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Mediation of Human Immunodeficiency Virus Type 1 Binding by Interaction of Cell Surface Heparan Sulfate Proteoglycans with the V3 Region of Envelope gp120-gp41

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The mechanism of heparan sulfate (HS)-mediated human immunodeficiency virus type 1 (HIV-1) binding to and infection of T cells was investigated with a clone (H9h) of the T-cell line H9 selected on the basis of its high level of cell surface CD4 expression. Semiquantitative PCR analysis revealed that enzymatic removal of cell surface HS by heparitinase resulted in a reduction of the amount of HIV-1 DNA present in H9h cells 4 h after exposure to virus. Assays of the binding of recombinant envelope proteins to H9h cells demonstrated a structural requirement for an oligomeric form of gp120/gp41 for HS-dependent binding to the cell surface. The ability of the HIV-1 envelope to bind simultaneously to HS and CD4 was shown by immunoprecipitation of HS with either antienvelope or anti-CD4 antibodies from $^{35}SO_4^{2-}$-labeled H9h cells that had been incubated with soluble gp140. Soluble HS blocked the binding of monoclonal antibodies that recognize the V3 and C4 domains of the envelope protein to the surface of H9 cells chronically infected with HIV-1_{IIIB}. The V3 domain was shown to be the major site of envelope-HS interaction by examining the effects of both antienvelope monoclonal antibodies and heparitinase on the binding of soluble gp140 to H9h cells.

Elucidation of the early molecular events associated with human immunodeficiency virus type 1 (HIV-1) infection is of major importance to AIDS therapeutics and vaccine development. Although cell surface CD4 is the primary receptor for HIV-1 (6, 23), evidence suggests that other cell surface molecules, either independently or in association with CD4, may participate in virus binding and entry (5, 17, 18, 35, 46). At least two CD4-dependent infection pathways are defined by tropic differences between macrophage-tropic and T-cell-line-tropic variants of HIV-1. Many of the phenotypic properties distinguishing these variants map to the V3 region of the gp120 envelope protein (7, 21).

We previously showed that cell surface heparan sulfate (HS) proteoglycans participate in infection of the $CD4^+$ T-cell lines MT4 and H9 (35). Removal of HS from the surface of the cells by heparitinase, a specific HS-degrading enzyme, reduced virus binding and infection as measured by cell surface virus attachment, cytopathogenicity, reverse transcriptase activity, and syncytium formation. The data supported the participation of both HS and membrane-bound CD4 in either initial virus attachment or postbinding events. Our previous studies on the antiviral effects of dextran sulfate (DS) on HIV-1 binding and infection showed that DS prevented monoclonal antibody (MAb) recognition of the V3 domain of envelope gp120 without interfering with the gp120-CD4 interaction directly (4). Together, these observations suggested that cell surface HS may interact with regions of the envelope near the V3 domain to mediate virus binding and entry.

We have now examined the direct molecular interaction between HS and the HIV-1 envelope protein. We present immunological and biochemical evidence for the simultaneous binding of envelope to both HS and CD4 to form a trimolecular complex. We further show that the V3 region of the oligomeric envelope gp120-gp41 complex mediates envelope-HS interactions.

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MATERIALS AND METHODS

Reagents. Recombinant vaccinia viruses expressing gp120, gp140, or gp140N/C HIV_{BH8} envelope (9) were gifts of P. Earl and C. Broder (National Institute of Allergy and Infectious Diseases, Bethesda, Md.). Density gradient-purified HIV-1_{IIIB} (39) was obtained from Pharmacia Diagnostics (Columbia, Md.). MAbs 110.3 and 110.4 (26, 47) were gifts of Genetics Institute (Seattle, Wash.). MAbs 10D8, 11G5, 13H8, 6E10, and 5B3 (32) were gifts of P. Berman (Genentech, San Francisco, Calif.). MAb 9284 was obtained from Dupont. MAb 1B1 was obtained from Waldheim Pharmazeutika. The following reagents were obtained through the AIDS Research and Reference Reagent Program (Division of AIDS, National Institute of Allergy and Infectious Diseases: MAb to HIV-1 gp41 (MAb 50.69) from Susan Zolla-Pazner (14), MAb F105 from Marshall Posner (37, 38), and 0.5β ascites fluid from Shuzo Matsushita (8, 27). MAbs G3.4, G3.136, G3.508, and BAT123 (12, 13, 20, 45) were gifts of Tanox Biosystems (Houston, Tex.). MAb L120 to CD4 (29) was a gift of Becton Dickinson. MAb ITM3 to LFA-1 (1, 48) was a gift of I. Andó (BRC, Szeged, Hungary), and the sheep polyclonal antiserum to HIV-1_{IIIB} gp120 was a gift of M. Phelan (36). The epitopes recognized by the various MAbs to gp160 are shown in Table 1.

