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Article *in* Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease · November 1997

Impact Factor: 4.88 · DOI: 10.1016/S0925-4439(97)00057-4

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Biochimica et Biophysica Acta 1362 (1997) 47–55

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Antiviral activity of derivatized dextrans on HIV-1 infection of primary macrophages and blood lymphocytes

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Received 11 July 1997; accepted 28 July 1997

Abstract

The present study demonstrates at the molecular level that dextran derivatives carboxymethyl dextran benzylamine (CMDB) and carboxymethyl dextran benzylamine sulfonate (CMDBS), characterized by a statistical distribution of anionic carboxylic groups, hydrophobic benzylamide units, and/or sulfonate moieties, interact with HIV-1 LAI gp120 and V3 consensus clades B domain. Only limited interaction was observed with carboxy-methyl dextran (CMD) or dextran (D) under the same conditions. CMDBS and CMDB (1 μM) strongly inhibited HIV-1 infection of primary macrophages and primary $CD4^+$ lymphocytes by macrophage-tropic and T lymphocyte-tropic strains, respectively, while D or CMD had more limited effects on M-tropic infection of primary macrophages and exert no inhibitory effect on M- or T-tropic infection of primary lymphocytes. CMDBS and CMDB (1 μM) had limited but significant effect on oligomerized soluble recombinant gp120 binding to primary macrophages while they clearly inhibit (> 50%) such binding to primary lymphocytes. In conclusion, the inhibitory effect of CMDB and the CMDBS, is observed for HIV M- and T-tropic strain infections of primary lymphocytes and macrophages which indicates that these compounds interfere with steps of HIV replicative cycle which neither depend on the virus nor on the cell. © 1997 Elsevier Science B.V.

1. Introduction

HIV-1 Env glycoprotein gp120 binding to CD4, the major virus receptor [1], initiates a cascade of events leading to virus–cell and cell–cell membrane fusion. Different cellular cofactors (glycolipids, proteins, glycoproteins) have been assumed to be involved in the latter events [2–4] but, in fact, chemokine receptors CCR-5 and CXCR4 play then the major role in the infection by macrophage (M)-tropic or T lymphotropic (T-tropic) strains, respectively [5]. The V3 region of gp120 appears to be involved in such post-CD4-binding events by: (i) binding to different cell surface membrane components, including the recently identified virus coreceptors [5]; (ii) interacting with CD4, possibly its CDR3-like domain [6–8]; and (iii) initiating thus Env

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PII S0925-4439(97)00057-4

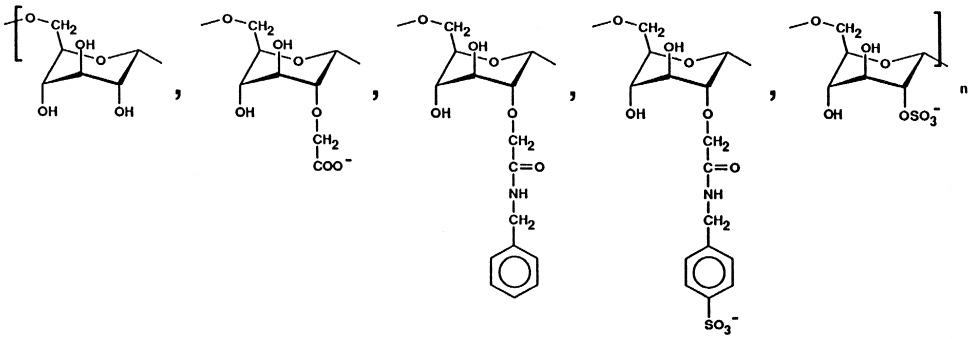
conformational changes necessary for unmasking gp41 fusogenic domain [9–11]. These possibilities are not exclusive, since V3 is positively charged and can interact with negatively charged cell surface molecules such as sulfated proteoglycans [12]. In this context, sulfated polyanions, such as dextran sulfate (DS) or heparin and other sulfated polysaccharides, may inhibit HIV-1 infection of $CD4^+$ lymphoid cells [13–15]. For example, recombinant gp160 (rgp160) specifically interacts with low molecular weight (MW) DS (8 kDa, DS 8000) [16] and inhibits HIV-1 infection of $CD4^+$ lymphoid cells by interfering with virus–cell interactions [17,18]. However, use of these compounds for therapy is limited by their anticoagulant activity [19–21], and DS may enhance rather than inhibit infection of primary macrophages by M-tropic viruses [15]. Derivatized dextrans, including a sulfonated molecule, also interact with rgp160 and rgp41 with stronger affinities than observed for DS 8000 [22].

