ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1994, p. 2910–2916
0066-4804/94/$04.00+0

Vol. 38, No. 12

Influence of Host Cell Type and V3 Loop of the Surface Glycoprotein on Susceptibility of Human Immunodeficiency Virus Type 1 to Polyanion Compounds

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Received 4 April 1994/Returned for modification 1 July 1994/Accepted 23 September 1994

Dextran sulfate is a potent inhibitor of human immunodeficiency virus (HIV) binding and replication in lymphocytic cell lines. In this study, we demonstrate that the effect of dextran sulfate and heparin depends on the host cell type and on the V3 loop, the principal neutralizing determinant of HIV gp120. In particular, when dextran sulfate was tested on primary human macrophages infected with macrophage-tropic viruses, enhancement of infection was observed in 6 of 11 independent macrophage preparations and with 5 of 13 primary HIV isolates. Our in vitro observations might explain why enhanced HIV replication was observed in HIV-infected patients treated with dextran sulfate.

Sulfated polyanions, such as dextran sulfate or heparin, have been shown to potently inhibit human immunodeficiency virus (HIV) infection in vitro in T-cell lines (4, 13). Later studies consistently demonstrated that sulfated polyanions reduced binding of virions to host cells (3, 22, 23). This led to the proposal that studies of polyanions should be pursued for their potential in the prophylaxis and therapy of retroviral infections in humans (3). Initially, studies of orally administered dextran sulfate produced little evidence for systemic absorption or antiviral effect (1, 17). A study of intravenously administered dextran sulfate revealed, besides a harrowing hematological toxicity, that all eight patients who received dextran sulfate for more than 3 days had significant increases in circulating p24 antigen (9). This apparently adverse effect on HIV replication was not explained and was especially puzzling because dextran sulfate levels in blood in those patients were maintained at up to 200-fold greater than the 50% inhibitory concentration for free HIV infectivity in vitro.

The exact mechanism of action of the sulfated polyanions is uncertain. Perhaps their large size and highly negatively charged nature enable them to interact in a relatively nonspecific fashion with positively charged molecules, which would explain the contradictory reports that polyanions interact with CD4 (16, 26), gp120 (5, 29), or even other cellular proteins (18, 36). However, dextran sulfate has been shown to block antibody binding to the V3 principal neutralizing determinant of HIV gp120 (5, 29), possibly through electrostatic interactions between dextran sulfate and the positively charged amino acids in this loop (5, 19). Cellular tropism determinants map also to the V3 loop (14, 24, 31), and the tropism conferred by the V3 loop appears to correlate with differences in the overall electric charge of the loop (7, 10, 32). To the best of our knowledge, the effect of dextran sulfate on HIV replication has not been tested in host cells other than T-cell lines. In the present study, we examined whether polyanions have differential effects on the replication of HIV that depend on which viral strain of HIV type 1 (HIV-1) and host cell type are used. Such information might better explain the paradoxical in vivo effect of polyanions.

The effect of dextran sulfate (molecular weight, about 500,000; Pharmacia, Uppsala, Sweden) and heparin (150 U/mg; Elkins-Simms Inc., Cherry Hill, N.J.) on the infection of primary human macrophages by the HIV-1_{BaL} strain was tested. Virus and cells were prepared as described previously (15). Macrophages were pretreated for 1 h with various concentrations of sulfated polyanions, then exposed to HIV-1_{BaL} at a multiplicity of infection of 0.025 for 1 h, washed, and cultured in the presence of the same concentration of polyanions. One week later, virus production, as reflected by the amount of p24 antigen produced in the supernatant, was assessed by an enzyme-linked immunosorbent assay (HIVAG-1; Abbott Laboratories, Chicago, Ill.), according to the instructions of the manufacturer. Dextran sulfate (10 μg/ml) in the medium did not affect the results of this assay.

Enhancement, defined as an increase in p24 antigen production of more than three times at at least two different polyanion concentrations, was observed with 6 out of 11 batches of monocyte-derived macrophages from independent donors, of which a representative result is shown in Fig. 1. Enhancement varied, depending on the experiment, with p24 antigen production by dextran sulfate-treated macrophages increasing 3-fold (0.5 log) to more than 10-fold (1 log) compared with that by untreated cells. Replicate cultures of macrophages infected with HIV-1_{BaL} typically produce p24 antigen in the supernatant in amounts with a coefficient of variation of less than 20% (see, for instance, reference 20). It is therefore obvious that the increased p24 antigen production by dextran sulfate-treated macrophages truly reflected a biological phenomenon and was not due to random variation. The typical cytopathic effect of syncytium formation by HIV-1_{BaL} on macrophages was markedly increased in those wells showing enhancement of p24 antigen production (data not shown). In the five other experiments, dextran sulfate had no clear-cut effect on HIV-1_{BaL} replication in macrophages. In three separate experiments, heparin was tested in parallel with dextran sulfate, and its

