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Susceptibility to highly sulphated glycosaminoglycans of human immunodeficiency virus type 1 replication in peripheral blood lymphocytes and monocyte-derived macrophages cell cultures

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Abstract

In the search for new drugs against human immunodeficiency virus type 1 (HIV-1), the replication of III_B and BaL strains, and of seven primary isolates from AIDS patients, cultured both in peripheral blood lymphocytes (PBLs) and in monocyte-derived macrophages (MACs), was investigated in the presence of two dermatan sulphate and heparin at 10 μg/ml. The three polysaccharides effectively inhibited the replication of III_B in PBLs and of BaL in MACs, while producing either a slight inhibition or an unexpected large increase in the replication of the seven primary isolates, especially in MAC cultures. In one case, stimulation was found in PBLs and, at lower doses, also with BaL in MACs. Co-receptor use, adaptation to C8166 T cell line, partial sequence of the gp120 V3 loop, variation in positive charge distribution and number of potential glycosylation sites along the V3 loop were assessed for each strain. No explanation could be found for the different susceptibility of the viruses to the polysaccharides. Their presence probably brings about both inhibitory and stimulatory effects, the final outcome depending on the virus, cells and polysaccharide.

Keywords: Oversulphated glycosaminoglycans; HIV; AIDS

1. Introduction

Polyanionic substances have been reported as potential antiviral drugs, since they inhibit the replication of a variety of viruses in vitro (Baba et al., 1988a). In particular, a number of sulphated polysaccharides, including heparin and dextran sulphate, have been shown to be potent inhibitors of human immunodeficiency virus type 1 (HIV-1) replication (Baba et al., 1988b; Nakashima et al., 1989). They do so, most probably, by hindering the binding between viral gp120 and cellular CD4, one of the first steps of the cell infection process (De Clercq, 1993).

We have previously shown that oversulphated glycosaminoglycan sulphates (GAGS), such as oversulphated chondroitin sulphate and oversulphated dermatan sulphate (DS), display antiviral activity in vitro against HIV-1, herpes simplex virus-type 1, and human cytomegalovirus, akin to what has been found with heparin, but without the anti-coagulant side effects, which are typical of heparin-like compounds and which can limit their therapeutic potential (Di Caro et al., 1999).

The extent of inhibition by GAGS appeared to be mainly dependent on their molecular mass and number of charged groups. It proved also dependent on both viral strain and on host cell type. In fact, the most active anti-HIV-1 III_B agent, an oversulphated DS of MW 23.2 kDa and sulphation index (SI: average number of sulphated groups per disaccharide unit) of 2.86, displayed an EC_{50} of 0.04 μg/ml, when the virus was grown in C8166 T cell line, but inhibition was 20-fold weaker when assessed in peripheral blood mononuclear cells (PBMCs). Similarly, inhibition of one primary isolate (Is-19) was also weak in PBMCs with an EC_{50} that

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was 40-fold greater than for the of HIV-1 III_B strain in C8166 cells.

These results prompted us to perform a more extended study on the susceptibility of HIV-1 replication to GAGS. For this purpose we chose as GAGS two chemically oversulphated DS (DS1: MW = 31.8 kDa, SI = 2.27; DS2: MW = 30.1 kDa, SI = 2.81) and heparin (HP: MW = 11.0 kDa, SI = 2.20). The two DS were expected to be both highly active inhibitors, having high MW and being highly charged, the latter more than the former (Di Caro et al., 1999). Heparin was chosen because it is chemically analogous to the other two compounds and it is the GAGS most commonly used as reference standard. HIV-1 lymphotropic III_B strain (III_B), macrophage-tropic BaL strain (BaL) and seven HIV-1 primary isolates (Is-4, Is-9, Is-10, Is-13, Is-17, Is-18, Is-19) obtained from AIDS patients, were used in the study. The viruses were grown in peripheral blood lymphocytes (PBLs) and monocyte-derived macrophages (MACs), two natural host cells for HIV-1.