The present study was undertaken in order to extend our previous findings and demonstrate at the molecular level that dextran (D) derivatives devoid of anticoagulant activity [23] and characterized by the statistical distribution of anionic carboxylic groups, hydrophobic benzylamide units, and/or sulfonate moieties, specifically interact with gp120 and its V3 domain, and inhibit HIV-1 infection of $CD4^+$ human primary macrophages and lymphocytes by M-tropic and T-tropic strains.

2. Materials and methods

2.1. Derivatized dextran

Water-soluble D derivatives were prepared as described [23–25] from D, T40, MW: 49900 (Pharmacia-LKB, St Quentin en Yvelines, France). The chemical composition of the compounds was characterized by acidimetric titration and elemental analy-



Name	D	CM	B	S + Su
CMDBS 1	0	58	18	26
CMDBS 2	0	72	14	22
CMDBS 3	0	96	22	39

Fig. 1. Chemical representation of derivatized dextrans. Dextrans (D) were substituted by a sequential reaction in three steps in order to obtain carboxymethyl groups (CM), coupled with benzylamine (B) and finally sulfonation (S) of some aromatic rings or sulfation (Su) of hydroxyl groups. Only the main substitution in the C2 position is depicted. The composition of three different CMDBS obtained by varying the chemical conditions is shown in the table. Values are % of chemical groups per glucoside unit.

sis. Carboxymethyl D (CMD: 78% CM; 22% D) was synthesized from native D by substituting glucosyl units with carboxymethyl groups. In a second step, benzylamine was coupled with some carboxylic groups to form benzylamide units (CMDB: 35% B; 59% CM; 6% D). Finally, benzylamide aromatic rings were sulfonated. Three CMDBS (1, 2 and 3, corresponding to three preparations of the derivative) were investigated (Fig. 1). The anticoagulant activities of the three tested CMDBS were of 1.4, 0.8, and 3.5 IU/mg, respectively, for CMDBS 1, CMDBS 2, CMDBS 3. The other dextran derivatives (CMDB, CMD) were as native dextran (D) devoid of anticoagulant activity. In comparison, the anticoagulant activity of heparin is in the range of 170 IU/mg. DS (90% D; 10% S), MW: 32700 (DS 32700), was synthesized from T40 as described [23–25].

The MW (Table 1) of derivatized D was determined by high performance steric exclusion chromatography in 0.2 M NaCl, using a Licrospher Si 500 Diol column (Merch–Clevenot, Nogent sur Marne, France) calibrated with sulfonated polystyrene standards (Polymer Laboratories, Montluçon, France).

In some experiments, DS 8000 (8 kDa) and D 10000 (10 kDa) were purchased from Sigma (St Louis, MO, USA).

2.2. Virus, rgp120 and V3 peptide

HIV-$1_{Ba\text{-}L}$ was a gift from Birgitta Asjö [26], and HIV-1_{LAI} was purchased from Diagnostics Pasteur (Marne la Coquette, France). Soluble rgp120 from HIV-1 LAI (> 90% pure) was from Intracell (London, UK), and clades B V3 consensus peptide (aa: CTRPNNNTRK SIHIGPGRAFYTTGEI-IGDIRQAHC) was from Neosystem (Strasbourg, France). Rgp120 and V3 were radiolabeled by the iodogen method, as described [27], or with iodobeads (Pierce, Oud-Beijerland, The Netherlands) according to the manufacturer's instructions. The iodinated molecule was separated from $Na^{125}I$ by filtration through a Sephadex G-25 (PD10) column (Pharmacia, Uppsala, Sweden). Specific activity of labeled rgp120 and V3 was about 0.1 MBq/μg and 0.05 MBq/μg, respectively.

