of infection by blood recipients who remain sexually active and by the small proportion of recipients who are women of child-bearing age.

No cases of transfusion-associated ATL have been reported to date. The absence of such reports, however, may not be an accurate indication of the impact of transfusion-transmitted HTLV-I infection in nonendemic areas of the world for the following reasons: first, a latency period of 10 to 30 years appears to exist between infection and clinical disease (*1*); and second, HTLV-I may play a role in indirectly mediating the development and growth of neoplasms other than ATL by mechanisms that are not fully understood (*3*).

Information obtained from this study and from continuing HIV-1 surveillance efforts indicates that the complete exclusion of potentially infective blood donors is not attainable solely by health history and self-deferral procedures (*14*). Refinements of donor selection methods are needed, as are continued efforts to develop blood sterilization technologies. In the meantime, the use of licensed assays to detect and exclude blood donors who have confirmed laboratory evidence of HTLV-I infection would appear to be the most prudent course of action.

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Dextran Sulfate Suppression of Viruses in the HIV Family: Inhibition of Virion Binding to CD4$^+$ Cells

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The first step in the infection of human T lymphocytes by human immunodeficiency virus type 1 (HIV-1) is attachment to the target cell receptor, the CD4 antigen. This step may be vulnerable to attack by antibodies, chemicals, or small peptides. Dextran sulfate (molecular weight approximately 8000), which has been given to patients as an anticoagulant or antilipemic agent for more than two decades, was found to block the binding of virions to various target T lymphocytes, inhibit syncytia formation, and exert a potent inhibitory effect against HIV-1 in vitro at concentrations that may be clinically attainable in human beings. This drug also suppressed the replication of HIV-2 in vitro. These observations could have theoretical and clinical implications in the strategy to develop drugs against HIV types 1 and 2.

DEXTRAN SULFATE, A LONG-CHAIN polymer of glucose that has a molecular weight of approximately 8000 and contains 17 to 20% sulfur, has been given orally to human beings for more than two decades as an anticoagulant or antilipemic agent (*1*). This agent was recently shown to be a potent agent against human immunodeficiency virus type 1 (HIV-1) in vitro (*2*). We have now investigated the possible mechanism of action of dextran sulfate against HIV-1 and related viruses.

When susceptible interleukin 2 (IL-2)–dependent helper T cells [strain ATH8 (*3*, *4*)] were exposed in vitro to HIV-1 in the form of cell-free virions, essentially all the cells were destroyed by the cytopathic effect of HIV-1 by day 7 (Fig. 1). However, when ATH8 cells were cultured in the presence of HIV-1 virions and more than 1.25 μM dextran sulfate, no cytopathic effects were observed and growth of the cells was comparable to that of the virus-free control ATH8 population.

Dextran sulfate may have two sulfate groups per glucose unit, and these sulfate groups appear to be associated with its antiviral activity (*2*). Indeed, nonsulfated dextran [molecular weight (MW) of 9400] showed no protective effect on ATH8 cells against the virus even at 125 μM.

Dextran sulfate also protected normal (tetanus toxoid–specific) helper/inducer clonal T cells (TM11 cells) against the cytopathic effect of HIV-1 (Fig. 1). At concentrations of 0.625 μM to 12.5 μM, this compound gave complete protection and did not suppress the growth of the T cells. At similar concentrations, dextran sulfate also inhibited the infectivity and cytopathic effects of human immunodeficiency virus type 2 (HIV-2), which may also cause an AIDS-like disease (*5*) (Fig. 1).

