Infection by HIV-1 blocked by binding of dextrin 2-sulphate to the cell surface of activated human peripheral blood mononuclear cells and cultured T-cells

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1 Structural analogues of a sulphated polysaccharide, dextrin sulphate, were synthesized and tested for their ability to block infection by HIV-1. Using the T-cell lines, C8166 and HPB-ALL, and the laboratory adapted strains of HIV-1.MN, HIV-1.IIb and HIV-1.RF, dextrin 2-sulphate (D2S) combined the best combination of high anti-HIV-1 activity (95% inhibitory concentration (IC_{95}) = 230 nM) and low anticoagulant activity. It also blocked infection of activated peripheral blood mononuclear (PBMN) cells by five primary viral isolates at an IC_{95} of 230–3700 nM depending upon the primary viral isolate tested.

2 In saturation binding studies, [³H]-D2S bound to a cell surface protein on HPB-ALL cells in a specific and saturable manner with a K_d of 82 ± 14 nM and a B_{max} of 4.8 ± 0.3 pmol/ 10^6 cells. It bound to other human T-cell lines in a similar manner.

3 There was very little binding of [³H]-D2S to freshly isolated PBMN cells (B_{max} 0.18 ± 0.03 pmol/ 10^6 cells) and these cells could not be infected by HIV-1. Culture of PBMN cells in lymphocyte growth medium (LGM) containing IL-2 did not significantly change the B_{max} of [³H]-D2S. In contrast, PBMN cells which had been cultured with phytohaemagglutinin (PHA; $5 \mu\text{g ml}^{-1}$) for 72 h had a B_{max} of [³H]-D2S binding of 7.2 ± 0.1 pmol/ 10^6 cells and these cells could be infected by HIV-1. Removal of the PHA and further culture of the PBMN cells in LGM containing IL-2 resulted in a fall in the B_{max} to 2.0 ± 0.1 pmol/ 10^6 cells. The K_d of binding did not change significantly during the course of these experiments.

4 [³H]-D2S did not bind to freshly isolated erythrocytes or to erythrocytes which had been cultured in PHA for 72 h.

5 These results suggest that there is a relationship between the expression of the [³H]-D2S binding protein on the plasma membrane of PBMN cells and the susceptibility of these cells to infection by HIV-1.

Keywords: HIV-1; laboratory adapted isolates of HIV-1; primary viral isolates of HIV-1; sulphated polysaccharides; chemical synthesis and characterization of sulphated polysaccharides; dextrin 2-sulphate; human cultured T-cells; human peripheral blood mononuclear cells

Introduction

Infection by the Human Immunodeficiency Virus-1 (HIV-1) is a prerequisite for the development of the Acquired Immune Deficiency Syndrome (AIDS) which is characterized by a progressive loss of CD4 positive T-cells. Infection of these cells is initiated by the binding of the viral envelope glycoprotein gp120 to the cell surface protein CD4, following which there is a temperature-dependent dissociation of gp120 from the viral envelope (Dagleish *et al.*, 1984; Klatzmann *et al.*, 1984; Maddon *et al.*, 1986; Hart *et al.*, 1991; Dimitrov *et al.*, 1992; Klasse & Moore, 1992). This exposes gp41 which is believed to be involved in the fusion of the virion with the cell membrane (Brasseur *et al.*, 1988). The mechanism of viral entry however remains obscure.

Endogenous sulphated polysaccharides have a role in cell adhesion and cell recognition (Rossignol *et al.*, 1984; Yamaguchi *et al.*, 1985; Cole *et al.*, 1986; Coombe *et al.*, 1987) and have been implicated in the binding of lymphocytes to endothelial venules (Stoolman & Rosen, 1983; Brenan & Parish, 1986; Coombe & Rider, 1989). More recently, sulphated polysaccharides have become the focus of renewed interest because they block infection of T-cell lines by laboratory adapted strains of HIV-1 *in vitro*. A variety of

compounds have been studied; fucoidan, dextran sulphate, pentosan polysulphate, mannan sulphate, lentinan sulphate, sulphated bacterial glycosaminoglycan, dextrin 2-sulphate, heparin and fragmented derivatives of heparin (Ito *et al.*, 1987; Ueno & Kuno, 1987; Baba *et al.*, 1988b; Bagasra & Lischner, 1988; McClure *et al.*, 1991; 1992; Beddows *et al.*, 1993). In previous studies, D2S was shown to block infection of human T-cell lines by a variety of laboratory adapted cell-free isolates of HIV-1 (McClure *et al.*, 1991; 1992; Beddows *et al.*, 1993).

It remains to be established whether the large family of sulphated polysaccharides which block HIV-1 infection share a common mechanism of action or whether several different mechanisms are responsible for their anti-HIV-1 activity. In the case of D2S, fucoidan and dextran sulphate, previous studies have suggested that they block HIV-1 infection by acting at the level of the cell surface but they did not characterize this binding (Mitsuya *et al.*, 1988; Baba *et al.*, 1988a; 1990; McClure *et al.*, 1991; 1992; Beddows *et al.*, 1993).

The identification and characterization of a cell surface receptor to which D2S binds and blocks HIV-1 infection has important implications for therapy. In this paper, we have synthesized structural variants of dextrin sulphate and used them to study the binding of these compounds to T-cells.

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Methods

Synthesis of D2S and its analogues

Limit dextrin (ML Labs, Waverley, Liverpool) was produced by enzymatic hydrolysis of starch and purified by ultra filtration and passage through absorptive filters. The product complies with the British Pharmacopoeia monograph for dextrin and consists predominantly of α -1, 4 linked glucan units. D2S was synthesized by sulphation of a solution of dextrin (40 g in 40 ml water) at room temperature using sulphur trioxide-trimethylamine complex (60 g) in the presence of sodium hydroxide (16.8 g). The stirred mixture was maintained alkaline over 12 h with 5 M sodium hydroxide. Excess reagent was removed by dialysis against water and the product recovered after freeze-drying. Dextrin 2,3,6-trisulphate was prepared in the same way as D2S but with a large excess of the sulphating agent.

Dextrin 3-sulphate was prepared from dextrin (16.2 g) after being acetylated over 2 d with acetic anhydride (20.4 g) and triethylamine (10 g) in dimethylformamide (150 ml). The product (12.3 g), predominantly the 2,6-diacetyl derivative, was collected by precipitation after the addition of water, dissolved in dimethylformamide (75 ml) and sulphated with trimethylamine-sulphur trioxide complex (25 g) over 24 h. The mixture was poured into acetone (500 ml) to yield a sticky residue and the supernatant decanted. The residue was dissolved in water (150 ml) and saponified with sodium hydroxide (5 g) to remove the acetyl groups. The dextrin 3-sulphate mixture was finally dialysed against water and freeze-dried.