2. Materials and methods

2.1. Chemistry

DS1 and DS2 were obtained by oversulphation of natural DS from porcine mucosa. A suspension of DS in *N*,*N*-dimethylformamide was treated for 5 h, with triethylamine–sulphurtrioxide in a 3:1 (DS1) or 7:1 (DS2) excess by weight (Whistler and Spencer, 1964). The work-up and the characterisation (MW, SI, absence of contamination by heparin, absence of anti-coagulant effects) of the two products were performed as previously described (Di Caro et al., 1999). HP sodium salt was from bovine intestinal mucosa.

2.2. Virology

2.2.1. Evaluation of cell toxicity

The absence of toxicity by GAGS, at concentrations up to 100 μg/ml, for PBL and MAC cultures was demonstrated as previously described (Di Caro et al., 1999).

2.2.2. Virus

Primary HIV-1 isolates were obtained from cultures of PBMCs from AIDS patients and expanded in activated PBMCs from healthy donors. All viral isolates were cultured by two passages in PBMCs at the most, and we have assumed that, under these conditions, these samples can still be considered representative of primary isolates.

The stock of lymphotropic HIV-1 III_B strain was obtained from the chronically infected cell line H9/III_B (Popovic et al., 1984), and the stock of macrophage-tropic HIV-1 BaL strain (Gartner et al., 1986) was obtained by collecting the supernatant of acutely infected macrophage cultures.

2.2.3. Phenotypic characterisation of viral isolates and T cell line tropism

The co-receptor use by viral isolates was assessed on U87·CD4·CCR5 and U87·CD4·CXCR4 cell lines. These cells are glioma cell lines stably expressing human CD4 and either CCR5 or CXCR4 chemokine receptors (Björndal et al., 1997). The test was performed infecting cells with a virus input equivalent to 20 ng of HIV-1-p24 and monitoring the appearance of syncitia.

T cell line tropism was assessed on C8166 cells as described below.

2.2.4. Evaluation of the effect of GAGS on viral replication

PBLs were isolated from healthy donors by subjecting non-coagulated blood samples to magnetic immune purging with M-450 CD14 Dynabeads (Dynal, Oslo, Norway) to deplete the blood of CD14+ monocytes. The immune purging procedure was carried out at a cell:bead ratio of 1:40, following the Manufacturer's instructions. The monocyte-depleted lymphocytes were purified in Ficoll–Hypaque gradient and activated by cultivating in RPMI 1640, 10% fetal calf serum (FCS) containing 2 μg/ml PHA, for 48 h, at 37 °C, 5% CO_2. The cell cultures were subsequently maintained by adding 100 U/ml of recombinant IL-2 (Sigma Chemical Co., St. Louis, MO) to the culture medium.

MACs were obtained by seeding PBMCs (2×10^6 cells/ml/well 2 cm) from healthy donors in plastic wells. Cells were maintained in RPMI 1640, 20% FCS. After 1 week, non-adherent cells were removed by extensive washing.

The infection of the different cell cultures was carried out by exposing the cells to the equivalent of 20 ng of p24 of each viral strain. After 2 h of incubation at 37 °C, in 5% CO_2, the cells were thoroughly washed and re-suspended in fresh medium.

The infected cultures were observed over a period of time for the appearance of syncytia and single cell lysis. HIV-1 production was evaluated by measuring viral p24 (Alliance HIV-1 p24 Elisa kit, NEN Life Science Products) in the culture medium 6 days post-infection (p.i.) for PBLs and C8166, and 10 days p.i. for MACs.

The activity of GAGS on HIV-1 replication was evaluated in PBLs (1×10^6 cells/ml/well) suspended in RPMI with 10% FCS. After 30 min of incubation at 37 °C, in 5% CO_2, in the presence of the compounds, the different HIV strains were added and the cultures were re-incubated. After 2 h, the medium was removed and the cells were repeatedly washed and re-suspended in fresh medium containing GAGS.

The activity of GAGS on HIV-1 replication was analogously assessed in MACs by infecting the cells with either BaL or with one of the primary isolates in the presence of the compounds.