2.3. Binding of rgp120 to sulfated D beads (SDB)

SDB (Sigma) were soaked for 24 h at 4°C in phosphate-buffered saline (PBS), 0.1% sodium azide, pH 7.4 (PBS-azide). After three washes in PBS-azide, SDB were suspended in an equal volume of 0.02 M Tris, 0.15 M NaCl, 0.01 M $CaCl_2$, 0.05% bovine serum albumin (BSA, Sigma) (Tris–Ca–BSA), pH 7.4. After one wash with 10 volumes Tris–Ca–BSA, 20 μl of matrix were incubated for 1 h at 37°C with 10^{-9} M [^{125}I]rgp120 in 60 μl buffer. Unbound glycoprotein was removed by two washes of the matrix in 500 μl buffer. Solid phase-bound radioactivity was counted in a γ counter (LKB, France). Results are expressed as mean cpm of duplicates. Specificity of the binding to the matrix was determined by incubating 10^{-9} M [^{125}I]rgp120 for 45 min at 37°C with μM concentrations of soluble DS 8000, DS 32700 or D

Table 1
Inhibition of [^{125}I]rgp120 binding to SDB by derivatized D

Compound	Bound rgp120 (cpm)	C_{50} (μM)	Maximum inhibiting concentration (μM)	Maximum % inhibition
Buffer	9000 ± 2280	–	–	–
D (10 kDa)	9200 ± 2757	–	266	0
D, T40 (49.9 kDa)	5950 ± 2720	–	78	36 ± 23 [a]
CMD (62.3 kDa)	5510 ± 2260	–	34	39 ± 21 [b]
DS 32700(32.7 kDa)	6080 ± 1060	2	65	76 ± 4 [d]
DS 8000 (8 kDa)	2860 ± 1350	16	266	68 ± 12 [d]
CMDB (61.7 kDa)	4050 ± 1850	34	34	51 ± 21 [c]
CMDBS1 (45 kDa)	2368 ± 1360	1.5	47	70 ± 16 [c]
CMDBS2 (57 kDa)	2730 ± 1450	2.5	37	57 ± 28 [d]

Statistical significance of percent inhibitions relative to controls: [a] $p < 0.05$, [b] $p < 0.02$, [c] $p < 0.01$, [d] $p < 0.001$. Results are means ± SD of 6 independent experiments.

10000, the latter used as negative control. Binding to 20 μl of SDB was then assayed. In parallel experiments to determine the effect of derivatized D, [^{125}I]rgp120 was preincubated with μM concentrations of D, CMD, CMDB, CMDBS1 or CMDBS2, which were kept in the mixture during the whole assay.

2.4. Binding to the V3 peptide of the derivatized D dotted onto nitrocellulose

CMDBS1, CMDBS2, CMDBS3, CMDB, CMD, D 10000 and Concanavalin A (ConA; Sigma; 10 μl, 10 μg) were dotted onto nitrocellulose filters (Sartorius, Göttingen, FRG). After 30 min at 20°C, strips were saturated for 30 min at 37°C with 2 ml PBS, 5% BSA. Excess BSA was washed out with PBS, 0.5% BSA, 0.2% Tween 20 (Sigma), and strips were incubated for 3–5 h at 37°C with 0.5–1×10^6 cpm of [^{125}I]V3. As control, labeled V3 was preincubated for 1 h at 37°C with a rabbit polyclonal anti-V3 antibody (1 : 100; Neosystem, Paris, France) before incubation with the strips. After six washes with the same buffer, strips were exposed at −20°C for 48 h or at −80°C for 3 h.

2.5. Cells

Blood mononuclear cells (PBMC) of healthy volunteers (Seine–Saint–Denis or Pitié–Salpêtrière blood banks) were obtained by Ficoll–Hypaque centrifugation. Cells were either: (i) stimulated with phytohemagglutinin (PHA, DIFCO, Detroit, MI) for 3 days in RPMI 1640, 50 μg/ml penicillin/streptomycin, 2 mM L-glutamine, (Gibco-BRL, Paisley, Scotland), 10% fetal calf serum (FCS; Boheringer, Mannheim, FRG) (R10); or (ii) cultured at $2–5 \times 10^6$ cells/ml for 5 days in the same medium but with 10% heat-inactivated normal human pooled AB serum (Pitié–Salpêtrière blood bank) and 20% FCS (20%), as described [28,29]. Nonadherent cells were then removed by several washes in Ca/Mg-free PBS. Adherent cells were cultured in medium without AB serum (R20) for another 24–48 h before exposure to HIV-1, or they were scrapped off with a rubber policeman for analysis. Procedure (ii) yielded > 90% CD14$^+$ monocyte-derived macrophages (MDM) with > 95% viability, which were viable for more than one month.