As an additional index of the antiretroviral effect of dextran sulfate, we looked for the presence of proviral DNA in susceptible ATH8 cells continuously exposed to HIV-1

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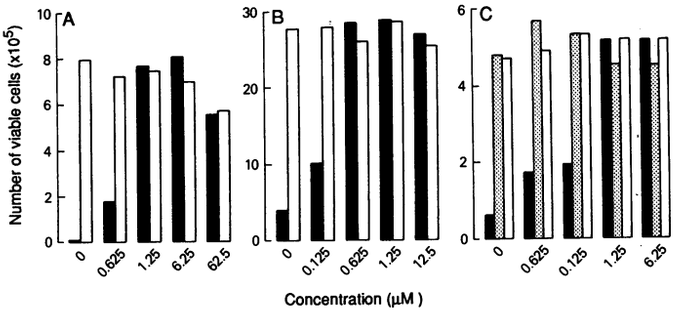

Fig. 1. Inhibition of the infectivity and cytopathic effects of HIV-1 and HIV-2 by dextran sulfate. (**A** and **B**) Inhibition of HIV-1 replication. AHT8 cells (2×10^5) (A) (*3, 4*) or normal clonal tetanus toxoid–specific helper T cells (TM11 cells; 2×10^5) (B) (*21*) were treated with polybrene, pelleted, exposed to HIV-1 (HTLV-III_B; 2000 virus particles per cell) (solid bars), and cultured in the presence or absence of various concentrations of dextran sulfate. Under the conditions used here, five virus particles per cell represented the minimum cytopathic dose of the virus. Control cells (open bars) were similarly treated but not exposed to the virus. On day 7 (A) or day 12 (B), viable cells were counted on a hemocytometer by the trypan blue exclusion method. Variability in cell number determination is ±10% of the value shown (*3, 4, 11*). (**C**) Inhibition of HIV-2 replication. ATH8 cells (2×10^5) were cocultured with 4×10^4 irradiated (10,000 rad) HIV-2 producing CEM cells (solid bars) or, as a control, uninfected CEM cells (stippled bars) in the presence or absence of dextran sulfate. ATH8 cells could be readily distinguished from neoplastic CEM cells by morphology. Control ATH8 cells were cultured without any cells added (open bars). When cultured alone, none of the irradiated HIV-2–producing CEM cells or uninfected CEM cells were alive on day 7 in culture. On day 7, total viable cells were counted.

in culture but protected by dextran sulfate. In the absence of the drug, proviral DNA was first detected on day 2, and on day 4 an abundant amount of proviral DNA was detected. In contrast, in ATH8 cells similarly exposed to the virus and cultured in the presence of 2.5 μ*M* dextran sulfate, no proviral DNA was detected throughout the study (Fig. 2).

We then studied the effect of dextran sulfate on HIV-1 DNA polymerase (reverse transcriptase, RT) activity, using a synthetic template (*6*). Dextran sulfate showed a potent inhibitory activity against purified HIV-1 RT at ≥1 μ*M*, and the RT-mediated DNA synthesis was completely inhibited. However, at similar concentrations this compound also suppressed the activity of mammalian DNA polymerase-α, an enzyme that has key DNA synthetic and repair functions in cells. Furthermore, the addition of a nonenzyme protein, bovine serum albumin, readily nullified the RT inhibition by dextran sulfate (*7*). In view of these results, the capacity of dextran sulfate to block the infectivity of HIV without cellular toxicity might be difficult to attribute to selective inactivation of viral RT per se.

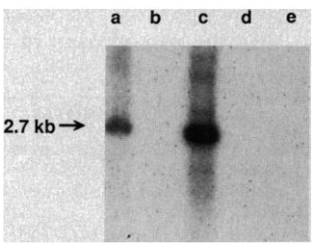

Fig. 2. Inhibition of HIV-1 DNA synthesis in ATH8 cells. ATH8 cells (10^7) were continuously exposed to HIV-1 (HTLV-III_B: 1000 virus particles per cell) and cultured in the presence or absence of 6.25 μ*M* dextran sulfate. On days 2 (lanes a and b) and 4 (lanes c and d) in culture, high molecular weight DNA was extracted, and 40 μg of such DNA was digested with Kpn I (Boehringer Mannheim) and subjected to Southern blot analysis. The DNA was hybridized with a radiolabeled insert of a molecular clone of the *env* region of BH10 containing a 1.3-kb Bgl II fragment. Lanes a and c, DNA from ATH8 cells that were exposed to HIV-1 and not protected by the drug; lanes b and d, DNA from ATH8 cells exposed to the virus and protected by dextran sulfate. Lane e, DNA of ATH8 cells not exposed to the virus. The 2.7-kb *env*-containing internal Kpn I fragment of the virus genome was detected in lanes a and c. We also rehybridized the same DNA with a T cell receptor β-chain probe, which showed that virtually the same amount of DNA was loaded in each lane and that they were completely digested.