2.2.5. Sequencing of V3 loop

HIV-1 RNA extracted from the supernatant of infected cultures was subjected to PCR amplification of the HIV-1 *env* gene (Donaldson et al., 1994). The product of amplifi-

cation was sequenced by the Big dye terminator labelling method (Perkin-Elmer) as previously described (Muscillo et al., 1999). Briefly, 30–90 ng of purified DNA, and 3.2 pmol of the sequencing primer were mixed in a reaction tube with the necessary amount of premixed Big Dye reagents. The mixture was transferred to a Centrisep column (Princeton Separations) and the purified labelled DNA recovered after a centrifugation step of 2 min at 750 × *g*. Five microliters of this solution were denatured with 15 μl of template suppressor reagents (TSR™, Perkin-Elmer). The sequencing was performed by capillary electrophoresis using the ABI-PRISM310 sequencing equipment (Perkin-Elmer).

For data analysis, forward and reverse electropherograms were further compared by the Navigator Sequence software (Perkin-Elmer) and ambiguous basis or compressed peaks were manually adjusted. The consensus sequences were exported in GCG (Genetics Computer Group, version 10, University of Wisconsin) format to a Sun Ultra Spark 10. The alignment was done with the PILEUP program of the GCG package and manually adjusted with LINEUP. Comparison was limited to the amplified sequence, corresponding to amino acids (a.a.) 267–395 of the gp120 region (HXB2).

The distribution of the charge on the side-chains and the number of potential glycosylation sites were determined by the PEP-Polypeptide Analysis System, version 5.4 of the Intelligenetics program.

3. Results

The viruses were characterised by analysing their replication efficiency in macrophages and PBLs, adaptation to a T cell line, such as the C8166, and their co-receptor use. Six of the seven primary isolates were able to replicate both in PBLs and in MACs, although they grew less well in the latter cells. Virus 19 was the exception, growing only in PBLs. The growth ability of the primary isolates in PBLs was in all cases higher than that of III_B, while in MACs it was lower than that of BaL (Table 1).

The growth of the two HIV-1 prototypic strains lymphotropic III_B and macrophage-tropic BaL was as expected in accordance with their co-receptor use (Table 1).

The adaptation of the primary isolates to the C8166 T cell line was in the following increasing order: Is-9, Is-17, Is-18, Is-4, Is-13, Is-10, Is-19. In fact, viruses 10, 13, and 19 displayed the best adaptation to the T cell line, 19 to the same extent as III_B, while viruses 9, 17 and 18 replicated poorly in these cells.

All isolates effectively replicated in U87·CD4·CXCR4, while only isolate 4 replicated also in U87·CD4·CCR5. Their co-receptor use was then assessed on the basis of these results (Table 1). Moreover, it has to be considered that all the primary isolates were obtained by culturing PBMCs of patients in an advanced stage of the disease (<200 CD4+ T cells/ml), a phase in which those viruses are more likely to be isolated that use CXCR4 as a co-receptor, either in addition to, or instead of, CCR5. Nevertheless, for example, the good replication efficiency in macrophages and the lack of adaptation to C8166 T cells would suggest that virus 9 was either a R5 or a dual tropic strain.

In accordance to our findings, it was previously described that many X4 tropic primary viruses were unable to grow efficiently in CXCR4-expressing CD4+ T cell lines, though they were able to grow in primary macrophages which did indeed express a low level of CXCR4 (Simmons et al., 1998; Verani et al., 1998; Yi et al., 1998, 1999).

The two oversulphated DS and heparin proved non-toxic up to 100 μg/ml for the cell cultures used in this study. It was thus possible to analyse their effect on virus growth at a

Table 1
Comparison of the replication rate of the different HIV-1 strains in PBLs, MACs and the C8166 T cell line, and characterisation of co-receptor use on U87·CD4 cells

