Human serum protein enhances HIV-1 replication and up-regulates the transcription factor AP-1

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In vitro studies on HIV (HIV-1) replication and neutralization are usually performed in human cell cultures supplemented with FBS instead of human serum (HS). Here we show that in contrast to FBS, addition of increasing amounts of human serum from noninfected donors to the cell culture directly correlates with an increase in HIV-1 replication in vitro. This effect is independent of cell line, virus strain, or batch of pooled human serum used. We found that human serum affects viral transcription in a dose-dependent manner by activating the activator protein-1 (AP-1) member proteins c-FOS, JunD, and JunB in TZM-bI cells. Analysis of the human serum component responsible for this effect indicates that it is a protein having a molecular mass between 250 and 300 kDa. This serum protein, HIV-1 enhancing serum protein (HESP), might promote viral transcription in vivo and consequently play a role in disease progression.

The need for fast and reliable results has driven the development of numerous methods to study HIV-1 infection in vitro. However, little is known about the impact that the artificial conditions in which the virus is expanded (e.g., cell background, genetic manipulation, growth media) might have on the final outcome of the infection, or if important effects on primary cells might be masked by the in vitro conditions.

In most assays, growth media is supplemented with FBS instead of human serum (HS). In a previous study, we supplemented our culture media with HS from uninfected donors as the source of natural antibodies, to show that such antibodies could be redirected toward HIV-1 and participate in the clearance of the viral infection by activation of the innate immune system (1). During the selection and optimization process of the neutralization assays used in these studies, we found that increasing concentrations of HS in the culture media consistently correlated with a higher readout of viral infectivity. Moreover, the effect was independent of virus strain, cell type, or batch of HS used.

An increase in HIV-1 infectivity promoted by HS has been previously described and it has been mainly attributed to anti-HIV-1 antibodies (antibody-dependent enhancement, ADE) and/or to the human complement activated by both the alternative and classical pathways. Anti-HIV-1 antibodies can coat virus particles and bind to fragment crystallizable (Fc) receptors on the cell surface, thereby facilitating virus anchoring and contact with the HIV-1 CD4 receptor and coreceptors (2, 3). In addition, proteins of the complement can recognize such antibodies and mediate immune adherence to complement receptor (CR) bearing cells (C-ADE) (4–6). The complement factors can also bind to virus particles or infected cells directly, i.e., independent of antibodies, and facilitate infection in a similar fashion (7, 8).

As our experiments on the redirection of natural antibodies were based on HS from uninfected individuals and because many of the cells used in our experiments did not express CR receptors, it was unlikely that ADE or the complement alone were responsible for our observations of augmentation of HIV-1 infectivity.

HIV-1 has also been described to interact with other proteins of the plasma such as mannose binding protein, fibronectin, apolipoprotein H, and chondroitin sulfate, but the binding to these molecules is either deleterious for viral infectivity or there is lack of evidence for their in vitro effect (9, 10). Human plasmin, on the other hand, may have a positive effect on viral infectivity as it has been shown to cleave the HIV-1 precursor envelope glycoprotein gp160 into gp120/gp41 (11).

The aims of the present study were to confirm that human serum indeed increases HIV-1 infectivity in vitro, to dissect which step in the viral replication cycle is affected, and whether the effect is virus and/or cell specific, as well as to characterize the factor(s) responsible for this phenomenon.

Results

Human Serum Enhances HIV-1 Infectivity. HIV-1 infectivity can be measured by different means, and one of the most accepted systems, the TZM-bI assay, provides fast viral readouts based on the expression of luciferase that is driven by the HIV-1 long terminal repeat (LTR) promoter. We found that infection of the cells with HIV-1 IIIB in the presence of increasing concentrations of heat inactivated human serum (HIHS) from normal donors was directly correlated to higher relative luminescence units (RLUs). In fact up to eight times higher values were observed in the presence of 40% (vol/vol) HIHS compared with controls cultured in advanced DMEM + 2% (vol/vol) FBS (Fig. 1A). The addition of indinavir to the culture of TZM-bI cells ensured that only one cycle of replication was monitored and because the readout was luciferase activity, the effects detected in this system did not include late steps in the viral cell cycle such as virion assembly, release, and maturation.