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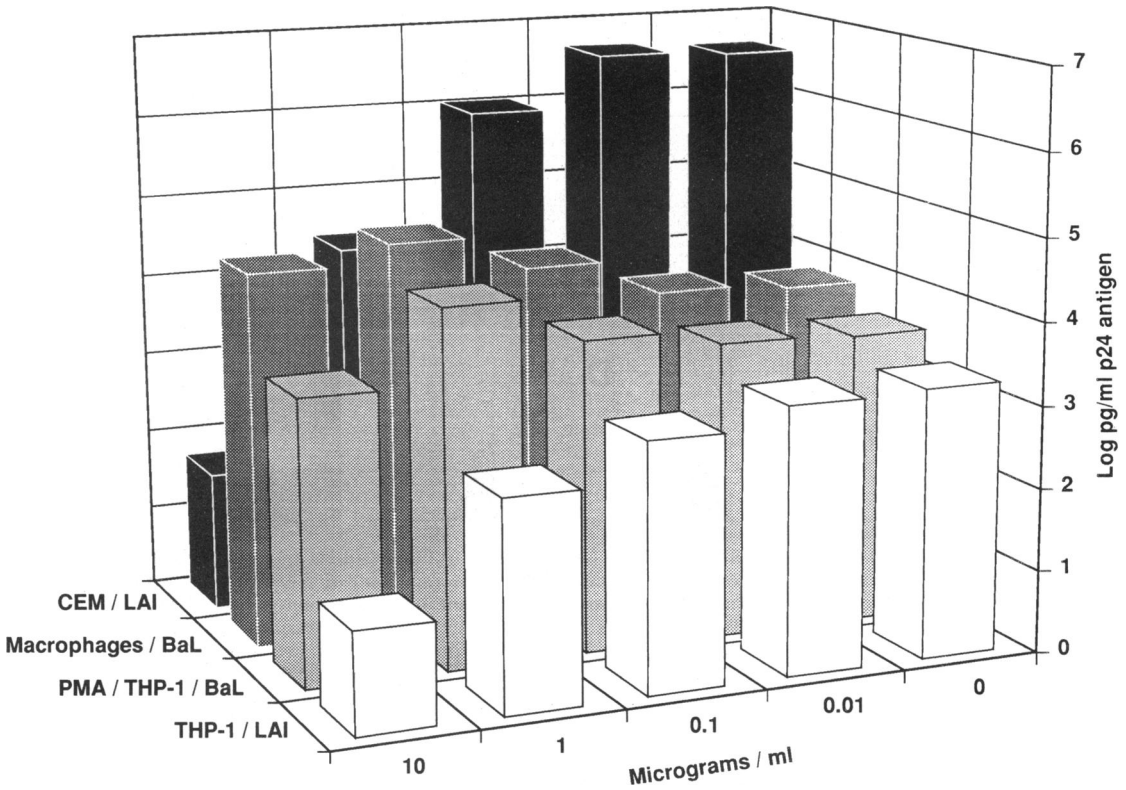

FIG. 1. Effect of dextran sulfate on the replication of HIV-1_{BaL} in monocyte-derived macrophages and in PMA-differentiated THP-1 cells and of HIV-1_{LAI} in CEM lymphocytic cells and in THP-1 cells. One-week-old macrophages and PMA-differentiated THP-1 cells in 48-well plates (4×10^5 cells per well) were treated for 1 h with the indicated polyanion concentration. HIV-1_{BaL} was then added at a multiplicity of infection of 0.025 50% tissue culture infective dose per cell and incubated for 1 h. The monolayers were then washed and cultured in RPMI 1640 medium with 10% fetal bovine serum. CEM and undifferentiated cells were infected in the same format as adherent cells, except that infection and washing of these nonadherent cells were performed in tubes. p24 antigen levels were determined after 3 days for CEM cells and after 1 week for the other cell types. Note the log scale for the p24 antigen levels. Results for monocyte-derived macrophages are representative for 6 of the total of 11 experiments performed with such cells. Results with other cell types are representative of at least three separate experiments giving reproducible results.

effect was found to closely reproduce the enhancing effect of dextran sulfate. A similar trend to enhancement was observed when the effect of dextran sulfate on THP-1 cells was tested after the cells had undergone differentiation with phorbol myristate acetate (PMA) and been infected with the HIV-1_{BaL} strain (Fig. 1). This cell line (ATCC TIB 202) is an early monocytic precursor (35) that can be differentiated to a macrophage-like phenotype through the use of various differentiating agents (34). We have recently reported that upon treatment with 10^{-8} M PMA, these cells become permissive to macrophage-tropic HIV strains (21).

In contrast, when we studied the effect of dextran sulfate on the replication of HIV-1_{LAI} in the CEM lymphocytic cell line (ATCC CCL 119) or in undifferentiated THP-1 cells which replicate lymphotropic HIV strains and were cultured as previously described (11, 21), the usual inhibitory effect was observed (Fig. 1). In lymphocytic systems, inhibition was observed with a 50% inhibitory concentration of around 0.1 μg/ml. Dextran sulfate did not render primary macrophages permissive for the lymphotropic HIV-1_{LAI} isolate.

To determine whether this reduced inhibition or enhancement was restricted to laboratory-adapted macrophage-tropic strains or was a more general phenomenon, we tested the effect of dextran sulfate on the replication of primary isolates (obtained by the coculture of patient peripheral blood mononuclear cells [PBMC] and healthy donor PBMC [28]) in monocyte-derived macrophages. Of 13 primary isolates tested on one batch of primary macrophages, 5 displayed enhancement (as defined above, with maximum increases in p24 antigen production in the presence of dextran sulfate amounting to, respectively, 8.5-, 9.5-, 10-, 12.8-, and 23.4-fold that of control cells), 6 showed the same replication independently of the dextran sulfate concentration, and 2 were clearly inhibited as dextran sulfate concentrations increased.