Virus	Replication rate[a]			Co-receptor use[b]
	PBL	MAC	C8166	U87·CD4
III_B	16 (3)	3 (0.3)	150 (27)	X4
BaL	6 (1)	139 (13)	n.d.	R5
Is-4	256 (33) (16.1)[c]	44 (6) (0.31)[d]	17 (2) (0.11)[e]	X4, R5
Is-9	163 (17) (10.1)[c]	90 (12) (0.65)[d]	≪1	X4
Is-10	171 (25) (10.7)[c]	20 (4) (0.14)[d]	48 (8) (0.32)[e]	X4
Is-13	108 (17) (6.7)[c]	23 (6) (0.17)[d]	36 (6) (0.24)[e]	X4
Is-17	128 (27) (8.0)[c]	27 (8) (0.20)[d]	≪1	X4
Is-18	186 (16) (11.7)[c]	17 (13) (0.12)[d]	<1 (0.1) (0.006)[e]	X4
Is-19	70 (12) (4.4)[c]	≪1	149 (16) (0.99)[e]	X4

Average of triplicate experiments for PBLs and MACs, with standard deviation in parentheses. Average of duplicate experiments for C8166, with deviation in parentheses. N.d.: not determined.

[a] Expressed as supernatant HIV-1 p24 antigen (ng/ml).

[b] Phenotype based on co-receptor use evaluated by culturing in U87·CD4 cells expressing either CXCR4 (X4) or CCR5 (R5) receptor.

[c] p24 produced by each isolate/p24 produced by III_B in PBLs.

[d] p24 produced by each isolate/p24 produced by BaL in MACs.

[e] p24 produced by each isolate/p24 produced by III_B in C8166 cells.

Table 2
Effects of GAGS (10 μg/ml) on the replication rate of HIV-1 isolates, expressed as HIV-1 p24 antigen (ng/ml), in various cell types

Virus	C+	DS1	DS2	HP
PBL				
III_B	16 (3)	7 (0.8)	<1	3 (0.6)
Is-4	256 (33)	170 (25)	155 (19)	208 (29)
Is-9	163 (17)	189 (16)	134 (27)	160 (8)
Is-10	171 (25)	126 (17)	111 (23)	162 (20)
Is-13	108 (17)	96 (11)	110 (23)	78 (12)
Is-17	128 (27)	137 (11)	110 (10)	68 (10)
Is-18	186 (16)	169 (42)	169 (12)	163 (18)
Is-19	70 (12)	429 (41)	317 (38)	49 (4)
MAC				
BaL	139 (13)	10 (0.4)	<1	<1
Is-4	44 (6)	70 (5)	106 (37)	98 (18)
Is-9	90 (12)	102 (8)	53 (18)	107 (23)
Is-10	20 (4)	40 (4)	76 (16)	48 (14)
Is-13	24 (6)	33 (3)	79 (21)	54 (7)
Is-17	27 (8)	39 (3)	80 (18)	61 (8)
Is-18	17 (13)	65 (9)	51 (6)	83 (17)

C+, untreated cultures, same as in Table 1. Averages of triplicate experiments, with standard deviations in parentheses.

concentration of 10 μg/ml. The viral replication was evaluated as p24 antigen production in MACs and PBL cultures, as reported in Table 2.

All three GAGS, at 10 μg/ml, were effective inhibitors of III_B replication in PBLs, DS2 over three times more effectively than HP and this over two times more than DS1. All three GAGS, at the same concentration, were also potent inhibitors of BaL in MAC, DS2 and HP over 10 times more than DS1 (Table 2).

The three GAGS, at 10 μg/ml, produced different effects on the replication of six of the seven primary isolates, displaying, in general, a lower or no inhibition of virus growth in PBLs. In detail, as reported in Table 2, taking into consideration the deviation of the data, the replication of viruses 9, 13, and 18 was not significantly influenced by the presence of any of the three GAGS. The replication of virus 4 was inhibited by all three GAGS in the range 20–40%; the replication of virus 10 was inhibited about 30% by DS1 and DS2, and not significantly influenced by the presence of HP; the replication of virus 17 was not significantly influenced by DS1 and DS2, and it was about 50% inhibited by HP. Virus 19 replication only was highly stimulated by both DS1 and DS2, while it was ca. 30% inhibited by HP.

The primary isolates were similarly tested in MACs, with the exclusion of virus 19, because of its too low replication rate in these cells (Table 1). In almost all cases there was a marked stimulation of viral replication with the exception of virus 9, whose replication was not significantly influenced by DS1 and HP, while it was ca. 40% inhibited by DS2 (Table 2).