2.6. Binding of [^{125}I]rgp120 to the cells

Cells (5×10^5–1×10^6) were incubated for 2 h at 4°C or at 37°C with 50 μl [^{125}I]rgp120 (1–4×10^5 cpm; 2–5×10^{-10} M) in 80 μl RPMI, 0.05% BSA, 0.05% azide, in order to avoid gp120 internalization, or in Hank's buffer supplemented with 0.05% BSA, 0.05% azide, 10 mM $CaCl_2$ in order, according to our previous data [30,31] to perform the experiments with an oligomerized gp120. After two washes (700 g, 10 min, 4°C), cell-bound radioactivity was counted. The effect of D, DS 32700, CMD, CMDB, CMDBS1 or CMDBS2 on rgp120 binding to the cells was analyzed as follows: [^{125}I]rgp120 was preincubated for 1 h at 37°C with different concentrations of the products diluted in medium, and the mixture was then incubated with cells as described above. It was verified that cell viability was not modified in the presence of Hank's buffer supplemented with 10 mM $CaCl_2$ as assessed by trypan blue exclusion dye.

2.7. HIV-1 infection of cells

HIV-$1_{Ba\text{-}L}$ (2×10^4 cpm reverse transcriptase activity) was added for 18 h at 37°C to 5×10^5 MDM in 900 μl culture medium, 20% FCS, as described [28,29]; unadsorbed virus was removed by washing, and cells were further cultured. In parallel, virus was pretreated for 45 min at 37°C with the different D derivatives, 5 μM Zidovudine (AZT) or 2.5 μg/ml anti-Leu3a monoclonal antibody (mAb) (Becton dickinson, Mountain View, CA), and the mixture was added to 5×10^5 MDM for 18 h at 37°C. Cells were then cultured in the presence of the compounds at the same concentrations than initially, after it was verified that this did not modify cell viability. Virus production in supernatants collected twice a week was assessed by measuring p24 by ELISA according to the manufacturer's instructions (Diagnostics Pasteur).

Peripheral blood lymphocytes (PBL) in R10 were stimulated for 3 days with PHA. Then, 100 $TCID_{50}$ of HIV-1_{LAI} or HIV-$1_{BA\text{-}L}$ (2×10^4 cpm reverse transcriptase activity) in IL-2-supplemented (10 U/ml; Boheringer) R10 were incubated for 45 min at 37°C in R10, or with 1 μM of the different D derivatives or

5 μM AZT in R10, and mixed thereafter with 5×10^5 PBL for 3 h at 37°C. In parallel, PBL were incubated for 30 min at 4°C with 2.5 μg/ml of anti-Leu3a mAb. Cells were then washed twice in culture medium (400 g, 10 min), and cultured at 5×10^5 cells/ml. Compounds were maintained at the initial concentration during the experiment. In some experiments, viruses were directly co-incubated with the different D derivatives and with the cells and washed out from the culture medium. Supernatants were collected twice a week to determine p24.

3. Result

3.1. Binding of rgp120 to derivatized D

We have shown that rgp120 specifically binds to SDB [16,22]. Here, we confirm that preincubating tetramerized rgp120, in the presence of 10 mM $CaCl_2$ [30,31] with DS 8000 or with DS 32700, inhibited this binding, the C_{50} of the rgp120–DS 8000 interaction being 16 μM, that of the rgp120–DS 32700 being 2 μM; these data therefore demonstrate the specificity of rgp120 binding to SDB (Table 1, and data not shown). We show in addition that derivatized D, CMDBS1, CMDBS2 and CMDB were also efficient in this respect (Table 1). The affinity of CMDBS1 and CMDBS2 interaction with rgp120, with C_{50} of 1.5–2.5 μM, were 8–10-fold higher than for DS 8000, but of the same order of magnitude than that noted in the presence of DS 32700. A lower affinity, with a C_{50} of 34 μM, was noted for CMDB. D 10000 had no effect on gp120 binding to SDB while D (T40) or CMD had limited but significant effects. This indicates that the D structure by itself or anionic CMD only slightly interact with rgp120.

3.2. Interaction of D derivatives with the clades B V3 consensus peptide

Clades B V3 consensus peptide interacted with CMDB, CMDBS1, CMDBS2 and CMDBS3, but not with CMD nor with D 10000 or ConA dotted onto nitrocellulose (Fig. 2); binding of V3 to CMDB or to CMDBS compounds was significantly inhibited by anti-V3 antibodies (data not shown), which indicates its specificity.