The mechanism of the effect of dextran sulfate on HIV replication has been linked to the binding of the virus to host cells. We therefore examined the effect of dextran sulfate on the binding of the virus to monocytic and lymphocytic cells by using a flow cytometry assay (21). We demonstrated that 10 μg of dextran sulfate per ml increased the binding of HIV-1_{BaL} to monocytic PMA-differentiated THP-1 cells (Fig. 2). In contrast, in parallel experiments, dextran sulfate decreased the binding of HIF-1_{LAI} to lymphocytic CEM cells (Fig. 2), a result similar to previous observations (3, 22, 23).

At this point, however, it was unclear whether differences in host cells or in virus strains were responsible for the different effects of dextran sulfate in lymphocytic and monocytic systems.

Primary lymphocytes are a major host cell type for HIV (8). In addition, because they are permissive to basically all strains

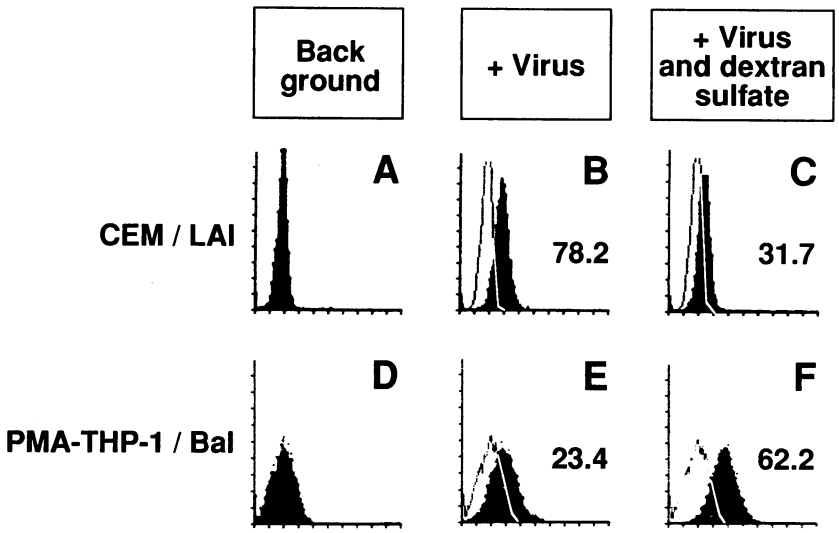

FIG. 2. Effect of dextran sulfate on the binding of HIV-1_{LAI} to CEM cells (A to C) and of HIV-1_{BaL} to THP-1 cells (D to F). Each graph represents the cell numbers (*y* axis) as a function of fluorescence intensity (200 channels spanning a 3.5 log scale on the *x* axis). (A and D) Unstained cells. Cells that were stained in the absence of the viruses did not display a significant increase in fluorescence (data not shown). (B and E) Cells exposed to the respective virus stocks and stained. The open areas display the background fluorescence, while the closed areas represent the fluorescence of virus-exposed cells. The percentages of positive cells (above background) are shown. (C and F) Fluorescence of cells exposed to the viruses in the presence of 10 μg of dextran sulfate per ml. In the case of HIV-1_{LAI}, the decreased fluorescence indicates decreased binding in the presence of dextran sulfate, while in the case of HIV-1_{BaL}, the increased fluorescence indicates enhanced binding of the virus. The open areas, closed areas, and percentages are as defined for panels B and E.

and clones of HIV-1 (in contrast to primary macrophages and T-cell lines), they offered the opportunity to compare the effects of dextran sulfate on different virus strains in the same host cells.

Primary lymphoblasts were prepared from buffy coats (kindly provided by the Centre de Transfusion Sanguine, Croix Rouge Suisse, Lausanne, Switzerland) by Ficoll-Hypaque separation and phytohemagglutinin (Wellcome; 1 μg/ml) stimulation of the PBMC at a cell concentration of 10^6/ml. Lymphoblasts were maintained in RPMI 1640 with 10% fetal bovine serum and 50 U of recombinant interleukin-2 (IL-2) per ml (kind gift of GianPietro Corradin, Institute of Biochemistry, University of Lausanne). After incubation with various dextran sulfate concentrations for 1 h, lymphoblasts were infected with HIV-1_{LAI} or HIV-1_{BaL} stocks for 1 h in the continued presence of dextran sulfate, washed three times by centrifugation, and cultured in the presence of dextran sulfate. HIV replication was assessed by the production of p24 antigen in the supernatant. Dextran sulfate-mediated inhibition was observed with both virus strains (Fig. 3), but the inhibitory effect of dextran sulfate on the replication of HIV-1_{LAI} was weaker in primary lymphocytes than that in CEM cells (a 50% inhibitory concentration more than 10-fold greater). No enhancement of the macrophage-tropic isolate was observed in these cells. These observations were consistent in five experiments involving separate stimulated primary lymphocyte preparations. Thus, dextran sulfate resulted in enhancement of the replication of the HIV-1 isolate in primary macrophages but in only weak inhibition in primary lymphocytes, demonstrating that the host cell type partly determines the effect of polyanions on HIV-1 replication.