The initial event in the infection of target cells by HIV-1 is the attachment of the HIV-1 envelope glycoprotein to the CD4 cellular protein. Such an attachment may be necessary, but not automatically sufficient, for effective binding and subsequent penetration. The cytopathic effect of HIV-1 is mediated, at least in part, by an interaction between the gp120 and CD4 molecule that brings about syncytia formation (*8*). A surface autofusion phenomenon that destroys the integrity of the cell membrane could also be related to the cytopathic effect of HIV-1. However, syncytia formation is not necessarily the only mechanism by which the virus exerts its cytopathic effects. Indeed, when susceptible ATH8 cells are exposed to HIV-1 in the form of cell-free virions, most ATH8 cells are destroyed without forming detectable syncytia (*9*). Fusion events are thought to be mediated by sequences in the gp41 portion of the envelope (*10*). The integrity of tertiary complexes of viral envelope protein is likely to be crucial for these processes. Dextran sulfate might function at any of these points. To determine if dextran sulfate could inhibit syncytia formation, we used a system permitting $CD4^+$ uninfected cells to interact with viral protein expressed on the surface of HIV-1 infected cells. When $CD4^+$ ATH8 cells were cocultured with HIV-1 infected H9 cells, substantial numbers of syncytia were formed in 48 hours (Fig. 3). Syncytia were still formed in the continuous presence of 3′-azido-2′,3′-dideoxythymidine (AZT) and 2′,3′-dideoxyadenosine, retroviral DNA chain terminators known to be effective against HIV-1 replication at the stage of reverse transcription (*3, 11*), but not thought to affect viral binding. Dextran sulfate (6.75 μ*M*), however, completely inhibited syncytia formation between ATH8 cells and HIV-1 infected H9 cells.

Fusion of uninfected $CD4^+$ cells with HIV-1 infected cells can be blocked by certain antibodies to CD4 (*8*). In the absence of such antibodies, the syncytia produced die shortly after formation and the surviving cells can be comprised of resistant $CD4^-$ cells (*12*). To determine whether dextran sulfate modulates or down-regulates CD4 antigen, we examined the amount of CD4 antigen expressed on normal helper T cells (TM11), susceptible helper T cells (ATH8), and relatively permissive cells (H9) by an immunofluorescence technique using OKT4A antibody. We found no significant change in the CD4 immunofluorescence intensities in any of these target T cells after

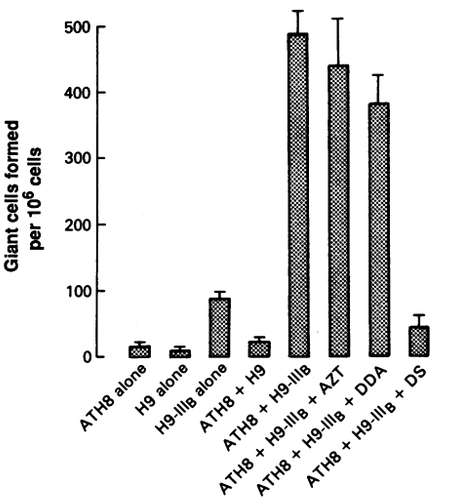

Fig. 3. Inhibition of syncytium (giant cell) formation by dextran sulfate. ATH8 cells (5×10^5) were cultured with or without an equal number of chronically HIV-1 infected H9 cells in Costar 12-well culture plates after 2 hours in the presence or absence of 10 μ*M* 3′-azido-2′,3′-dideoxythymidine, 50 μ*M* 2′,3′-dideoxyadenosine, or 6.75 μ*M* dextran sulfate. After 48 hours of coculture, the number of giant cells was assessed on the inverted microscope. Bars represent the means (±1 SD) of duplicate determinations.