3.3. rgp120 binding to MDM and PBL

No inhibition of rgp120 binding to MDM or to PBL was observed, in RPMI medium at +4°C, in the presence of the tested compounds: D, DS 32700, CMD, CMDB and CMDBS (1 and 2); mAb anti-Leu3a, used as control, induced 2-fold inhibition of rgp120 binding to PBL but had no effect on the binding to MDM (data not shown), in agreement with previous findings [28,29,32]. However, when the experiments were performed at 37°C, in Hank's buffer supplemented with 10 mM $CaCl_2$, in order to approach the effects of the various dextran derivatives on oligomerized gp120 binding to the cells [30,31], 6 μM CMD, CMDBS1 and CMDBS2 significantly and strongly (> 50%) inhibited rgp120 binding to MDM; however, 1 μM of these compounds had significant but more limited effects (mean % of inhibition = 29–30%). In the same conditions, T40, DS (32700) or CMD had no effect (Table 2). Nevertheless, CMDB, CMDBS1 and CMDBS2, at 1 μM concentrations, significantly and rather strongly inhibited rgp120 binding to PBL (mean percentages of inhibition: 51–58%); DS(32700) had more limited but significant effect; on the contrary, T40 or CMD had no effect.

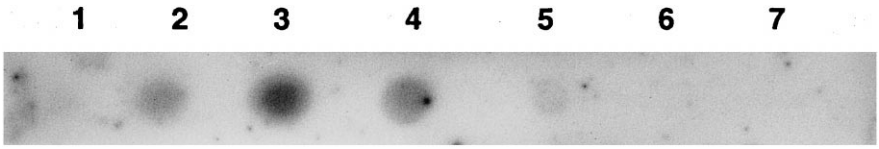


Fig. 2. Interactions of derivatized D (10 μg) with the V3 consensus peptide. 1: ConA; 2: CMDB; 3: CMDBS1; 4: CMDBS2; 5: CMDBS3; 6: CMD; 7: D 10000. Data are from one out of three experiments.

Table 2
^{125}I rgp120 binding to monocyte-derived macrophages (MDM) and peripheral blood lymphocytes (PBL) in 10 mM $CaCl_2$ supplemented medium

	Bound radioactivity (cpm)	% inhibition
MDM		
Control	25500 ± 5350	–
T40 (14 μM)	24800 ± 3150	–
DS (47 μM)	22680 ± 6500	–
CMD (6 μM)	24300 ± 4900	–
CMDB (6 μM)	10230 ± 5800 [a]	64 ± 12 [a]
CMDB (1 μM)	17700 ± 5900 [b]	29 ± 7 [b]
CMDBS1 (8 μM)	7634 ± 3900 [c]	71 ± 9 [a]
CMDBS1 (1 μM)	17340 ± 4360 [c]	23 ± 14 [c]
CMDBS2 (6 μM)	6780 ± 4300 [a]	75 ± 100 [a]
CMDBS2 (1 μM)	15526 ± 4110 [c]	30 ± 22 [c]
PBL		
Control	5900 ± 3600	–
T40 (1.8 μM)	4400 ± 2370	29 ± 29
DS (1 μM)	2745 ± 1600 [c]	44 ± 17 [c]
CMD (1 μM)	3500 ± 1700	23 ± 23
CMDB (1 μM)	2170 ± 695 [c]	51 ± 20 [c]
CMDBS1 (1 μM)	2220 ± 890 [c]	55 ± 14 [c]
CMDBS2 (1 μM)	2250 ± 990 [b]	58 ± 8 [b]

Results are means ± S.D. of 3–7 independent experiments. Statistical significance of the differences relative to control as determined by the paired Student's t-test.
[a] $p < 0.01$; [b] $p < 0.02$.; [c] $p < 0.05$.

3.4. Effect of dextran derivatives on HIV-1 infection of MDM and PBL

We then tested the effect of the three CMDBS preparations, CMDB, CMD, D and DS 32700 on HIV-$1_{Ba\text{-}L}$ infection of MDM. The compounds were preincubated with the virus before addition to the cells, and were then kept at initial concentrations for the whole culture. AZT or mAb anti-Leu3a were used as positive control of inhibition. The CMDBS preparations and CMDB strongly inhibited in a dose-dependent manner HIV-$1_{Ba\text{-}L}$ infection (p24 levels ranging from 180 to 2200 pg/ml on day 18 post-infection with 1 μM of compound, relative to 9300–11200 pg/ml without compound), while CMD, DS 32700 and D (50 μg/ml) had more limited effects (Fig. 3(a),(b)).