To test whether viral determinants, particularly the V3 loop of HIV-1 gp120, were involved in the differential effects of dextran sulfate in various virus-host cell systems, we used chimeric isogenic clones of HIV-1 that differ only by the envelope V3 loop region in an HXB-2 background (7). This fragment has been shown to confer tropism and cytopathic effect. Infectious clones 168.1, 168.3, and 168.10 contain the V3 loop of sequential isolates from the same patient. The V3 fragments of gp120 have been cloned from these isolates by PCR and introduced in an HXB-2 background to produce isogenic infectious clones (kindly provided by J.-J. De Jong [7]). Clones 168.1 and 168.3 are both noncytopathic, macrophage-tropic clones with, respectively, 3+ and 4+ overall V3 loop electric charges, while 168.10 is a cytopathic lymphotropic clone with a 6+ overall electric charge. In experiments involving infections with a cloned virus, pNL43 (2) was used as a prototypic lymphotropic cytopathic virus while pNL43BE was used as a macrophage-tropic clone. The pNL43BE infectious proviral clone was constructed by replacing the *Sal*I-*Bam*HI envelope fragment of pNL43 with the corresponding fragment from pBaL, a plasmid containing the envelope gene of the HIV-1_{BaL} isolate cloned by PCR (kindly provided by Bryan Cullen [12]). To infect PBMC with these clones, DNA was directly transfected into PBMC with Lipofectin (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) at a Lipofectin/DNA weight ratio of 3 for 4 h. Alternatively, DNA was transfected into COS-7 cells, also with Lipofectin by a similar protocol, to produce virus stocks. These stocks, containing from 17 to 38 ng of p24 antigen per ml, were in turn used to infect PBMC.

In one set of experiments, the various HIV clones were transfected directly into primary lymphocytes. After transfection, cells were diluted to 5×10^5 cells per ml and aliquotted with IL-2 in 1-ml cultures in 48-well plates. Dextran sulfate was added 24 h later to give the indicated final concentrations (Fig. 4). The cells were diluted weekly fivefold with medium containing the various dextran sulfate concentrations. Fifteen days later, HIV replication was measured by determining the p24 antigen concentrations in the culture supernatants. In this type of experiment, dextran sulfate could have an effect only on secondary rounds of infection. Alternatively, stocks of virus

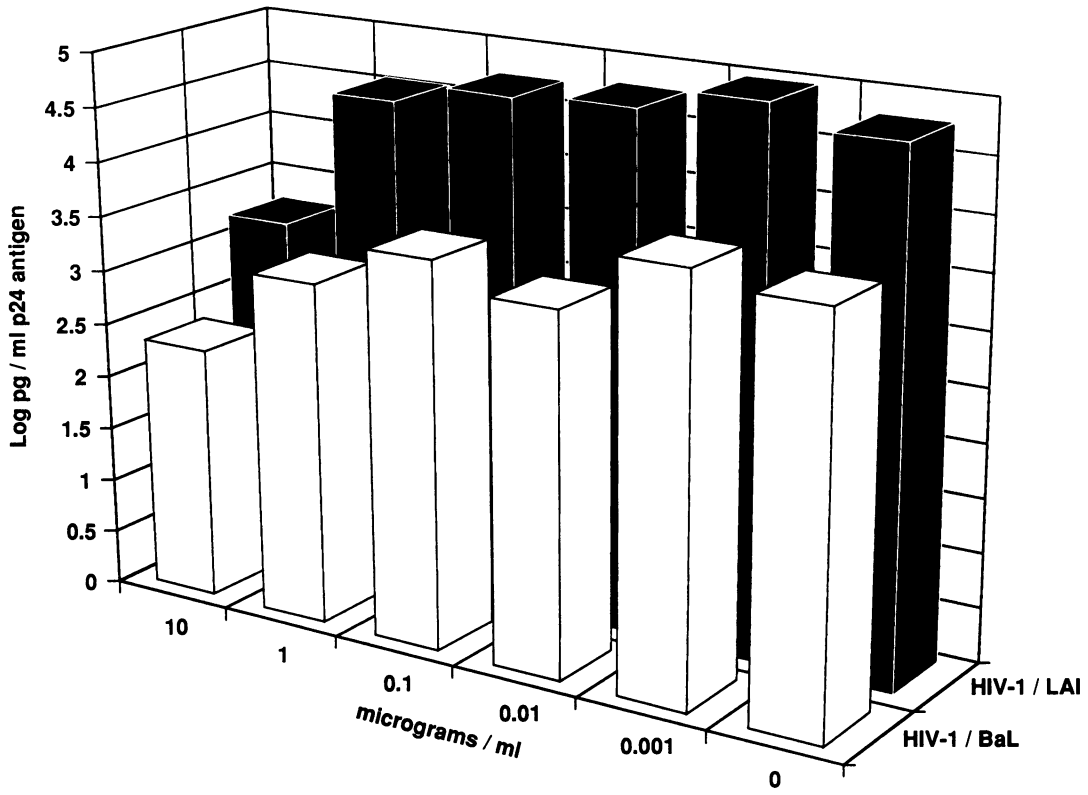

FIG. 3. Effect of dextran sulfate on HIV-1_{BaL} and HIV-1_{LAI} replication in primary lymphocytes. Phytohemagglutinin-stimulated, IL-2-maintained primary lymphocytes were treated for 1 h with the indicated polyanion concentrations and infected with the viruses at multiplicities of infection of 0.1 and 0.3, respectively, in round-bottom tubes. After 1 h, the cells were washed three times and resuspended in RPMI 1640 with 10% fetal bovine serum, 50 U of IL-2 per ml, and dextran sulfate at the indicated concentrations. At day 6, culture supernatants were harvested for p24 antigen determination.