Dextrin 6-sulphate was prepared by sulphation of dextrin (10 g) in dimethylformamide (100 ml) with cyclamic acid (22.5 g) at 78°C over 1.5 h. The mixture was made alkaline with sodium hydroxide in aqueous ethanol (10%; 50 ml) and then poured into diethyl ether (400 ml). The resultant solid was washed with ether, dissolved in sodium acetate solution (50%; 100 ml), dialysed against water and freeze-dried.

Dextrin 2-(2-hydroxypropyl-3-trimethylammonium salt) was prepared by stirring dextrin (81 g) in 5% sodium hydroxide (240 ml) with glycidyltrimethylammonium chloride (120 g) for 12 h at 20°C. The resulting solution was dialysed against water and freeze-dried. Dextrin 2-(2-hydroxypropyl-3-triethylammonium salt) was prepared in the same way except that glycidyltriethylammonium chloride was used in place of glycidyltrimethylammonium chloride.

Dextrin and its sulphated derivatives were characterized by sulphate analysis, Fourier transformed infra red spectra recorded in KBr discs and ^{13}C n.m.r.

Synthesis of radiolabelled D2S

[^3H]-D2S was prepared by partial alkylation of D2S with [^3H]-methyl iodide in dimethylformamide in the presence of a silver (I) oxide catalyst. A stirred solution of D2S (27.7 mg) and silver (I) oxide (10 mg) in dimethylformamide (2 ml) was treated dropwise with [^3H]-methyl iodide (3.74 mg in 1 ml of toluene; specific activity 80 Ci mmol $^{-1}$, Amersham, Bucks) over 4 h at room temperature. Distilled water (5 ml) was then added with vigorous stirring before the mixture was allowed to separate into aqueous and organic layers. The organic layer was removed. The residual aqueous phase was dialysed against distilled water (8×10 l) followed by gel filtration (Sephadex G-25) to yield D2S in which it was estimated that 1 in 500–1000 glucan units was [^3H]-methylated (specific activity 70–200 mCi mmol $^{-1}$). This degree of methylation did not affect its anti-HIV-1 activity.

Cell lines and primary cells

The human T-cell lines studied were HPB-ALL (Morikawa *et al.*, 1978), CEM (Foley *et al.*, 1965), C8166 (Salahuddin *et al.*, 1983) and H9 (Popovic *et al.*, 1984). The human

epithelial-like cell line HeLa (Scherer *et al.*, 1953) and the HeLa cell line transfected with the gene for human CD4 (Chesebro & Wehrly, 1988) were also studied. HPB-ALL cells were maintained in Iscove's modified Dulbecco's medium and C8166 cells, H9 cells, HeLa cells and HeLa CD4 cells were maintained in RPMI 1640 medium. The culture medium was supplemented with 10% (v/v) foetal calf serum (FCS), 2 mM glutamine, 250 iu ml $^{-1}$ penicillin and 250 μg ml $^{-1}$ streptomycin. Cell lines were obtained from the MRC AIDS Directed Programme.

PBMN cells were isolated from whole blood using Ficoll-Paque (Pharmacia). The cells were suspended at 2×10^6 cells ml $^{-1}$ in lymphocyte growth medium (LGM) which contains RPMI 1640, 2 mM L-glutamine, 15% (v/v) FCS, 250 iu penicillin ml $^{-1}$ and 250 μg streptomycin ml $^{-1}$. In some experiments, cells were cultured with 5 μg ml $^{-1}$ phytohaemagglutinin (PHA; Sigma) for up to 72 h, washed with phosphate buffered saline (PBS)/5% (v/v) FCS, and then resuspended in fresh LGM containing 20 iu recombinant IL-2 ml $^{-1}$ (MRC AIDS Directed Programme). In other experiments, cells were cultured with 5 μg PHA ml $^{-1}$ for up to 96 h, washed with PBS/5% (v/v) FCS and then resuspended in fresh lymphocyte growth medium containing 20 iu recombinant IL-2 ml $^{-1}$ for up to 96 h.

Freshly isolated PBMN cells were also incubated with LGM containing 1.0 μg ml $^{-1}$ of the monoclonal antibody OKT3 (anti-CD3, a T-cell specific activator antibody) (Roosnek *et al.*, 1990) or with LGM containing 20 iu recombinant IL-2 ml $^{-1}$ and one of several cytokines (Schrier *et al.*, 1993) (1 ng ml $^{-1}$ human recombinant IL-1 α , 1 ng ml $^{-1}$ human recombinant IL-1 β , 50 ng ml $^{-1}$ human recombinant IL-4 and 50 ng ml $^{-1}$ human recombinant IL-6; Genzyme Diagnostics, Kent) for up to 72 h prior to being washed with PBS/2% (v/v) FCS and binding studies being performed.

Infectivity assays

The HIV-1 isolates HIV-1.MN, HIV-1.IIIB and HIV-1.RF were obtained through the MRC AIDS Directed Programme and were propagated in H9 cells. Cell free supernatants containing HIV-1 were prepared by lysis of acutely infected H9 cells and centrifugation of the cellular debris at 400 g for 5 min. The titre of virus was determined by endpoint dilution of the viral supernatant on C8166 cells (Lifson *et al.*, 1986; McClure *et al.*, 1991). Productive infection resulted in the formation of syncytia in the C8166 cells and the result was recorded as the tissue culture infectious dose (TCID). Syncytial assays and/or measurement of the major core protein of HIV-1 (p24) were used to determine the anti-HIV-1 activity of D2S and its analogues. Cells were incubated with the test compound for 1 h at 37°C prior to the addition of HIV-1. The cultures were then assessed by light microscopy on a daily basis for 5 days for the formation of syncytia and/or cell free culture supernatants collected for measurement of p24 (EIA, Coulter, Luton, Beds).

The ability of D2S to block infection of PBMN cells by primary viral isolates of HIV-1 was also determined. These isolates were grown from HIV-1 positive patients by culturing their PBMN cells (2×10^6 ml $^{-1}$) with an equal number of PHA activated PBMN cells from seronegative donors (Hollinger *et al.*, 1992). The co-culture was fed every 4 days with fresh LGM containing IL-2 and fresh PHA activated PBMN cells (0.5×10^6 cells ml $^{-1}$). Cell free supernatants were collected 10 days after the start of the co-culture and stored in liquid nitrogen until use. The titre of the primary viral isolate was determined by endpoint dilution of the viral supernatant on PHA stimulated PBMN cells. Productive infection was determined by measurement of p24 in cell free culture supernatants and the result recorded as the TCID.