To examine whether CMDB and CMDBS could protect other primary cells from infection with the same as well as with another virus strain, we analyzed their effect on HIV-1_{LAI} and HIV-$1_{Ba\text{-}L}$ infection of primary PBL, using mAb anti-Leu3a as control as previously. The CMDBS and CMDB also strongly inhibited HIV-1_{LAI} as well as HIV-$1_{Ba\text{-}L}$ infection of PBL, whereas no inhibition was observed in the presence of CMD, DS 32700 or D. Similar results were observed whether virus was pre-treated or not with the CMDBS. However, when CMDB or CMDBS1 were washed out from the culture medium, p24 production returned to the levels observed for the control (Fig. 4(a)–(c)). Taken together, these results demonstrate that CMDB and CMDBS compounds inhibit M-tropic and T-tropic HIV infections of pri-

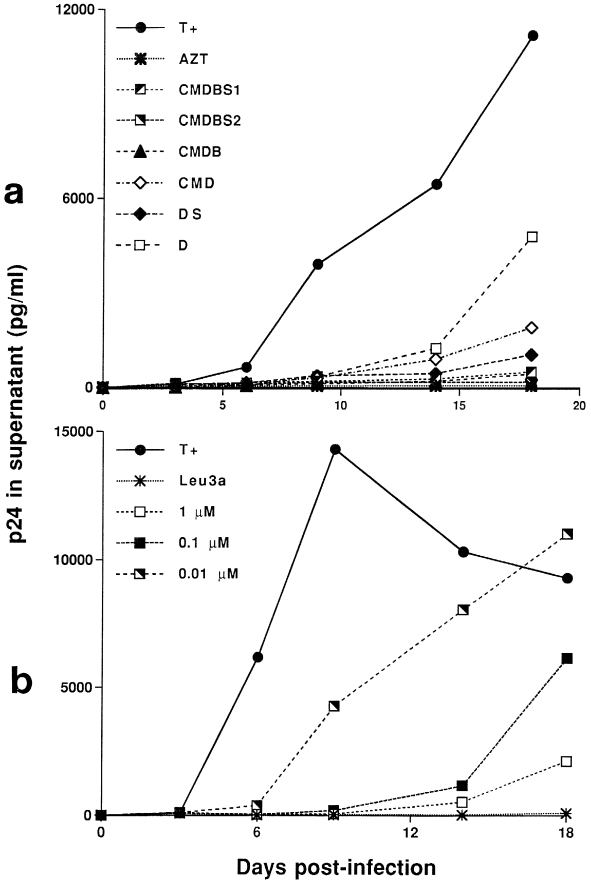
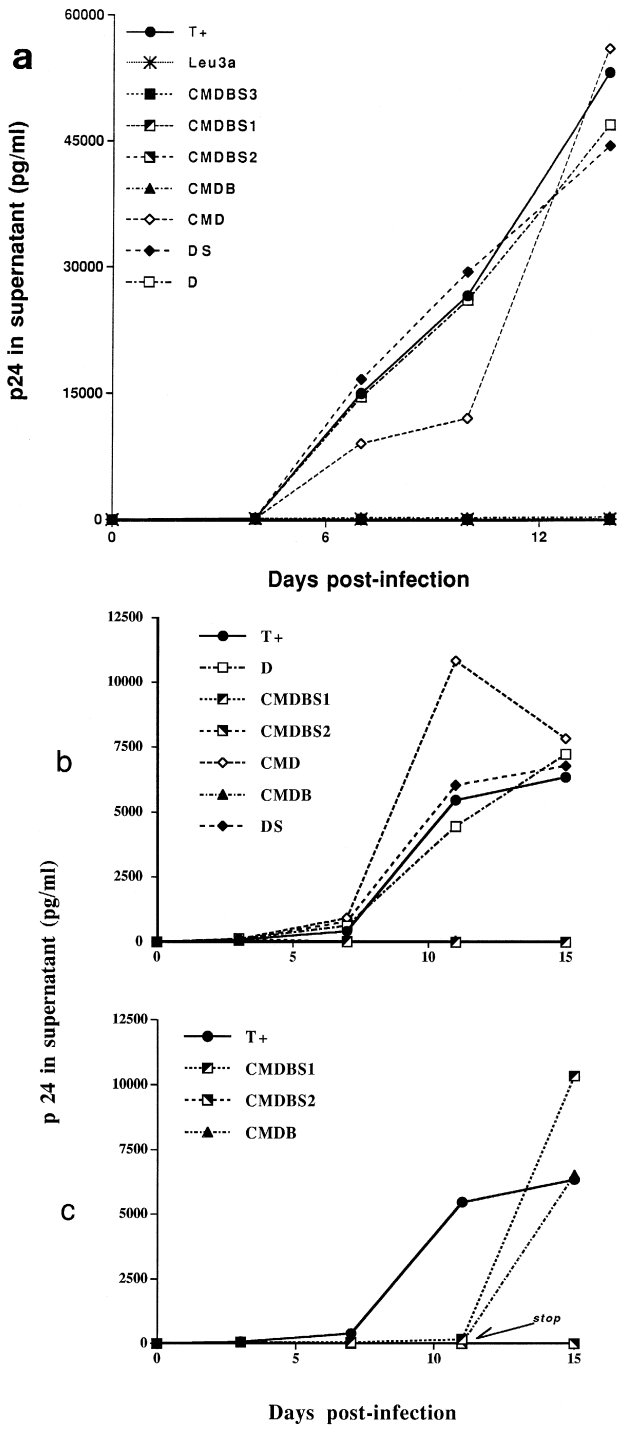