were prepared in COS-7 cells, and similar amounts of virus, standardized by p24 antigen content, were added to 3×10^5 primary lymphocytes that had been pretreated for 1 h with various concentrations of dextran sulfate. After 1 h, the cells were washed and cultured in medium containing the same concentration of dextran sulfate. The cells were diluted weekly fivefold with medium containing the various dextran sulfate concentrations. HIV replication was measured weekly by assaying the p24 antigen concentrations in the culture supernatants. Figure 4 shows the representative results of three such experiments. Clone 168.10 was particularly susceptible to dextran sulfate inhibition, while clone 168.1 was not at all susceptible, showing a hint of enhancement. Clone 168.3 was intermediate in susceptibility. Clones pNL43 and pNL43BE, with envelope genes derived from lymphotropic and macrophage-tropic isolates, respectively, were also tested in primary lymphocytes. Clone pNL43 was only slightly more susceptible to dextran sulfate inhibition than pNL43BE (note the slight, about half-log inhibition of pNL43 at 1 μg/ml, in contrast to that of pNL43BE), suggesting that in this case, the difference in V3 loop amino acid composition had a reduced influence on dextran sulfate susceptibility. Similar results were obtained repeatedly in both experiment formats. These results demonstrated that the effect of dextran sulfate on HIV-1 replication depends significantly upon the amino acid composition of the V3 loop.

In the present study, the effect of polyanions on HIV replication depended upon the host cell type and on the viral strain studied. Indeed, while we observed a strong inhibitory effect of polyanions on the replication of cytopathic, T-cell-line-adapted viruses such as HIV-1_{LAI} in the CEM T-cell line, similar to those in reported observations (3, 4, 13, 22, 23), different patterns were observed with other host cell types and other viral strains. In primary lymphocytes (perhaps the most important host cell type in vivo [25]), a much reduced inhibitory effect was observed, with only the highest concentrations tested having an inhibitory effect on the replication of both lymphotropic and macrophage-tropic viruses. With monocyte-derived macrophages as host cells, polyanions enhanced the replication of macrophage-tropic viruses in a majority of experiments. The enhancement observed here was typically severalfold, i.e., of the same magnitude as the antibody-mediated enhancement that has been described in monocytic cells infected with HIV (33). In the remaining experiments, very weak or no inhibition was observed at even the highest concentrations tested. In particular, the effects of dextran sulfate on the replication of HIV-1_{BaL} could be compared in primary lymphocytes and macrophages. Enhancement was observed only in the latter host cell type. Also, the inhibitory effect of dextran sulfate on the replication rates of HIV-1_{LAI} was much reduced in primary lymphocytes compared to those in cell lines. Thus, the effect of polyanions on prototypic lymphotropic or macrophage-tropic viruses varied on the basis of the host cells.

To assess whether viral factors are important in determining the effect of polyanions, the effect of dextran sulfate on various