As a further investigation, prompted by the discovery of these unexpected stimulatory effects, experiments aimed at verifying the influence of the dose of GAGS were then performed in MACs, the cell culture in which such effects appeared more frequently.

The replication of BaL and of viruses 9 and 18 in MACs was studied in the presence of DS2 and HP at 0.1, 1, and 10 μg/ml. BaL was chosen as reference virus. Isolates 9 and 18 were chosen for having displayed both a very different growth rate in MAC, Is-9–Is-18 (Table 1), and a different susceptibility to GAGS (Table 2). The choice of the GAGS was limited to DS2 and HP since DS1 and DS2 had shown similar properties, in general, more pronounced in DS2.

The results are reported in Table 3 and show a trend in accordance with the results reported in Table 2, also in consideration of the varying data and of the variability connected with the use of different batches of MACs. In particular, the replication of BaL was inhibited in a dose-dependent way by DS2, while HP strongly inhibited the replication of BaL only at 10 μg/ml, but exerted a stimulatory effect at the lower concentrations. The replication of virus 9 was not significantly influenced by the presence of DS2, as is already apparent in Table 2, while the same virus was stimulated, although to a little extent, by the presence of HP (Table 3). The replication of virus 18, instead, was highly stimulated by the presence of both DS2 and HP at all tested concentrations (Table 3), much more than in the experiments reported in Table 2.

In order to investigate the molecular basis of this peculiar behavior, we sequenced a segment of the *env* gene of each virus, encompassing the gp120 V3 loop. Some variations in the amino acid sequence were found with respect to III_B and BaL (Fig. 1a), but none appeared relevant for

Table 3
Dose effects, expressed as HIV-1 p24 (ng/ml), for DS2 and HP on the replication of HIV-1 BaL and clinical isolates 9 and 18 grown in MACs, 10 days post-infection

Virus	C+	DS2 (μg/ml)			HP (μg/ml)		
		10	1	0.1	10	1	0.1
BaL	148 (13)	<1	68 (11)	110 (11)	<1	232 (54)	233 (62)
Is-9	91 (11)	76 (10)	100 (18)	64 (19)	153 (27)	139 (24)	126 (28)
Is-18	15 (7)	263 (69)	250 (78)	146 (68)	257 (78)	602 (56)	266 (56)

C+, untreated cultures. Averages of duplicate experiments, with standard deviations in parentheses.

III_B	N	C	T	R	P	N	N	N	T	R	K	S	I	R	I	Q	R	G	P	G
BaL														N		*	*			
Is-4								H		V	R			A		*	*			
Is-9								T				G		Y	V	*	*			
Is-10								H				R	M	T	L	*	*			
Is-13											X	R	M	T	A	*	*			
Is-17						R	K	I	K	I	R	H	M	H		*	*			
Is-18												G			V	*	*			
Is-19							Y		K	G		K		P		*	*			

(i)

III_B	R	A	F	V	T	I	G	K	I	*	G	N	M	R	Q	A	H	C	N
BaL				Y		T		E		I		D	I						
Is-4		S		Y		T		R		I			I						
Is-9		R	V	Y	A	T	D			I		D	I		K		Y		
Is-10	K	V	Y	Y		T		E		V		D	V	K	R				
Is-13		V	Y	Y		T		Q		V		D	I		K				
Is-17				H	A	T		E	T	*	*	D		K	K		Y		
Is-18			I	Y		A	R	R		I		D	V						
Is-19				Y		T	S	L		K		D	I						S

(a) (ii)

HIV-1 strain	Total positive charge	No. of potential glycosylation sites
III_B	9	8
Is-4	7	9
Is-9	8	8
Is-10	9	8
Is-13	7	9
Is-17	11	6
Is-18	9	8
Is-19	8	6

(b)