Fig. 3. Effect of derivatized D on HIV-$1_{Ba\text{-}L}$ infection of MDM. (a) Infection was conducted in the absence (T+) or in the presence of CMDBS1, CMDBS2, CMDB, CMD, D, DS 32700 (each at 1 μM) or of AZT (5 μM). (b) Effect on infection of different amounts of CMDBS3 (0.01, 0.1 and 1 μM) or of mAb anti-Leu3a (2.5 μg/ml). Data in (a) and (b) are from two different experiments out of four.

mary macrophages and lymphocytes and indicate that these compounds may interfere with post-binding events.



4. Discussion

Several soluble derivatized D with different percentages of carboxymethyl, benzylamide and sulfonate/sulfate groups were evaluated for possible inhibitory effects on HIV-1 infection of primary MDM and PBL by M-tropic and T-tropic HIV-1 strains, respectively. We first observed, in accordance with our previous results, specific interactions between soluble tetramerized rgp120 in the presence of 10 mM $CaCl_2$ and SDB inasmuch as these interactions were inhibited by DS 8000 and by DS 32700. We also observed specific interactions between rgp120 and two CMDBS compounds (CMDBS1 and 2) at the molecular level, with C_{50} values (1.5–2.5 μM) similar to those of rgp160 interacting with CMDBS3 [22].

The affinity of CMDB binding to rgp120 (C_{50} = 34 μM) noted here was lower than that noted previously using rgp160 (C_{50} = 2 μM) [22], which suggests gp41 involvement in this interaction. In addition, no interaction occurred between rgp120 and D 10000, which indicates specificity of the interactions, while limited interactions occurred in the presence of D, T40 or CMD (with maximum % of inhibition of gp120 binding to SDB of 36–39%). Furthermore, specific interactions were also observed, here, between a peptide mimicking the clades B consensus V3 loop of gp120 and CMDB or CMDBS, but not CMD or D.

The CMDBS and CMDB also strongly inhibited HIV infection of MDM by a M-tropic strain and of PBL by M- and T-tropic strain. These results indicate that the inhibitory effects of the compounds do not depend on the virus strain nor on the target cells. This

Fig. 4. Effect of derivatized D on HIV-1_{LAI} or of HIV-$1_{Ba\text{-}L}$ infection of PBL. Infection was conducted in the absence (T+) or in the presence of CMDBS1, CMDBS2, CMDB, CMD, D, DS32700 (each at 1 μM) which were preincubated with the virus and maintained at initial concentration in the culture medium, or of mAb anti-Leu3a (2.5 μg/ml). (a) HIV-1_{LAI} or (b) HIV-$1_{Ba\text{-}L}$ infection of PBL. (c) HIV-$1_{Ba\text{-}L}$ infection of PBL in the presence of CMDBS1, CMDBS2, CMDB which were not preincubated with the virus but only coincubated with the virus and with the PBL. CMDB and CMDBS1 were washed out from the culture medium. Data are from one out of two experiments.