We tested other cell lines and other methods for quantification of viral infectivity. As the TZM-bI cells are derived from HeLa cells (cervical cancer), we also tested cell line CEM-GFP cells and H9 cells (both with T-cell background) and glioma-derived U87 cells. In all cases, the increasing amounts of HIHS correlated with a higher viral readout, i.e., green fluorescent protein (GFP) expression, p24 levels in culture supernatant, and copies per milliliter of viral RNA in culture supernatant, respectively. In these systems, however, unlike the TZM-bI cells, several cycles of replication are needed for progeny virus to be detected (Fig. S1).

To test whether HS had an effect on attachment and fusion, we tested whether we could observe the effect of HIHS in chronically infected cell line ACH-2 cells. These cells cannot be reinfected as they lack the CD4 receptor. In Fig. 1B we show that ACH-2 cells stimulated with phorbol 12-myristate 13-acetate (PMA) and cultured in the presence of 10 or 20% (vol/vol) HIHS produced twice as much virus particles as the control cells cultured in the presence of 10% (vol/vol) FBS. Thus, there seems to be an effect of HS on HIV-1 beyond viral entry, reverse transcription, and integration.

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The authors declare no conflict of interest.

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infectivity values varied between donors (Fig. S2). We directly compared the effect of increasing concentrations of either HIHS or FBS on HIVIIIB infectivity in the TZM-bl cells. As seen in Fig. 2C, none of the other sera were able to increase viral production at a 5% (vol/vol) concentration compared with HS ($P < 0.01$). It should be mentioned, that higher concentrations of the sera from rabbit, pig, and goat induced visible cell cytotoxicity.

We then studied the impact that increasing concentrations of NHS had on cell proliferation, cell size, cell cycle progression, as well as apoptosis on the uninfected TZM-bl cells. In Fig. S3A, we show that there was no difference in the proliferation pattern between the samples cultured in the presence of 5, 10, or 20% (vol/vol) NHS after 24 or 48 h in culture, as determined by the passive transfer to progeny cells of the intracellular dye carboxyfluorescein diacetate succinimidyl ester (CFSE). A general increase in the cell diameter was observed after 48 h in culture but there was no difference in the cell size between samples cultured with increasing amounts of NHS (Fig. S3B). To study cell cycle progression we measured the incorporation of the thymidine-nucleoside analog 5-ethyl-2'-deoxyuridine (EdU), which is incorporated during active DNA synthesis (S phase). We found no significant differences in the percentage of EdU-positive cells in the samples cultured with 5, 10, or 20% (vol/vol) NHS after 48 h in culture (Fig. S3C). We also studied the amount of apoptotic cells and/or necrotic TZM-bl cells after treatment with NHS. We did so by staining the cells with annexin V and propidium iodide (PI), respectively. We found no significant differences in the expression of either of the markers between the samples cultured with increasing amounts of NHS (Fig. S3D).

**Effect of Human Serum on HIV-1 Replication Cycle.** We showed in Fig. 1B that HS increases viral production in chronically infected ACH-2 cells. To confirm that NHS does not affect virus binding and fusion, we preinfected TZM-bl cells with HIV-1HXB virus for 2.5 h and washed off unbound virus before the addition of NHS. In Fig. 3A we show that compared with those samples in which NHS and HIHS in relation to the controls cultured with 2% FBS ($P < 0.01$). However, it was with the NHS that higher luminescence values (~1.5 times higher at each serum concentration) were obtained. Subsequent experiments were therefore performed in the presence of NHS.

We also tested sera from three other species (rabbit, pig, and goat) in HIVinfl-infected TZM-bl cells. As seen in Fig. 2C, none of the other sera were able to increase viral production at a 5% (vol/vol) concentration compared with HS ($P < 0.01$). It should be mentioned, that higher concentrations of the sera from rabbit, pig, and goat induced visible cell cytotoxicity.

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**Fig. 1.** Effect of human serum on HIV-1 infectivity in different cell lines. Virus expression was measured in cell cultures supplemented with pooled HIHS from normal donors. Infectious titers are presented as (A) relative luminescence units in TZM-bl at 48 h postinfection with HIVIIIB, (B) copies of viral RNA in culture supernatant of PMA-stimulated ACH-2 cells after 48 h incubation, (C) RT activity of PBMCs infected with three primary isolates in autologous plasma at day 11 postinfection. 0 represents controls of infected cells cultured in maintenance medium. In all cases the differences between the samples cultured at the highest serum concentration and controls were statistically significant ($P < 0.01$). All values shown are the mean values ± SD from four replicates.