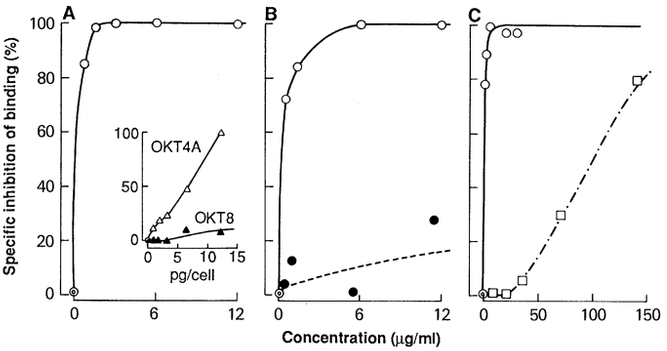

Fig. 4. Inhibition of HIV-1 binding by dextran sulfate. Radiolabeled virus: two-week-HX10-infected H9 cells were incubated in medium containing 2 n*M*/ml [5-^{3}H]uridine (26.7 Ci/mmol, New England Nuclear) for 48 hours. The cell-free supernatant was filtered (HVLP, Millipore), centrifuged at 30,000 rpm for 1 hour, and dialyzed in NTE buffer (0.1*N* NaCl, 0.1*M* tris, 0.001*M* EDTA, *p*H 7.4) for 1 to 2 hours at 4°C. Preparations with high nonspecific binding were further clarified by centrifugation at 10,000*g* for 10 to 20 minutes. Preparations that showed >30% control binding after maximal OKT4A inhibition were not used. Virus preparations usually contained 0.83×10^4 to 2.5×10^4 Bq/ml or 0.5×10^{-6} to 2.5×10^{-5} Bq per virion [1 Ci = 3.7×10^{10} Becquerel]. Binding inhibition assay: serial twofold dilutions of OKT4A and OKT8 (Ortho-Diagnostics) (A, inset) or drug solutions (○, dextran sulfate; ●, nonsulfated dextran; □, suramin) were made in binding assay buffer (BAB: 2 mg/ml of bovine serum albumin, 2% heat-inactivated calf serum, and 0.1% sodium azide in Dulbecco's phosphate-buffered saline). Labeled virus was diluted and 10 μl (4500 to 9000 cpm) was added to 2×10^5 H9 cells suspended in BAB; after incubation for 1 hour at 37°C in 5% CO_2, the cells were washed twice with BAB and lysed with distilled water, and radioactivity was counted. Actual mean counts (± 1 SD) of radioactivity bound to CD4$^+$ cells were 671 ± 29 and 683 ± 24 (cpm) and those upon the maximal inhibition by OKT4A were 168 ± 23 and 183 ± 24 for (**A**), (**B**), and (**C**), respectively. The specific binding of CD4$^-$ RAMOS (B-lymphoid) or CD4$^-$ K562 (erythroid) was almost negligible. In one experiment, H9 cells bound 12,300 cpm while RAMOS bound 6,200 cpm. However, under the conditions used here, CD4$^+$ cell lines (H9, MOLT-3, and Sub T1) always showed 65 to 90% decreases in bound radioactivity in the presence of OKT4A, and CD4$^-$ cell lines (RAMOS and K562) showed only <15% decreases. Specific inhibition was calculated according to the formula: % specific inhibition = $100 \times (CPM_{control} - CPM_{sample})/(CPM_{control} - CPM_{max})$; where CPM_{max} is the radioactivity observed with the supramaximal concentration of OKT4A (50 μg/ml), $CPM_{control}$ is the radioactivity in the absence of drug or antibody, and CPM_{sample} is the observed experimental radioactivity. Each symbol represents the arithmetic mean of triplicate determinations using one virus preparation. Standard deviation of the bound radioactivity did not exceed 15% of the mean value.

exposure to dextran sulfate (*13*). This suggests that dextran sulfate might inhibit the step in which the virion binds to the target T cells.