The anti-HIV-1 activity of the compounds studied was determined using PHA stimulated PBMN cells which, after washing, had been resuspended in fresh LGM containing IL-2 (20 iu ml $^{-1}$). They were then incubated with D2S

(20–100 $\mu\text{g ml}^{-1}$) for 1 h at 37°C. Following this, either HIV-1.MN (10^2 TCID) or one of 5 primary viral isolates of HIV-1 (10^1 – 10^2 TCID) was added to the culture. After 24 h, the cells were washed with PBS/2% (v/v) FCS and cultured in LGM containing IL-2 (20 iu ml^{-1}) for a further 4 days. Cell free culture supernatants were then collected, filtered and p24 measured. The inhibitory concentration (IC_{95}) of each compound was defined as the concentration which reduced the level of p24 in cell free supernatants by 95% as compared to the positive control. The IC_{95} was measured in preference to the IC_{50} because studies with recombinant soluble CD4 have suggested that its inhibitory effect on plasma viraemia correlates with the *in vitro* IC_{95} of the viral isolate and not its IC_{50} (Schacker *et al.*, 1994).

Anticoagulant assays

Thrombin mediated fibrin formation was measured in pooled human plasma. Compounds were diluted in plasma/veronal buffer (2/1, pH 7.35) and incubated at 37°C for 30 min. Fractions of each solution (200 μl) were then mixed with bovine thrombin (7 u) and the time to clot formation recorded. The normal thrombin time was 17 s.

Receptor-ligand binding studies

C8166 cells were used to determine the anti-HIV-1 activity of the compounds synthesized. However, although they are an ideal T-cell line for infection screening assays, they were not used for the binding assays because they grow in clumps which are difficult to separate into individual cells. HPB-ALL cells (which are also very sensitive to infection by HIV-1) were used for the binding studies because they grow predominantly as single cells and the small percentage of cells which grow in clumps can be easily separated. For the purpose for which these cell lines were used, there was no difference between them; the K_d and B_{max} values of the two cell types were similar and D2S blocked infection of both C8166 and HPB-ALL cells at the same concentration (see results).

All binding studies were performed at 4°C. Competition binding studies were performed in triplicate using HPB-ALL cells at a concentration of $2 \times 10^6 \text{ ml}^{-1}$ (Coulter ZM counter) and 0.2 μM [^3H]-D2S. Dextran sulphate (8 kDa), fucoidan, pentosan polysulphate, auran tricarboxylic acid, sodium sulphate and glucose 6 phosphate were purchased from Sigma, UK. The total reaction volume was 250 μl . After 1 h at 4°C, the cells were washed with PBS containing 9 mM calcium chloride and 4.9 mM magnesium chloride and collected on Whatman GF/C filters using a Brandel cell harvester. The [^3H]-D2S bound to cells was measured in a Tricarb 2000A (Packard, UK) liquid scintillation counter.

The saturation binding curves for [^3H]-D2S were established using at least 8 different concentrations and tested in triplicate. Adherent cells were removed in PBS containing 2 mM EDTA/2% (v/v) FCS, counted and resuspended in RPMI 1640 at a concentration of $2 \times 10^6 \text{ cells ml}^{-1}$. Non-specific binding was determined using at least a 100 fold excess of unlabelled D2S. After 1 h at 4°C, the cells were washed, collected and counted as described above.

In several experiments, HPB-ALL cells were preincubated with the antibody Leu 3a (an anti-CD4 monoclonal antibody which blocks the interaction of the HIV-1 envelope glycoprotein gp120 with CD4) for 1 h. Anti-CD 14 and anti-CD 26 monoclonal antibodies were used as control antibodies. In other experiments, HPB-ALL cells were incubated with 0.05% (w/v) trypsin for 15 min at 37°C or 1.25 units neuraminidase ml^{-1} for 30 min at 37°C prior to the binding studies.

Binding curves were modelled by computer using an iterative non-linear least squares regression (Graphpad, Inplot, U.S.A.) from which the dissociation constant (K_d) and the B_{max} were determined. A 1:1 receptor-ligand

stoichiometry and no co-operativity between receptors were assumed. The ED_{50} (concentration of the competing drug which displaced 50% of the [^3H]-D2S bound) was determined from the competition binding curves. The inhibitory constant (K_i) was calculated from $K_i = \text{ED}_{50} / \{1 + (*L/K_d)\}$ where $*L$ is the concentration of [^3H]-D2S in the reaction and K_d is the dissociation constant of [^3H]-D2S.

Statistical analysis

This was performed with Student's unpaired *t* test.

Results

Synthesis of modified dextrans

Since dextrin is a polymer comprising repeat units, chemical modification such as sulphation yields a product which is predominantly (but not exclusively) modified at the same site of each repeating unit. Elemental and spectroscopic analysis provide evidence for, and distinguished between, the various sites of modification. The mol. wt. of D2S was calculated as 27 kDa on the basis of the incorporation of 1 sulphate per glucan unit (see below).

The sulphate content of the dextrin 2-, 3-, and 6-sulphates was determined by elemental analysis as 37%, being equivalent to one sulphate per glucan moiety. In the case of dextrin 2,3,6-trisulphate, the sulphate content was determined as 70%, this being equivalent to complete hydroxyl substitution (three sulphate groups per glucan).

Sulphated dextrans all yielded infrared spectra with a band at about 1220 cm^{-1} characteristic of the presence of sulphate and another band between 820 cm^{-1} and 840 cm^{-1} . Neither of these bands were present in the infrared spectrum of dextrin itself. A peak at 820 cm^{-1} has been reported as typical for primary sulphates of sugars, i.e. for 6-substitution, whereas equatorial secondary sulphate gives a peak at 835 cm^{-1} (Turvey, 1965).

By comparison to authentic reference sugars, the ^{13}C n.m.r. spectrum of dextrin was assigned as follows; the C-1 acetal carbon is to lowest field, 100.3 p.p.m. and the primary C-6 is to the highest field near 61 p.p.m. Between these two are C-4 (77.6 p.p.m.), C-3 (73.9 p.p.m.), C-2 (72.2 p.p.m.) and C-5 (71.8 p.p.m.).