We then tested whether the enhancement could be reproduced in freshly isolated peripheral blood mononuclear cells (PBMCs) using three primary isolates cultured with autologous plasma. As seen in Fig. 1C, at day 11 postinfection, the reverse transcriptase (RT) activity increased in cell culture supernatants in relation to the human plasma concentration. At a 10% (vol/vol) plasma concentration the RT activity was 1.8-fold higher for BaL compared with the controls cultured with 10% (vol/vol) FBS (designated as 0 in Fig. 1C).

The difference between infectivity units at the highest HS concentration and controls cultured in the presence of FBS, as well as for the samples cultured in the same amount of either FBS or HIHS, for all cell-based assays were all statistically significant ($P < 0.01$). The assays mentioned above were repeated at least three times with different pools of HS batches and in all cases the enhancement of infectivity was observed.

We also tested individual serum samples from uninfected donors in TZM-bl cells infected with HIVIIIB and we found that for all subjects there was an increase in infectivity in relation to the amount of HIHS; yet, at the same serum concentration, the infectivity values varied between donors (Fig. S2).

We directly compared the effect of increasing concentrations of either HIHS or FBS on HIVIIIB infectivity in the TZM-bl cells. As seen in Fig. 2A, the presence of 30% (vol/vol) HIHS clearly enhanced viral production, with a fivefold increase in the RLU values compared with the controls cultured in 2% (vol/vol) FBS ($P < 0.01$). Increasing concentrations of FBS had a much lower impact on the virus infectivity. At a 30% (vol/vol) concentration, the infectious titers were 2.3-fold higher in those cells cultured in the presence of HIHS than in those cultured in the same amount of FBS ($P < 0.01$).

As heat inactivation of HS (56 °C for 1 h) nonspecifically denatures heat-sensitive proteins, we tested the difference in effect between heat inactivated and nonheat inactivated HS (NHS). We found that the relative increase in infectivity was similar for both in relation to the controls (Fig. 2B). At 30% (vol/vol) HS a 2.6-fold increase in infectivity was observed for both rabbit, pig, and goat sera as compared with 0.01). All values shown are the mean values ± SD from four replicates.
As Tat plays a key role in orchestrating the transcription of HIV-1, we evaluated the contribution of NHS in a Tat-free environment, by infecting SupT1 cells with a Δtat IIIB virus. As seen in Fig. 3C, the amount of virus particles produced by the cells was almost twofold higher already at a 10% (vol/vol) NHS concentration compared with the controls cultured in 10% FBS ($P < 0.01$).

We then checked the specificity for LTR by transfecting 293T cells with either LTR-CAT or CMV-CAT plasmids and cultured them in the presence of 10% FBS, 5% (vol/vol) NHS, or 10% NHS. In Fig. 3E we show that, whereas the CAT produced in the CMV-CAT transfected cells remained constant regardless of the amount of serum added to the cells, the CAT expression in those cells transfected with LTR-CAT was augmented in the presence of NHS. We found a fivefold difference in CAT expression between cells cultured with 10% NHS and 10% FBS ($P < 0.01$).

To confirm the effect of NHS on the LTR, we measured the luciferase expression in uninfected TZM-bl cells. As shown in Fig. 3F, we observed that indeed the luciferase protein was produced in higher amounts in the presence of NHS ($P < 0.01$), albeit at much lower levels than when infected with HIV-1.

**Effect of Human Serum on HIV-1 Transcription.** Several transcription factors can act on the LTR and the precise mapping of the binding sites for different molecules has already been described (12–14). Factors like nuclear factor kappa B (NFκB), the activator protein-1 (AP-1) family, and specificity protein 1 (SP-1) have been shown to play important roles on HIV-1 transcription.

To test whether HS would have an effect on the levels of these proteins we cultured TZM-bl cells in the presence of 5% FBS, 5% NHS, or 10% (vol/vol) NHS and the nuclear extracts were collected at 1, 6, 24, and 48 h. In Fig. 4A we show that the AP-1 member proteins c-FOS, JunD, and JunB exhibited a time and dose dependent increase in expression, with 10% NHS resulting in the highest increase ($P < 0.01$). We also performed a transfection of the TZM-bl with the HIV-1 infectious plasmid pNL4-3 to circumvent the fusion step. As seen in Fig. 3B, we show that indeed the luciferase protein was produced in higher amounts in the presence of NHS ($P < 0.01$), albeit at much lower levels than when infected with HIV-1.