[5-^{3}H]Uridine–labeled HIV-1 virions derived from the molecular clone HX10 (*14*) were incubated with uninfected H9 cells in the presence or absence of various concentrations of dextran sulfate, and the radioactivity of virions bound to the cells was determined. We assumed that the capacity of certain compounds to block that amount of virion binding which can be inhibited by OKT4A is also essential for blocking the interaction of gp120 and CD4 protein, thereby blocking infection of HIV-1. To express the level of virion binding inhibition displayed by various compounds, we calculated the specific inhibition in such a way that the amount of labeled virions bound in the presence of a supramaximal concentration of OKT4A (50 μg/ml) represented 100% specific inhibition, and the amount bound in the absence of drug or antibody represented 0% specific inhibition (see Fig. 4A, inset). By analyzing the bound radioactivity on a per-cell basis, we generally found 500 to 2000 virus particles bound to a cell in the absence of drug under the conditions used (*15*). Similar data have been obtained with Jurkat cells and Molt-3 cells and with a different molecularly cloned virus strain WMJ-1 (*16*). At comparable concentrations of antibody to CD8 (OKT8) we observed almost no inhibitory effect on viral binding. As shown in Fig. 4A, dextran sulfate completely inhibited the binding of radiolabeled virus to H9 cells. The level of binding inhibition by dextran sulfate was equal to the maximum inhibition achieved by OKT4A at concentrations as low as 1 μ*M*. Nonsulfated dextran (MW 9400), which is inactive against HIV-1, again showed no inhibition (Fig. 4B). Suramin, a compound (MW 1429) with six sulfate groups, suppresses the infectivity and replication of HIV-1 at 35 μ*M* in vitro (*17*), but even at 140 μ*M* suramin did not completely inhibit virion binding (Fig. 4C), suggesting that this polyanionic substance inhibits HIV-1 by other mechanisms.

In our [5-^{3}H]uridine–labeled virus binding assay, binding inhibition has been only modest in sera from a number of HIV-1 infected patients and has not been found in all sera showing HIV-1 neutralizing activity (*16*). These data appear to be consistent with observations by Lifson *et al.* that sera from certain HIV-1 infected individuals, while capable of neutralizing the infectivity, have only a limited capacity to inhibit HIV-1–induced cell fusion (*18*). It is possible that a CD4 contact site of gp120 represents an immunologically tolerated epitope, or is hidden by another portion of the virion envelope.

At least two viral structural determinants are involved in the penetration of HIV-1 that results from fusion of viral and cellular membranes and the giant cell–inducing activity of HIV-1: the CD4 binding site within gp120 and a fusogenic domain believed to reside within the transmembrane *env* protein of HIV-1, gp41 (*10*). Our current data suggest that dextran sulfate is capable of inhibiting virion attachment and fusion-dependent events, although other mechanisms (for example, interference with retroviral uncoating) could be involved in its anti–HIV-1 effect. The way in which dextran sulfate affects virion binding is not established and will require further research.

We have observed that combinations of dextran sulfate and certain DNA chain–terminating dideoxynucleosides, including AZT, 2′,3′-dideoxycytidine and 2′,3′-dideoxyadenosine, work against HIV-1 better than each drug does alone in vitro (*19*). Studies completed in Japan suggest that concentrations higher than those that show potent antiviral activity in vitro are achievable by oral administration in normal volunteers (*20*). While it is premature to assess clinical results on dextran sulfate, it is conceivable that therapies that alter virion binding will alter the pathogenesis of HIV-1 infection in patients.

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7. The addition of 50 μg/ml and 100 μg/ml of bovine serum albumin nullified the RT inhibition by 1.25 μ*M* dextran sulfate from 99% to 47% and 14%, respectively (bovine serum albumin at these concentrations did not affect the RT-mediated DNA syn-

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Sustained Dendritic Gradients of Ca^{2+} Induced by Excitatory Amino Acids in CA1 Hippocampal Neurons

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Spatially resolved measurements of intracellular free calcium and of the changes produced by excitatory amino acids were made in neurons isolated from adult mammalian brain. Extremely long-lasting (minutes) Ca^{2+} gradients were induced in the apical dendrites of hippocampal CA1 neurons after brief (1 to 3 seconds), local application of either glutamate or *N*-methyl-D-aspartate (NMDA). These gradients reflect the continuous flux of Ca^{2+} into the dendrite. The sustained gradients, but not the immediate transient response to the agonists, were prevented by prior treatment with the protein kinase C inhibitor sphingosine. Expression of the long-lasting Ca^{2+} gradients generally required a priming or conditioning stimulus with the excitatory agonist. The findings demonstrate a coupling between NMDA receptor activation and long-lasting intracellular Ca^{2+} elevation that could contribute to certain use-dependent modifications of synaptic responses in hippocampal CA1 neurons.