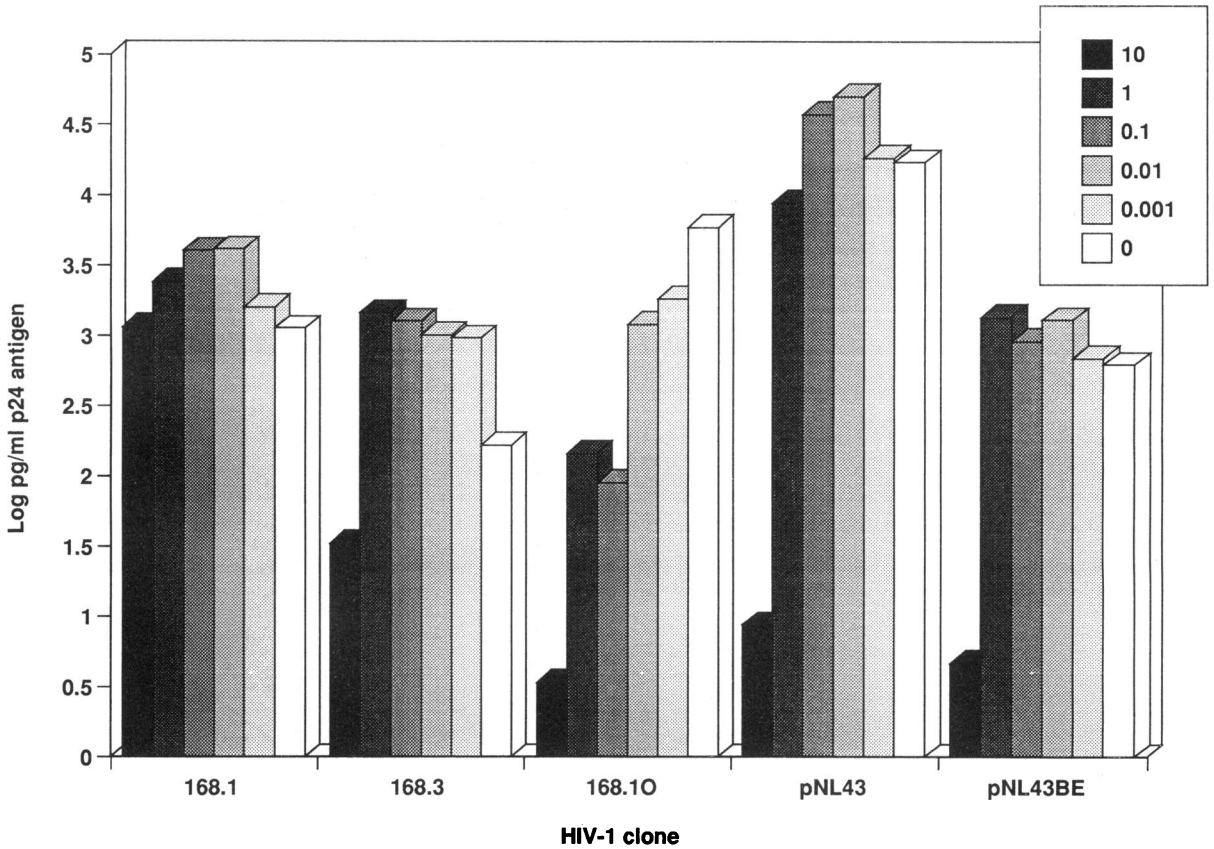

FIG. 4. Effect of dextran sulfate on the replication of various HIV-1 clones in stimulated primary human lymphocytes. Aliquots of 3×10^5 lymphocytes were incubated for 1 h at 37°C with various concentrations of dextran sulfate 3 days after they were isolated. The cells were then infected with 160 μl of virus stocks produced in COS-7 cells, representing from 3 to 6 ng of p24 antigen depending on the clone, in the continued presence of dextran sulfate. After incubation for 1 h, the cells were washed and resuspended in 1 ml of RPMI 1640 with 10% fetal bovine serum, 50 U of IL-2 per ml, and dextran sulfate at the indicated concentrations in 48-well plates. Twice a week, the cells were counted and diluted to a density of 3×10^5 ml. At day 7 (pNL43 and pNL43BE) or day 14 (168.1, 168.3, and 168.10), culture supernatants were harvested for p24 antigen determination.

viral clones was tested in the same host cell: primary lymphocytes. These cells are basically permissive to every HIV isolate. Because the cytopathic effect and tropism of HIV are largely determined by the V3 loop of gp120 (10, 14, 24, 31, 32), we investigated whether differences in V3 loop amino acid composition would affect the antiviral effect of dextran sulfate. We therefore chose HIV clones that were isogenic except for the V3 loop of gp120, which was cloned from sequential isolates from the same patient and which had increasing cytopathic effect and T-cell line tropism. Dextran sulfate (5, 19) and negatively charged peptides (19) have been shown to bind to this positively charged structure. The overall positive electric charge of this loop has been shown to increase with the T-cell line-tropic, cytopathic phenotype it confers (10, 32). Our observations of different susceptibilities of these clones to the inhibitory effect of dextran sulfate confirm that this molecule acts in some way at the level of the V3 loop to inhibit HIV replication. Perhaps the lower positive charge of the V3 loop of noncytopathic viruses confers a reduced affinity for dextran sulfate, resulting in turn in a reduced inhibitory effect.

How dextran sulfate can enhance infection in macrophages is unclear. Studies with T-cell lines suggested that the inhibitory effect of dextran sulfate on HIV replication was related to a decreased binding of the virus to the host cell (3, 22, 23). In our experiments, dextran sulfate had opposite effects on the binding of HIV-1 to host cells, inhibiting the binding of HIV-1_{LAI} to lymphocytes while enhancing the binding of HIV-1_{BaL} to monocytic cells (Fig. 3), suggesting again that the effect of dextran sulfate on HIV replication is due to an effect at the level of virus binding.

The present observation might be a potential explanation for the surprising clinical observation made by Flexner et al. (9). These authors administered dextran sulfate intravenously to HIV-infected patients in doses sufficient to obtain sustained plasma drug concentrations up to 200-fold higher than the 50% inhibitory concentrations for HIV infectivity in vitro in T-cell lines. Nevertheless, circulating HIV p24 antigen increased in all subjects who received the drug for more than 3 days. The authors argued that this was unlikely to be an artifact of the dextran sulfate on the p24 antigen capture assay, especially as the paradoxical effect increased with the duration of the dextran sulfate treatment. We indeed demonstrated no such artifactual effect of dextran sulfate on the p24 antigen assay. Enhancement in macrophages was observed in the 0.1- to 10-μg/ml range, typically the concentrations achieved in patients treated intravenously with dextran sulfate (9). It is therefore possible that the increasing p24 antigen levels during dextran sulfate treatment were due to the enhancement of HIV-1 replication in monocytic cells in these patients. This would indicate that steady-state virological markers like the

p24 antigen level in serum might reflect a very active replication rate in macrophages rather than T cells and that this rate can be changed within days by interventions affecting replication.

Heparin is a polyanion very similar to dextran sulfate. Our data show an ability of heparin to enhance HIV replication similar to that of dextran sulfate. Therefore, we advise caution when heparin is used to treat HIV-infected patients, as it might enhance HIV replication in vivo as well.

It is interesting to relate our data to the recent report by Pettoello-Mantovani et al. (27) that *Cryptococcus neoformans* capsular polysaccharide can enhance HIV infection in H9 cells and in PBMC at concentrations that are similar to those found in the blood or cerebrospinal fluid of patients with cryptococcal meningitis or disseminated infections. While these polysaccharides obviously have effects different from those of dextran sulfate, our results suggest that the enhancement observed in vitro might indeed predict increased viral replication in vivo in patients with disseminated cryptococcal infection.

In a manner reminiscent of that for soluble CD4 (6, 30), dextran sulfate was deemed a promising candidate drug on the basis of a preclinical evaluation limited to the effect of the drug on the replication of laboratory-adapted HIV strains in T-cell lines. Clinical studies were then performed that showed a lack of clinical efficacy. This could have been suspected by more extensive in vitro testing, as shown in the present study. Our data therefore demonstrate once again that the preclinical evaluation of candidate anti-HIV drugs should not be based only upon an investigation of their antiviral effect on T-cell lines infected with laboratory-adapted viral strains but should also include the evaluation of their effect on primary HIV isolates infecting both primary lymphoblasts and macrophages.