**Effect of Human Serum on HIV-1 Transcription.** Several transcription factors can act on the LTR and the precise mapping of the binding sites for different molecules has already been described (12–14). Factors like nuclear factor kappa B (NFκB), the activator protein-1 (AP-1) family, and specificity protein 1 (SP-1) have been shown to play important roles on HIV-1 transcription.
dose-dependent response to the HS, whereas c-JUN, FOS B, SP-1, and NFκB were not altered by the treatment. Similar results were obtained with HIV-1α-infected TZM-bl cells cultured in the presence of NHS (Fig. S4A). We proceeded to confirm these results by transfecting TZM-bl cells with a plasmid encoding the complete LTR sequence of pNL4-3, which was serving as a promoter of enhanced green fluorescent protein (EGFP) expression (wLTR-EGFP) and another plasmid containing in total seven point mutations in the AP-1 binding sites of LTR (ΔAP-1LTR-EGFP) that have been described to hinder binding of the respective transcription factors (15). In Fig. 4B we show that the cells transfected with the mutant vector had a twofold lower expression of EGFP compared with those of the wLTR vector (P < 0.01) and there was no difference across serum concentrations. As a control of functional LTR we used PMA. This compound potently activates signal transduction and promotes viral transcription by activation mainly of NFκB but an effect on c-FOS, JunD, and JunB has also been described (16). In Fig. 4B we show that there was a 1.7-fold difference in EGFP expression in the cells transfected with the mutant LTR, thus, underlining the importance that AP-1 factors have on the PMA-induced transcription initiation. We then measured the actual input of NHS on viral transcription by culturing ACH-2 cells in the presence or absence of PMA. In Fig. 4C we show that the RT activity increased in relation to the concentration of NHS in the culture both in the presence or absence of PMA, but the viral production was 7–10 times lower in those samples cultured with NHS alone. Nevertheless, in the absence of PMA there was a dose-dependent response to NHS. The viral production at 10% (vol/vol) NHS was twice as high as in the control wells cultured in the same concentration of FBS (P < 0.01).

We also tested the impact that U0126, a potent MEK1/2 inhibitor, would have on the NHS-induced AP-1 activation by pretreating the cells with 20 μM of the drug before the addition of NHS. We found that the drug reduced by over twofold the NHS-induced cFOS up-regulation and it had also a lower impact on JunB and JunD expression (Fig. S4B).

Test of HS Components and Fractionation. In Fig. 2B we showed that heat inactivation only partially compromised the effect of HS. However, as proteins of the complement (particularly C2 and C3) have been implicated in the enhancement of HIV-1 infectivity, we tested sera depleted from C3 and C2 in the TZM-bl system. Increasing luminescence values were also observed with the C2- and C3-depleted HS giving three- and fourfold higher RLU values, respectively, with the HIV-1α-infected cells cultured with 2% FBS (P < 0.01) (Fig. 5A and B). Also, the HS enhancement was still observed using serum depleted of either its lipid fraction or hormones (charcoal stripped). In Fig. 5 C and D, respectively, we observe that at a 20% (vol/vol) concentration, there was a threefold and a 1.5-fold increase in infectivity in relation to the controls (P < 0.01).

We also tested purified human plasma for its potential effect on increasing gp120 availability in the viral surface, as well as, albumin, IgG, and IL-2, which have been described to aid the infectivity of other pathogens (17–21). Contrary to what is known, plasmin dramatically reduced infectivity as did IgG. Albumin and IL-2 did not show a dose-dependent increase of infectivity (Fig. S5).

Because the depletion of the lipid and hormone fractions did not seem to affect the enhancing ability of HS in a major way, the factor(s) responsible is most likely a protein. To test this, we treated NHS with increasing amounts of trypsin. Following digestion of the HS, the trypsin was inactivated and the serum was tested in the TZM-bl assay. In Fig. 6A we show that after trypsin digestion the HS did not retain its enhancing activity (P < 0.01).

To test whether the enhancement was due to one protein or to a protein complex, we added increasing concentrations of a mild detergent (CHAPS) to disaggregate possible complexes. Before adding the treated NHS to the TZM-bl cells, it was centrifuged on a 100-kDa filter to rid it of the detergent and also of possible smaller complex subunits. As seen in Fig. 6B, this treatment did not affect the enhancement in infectivity, indicating that a single protein and not a complex was responsible for the effects on HIV-1 infectivity by HS. The difference between samples cultured with 20% (vol/vol) of detergent-pretreated NHS and controls was statistically significant (P < 0.01).