NEURONS OF THE MAMMALIAN HIPpocampus have become an important focus for the investigation of putative memory mechanisms, including long-term potentiation (LTP) of excitatory synaptic connections, kindling phenomena, and other persistent effects of conditioning paradigms (*1*). Both LTP and certain kindling phenomena show dependence on Ca^{2+} levels [Ca^{2+}] or fluxes (*2*) and Ca^{2+}-dependent enzymes (*3, 4*) for expression. Several studies have linked *N*-methyl-D-aspartate (NMDA) receptor activation with LTP induction, presumably through Ca^{2+}-dependent mechanisms (*5*). There have been no direct measurements, in hippocampus or in any other fully differentiated mammalian neurons, of the Ca^{2+} changes that result from stimulation with the putative natural excitatory transmitter glutamate or the analog NMDA, although in cultured neurons NMDA induces a large transient influx of Ca^{2+} through the receptor-operated channels (*6*).

We measured [Ca^{2+}] and the changes produced by excitatory amino acids in isolated CA1 neurons (*7*) from adult guinea pig hippocampus by using the fluorescent Ca indicator fura-2 (*8*). These completely isolated cells have been used in elucidating the membrane properties of neurons, independent of presynaptic and hormonal input (*9*). The preparation is also ideally suited to optical studies because of the freedom from absorbance and scattering by extraneous tissue. Resting [Ca^{2+}] in 120 CA1 neurons tested averaged 84 ± 3 n*M* (SEM). Neurons in the range below 100 n*M* were stable for periods of more than 30 minutes (*10*).

Figure 1 illustrates the basic finding of this study: repeated stimulation of the apical dendrite leads to localized Ca^{2+} increases of long duration. The bathing saline contained the Na^+-channel blocker tetrodotoxin (TTX) [1 μ*M*, see (*11*)]. A glutamate pulse was applied to the dendrite at two different locations. The first pulse, applied at the leftmost end of the dendrite (Fig. 1A), produced a large change in Ca^{2+} that recovered rapidly and remained stable for the next 3 minutes (Fig. 1, B through E). The second application (Fig. 1H) produced a smaller response than the first one, and there was an initial small recovery after the stimulus (Fig. 1I), but over the next 3 minutes [Ca^{2+}] progressively increased at the site of the second application, generating a gradient that tailed off to either side (Fig. 1, J and K). We emphasize that [Ca^{2+}] decreased prior to the secondary rise and the generation of the standing gradient. This secondary rise in [Ca^{2+}] occurred in 93% of the cells examined (n = 49). In most other experiments the successive glutamate applications were made at or near the initial site. Removing Ca^{2+} from the bathing medium abolished the standing gradients as rapidly as the solution change could be made and allowed the cell to restore low levels of Ca^{2+} (Fig. 1L). Glutamate stimuli did not produce changes in Ca^{2+}-free saline.

In 29% of the 34 neurons tested for glutamate response, two stimuli were required before the long-lasting response was expressed. Once the long-lasting response was established, [Ca^{2+}] almost never recovered spontaneously to their initial values during the usual experimental time course of 20 to 25 minutes. When the response was allowed to persist for ten or more minutes, [Ca^{2+}] in the soma and dendrite gradually increased to 300 to 500 n*M*. At this stage of the response the dendritic Ca^{2+} gradient was gone and the nuclear region of the soma showed higher levels than other parts of the cell. In 32% of the neurons, the extended response was elicited after the first application of glutamate; however, most of these cells already showed a small Ca^{2+} gradient, dendrite higher than soma. The rest of the cells showed the extended response only after three or more applications. Spacing between stimuli was a necessary part of the

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