We thank Jean-Jacques de Jong and Brian Cullen for providing plasmids, Philippe Schneider for providing buffy coats, GianPietro Corradin for IL-2, Judy Nordberg for the flow cytometry analysis, Sandra Rasad and Pat Ley for technical help, and Roland Sahli for invaluable discussions.

P.R.A.M. was supported by grants 31-36164.92 and 31-31955.91 of The Swiss Committee for AIDS Research of the Federal Office for Public Health.

REFERENCES

1. **Abrams, D. I., S. Kuno, R. Wong, K. Jeffords, M. Nash, J. M. Molaghan, R. Gorter, and R. Ueno.** 1989. Oral dextran sulfate (UA001) in the treatment of the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. Ann. Intern. Med. **110:**183–188.
2. **Adachi, A., H. E. Gendelman, S. Koenig, T. Folks, R. Willey, A. Rabson, and M. A. Martin.** 1986. Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. J. Virol. **59:**284–291.
3. **Baba, M., R. Pauwels, J. Balzarini, J. Arnout, J. Desmyter, and E. De Clercq.** 1988. Mechanism of inhibitory effect of dextran sulfate and heparin on replication of human immunodeficiency virus in vitro. Proc. Natl. Acad. Sci. USA **85:**6132–6136.
4. **Baba, M., R. Snoeck, R. Pauwels, and E. De Clercq.** 1988. Sulfated polysaccharides are potent and selective inhibitors of various enveloped viruses, including herpes simplex virus, cytomegalovirus, vesicular stomatitis virus, and human immunodeficiency virus. Antimicrob. Agents Chemother. **32:**1742–1745.
5. **Callahan, L. N., M. Phelan, M. Mallinson, and M. A. Norcross.** 1991. Dextran sulfate blocks antibody binding to the principal neutralizing domain of human immunodeficiency virus type 1 without interfering with gp120-CD4 interactions. J. Virol. **65:** 1543–1550.
6. **Daar, E. S., X. L. Li, T. Moudgil, and D. D. Ho.** 1990. High concentrations of recombinant soluble CD4 are required to neutralize primary human immunodeficiency virus type 1 isolates. Proc. Natl. Acad. Sci. USA **87:**6574–6578.
7. **de Jong, J.-J., J. Goudsmit, W. Keulen, B. Klaver, W. Krone, M. Tersmette, and A. de Ronde.** 1992. Human immunodeficiency virus type 1 clones chimeric for the envelope V3 domain differ in syncytium formation and replication capacity. J. Virol. **66:**757–765.
8. **Embretson, J., M. Zupancic, J. L. Ribas, A. Burke, P. Racz, K. Tenner-Racz, and A. T. Haase.** 1993. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. Nature (London) **362:**359–362.
9. **Flexner, C., P. A. Barditch-Crovo, D. M. Kornhauser, H. Farzadegan, L. J. Nerhood, R. E. Chaisson, K. M. Bell, K. J. Lorentsen, C. W. Hendrix, B. G. Petty, and P. S. Lietman.** 1991. Pharmacokinetics, toxicity, and activity of intravenous dextran sulfate in human immunodeficiency virus infection. Antimicrob. Agents Chemother. **35:**2544–2550.
10. **Fouchier, R. A. M., M. Groenink, N. A. Kootstra, M. Tersmette, H. G. Huisman, F. Miedema, and H. Schuitemaker.** 1992. Phenotype-associated sequence variation in the third variable domain of the human immunodeficiency virus type 1 gp120 molecule. J. Virol. **66:**3183–3187.
11. **Haertle, T., C. J. Carrera, J. S. McDougal, L. C. Sowers, D. D. Richman, and D. A. Carson.** 1988. Metabolism and anti-HIV activity of 2′-halo-2′,3′-dideoxyadenosine derivatives. J. Biol. Chem. **263:**5870–5875.
12. **Hwang, S. S., T. J. Boyle, H. K. Lyerly, and B. R. Cullen.** 1991. Identification of the envelope V3 loop as the primary determinant of cell tropism in HIV-1. Science **253:**71–74.
13. **Ito, M., M. Baba, A. Sato, R. Pauwels, E. De Clercq, and S. Shigeta.** 1987. Inhibitory effect of dextran sulfate and heparin on the replication of human immunodeficiency virus (HIV) in vitro. Antiviral Res. **7:**361–367.
14. **Johnson, M. A., and A. J. Cann.** 1992. Molecular determination of cell tropism of human immunodeficiency virus. Clin. Infect. Dis. **14:**747–755.
15. **Kornbluth, R. S., P. S. Oh, J. R. Munis, P. H. Cleveland, and D. D. Richman.** 1989. Interferons and bacterial lipopolysaccharide protect macrophages from productive infection by HIV in vitro. J. Exp. Med. **169:**1137–1151.
16. **Lederman, S., R. Gulick, and L. Chess.** 1993. Dextran sulfate and heparin interact with CD4 molecules to inhibit the binding of coat protein (gp120) of HIV. J. Immunol. **143:**1149–1154.
17. **Lorentsen, K. J., C. W. Hendrix, J. M. Collins, D. M. Kornhauser, B. G. Petty, R. W. Klecker, C. Flexner, R. H. Ecklel, and P. S. Lietman.** 1989. Dextran sulfate is poorly absorbed after oral administration. Ann. Intern. Med. **111:**561–566.
18. **McClure, M. O., J. P. Moore, D. F. Blanc, P. Scotting, G. M. W. Cook, R. J. Keynes, J. N. Weber, D. Davies, and R. A. Weiss.** 1992. Investigations into the mechanism by which sulfated polysaccharides inhibit HIV infection in vitro. AIDS Res. Hum. Retroviruses **8:**19–26.
19. **Meshcheryakova, D., S. Andreev, S. Tarasova, M. Sidorova, M. Vafina, G. Kornilaeva, E. Karamov, and R. Khaitov.** 1993. CD4-derived peptide and sulfated polysaccharides have similar mechanisms of anti-HIV activity based on electrostatic interactions with positively charged gp120 fragments. Mol. Immunol. **30:**993–1001.
20. **Meylan, P. R., J. R. Munis, D. D. Richman, and R. S. Kornbluth.** 1992. Concurrent human immunodeficiency virus and mycobacterial infection of macrophages in vitro does not reveal any reciprocal effect. J. Infect. Dis. **165:**80–86.
21. **Meylan, P. R. A., C. A. Spina, D. D. Richman, and R. S. Kornbluth.** 1993. In vitro differentiation of monocytoid THP-1 cells affects their permissiveness for HIV strains: a model system for studying the cellular basis of HIV differential tropism. Virology **193:**256–267.
22. **Mitsuya, H., D. J. Looney, S. Kuno, R. Ueno, W. Wong-Staal, and S. Broder.** 1988. Dextran sulfate suppression of viruses in the HIV family: inhibition of virion binding to CD4+ cells. Science **240:** 646–648.
23. **Nakashima, H., O. Yoshida, M. Baba, E. De Clercq, and M. Yamamoto.** 1989. Anti-HIV activity of dextran-sulphate as determined under different experimental conditions. Antiviral Res. **11:** 233–246.