The structure of D2S was confirmed by comparison with dextrin. A major signal for unsubstituted C-6-OH at 61.1 p.p.m. was present and a C-4 signal at 78.1 p.p.m. The major C-1 peak moved upfield to 99.8 p.p.m. (100.3 p.p.m. for dextrin) due to the 2-O-sulphation. The spectrum of dextrin 3-sulphate had a strong signal at 61.1 p.p.m. characteristic of unmodified C-6-OH. Prominent new signals were present at 82.2 and 82.5 p.p.m. and are assigned to C-3 (a shift of 8.3 and 8.6 p.p.m. downfield compared to dextrin). These are close to the chemical shifts for C-3 in glucose 3-sulphate (8.5 and 9.5 p.p.m. downfield compared to glucose). The assignment is supported by the virtual disappearance of the C-4 signal (77.6 p.p.m. in dextrin) since substitution at C-3 would be expected to cause an upfield shift (eg. 2.2 p.p.m. upfield shift in glucose 3-sulphate relative to glucose), taking it under the envelope of other signals. The C-2 and C-5 peaks shifted upfield to 70.2 and 70.8 p.p.m., respectively (from 72.2 or 71.8 p.p.m. in dextrin). This is similar to the 1.1 p.p.m. upfield shift for C-2 in glucose 3-sulphate compared to glucose. The C-1 region showed six closely spaced lines between 100.1 to 98.3 p.p.m. slightly upfield from that in dextrin (100.3 p.p.m.).

In the spectrum of dextrin 6-sulphate the original C-6 peak at 61.1 p.p.m. was greatly diminished and a new signal was present at 67.5 p.p.m., a 6.4 p.p.m. downfield shift from C-6 in dextrin. This is similar to the reported 6.2 p.p.m. downfield shift seen in glucose 6-sulphate compared to glucose. The signal for C-5 was shifted upfield (2.5 p.p.m.) to

69.3 p.p.m. A similar upfield shift of 1.7 p.p.m. has been reported for the 6-O-sulphation of glucose (Bock & Pedersen, 1983).

In the ^{13}C n.m.r. spectrum of dextrin 2-(2-hydroxypropyl-3-trimethylammonium salt), the sharp singlet at 54.8 p.p.m. confirmed the pendent trimethylammonium group. The hydroxypropyl bridge gave signals at 65.6 and 68.5 p.p.m. and the C-1 signal was shifted 3.3 p.p.m. upfield to 97 p.p.m. (from 100.3 p.p.m. in dextrin) confirming C-2 substitution. The spectrum of dextrin 2-(2-hydroxypropyl-3-triethylammonium salt) contained signals at 7.9 and 54.2 p.p.m. demonstrating the presence of the methyl and methylene groups in the triethylammonium moiety. The hydroxypropyl bridge gave signals at 59.2 and 64.8 p.p.m. The unsubstituted C-6 gave a peak at 60.9 p.p.m. (dextrin C-6, 61.1 p.p.m.). The C-1 signal was shifted 3 p.p.m. upfield to 97.3 p.p.m. (100.3 p.p.m. in dextrin) confirming C-2 substitution.

Infectivity and anticoagulant measurement

The anti-HIV-1 activity (as determined in C8166 cells and 3 laboratory adapted strains of HIV-1) and the anticoagulant activity of dextrin sulphate were affected by the number and position of the sulphate groups present. D2S, dextrin 6-sulphate and dextrin 2,3,6-trisulphate were potent anti-HIV-1 compounds with similar IC_{95} values (Table 1). Amongst the compounds tested, D2S had the shortest thrombin time and dextrin 2,3,6-trisulphate the longest (Table 1). The 3-sulphated derivative had poor anti-HIV-1 activity (Table 1) although its thrombin time was similar to that for dextrin 6-sulphate (D6S) (Table 1). Dextrin 2-(2-hydroxypropyl-3-trimethylammonium salt) and dextrin 2-(2-hydroxypropyl-3-triethylammonium salt) had no anti-HIV-1 activity (Table 1). The inhibition of infection by HIV-1 was confirmed by measurement of HIV-1 p24 in cell free culture supernatants. Amongst the sulphated derivatives of dextrin, D2S combined high anti-HIV-1 activity with low anticoagulant activity. Neither D2S nor [^3H]-D2S was toxic to C8166 or to HPB-ALL cells (as determined by cell viability) at a concentration of 7.4 μM , even after several weeks of culture.

Freshly isolated PBMN cells can only be infected by HIV-1 (as determined by p24) after they have been activated by culturing the cells in LGM containing PHA for 24–72 h. Gowda *et al.* (1989) have also shown, using PCR for HIV-1 proviral DNA, that activated but not resting PBMN cells can be infected by HIV-1. The susceptibility of these cells to

infection by HIV-1 following activation is not due to an increased cell surface expression of CD4 (Gowda *et al.*, 1989). Dextrin 2-sulphate was found to block infection of PHA activated PBMN cells by 5 primary viral isolates with an IC_{95} of 230–3700 nM depending upon the primary viral isolate tested as determined by the measurement of p24 in cell free culture supernatants.

Receptor-ligand binding studies

Competition binding studies In competition binding studies using HPB-ALL cells, dextrin 6-sulphate, dextrin 3-sulphate and dextrin 2,3,6-trisulphate were all effective inhibitors of [^3H]-D2S binding (Table 1; Figure 1). The positively charged molecules, dextrin 2-(2-hydroxypropyl-3-trimethylamine) and dextrin 2-(2-hydroxypropyl-3-triethylamine), did not inhibit [^3H]-D2S binding and had no anti-HIV-1 activity. Similarly, dextrin, glucose 6-phosphate and sodium sulphate had no anti-HIV-1 activity and did not displace [^3H]-D2S (Table 1).

Competition binding studies were also performed using several naturally occurring sulphated polysaccharides and polyanionic compounds which have anti-HIV-1 activity. Of those tested, fucoidan was the most effective competitor (Table 1). Dextran sulphate (8 kDa) was less effective than the sulphated derivatives of dextrin. Pentosan polysulphate did not compete at all whilst auran tricarboxylic acid competed very poorly.

Saturation binding studies on T-cell lines [^3H]-D2S bound to HPB-ALL cells in a specific and saturable manner (Figure 2). The dissociation constant (K_d) for HPB-ALL cells was 82 ± 14 nM with a B_{max} of 4.8 ± 0.3 pmol per 10^6 cells. CEM, C8166 and H9 cells had similar K_d values for the binding of [^3H]-D2S (Table 2).