We then fractionated the HS proteins by size. We fractionated NHS by centrifugation through filters of different pore size. First we added the NHS to a 1,000-kDa filter and after centrifugation the flow-through was added to a 300-kDa filter and subsequently to 100-, 50-, and 30-kDa filters. An aliquot from each flow-through was collected and tested in the TZM-bl assay. Similar enhancement in infectivity, as observed with unfiltered NHS, was seen with those aliquots that passed through the 1,000 kDa and the 300-kDa cutoff filters, but was markedly reduced when collected after passing filters with cutoffs of 100 kDa or less (Fig. 6C). Dialysis of NHS also reproduced an increased infectivity in the fraction above 100 kDa (Fig. S6A).

We also carried out size exclusion chromatography using Superdex 200 (effective range 10–600 kDa). We found the increase in infectivity with the pooled fractions 15–21 (Fig. S6B) and with the split pool particularly in fraction 18 (Fig. 6D), indicating a molecular mass of 250–300 kDa. We name this HS protein: HIV-1 enhancing serum protein (HESP).

Discussion

In the present study we could show that HS from uninfected individuals increases the in vitro production of HIV-1. This effect was reproducible in five different cell lines infected with the HIV-1α virus, as well as in PBMC infected with three primary isolates, the latter when cultured in syngenic plasma. Moreover, an enhancement of HIV-1 infectivity was seen independent of batch of pooled serum used and was present in all tested individual serum samples, although we observed variations in the infectious levels reached by the different donors. We also showed that the effect was specific to HS at least compared with bovine, porcine, rabbit, or goat serum and that it was not dependent on complement factors C2 or C3.

To dissect which step within the viral replication cycle was being affected by the NHS, we used two different single virus replication cycle cell-based assay systems, the ACH-2 and TZM-bl cell lines. We were able to show that neither the early steps (fusion, reverse transcription, and integration) nor the late steps (virus assembly, release, and maturation) seem to be affected by HS in these two cell lines. Wu et al. (22) previously reported on a possible effect of
Further studies to try to define and characterize HESP using inter alia mass spectrometry are under way.

Because viral load is correlated to disease progression in infected individuals (28, 29), it would be interesting to evaluate possible differences in the HIV-1 enhancing effect of sera from rapid progressors to those from elite controllers. The paper also highlights the importance of having proper controls when, e.g., studying HIV-neutralizing antibodies in serum from patients in clinical vaccination studies.

In summary, we present here results indicating that a protein in HS (HESP) increases the transcription of the proviral HIV-1 genome, possibly by signal transduction and activation of AP-1.

Materials and Methods

Cells, Virus, and Serum. The following cell lines were obtained from the National Institutes of Health (NIH) AIDS Research and Reference Program, Division of AIDS, National Institute of Allergy and Infectious Diseases (NIAID), NIH: H9, ACH-2, CEM-GFP, SupT1, TZM-bl, and U87.CXCR4 cells. The 293T cells were purchased from the Interlab Cell Line Collection. Maintenance medium for suspension cells was RPMI + 10% (vol/vol) FBS and for adherent cells was advanced DMEM + 2% FBS. The PBMCs were obtained as buffy coats from the blood bank at Karolinska University Hospital and isolated by Ficolli-Paque plus gradient (GE Healthcare) according to the manufacturer’s protocol. The viruses used in this study, i.e., HIV-1IIIB, primary isolate 90SE364, A083M411, and BaL; and the HIV-1 MC9911BaTat-Rev (referred as Δtat IIIB virus in the text) were all obtained from the NIH AIDS Research and Reference Program, Division of AIDS, NIAID, NIH.

The HS was of type AB from a pool of uninfected individuals and several batches were tested from different sources: Cambrex, Innovative Research, and 3H Biomedicals. Individual AB serum samples were obtained from 3H Biomedicals. For the studies on PBMCs, the plasma collected in citrate buffer from the same anonymous donor was purchased from the blood bank of Karolinska Hospital. The C3- and C2-depleted sera were obtained from Sigma-Aldrich, whereas the charcoal stripped and delipidized sera were obtained from Innovative Research. Rabbit, porcine, and goat sera were purchased from Invitrogen. Complement inactivation of the sera was done by heat inactivation at 56 °C in a water bath for 1 h.

