calculated together (Fig. 4), levels sufficient enough to show in vitro inhibition (Fig. 2) or increasing in vitro natural killer cell activity (35). The significant differences found between the long term non-progressors and asymptomatic and symptomatic patients might be the result of a higher cytotoxic activity of the CD8+ T-cells of the long term non-progressors. Because the amount of protein released is independent from a secretion process, a higher rate of killing during the cytotoxic response might be the reason for the higher amount of protein in the plasma.

Our data also indicate that T-cells overexpressing NKEF-A or NKEF-B inhibited HIV-1 replication (Fig. 5, C and D). It has been shown previously that overexpressing peroxiredoxin in T-cells inhibits HIV-1 transcription through inactivation of the NF-kB-dependent initiation of HIV-1 LTR replication (48). Additionally, this might be because of an inhibition of the AP-1 transcription factor-dependent HIV-1 replication, because it was already shown that overexpression of NKEF-B can block the TNF-α activation of AP-1 (38).

In summary, we report that NKEF-A and NKEF-B, members of the peroxiredoxin gene family are released by activated CD8+ T-cells and are capable of inhibiting HIV-1 replication. Although chemokine-like features are described for this protein family, including heparin binding (44) and chemotaxis (61), the antiviral mechanism of NKEF-A and NKEF-B is likely to be very different from the chemokines thus making their potential as a synergistic therapeutic agent or a marker for disease progression attractive and warranting further investigation.

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Natural Killer Cell Enhancing Factors Inhibit HIV-1

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