Pre-treatment of HPB-ALL cells with trypsin reduced the saturable component of [^3H]-D2S binding by 91%, in contrast to pretreatment with neuraminidase which did not reduce the binding of [^3H]-D2S as compared to PBS-treated control cells. Exposure of HPB-ALL cells to an antibody against CD4 (Leu 3a) or with the control antibodies (anti-CD14 and anti-CD26) at a concentration of $2 \mu\text{g ml}^{-1}$ for 1 h prior to performing the binding studies made no difference to either the B_{max} or the K_d of [^3H]-D2S binding. Recombinant gp120 also failed to displace [^3H]-D2S in competition binding studies.

The binding of [^3H]-D2S to HeLa cells was also compared

Table 1 The anti-HIV-1 activity, anticoagulant activity and inhibition of [^3H]-dextrin 2-sulphate ([^3H]-D2S) binding

	Thrombin time (s) {n = 3}	anti-HIV-1 activity (IC_{95} , nM) {n = 9}	[^3H]-D2S binding (K_i , nM) {n = 6}
Dextrin 2-sulphate	32 ± 1	230 ± 15	66 ± 10
Dextrin 6-sulphate	56 ± 2	230 ± 15	55 ± 12
Dextrin 3-sulphate	63 ± 2	1850 ± 67	198 ± 6
Dextrin 2,3,6 trisulphate	> 180	300 ± 14	50 ± 3
Dextrin 2-(2-hydroxypropyl-3-trimethylamine)	ND	Not active	No competition
Dextrin 2-(2-hydroxypropyl-3-triethylamine)	ND	Not active	No competition
Dextrin	17 ± 1	Not active	No competition
Glucose 6-phosphate	ND	Not active	No competition
Sodium sulphate	ND	Not active	No competition
Fucoidan	80 ± 2	80 ± 6	190 ± 8
Dextran sulphate (8 kDa)	27 ± 1	780 ± 49	410 ± 14
Pentosan polysulphate	ND	2000 ± 128	No competition
Aurin tricarboxylic acid	ND	3700 ± 196	29000 ± 970

The inhibitory concentration (IC_{95}) is the mean concentration of the compound which blocked infection by HIV-1.MN, HIV-1.IIIb and HIV-1.I.RF of C8166 cells by 95% as measured by syncytia formation and HIV-1 p24. The thrombin time was measured using the concentration of each compound which corresponded to the IC_{95} for its anti-HIV-1 activity. Competition binding studies were performed with HPB-ALL cells. The inhibitory constant (K_i) was determined from these experiments using the concentration of the competing compound which inhibited the binding of [^3H]-D2S by 50%.

Results are shown as the mean $\pm$ s.e.mean. ND = not done.

Figure 1 Inhibition of the binding of $0.2 \mu\text{M}$ [^3H]-D2S to HPB-ALL cells. Competition curves of specific binding of dextrin 2-sulphate (●), fucose (■), aurotricarboxylic acid (○), pentosan polysulphate (▲) and glucose 6-phosphate (□).

with that to HeLa cells transfected with the gene for human CD4. Although the K_d for [^3H]-D2S binding did not change, the B_{max} value for HeLa cells was $4.1 \pm 0.2 \text{ pmol}/10^6$ cells compared to HeLa cells transfected with CD4 for which it was $2.2 \pm 0.1 \text{ pmol}/10^6$ cells; $n = 9$, $P < 0.05$.

Saturation binding studies on PBMN cells There was little binding of [^3H]-D2S to freshly isolated PBMN cells ($B_{\text{max}} = 0.18 \pm 0.03 \text{ pmol}/10^6$ cells; Figure 3). Binding increased 2–3 fold after 4 h of culture in LGM containing IL-2 ($B_{\text{max}} = 0.5 \pm 0.1 \text{ pmol}/10^6$ cells; K_d $134 \pm 59 \text{ nM}$), but no further increase in binding was seen even where the cells were maintained in culture with IL-2 for up to 72 h (Figure 3). In contrast, when PBMN cells were cultured in the presence of PHA, the B_{max} increased over 72 h to a maximum of $7.2 \pm 0.1 \text{ pmol}/10^6$ cells (K_d $198 \pm 18 \text{ nM}$). The binding of [^3H]-D2S to PHA-activated PBMN cells was specific and saturable within the concentration range studied. Culture of PBMN cells in PHA beyond 72 h to 96 h resulted in a small reduction in cell viability (as measured by Trypan blue exclusion) and in the B_{max} to $6.0 \pm 0.4 \text{ pmol}/10^6$ cells. The K_d did not change during the course of these cultures. There was no difference in the binding constants of [^3H]-D2S to PBMN cells from men as compared to women with no variation seen during the menstrual cycle (data not shown).

The binding of [^3H]-D2S to activated PBMN cells fell following removal of PHS, washing with PBS/5% (v/v) FCS and resuspension in LGM containing IL-2 (Figure 3). Twenty-four hours after the change in the culture medium, the B_{max} had fallen to $2.0 \pm 0.1 \text{ pmol}/10^6$ cells and it then remained at this level with further culture. Cell viability was $>90\%$ at all times except where indicated in Figure 3.

In order to determine whether the PHA-dependent increase in the binding of [^3H]-D2S to activated PBMN cells could have been mediated by the adherence of cellular products in the culture medium to the surface of PBMN cells, freshly isolated PBMN cells were incubated for 1 h at 4°C with cell free culture media from 96 h old cultures of HPB-ALL cells, washed with PBS/2% (v/v) FCS and binding studies performed. The B_{max} of binding ($0.18 \pm 0.03 \text{ pmol}/10^6$ cells) did not change as compared to control cells ($0.18 \pm 0.03 \text{ pmol}/10^6$ cells) despite the high B_{max} of [^3H]-D2S binding to the HPB-ALL cells ($4.8 \pm 0.3 \text{ pmol}/10^6$ cells). Addition of cell free supernatants from 96 h old cultures of PBMN cells in LGM containing PHA also failed to increase the binding of [^3H]-D2S to freshly isolated PBMN cells under similar conditions. The same result was obtained with supernatants from PBMN cells cultured without PHA.