Cell-Based Assays.

i) TZM-bl cells: performed as previously specified (30) and infected with 150 50% tissue culture infective dose (TCID50) of the HIV-1IIIB virus in advanced DMEM (Gibco) containing 1 mM sodium butyrate. Each serum dilution concentration was tested in quadruplicate in the same plate and in at least five independent experiments. When testing the effect of HS on uninfected TZM-bl cells, the cells were seeded in six-well plates at a concentration of 400,000 cells per well and total volume of 1 mL.

ii) ACH-2 cells: 500,000 cells per well were incubated with HS in the absence or presence of 100 nM PMA for 48 h. Each concentration was run in triplicate in the same plate and tested in at least four separate experiments.

The supernatant tested for quantification of viral RNA using the COBAS AmpliPrep/COBAS TaqMan HIV-1 test (Roche) or by RT quantification with RT activity kit (Cavidi).

iii) H9 cells: Assays were performed as previously specified (1). The cultures were infected with 150 TCID50 of the HIV-1IIIB plus HIVS. At day 7, the supernatants were evaluated for p24 levels with an in-house ELISA (31). All conditions were run in quadruplicates in the same plate and on three separate occasions.

iv) U87.CXCR4 cells: Assays were performed as previously specified (1). The cultures were infected with 150 TCID50 of the HIV-1IIIB plus HIVS in advanced DMEM. Each virus/serum dilution was tested in quadruplicate in the same plate. The viral supernatant was collected and tested for quantification of viral RNA using the COBAS AmpliPrep/COBAS TaqMan HIV-1 test. The viral supernatant was collected and tested for quantification of viral RNA using the COBAS AmpliPrep/COBAS TaqMan HIV-1 test.

v) CEM-GFP cells: 500,000 cells were infected with 150 TCID50 of HIV-1IIIB plus HIVS at a final volume of 2 mL/well. Each HS concentration was run in triplicate and in three separate experiments. GFP expression was measured at day 7 postinfection in a FACSCalibur flow cytometer (BD Biosciences).

vi) PBMC: 200,000 freshly isolated, phytohemagglutinine-stimulated PBMCs were infected with 200 TCID50 of the primary isolate and cultured with human plasma and 200 IU interleukin-2. Control wells were cultured in 10% FBS. All samples were measured in quadruplicates in the same plate and in three different plates. RT was quantified at days 7 and 11 post-infection using the RT activity kit (Cavidi).
Cell proliferation, cell cycle, and apoptosis were measured in the TZM-bl cells at 0 and 48 h postculture with NHS, following the same culture conditions as previously specified. They were measured using CellTrace CFSE cell proliferation kit, Click-IT Edu kit, Alexa Fluor 488, and Dead Cell Apoptosis kit with Annexin Alexa Fluor 488 and PI, respectively, following manufacturer protocol (Invitrogen) and analyzed by flow cytometry in FACScalibur (BD Biosciences). The experiment was performed in triplicates in two separate occasions.

**Plasmids and Transfections.** The following plasmids were used in this study: pNL4-3 (NIH AIDS reagent facility), pCMVCAT (Invitrogen), pCMVtat (provided by S. Schwartz, Lund University, Lund, Sweden), pNL2CAT (referred as LTR-CAT in the text) (23). The latter contains LTR nucleotides from the first nucleotide in U3 through the nucleotide at position 743. The complete LTR sequence derived from pNL4.3 (789 bp) and one including seven point mutations as previously specified (15) were synthesized and cloned into the pEGFP-N1 vector (Clontech) by Geneart (wltLTR-EGFP and p-AP-LTR-EGFP, respectively). The LTR sequence was expressed as a transcript of EGFP in replacement of the CMV promoter and multiple cloning site.

For the transfections, 400,000 TZM-bl per well were preseeded in six-well plates and transfected with 1 μg/well of the plasmids + 8 μL Fugene HD (Roche) and incubated for 7 h at 37 °C. Then the cells were washed twice and incubated in advanced DMEM with the appropriate concentration of NHS for 48 h at 37 °C. Each dilution was run in triplicate. The cells were lysed with Glo Lysis buffer at 4 °C and the lysate was either tested for luciferase as mentioned above or tested for CAT concentration with CAT ELISA kit (Roche) as per manufacturer protocol. The fractions were collected over an 8- to 12-h period at a flow rate of 0.5 mL/min using PBS as running buffer. Samples were concentrated by centrifugation at 3,200 × g in a Vivaspin 240 (Sartorius) before test in the TZM-bl cells.