Fig. 1. (a) Amino acid sequence of the V3 region of gp120 of HIV-1 strains. Amino acids (a.a.) are indicated by the single-letter codes, deletions are marked with *. The a.a. sequence in frame (ii) follows the one in frame (i). (b) Total positive charge calculated in a.a. residues of gp120 V3 loop and number of potential glycosylation sites calculated on 138 a.a. residues of gp120, including the V3 loop of primary HIV-1 strains in comparison to HIV-1 III_B.

tropism (Chesebro et al., 1992; De Jong et al., 1992; Shioda et al., 1992; Westervelt et al., 1992). The distribution of the hydrophilic and lipophilic amino acid side-chains (data not shown), and the total positive charge of the V3 loop were maintained (Fig. 1b). Furthermore, the number of potential glycosylation sites was calculated on 138 residues of the gp120 sequence including the V3 loop (Fig. 1b). No correlation could be drawn from these data.

An apparent correlation observed was that the poorer the growth of a primary isolate in MACs (Table 1), the more its growth was stimulated by the presence of GAGS (Table 2). The combination of results reported in Tables 1 and 2 leads to the plot represented in Fig. 2.

In conclusion, the most evident effects produced by the presence of GAGS on the replication of HIV-1 were the following:

(a) a clear inhibition of the replication of III_B in PBLs and a strong inhibition of BaL in MACs by all three GAGS, at 10 μg/ml (Table 2);
(b) a general relevant enhancement of the replication of five primary isolates (Is-4, Is-10, Is-13, Is-17, Is-18) in MACs by the three GAGS, but also of one primary isolate (Is-19) in PBLs by DS1 and DS2, at 10 μg/ml (Table 2); the primary isolates growing less well in MACs were those that were more stimulated by the presence of GAGS;
(c) a dose-dependent decrease of the inhibitory effect of DS2 on BaL (Table 3);
(d) a variability in the appearing of inhibitory–stimulatory effects in MACs with the concentration of the GAGS, depending on the MAC batch, on the GAGS and on the virus strain (Tables 2 and 3).