Culture of freshly isolated PBMN cells with anti-CD3 for 72 h increased the B_{max} of [^3H]-D2S to $2.0 \pm 0.2 \text{ pmol}/10^6$ cells ($K_d = 138 \pm 26 \text{ nM}$; $n = 6$) and made these cells suscepti-

Figure 2 The binding of [^3H]-D2S to HPB-ALL cells. (a) Total binding (■) and non-specific binding (□), (b) the specific component of binding of [^3H]-D2S. The specific binding (○) is shown as a Scatchard plot in (c), where bound = pmol [^3H]-D2S/ 10^6 cells and free = free ligand (nM).

ble to infection by HIV-1 as measured by p24 and as previously shown by Gowda *et al.* (1989). In contrast, incubating PBMN cells in LGM containing the cytokines IL-1 α , IL-1 β , IL-4 or IL-6 for 72 h did not change the B_{max} of [^3H]-D2S as compared to PBMN cells which had been cultured in LGM containing IL-2 only. These cells could not be infected by HIV-1.

[^3H]-D2S did not bind to fresh human erythrocytes, nor to erythrocytes after 72 h of culture in LGM with PHA. Furthermore, [^3H]-D2S did not bind to erythrocytes even when

Table 2 Binding constants for [³H]-dextrin 2-sulphate to human T-cells

Cell line	Dissociation constant K_d (nM)	B_{max} (pmol/10 ⁶ cells)	
HPB-ALL	82 ± 14	4.8 ± 0.3	<i>n</i> = 7
CEM	163 ± 56	5.3 ± 1.2	<i>n</i> = 3
C8166	190 ± 11	4.2 ± 0.2	<i>n</i> = 3
H9	137 ± 18	2.2 ± 0.1	<i>n</i> = 3

These cell lines are all susceptible to infection by HIV-1. Infection of these cells is blocked by D2S (McClure *et al.*, 1991; 1992; Shaunak, 1993). The saturation binding curves were determined with at least 8 different concentrations and tested in triplicate. The results are given as the mean ± s.e.mean. The mean K_d of these T-cell lines is 143 ± 23 nM with a mean B_{max} of 4.1 ± 0.7 pmol/10⁶ cells.

Figure 3 The binding of [³H]-D2S to PBMN cells. Following isolation from whole blood, PBMN cells were cultured in either LGM with PHA (5 µg ml⁻¹) (●) or in LGM with IL-2 (20 iu ml⁻¹) (○) for up to 96 h. In some experiments, PBMN cells were first cultured in LGM with PHA for 48 h (▽) or 72 h (◇), washed with PBS/2% (v/v) FCS and then resuspended in LGM with IL-2 (20 iu ml⁻¹) and cultured. Cell viability was 90–99% at all times except as shown; * (77%) and ** (80%). All points are shown as the mean ± s.e.mean (*n* = 12).

their isolation from PBMN cells was performed after whole blood had first been cultured in LGM containing PHA for 48 h.

Discussion

In this study, the relationship between the structure of sulphated dextrans and their ability to block infection of T-cells by HIV-1 was examined. The position of the sulphate group on the glucan moiety was found to be important for their anti-HIV-1 activity. Sulphation at the 2-position or 6-position of dextran resulted in considerably better anti-HIV-1 activity compared to sulphation at the 3-position. Increasing the number of sulphate groups per glucan molecule (dextran 2,3,6-trisulphate) resulted in a considerable increase in the anticoagulant activity of the molecule. Neither dextran nor 2-substituted quaternary ammonium dextran had any anti-HIV-1 activity. Thus the cell surface binding of these compounds and their anti-HIV-1 activity is dependent upon the spatial arrangement of the negatively charged sulphate groups.

Competition binding experiments were used to compare [³H]-D2S with other sulphated polysaccharides and polyanionic compounds which have anti-HIV-1 activity. Fucoidan was a good competitor of [³H]-D2S in contrast to pentosan polysulphate which did not compete at all.

Fucoidan is thought to share a similar mechanism of action to D2S for its anti-HIV-1 activity (McClure *et al.*, 1992) in contrast to pentosan polysulphate which binds to CD4 (Parish *et al.*, 1990). Aurin tricarboxylic acid, which also blocks HIV-1 infection by binding to CD4 (Schols *et al.*, 1989; Szabo *et al.*, 1992), was a very poor competitor of [³H]-D2S.

The results of saturation and competition binding studies suggest that [³H]-D2S is binding to a cell surface molecule with a K_d in the range of 82–198 nM and a B_{max} of 0.18–7.2 pmol/10⁶ cells. The B_{max} was affected by the state of activation of the cells. The reduction in the binding of [³H]-D2S to the cell surface following treatment with trypsin indicates that the cell surface molecule is a protein. Furthermore, competition experiments with the anti-CD4 monoclonal antibody, Leu 3a, and with gp120, together with the binding observed to HeLa and HeLa CD4 cells suggests that this binding is independent of CD4.

D2S blocked infection of several human T-cell lines by a variety of cell free, laboratory adapted isolates of HIV-1 at an IC₉₅ of 230 nM as judged by the inhibition of syncytia formation in C8166 cells and HIV-1 p24 levels. We have also shown that HIV-1 proviral DNA cannot be detected by PCR in cells incubated with D2S and then exposed to HIV-1 (Bieniasz *et al.*, 1991; Shaunak, 1993), providing evidence that D2S prevents HIV-1 infection by blocking viral entry into cells. In view of the recent observation that the concentration of recombinant soluble CD4 which blocks infection of PBMN cells by primary viral isolates of HIV-1 is 200–2700 times greater than that required to block infection of T-cell lines by laboratory adapted isolates of HIV-1 (Daar *et al.*, 1990), the activity of D2S against primary viral isolates of HIV-1 was also tested. D2S blocked infection of PHA activated PBMN cells by several different primary viral isolates at concentrations that were up to 16 times higher than those required to block laboratory adapted isolates. D2S therefore has potential as a clinically useful compound.

All the T-cell lines examined bound [³H]-D2S and the addition of PHA or IL-2 to the culture media did not alter the B_{max} of binding (data not shown). In contrast, the binding of [³H]-D2S to freshly isolated PBMN cells increased when the cells were cultured in LGM with PHA to a B_{max} that was almost double that for the T-cell lines that we studied (Table 2). When the cells were then transferred to LGM containing IL-2, the B_{max} fell to 2.0 ± 0.1 pmol/10⁶ cells and it remained at this level with further culture. Several experiments were performed to ensure that the binding of [³H]-D2S to the cell surface was not an artifact of *in vitro* culture, for example, with intracellular material from disrupted PBMN cells being adsorbed onto the surface of intact PBMN cells. Freshly isolated PBMN cells did not bind [³H]-D2S following addition of conditioned media either from 96 h old cultures of HPB-ALL cells or from 96 h old cultures of PBMN cells. Furthermore, erythrocytes did not bind [³H]-D2S under any of the experimental conditions tested.