24. **O'Brien, W. A., Y. Koyanagi, A. Namazie, and J.-Q. Zhao.** 1990. HIV-1 tropism for mononuclear phagocytes can be determined by regions of gp120 outside the CD4-binding domain. Nature (London) **348**:69–73.
25. **Pantaleo, G., C. Graziosi, and A. S. Fauci.** 1993. The immunopathogenesis of human immunodeficiency virus infection. N. Engl. J. Med. **328**:327–335.
26. **Parish, C. R., L. Low, H. S. Warren, and A. L. Cunningham.** 1990. A polyanion binding site on the CD4 molecule; proximity to the HIV-gp120 binding region. J. Immunol. **145**:1188–1195.
27. **Pettoello-Mantovani, M., A. Casadevall, T. R. Kollmann, A. Rubinstein, and H. Goldstein.** 1992. Enhancement of HIV-1 infection by the capsular polysaccharide of Cryptococcus neoformans. Lancet **339**:21–23.
28. **Richman, D. D., D. Havlir, J. Corbeil, D. Looney, C. Ignacio, S. A. Spector, J. Sullivan, S. Cheeseman, K. Barringer, D. Pauletti, C.-K. Shih, M. Myers, and J. Griffin.** 1994. Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. J. Virol. **68**:1660–1666.
29. **Schols, D., R. Pauwels, J. Desmyter, and E. De Clercq.** 1990. Dextran sulfate and other polyanionic anti-HIV compounds specifically interact with the viral gp120 glycoprotein expressed by T-cells persistently infected with HIV-1. Virology **175**:556–561.
30. **Schooley, R. T., T. C. Merigan, P. Gaut, M. S. Hirsch, M. Holodniy, T. Flynn, S. Liu, R. E. Byington, S. Henochowicz, E. Gubish, D. Spriggs, D. Kufe, J. Schindler, A. Dawson, D. Thomas, D. G. Hanson, B. Letwin, T. Liu, J. Gulinello, S. Kennedy, R. Fisher, and D. D. Ho.** 1990. Recombinant soluble CD4 therapy in patients with the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. Ann. Intern. Med. **112**:247–253.
31. **Shioda, T., J. A. Levy, and C. Cheng-Mayer.** 1991. Macrophage and T cell-line tropisms of HIV-1 are determined by specific regions of the envelope gp120 gene. Nature (London) **349**:167–169.
32. **Shioda, T., J. A. Levy, and C. Cheng-Mayer.** 1993. Small amino acid changes in the V3 hypervariable region of gp120 can affect the T-cell-line and macrophage tropism of human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA **89**:9434–9438.
33. **Takeda, A., C. U. Tuazon, and F. A. Ennis.** 1988. Antibody-enhanced infection by HIV-1 via Fc receptor-mediated entry. Science **242**:580–583.
34. **Tsuchiya, S., Y. Kobayashi, Y. Goto, H. Okumura, S. Nakae, T. Konno, and K. Tada.** 1982. Induction of maturation in cultured human monocytic leukemia cells by a phorbol diester. Cancer Res. **42**:1530–1536.
35. **Tsuchiya, S., M. Yamabe, Y. Yamaguchi, Y. Kobayashi, T. Konno, and K. Tada.** 1980. Establishment and characterization of a human acute monocyte leukemia cell line (THP-1). Int. J. Cancer **26**:171–176.
36. **Weaver, J. L., P. Gergely, P. S. Pine, E. Patzer, and A. Aszalos.** 1990. Polyionic compounds selectively alter availability of CD4 receptors for HIV coat protein rgp120. AIDS Res. Hum. Retroviruses **6**:1125–1130.