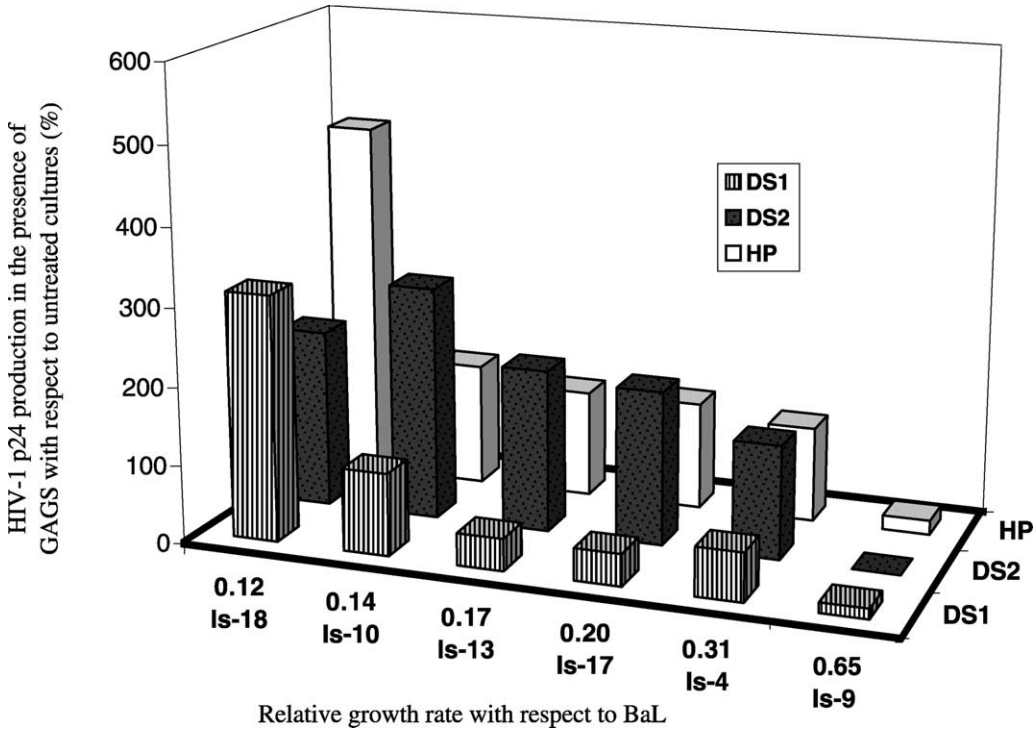

Fig. 2. Comparison of the growth ability of six HIV-1 primary isolates (Is) in macrophages with respect to BaL, derived from Table 1, with the susceptibility of their growth to GAGS (DS1, DS2, HP), derived from Table 2 as: 100 × p24 produced by treated cultures/p24 produced by control cultures.

4. Discussion

The already known inhibitory effects produced by GAGS on HIV-1 replication can be ascribed to their large size and highly negatively charged nature, which enable them to hinder, in a relatively non-specific manner, the function of positively charged macromolecules participating in the infection process.

The unexpected effect of enhancement mainly seen with HIV-1 primary isolates replication in MACs (Tables 2 and 3), but in one case also in PBLs (Table 2), is not easily explained at present and can only be tentatively interpreted on the basis of the following observations.

HIV-1 attachment to host cells is considered to take place mainly via high affinity binding between host cell CD4 and virion-associated gp120, but this only event is not sufficient for infection. There are other interactions between the virion and the cell surface relevant for the attachment and entry processes, like the complex interactions with the chemokine receptors.

Macrophage-tropic HIV-1 strains use surface CCR5 co-receptor in conjunction with CD4, while the CD4+ T lymphotropic strains use CXCR4. Other chemokine receptors, such as CCR3 can play similar roles in facilitating HIV-1 entry.

Studies with high MW dextran sulphate already revealed an enhancement in the infection of MACs by HIV-1 isolates (Meylan et al., 1994). It was suggested that probably this compound enhances the expression of HIV-1 co-receptor CCR5 at the transcriptional level (Jagodzinski and Trzeciak, 1998).

GAGS themselves are also attractive candidates as additional partner receptors. Many viruses, such as HSV-1 (Shieh et al., 1992; Shieh and Spear, 1994), Dengue virus, Foot and Mouth Disease virus, Sindbis virus (Byrnes and Griffin, 1998) have evolved to exploit cell-surface GAGS, particularly heparan sulphate (HS), to facilitate their attachment and infection of the host cells.

It is also already known that the quantitative contribution of GAGS to attachment and infection is highly dependent on the cell line. In some permissive cell lines HIV-1 infection is facilitated by cell-surface HS (Ibrahim et al., 1999) so that the treatment of lymphoblastic T cell lines MT-4 and H9 with heparinase significantly prevented HIV-1 III_B infection (Patel et al., 1993). The removal of GAGS by heparinase was less effective in inhibiting the binding of the macrophage-tropic strain HIV-1 SF162 than that of the T cell line-tropic III_B (Oshiro et al., 1996). In CD4+ T cells from normal PBMCs, III_B attachment was not significantly affected by GAGS removal (Oshiro et al., 1996).

In fact, cell surface GAGS-mediated enhancement of infection is confined to isolates that contain a highly positively charged V3-loop sequence, while infection by most strains is apparently inhibited by the presence of GAGS (Zhang et al., 2002).

It was also shown (Roderiquez et al., 1995) that in a clone (H9 h) of H9 T cell line, selected on the basis of its high level of cell surface CD4 expression, interactions with the cell surface occurred through both CD4 and HS. In fact it is postulated that such sulphated polyanions participate in virus binding to host cell and penetration, but the exact mechanism is uncertain.

Binding through HS should localize the virus to the membrane and thus increase the occurrence of a secondary interaction with CD4. Virus–cell interactions would be stabilised by the formation of a trimolecular complex via two different binding sites on the same envelope spike. It is possible that linear HS chains interact with positively charged residues of the V3 and CD4 domains of two oligomerised envelope subunits (Roderiquez et al., 1995).

Primary macrophage-tropic strains of the virus show variations in the amino acid sequence of the V3 domain, which markedly reduce the positive charge of this region; these strains may thus differ in their dependence on HS or other charged molecules during virus binding and infection (Roderiquez et al., 1995).