PBMN cells which have been activated by culturing them either in LGM containing PHA or with an anti-CD3 antibody were susceptible to infection by HIV-1. Infectivity correlated with an increased expression of the binding [³H]-D2S. In contrast, culture of fresh PBMN cells with LGM and each of the cytokines interleukin-1α (IL-1α), IL-1β, IL-2, IL-4 and IL-6 for up to 72 h failed to increase the expression of the [³H]-D2S binding protein significantly or to make the cells susceptible to infection by HIV-1. These results suggest a relationship between the expression of the [³H]-D2S binding protein and the susceptibility of PBMN cells to infection by HIV-1.

In conclusion, we have synthesized several structural analogues of sulphated dextran. Of these, D2S combines high anti-HIV-1 activity against both laboratory-adapted and primary viral isolates of HIV-1 with low anticoagulant activity. Our experiments with trypsin suggest that it binds to a cell surface protein which is expressed on T-cell lines. D2S

blocks infection of these cells by both laboratory-adapted isolates of HIV-1 and primary viral isolates of HIV-1. Unstimulated PBMN cells, which cannot be infected by HIV-1, bound little [3 H]-D2S. However, after activation by PHA or anti-CD3 antibody, these cells could be infected by HIV-1 and they bound [3 H]-D2S. Infection of these cells was blocked by D2S. These results suggest that there is a relationship between the expression of the [3 H]-D2S binding protein

on the plasma membrane of PBMN cells and their susceptibility to infection by HIV-1.

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References

- BABA, M., DE CLERCQ, E., SCHOLS, D., PAUWELS, R., SNOECK, R., VAN BOECKEL, C., VAN DEDEM, G., KRAAIJEVELD, N., HOBELLEN, P., OTTENHEIJM, H. (1990). Novel sulfated polysaccharides: dissociation of anti-human immunodeficiency virus activity from antithrombin activity. *J. Infect. Dis.*, **161**, 208–213.
- BABA, M., NAKAJIMA, M., SCHOLS, D., PAUWELS, R., BALZARINI, J. & DE CLERCQ, E. (1988a). Pentosan polysulfate, a sulfated oligosaccharide, is a potent and selective anti-HIV agent *in vitro*. *Antiviral Res.*, **9**, 335–343.
- BABA, M., PAUWELS, R., BALZARINI, J., ARNOUT, J., DESMYTER, J. & DE CLERCQ, E. (1988b). Mechanism of inhibitory effect of dextran sulfate and heparin on replication of human immunodeficiency virus *in vitro*. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 6132–6136.
- BAGASRA, O. & LISCHNER, H.W. (1988). Activity of dextran sulfate and other polyanionic polysaccharides against human immunodeficiency virus. *J. Infect. Dis.*, **158**, 1084–1087.
- BEDDOWS, S., BIENIASZ, P., SHAUNAK, S. & WEBER, J.N. (1993). HIV replication in CD4-negative cell lines: effect of cloning, CD4 expression and inhibition by dextrin sulphate. *Antiviral Chem. Chemother.*, **4**, 173–177.
- BIENIASZ, P.D., BEDDOWS, S.A. & WEBER, J.N. (1991). Quantitative assessment of CD4 independent HIV-1 infection *in vitro*. *MRC AIDS Directed Programme Workshop*. University of Warwick. Abstract Number 28.
- BOCK, K. & PEDERSEN, C. (1983). C-13 nuclear magnetic-resonance spectroscopy of monosaccharides. *Adv. Carbohydr. Chem. Biochem.*, **41**, 27–66.
- BRASSEUR, R., CORNET, B., BURNY, A., VANDENBRANDEN, M. & RUYSSCHAERT, J.M. (1988). Mole of insertion into a lipid membrane of the N-terminal HIV gp41 peptide segment. *AIDS Res. Hum. Retroviruses*, **4**, 83–90.
- BRENAN, M. & PARISH, C.R. (1986). Modification of lymphocyte migration by sulfated polysaccharides. *Eur. J. Immunol.*, **16**, 423–430.
- CHESEBRO, B. & WEHRLY, K. (1988). Development of a sensitive quantitative focal assay for human immunodeficiency virus infectivity. *J. Virol.*, **62**, 3779–3788.
- COLE, G.J., LOEWY, A. & GLASER, L. (1986). Neuronal cell-cell adhesion depends on interactions of N-CAM with heparin-like molecules. *Nature*, **320**, 445–447.
- COOMBE, D.R., JAKOBSEN, K.B. & PARISH, C.R. (1987). A role for sulfated polysaccharide recognition in sponge cell aggregation. *Exp. Cell Res.*, **170**, 381–401.
- COOMBE, D.R. & RIDER, C.C. (1989). Lymphocyte homing receptors cloned – a role for anionic polysaccharides in lymphocyte adhesion. *Immunol. Today*, **10**, 289–291.
- DAAR, E.S., LI, X.L., MOUDGIL, T. & HO, D.D. (1990). High concentrations of recombinant soluble CD4 are required to neutralize primary human immunodeficiency virus type 1 isolates. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 6574–6578.
- DALGLEISH, A.G., BEVERLEY, P.C., CLAPHAM, P.R., CRAWFORD, D.H., GREAVES, M.F. & WEISS, R.A. (1984). The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature*, **312**, 763–767.
- DIMITROV, D.S., HILLMAN, K., MANISCHEWITZ, J., BLUMENTHAL, R. & GOLDING, H. (1992). Correlation between kinetics of soluble CD4 interactions with HIV-1-Env-expressing cells and inhibition of syncytia formation: implications for mechanisms of cell fusion and therapy for AIDS. *AIDS*, **6**, 249–256.
- FOLEY, G.E., LAZARUS, H. & FARBER, S. (1965). Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukaemia. *Cancer*, **18**, 522–529.
- GOWDA, S.D., STEIN, B.S., MOHAGHEGHPOUR, N., BENIKE, C.J. & ENGLEMAN, E.G. (1989). Evidence that T-cell activation is required for HIV-1 entry in CD4+ lymphocytes. *J. Immunol.*, **142**, 773–780.
- HART, T.K., KIRSH, R., ELLENS, H., SWEET, R.W., LAMBERT, D.M., PETTEWAY, S.R. Jr., LEARY, J. & BUGELSKI, P.J. (1991). Binding of soluble CD4 proteins to human immunodeficiency virus type 1 and infected cells induces release of envelope glycoprotein gp120. *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 2189–2193.
- HOLLINGER, F.B., BREMER, J.W., MYERS, L.E., GOLD, J.W. & MCQUAY, L. (1992). Standardization of sensitive human immunodeficiency virus coculture procedures and establishment of a multicenter quality assurance program for the AIDS Clinical Trials Group. The NIH/NIAID/DAIDS/ACTG Virology Laboratories. *J. Clin. Microbiol.*, **30**, 1787–1794.
- ITO, M., BABA, M., SATO, A., PAUWELS, R., DE CLERCQ, E. & SHIGETA, S. (1987). Inhibitory effect of dextran sulfate and heparin on the replication of human immunodeficiency virus (HIV) *in vitro*. *Antiviral Res.*, **7**, 361–367.
- KLASSE, P.J. & MOORE, J.P. (1992). Kinetics of the HIV-CD4 interactions and virus-cell fusion. *AIDS*, **6**, 325–327.
- KLATZMANN, D., CHAMPAGNE, E., CHAMARET, S., GRUEST, J., GUETARD, D., HERCEND, T., GLUCKMAN, J.C. & MONTAGNIER, L. (1984). T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. *Nature*, **312**, 767–768.
- LIFSON, J.D., FEINBERG, M.B., REYES, G.R., RABIN, L., BANAPOUR, B., CHAKRABARTI, S., MOSS, B., WONG-STAAAL, F., STEIMER, K.S. & ENGLEMAN, E.G. (1986). Induction of CD4-dependent cell fusion by the HTLV-III/LAV envelope glycoprotein. *Nature*, **323**, 725–728.
- MADDON, P.J., DALGLEISH, A.G., MCDUGAL, J.S., CLAPHAM, P.R., WEISS, R.A. & AXEL, R. (1986). The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell*, **47**, 333–348.
- MCCLURE, M.O., MOORE, J.P., BLANC, D.F., SCOTTING, P., COOK, G.M., KEYNES, R.J., WEBER, J.N., DAVIES, D. & WEISS, R.A. (1992). Investigations into the mechanism by which sulfated polysaccharides inhibit HIV infection *in vitro*. *AIDS Res. Hum. Retroviruses*, **8**, 19–26.
- MCCLURE, M.O., WHITBY, D., PATIENCE, C., GOODERHAM, N.J., BRADSHAW, A., CHEINGSONG-POPOV, R., WEBER, J.N., DAVIES, D.S., COOK, G.M.W., KEYNES, R.J. & WEISS, R.A. (1991). Dextrin sulphate and fucoidan are potent inhibitors of HIV infection *in vitro*. *Antiviral Chem. Chemother.*, **2**, 149–156.
- MITSUYA, H., LOONEY, D.J., KUNO, S., UENO, R., WONG-STAAAL, F. & BRODER, S. (1988). Dextran sulfate suppression of viruses in the HIV family: inhibition of virion binding to CD4+ cells. *Science*, **240**, 646–649.
- MORIKAWA, S., TATSUMI, E., BABA, M., HARADA, T. & YASUHIRA, K. (1978). Two E-rosette-forming lymphoid cell lines. *Int. J. Cancer*, **21**, 166–170.
- PARISH, C.R., LOW, L., WARREN, H.S. & CUNNINGHAM, A.L. (1990). A polyanion binding site on the CD4 molecule. Proximity to the HIV-gp120 binding region. *J. Immunol.*, **145**, 1188–1195.
- POPOVIC, M., SARNGADHARAN, M.G., READ, E. & GALLO, R.C. (1984). Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science*, **224**, 497–500.
- ROOSNEK, E., TUNNACLIFFE, A. & LANZAVECCHIA, A. (1990). T-cell activation by a bispecific anti-CD3/anti-major histocompatibility complex class I antibody. *Eur. J. Immunol.*, **20**, 1393–1396.
- ROSSIGNOL, D.P., EARLES, B.J., DECKER, G.L. & LENNARZ, W.J. (1984). Characterization of the sperm receptor on the surface of eggs of *Strongylocentrotus purpuratus*. *Dev. Biol.*, **104**, 308–321.
- SALAHUDDIN, S.Z., MARKHAM, P.D., WONG-STAAAL, F., FRANCHINI, G., KALYANARAMAN, V.S. & GALLO, R.C. (1983). Restricted expression of human T-cell leukemia-lymphoma virus (HTLV) in transformed human umbilical cord blood lymphocytes. *Virology*, **129**, 51–64.