means that the inhibitory effect of these compounds does not depend on the HIV-1 co-receptors used, CXCR4 during HIV-1_{LAI} infection of PBL or CCR5 during HIV-1_{BA-L} infection of PBL or MDM [5]. As other HIV-1 subtypes, C, D, E or O also use CXCR4 and/or CCR5 [33], an inhibitory effect of their infection by the presently tested compound cannot be excluded. Our results extend findings by Neyts et al. [34] using different experimental conditions, that CMDB and the CMDBS inhibited HIV-1-induced cytopathicity for lymphoid MT4 cells, whereas D or CMD had no activity. Our present results, obtained on primary lymphocytes, which report that D, T40 and CMD are devoid of effects on T-tropic infection of primary lymphocytes are therefore in agreement with those observed by Neyts et al. [34] on a lymphoïd cell line. It is to note that D, T40 or CMD which are devoid of any inhibitory effect on HIV-1 infection of primary lymphocytes and exert limited effect on HIV-1 infection of primary macrophages are able, according to the present data, to slightly interact with rgp120. In this respect, it must be considered that some high affinity gp120 ligands, such as galactosylceramide or sulfatide, have no role on HIV infection of primary cells [29]. On the other hand, enhancement of HIV infection of primary cells from some blood donors has been described in the presence of DS [15]; therefore, the purpose of the present study was not to investigate again its effects on HIV infection, but rather to analyze those of other dextran derivatives, especially CMDB and CMDBS on T- or M-tropic infection of primary cells. Indeed, in the present study, DS 32700, characterized by only 10% sulfated groups, had some inhibitory effects on HIV-1 infection of MDM, while it was devoid of effect on M- or T-tropic HIV-1 infection of PBL; this may be related to its MW, negative charge, low amount of sulfated groups and/or the blood donor target cells used. Indeed it has been suggested [15,35] that there is a critical MW required for DS anti-HIV-1 activity, that interactions of these compounds with the cells has also to be considered and that different results, according to the blood donors can be observed [15].

Here, CMDB and the CMDBS, at 1 μM concentration, only slightly, but significantly, inhibit oligomeric rgp120 binding to MDM, which rules out that they mainly act on primary gp120 binding to MDM and rather suggest that they interfere with some of other steps occurring during HIV M-tropic viral cycle. However, at 1 μM concentration, these compounds inhibit by 51–58% oligomerized gp120 binding to PBL which strongly indicates that during T-tropic infection of primary lymphocytes, CMDB and CMDBS interfere with some HIV env binding steps to the cells. In addition, we report, here, that CMDB and CMDBS, but not CMD or D 10000, specifically interact with clades B V3 consensus domain which indicates that these compounds may interfere with post-binding events necessary for viral entry. Indeed, strong inhibitory effects of CMDB or CMDBS on HIV infection were only observed when these compounds were maintained in the culture medium at initial concentration, which further strongly suggests that they interfere with some post-binding events.

That D and CMD had no or limited effect on HIV-1 infection of MDM and PBL, as compared to the inhibitory effect of CMDB and the CMDBS, indicates that compounds that contain sufficiently high combined percentages of benzylamide and benzylamide sulfonate groups elicit anti-HIV-1 activity. Of note, a prototype compound (T1C4E5-tribenzyl-CD4) from the CDR3 region of CD4 inhibited at μM concentration HIV-1-induced cell fusion and infection of transformed T lymphoid cells [36]. These results suggest that the cyclic chemical presentation of these compounds (benzyl) is necessary to observe any inhibition of infection.

In conclusion, the inhibitory effect of CMDB and the CMDBS which are devoid of toxicity and anticoagulant activity when injected to animals [37,38], is observed for HIV M- and T-tropic strain infection of primary lymphocytes and macrophages which indicates that they interfere with steps of HIV replicative cycle which do not depend on the virus nor on the cell. Their use as anti-HIV therapeutic agents can therefore be proposed.

Acknowledgements

We thank Birgitta Äsjo (Bergen, Norway) for the kind gift of HIV-1_{Ba-L} strain. This work was supported by the Agence Nationale de Recherche sur le SIDA, the Direction de la Recherche et des Enseignements Doctoraux (Ministère de l'Enseignement Supérieur et de la Recherche), Université Paris XIII

and Université Paris VI, the Centre National de la Recherche Scientifique (ERS 107), and the Association de Recherche sur les Déficits Immunitaires Viro-Induits (Paris, France). The authors thank J. Champion and F. Chaubet (LRM, Villetaneuse) for the synthesis and characterization of some dextran derivatives.

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