A recent report indicates that heparan sulphate of the T-lymphocyte cell surface interacts specifically with the fusion domain of HIV gp41. The interaction is not influenced by the presence of heparin (Cladera et al., 2001).

Effects by the addition of GAGS on the growth of various HIV-1 viruses in different host cell systems have already been reported (Meylan et al., 1994). The enhancement of HIV-1 infection in MACs by high MW dextran sulphate is also associated with conformational changes within the V2 region of gp120, leading to an increase in the interactions between gp120 and the CCR5 co-receptor on the target cell (Jagodzinski et al., 1999).

Summarising the considerations above, it can be hypothesised that the increase in viral replication, seen in the presence of GAGS, can be ascribed to one or more of the following factors, connected with virus–host cell interactions leading to virus binding and entry:

1. Facilitation of the participation of host cell membrane co-receptors, i.e. co-operative effects by the GAGS on co-receptors function.
2. Participation of the GAGS in the stabilisation of complexes between viral and host cell components. Specific conformational effects on V2 loop of viral gp120.
3. Bridging effects by the GAGS between virus and host cell.
4. Increase of the expression of host cell co-receptors by the action of GAGS at transcriptional level.

The occurrence of some of these additional interactions, connected with the presence of the GAGS, would operate in such a way that the stimulatory effects could, in some cases, overwhelm the inhibitory hindrance effects, which must as well be present.

On the contrary, the inhibitory effects of GAGS could be explained if the inhibited strains should be less dependent on these additional interactions for their entry, thus allowing inhibition to prevail.

In particular, laboratory viral strains like III_B and BaL, having adapted themselves to grow in these tissue cultures, might have optimised their ability to penetrate their host cells and might gain a limited advantage by the co-operative effects, which can be exerted by the GAGS. The GAGS then can interact and cause inhibition by hindrance effect. The inhibitory effects reported in this paper were in fact mainly seen with III_B in PBLs and with BaL in MACs, depending on the GAGS used and on its concentration.

In accordance to this, it was reported that the laboratory adaptation of a X4-tropic primary virus, i.e. the ability to replicate in CD4+ T cell lines such as H9, involves acquisition of a higher affinity for CD4 receptor. This may result from a concerted structural rearrangement of the gp120–gp41 complexes (Platt et al., 2000). A similar phenomenon could be involved also in laboratory adaptation of a R5 strain. Conversely, it has been speculated that the inability of primary X4-tropic isolates to replicate in T cells reflects a low affinity for CD4 (Cullen, 2001).

In conclusion, the results reported in this paper show that there can be a high variability in the susceptibility of the replication of HIV-1 to the presence of polyanionic compounds such as GAGS. Viral replication can be more or less inhibited, but even highly stimulated, depending on the virus, on the cell culture type, particularly for MACs even on the cell batch used in the experiments (Meylan et al., 1994), on the type of the GAGS (chemical composition, charge density, size, structure, presence of particular sequence domains) and on its concentration.

As for the GAGS used in this study, in general, HP and DS2 both displayed stronger inhibitory ability than DS1, with the inhibitory effects mostly produced at the highest concentration used.

GAGS are being considered as potential topic drugs for the prevention of AIDS transmission. In consideration of what has been discussed above, in the evaluation of their potentiality to interfere with the HIV-1 entry step of infection, the importance has to be underlined of using models which entail virus primary isolates, and PBMC and MAC cultures, besides the usual virus laboratory strains and CD4+ cell line.

However, the overall potential of the GAGS as anti-HIV-1 drugs appears questioned by the present findings. This is in accordance with the clinical data (Meylan et al., 1994) reporting an increase in p24 in the serum of HIV-1 positive patients treated with dextran sulphate.

Nevertheless, GAGS still maintain potential as drugs against other viruses, for which such growth-stimulatory effects have not yet been shown. Additionally, further studies can evaluate the use of GAGS, as growth stimulators, for facilitating the isolation of HIV-1 from plasma or liquor containing a low viral load.

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