- SCHACKER, T., COOMBS, R.W., COLLIER, A.C., ZEH, J.E., FOX, I., ALAM, J., NELSON, K., EGGERT, E. & COREY, L. (1994). The effects of high-dose recombinant soluble CD4 on HIV-1 viraemia. *J. Infect. Dis.*, **169**, 37–40.
- SCHERER, W.F., SYVERTON, J.T. & GEY, G.O. (1953). Studies on propagation *in vitro* of poliomyelitis viruses. *J. Exp. Med.*, **97**, 695–710.
- SCHOLS, D., BABA, M., PAUWELS, R., DESMYTER, J. & DE CLERCQ, E. (1989). Specific interaction of aurin tricarboxylic acid with the human immunodeficiency virus/CD4 cell receptor. *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 3322–3326.
- SCHRIER, R.D., MCCUTCHAN, J.A. & WILEY, C.A. (1993). Mechanisms of immune activation of human immunodeficiency virus in monocytes/macrophages. *J. Virol.*, **67**, 5713–5720.
- SHAUNAK, S. (1993). Cell surface receptors for HIV-1. *PhD thesis. University of London.*
- STOOLMAN, L.M. & ROSEN, S.D. (1983). Possible role for cell-surface carbohydrate-binding molecules in lymphocyte recirculation. *J. Cell Biol.*, **96**, 722–729.
- SZABO, G. Jr., PINE, P.S., WEAVER, J.L., RAO, P.E. & ASZALOS, A. (1992). CD4 changes conformation upon ligand binding. *J. Immunol.*, **149**, 3596–3604.
- TURVEY, J.R. (1965). Sulfates of the simple sugars. *Adv. Carbohydr. Chem. Biochem.*, **20**, 183–212.
- UENO, R. & KUNO, S. (1987). Dextran sulphate, a potent anti-HIV agent *in vitro* having synergism with zidovudine. *Lancet*, **i**, 1379.
- YAMAGUCHI, M. & KINOSHITA, S. (1985). Polysaccharides sulfated at the time of gastrulation in embryos of the sea urchin *Clypeaster japonicus*. *Exp. Cell Res.*, **159**, 353–365.

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