**Tryptic digestion.** Tryptic digestion was performed using Trypsin gold (Promega) at substrate/trypsin ratios of 30:1, 10:1, 5:1 and 1:1 followed by overnight incubation at 37 °C on gentle agitation. To stop digestion, soybean protease inhibitor (Sigma-Aldrich) was used at an inhibitor:trypsin ratio of 2:1.

**Statistical Analysis.** A Student t test was used to assess statistical differences between two sets of data. In most cases, it was used to compare samples cultured at the highest concentration of HS and controls cultured in maintenance medium.

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Supporting Information
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Fig. S1. Effect of human serum (HS) on HIV-1 infectivity. The HIV$_{\text{IIIb}}$ viral output was studied in different cell lines after supplementation with heat-inactivated human serum (HIHS) and read as (A) p24 concentration in culture supernatant of H9 cells at day 7 postinfection. (B) Copies of viral RNA in culture supernatant of U87 cells at day 5 postinfection. (C) Green fluorescent protein (GFP) expression of cell line CEM-GFP cells at day 7 postinfection. All values shown are the mean values ± the SD from four replicates. Comparisons of samples cultured at the highest serum concentration and controls were statistically significant ($P < 0.01$).

Fig. S2. Interindividual variation of HS. TZM-bl cells were infected with HIV$_{\text{IIIb}}$ and cultured in the presence of 15 individual serum samples from uninfected individuals. Each serum sample was tested at 3% (squared bars), 5% (white bars), 10% (diagonal-line bars), 20% (black bars), or 30% (horizontal-line bars) (vol/vol). All values shown are the mean values ± the SD from four replicates.
Fig. S3. Effect of human serum (HS) on cell cycle, cell proliferation, and apoptosis of uninfected TZM-bl cells. (A) TZM-bl cells were stained with CFSE and cultured in the presence of 5, 10, or 20% nonheat-inactivated human serum (NHS). Cell proliferation was followed after 24 and 48 h in culture by flow cytometry. Represented is the overlay of the carboxyfluorescein succinimidyl ester (CFSE) expression by progeny cells cultured at the different serum concentrations. (B) Cell size measured as mean of forward scatter at 24 h and 48 h in culture with NHS. (C) Percentage of cells on S phase of the cell cycle as determined by incorporation of the fluorescent thymidine analog 5-ethynyl-2′-deoxyuridine (Edu) after 48 h in culture with NHS. (D) Percentage of cells expressing annexinV (as apoptosis marker) and/or propidium iodide (PI) (as necrosis marker) after 48 h in culture with NHS.

Fig. S4. Effect of HS on transcription. (A) TZM-bl cells were infected with HIVIIIB and cultured in the presence of 2% FBS (horizontal-line bars), 5% NHS (white bars), or 20% (vol/vol) NHS (black bars). Nuclear fractions were extracted at the specified time points and tested for expression of activator protein-1 (AP-1) family, specificity protein-1 (SP-1), and nuclear factor kappa beta (NFκB) transcription factors. (B) TZM-bl cells were pretreated for 30 min with 20 μM of U0126 before the addition of NHS. Represented are U0126-treated cells cultured in 2% FBS (black bars), U0126-treated cells cultured with 20% NHS (white bars), and, as controls, untreated cells cultured with 20% NHS (gray bars). The nuclear extracts were collected at the specified time points and tested for AP-1 activation.
Fig. S5. Effect of individual serum components on HIV$_{ova}$ infectivity. (A) Purified human plasmin, (B) purified albumin, (C) total purified human IgG, and (D) IL-2 were tested in the TZM-bl assay for their potential role in enhancing HIV infectivity. All values shown are the mean values ± the SD from four replicates.

Fig. S6. Fractionation of human serum. (A) NHS was dialyzed with membranes of different pore size followed by test of the fractions at 5% (white bars), 10% (black bars), or 20% (diagonal-line bars) (vol/vol) on the TZM-bl cells. As a control dialyzed FBS (squared bars) was tested at 5% concentration. (B) Fractionation by size exclusion chromatography and test of pooled fractions in the TZM-bl assay. All values shown are the mean values ± the SD from four replicates.