Measurement of HIV-1 and envelope protein binding to H9h cells. H9h cells, obtained by cloning H9 cells and selecting for high levels of surface CD4 expression, were cultured in RPMI 1640 (Gibco-BRL) supplemented with 10% fetal bovine serum. Cells (10^6/ml) were treated with heparitinase (8 mU/ml; Seikagaku, Tokyo, Japan) or chondroitinase (4 mU/ml; Seikagaku) for 1 h at 37°C and then exposed to HIV-1_{IIIB} (2 μg/ml) that had been incubated for 30 min at room temperature in the absence or presence of soluble CD4 (sCD4; 20 μg/ml; gift of R. Sweet, Smith, Kline and Beecham). Bound virus was detected by indirect immunofluorescence with sera from HIV-1-positive humans and fluorescein isothiocyanate (FITC)-conjugated goat antibodies to human immunoglobulin G (IgG) (Cappel) as described previously (35). The vaccinia virus-produced recombinant proteins, isolated from the supernatants of vaccinia virus-infected BSC-1 cells (9), were incubated at 2.0 μg/ml for 1 h with H9h cells. Envelope binding was then detected by incubation first with polyclonal sheep antibodies to HIV-1_{IIIB} gp120 (36) and then with biotinylated rabbit antibodies to sheep IgG and FITC-conjugated avidin (Vector Laboratories). In experiments in which MAbs were used to block gp140 binding to cells, gp140 was incubated with MAbs (10 μg/ml) for 1 h prior to addition to cells. MAbs 110.3, 110.4, and 0.5β were added as ascites fluid at a final dilution of 1:500.

Measurement of anti-gp120 MAb binding to chronically infected H9 cells. H9

TABLE 1. Epitope specificity of MAbs to gp160

Epitope	MAb	Reference(s)
C1	5B3	32
V2	G3.4	20
V2	G3.136	13
V2	6E10	32
V3	110.3	26, 47
V3	110.4	26, 47
V3	BAT123	12
V3	0.5β	8, 27
V3	10D8	32
V3	11G5	32
V3	9284	Dupont
C4	13H8	32
C4	G3.508	45
gp41	50.69	14
CD4[a]	1B1	Waldheim Pharmazeutika
CD4[a]	F105	37, 38

[a] Binding site.

cells chronically infected with HIV-1_{IIIB} were incubated for 30 min with DS (M_r, 5,000; Sigma), HS (bovine intestinal mucosa; M_r, 7,500; Sigma), or dextran (M_r, 10,000; Sigma) at the indicated concentrations (Fig. 5). The cells were then incubated with antienvelope MAbs (10 μg/ml) or sCD4 (1 μg/ml) for 30 min, washed, and then incubated with FITC-conjugated goat antibodies to either human IgG or goat F(ab′)$_2$ to mouse antibodies (Cappel). The sCD4 binding was detected with phycoerythrin-conjugated MAb OKT4 (Ortho Diagnostic Systems). Fluorescein-activated cell scanning analysis was performed as described previously (35).

Semiquantitative PCR. H9h cells (10^6/ml) were incubated in the absence or presence of heparitinase (8 mU/ml) for 1 h at 37°C before the addition of HIV-1_{IIIB} (50 ng/ml). At 0 and 4 h after infection, 5×10^5 cells were removed from the culture and washed with phosphate-buffered saline (PBS), and crude lysates (0.5 ml) were prepared. A control lysate was prepared from the cell line 8E5 (10), which carries one copy of the HIV genome per cell, and diluted for PCR amplification to obtain the copy numbers indicated (Fig. 2). PCR amplification was performed with 10 μl of lysate, the *gag*-specific primers SK38 and SK39, and 1 U of AmpliTaq (Perkin-Elmer Cetus) for 30 cycles (92°C for 1 min, 55°C for 1 min, 65°C for 1.5 min). The PCR products were detected by oligomer hybridization with an excess of SK19 probe end labeled with ^{32}P (34). Products were resolved on a native 10% polyacrylamide gel and visualized by autoradiography.

Immunoprecipitation of ^{35}S-labeled HS with MAbs to gp120 and CD4. H9h cells (10^8) were incubated for 18 h at 37°C with 3 mCi of $Na_2{}^{35}SO_4$ (ICN Radiochemicals) in 50 ml of sulfate-free RPMI 1640 (Gibco-BRL) supplemented with 10% fetal bovine serum. Cells (5×10^6/ml) were then washed, resuspended in serum-free medium with or without gp140 (5 μg/ml), and incubated for 15 min at 37°C followed by 30 min at 4°C. Cells were washed with PBS, lysed in a solution containing 10 mM Tris-HCl (pH 7.4), 1% Nonidet P-40, 150 mM NaCl, 1 mM EDTA, bovine serum albumin (1 mg/ml), 5 mM iodoacetamide, and 1 mM phenylmethylsulfonyl fluoride, and then centrifuged at 10,000 × *g* for 15 min at 4°C. The supernatants were first incubated with protein G-Sepharose beads (Pharmacia) coated with irrelevant immunoglobulin and then incubated overnight at 4°C with beads coated with specific antibody. The beads were washed in lysis buffer without albumin, resuspended in 20 μl of PBS with or without 10 mU of heparitinase, and incubated for 2 h at 37°C. Samples were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 8% gels, and labeled molecules were detected by autoradiography.

RESULTS

Role of HS in HIV-1 binding to and entry into H9h cells. We previously showed that removal of HS by heparitinase treatment of CD4$^+$ T-cell lines resulted in a decrease in both the amount of HIV-1 binding to the cell surface and the level of infection (35). We have now further examined the role of HS in HIV-1 binding and entry with H9h cells, an H9 T-cell clone selected on the basis of its high level of surface CD4 expression. Treatment of H9h cells with heparitinase inhibited the binding of HIV-1_{IIIB} to the cell surface by 50% (Fig. 1). Preincubation of the virus with sCD4 inhibited virus binding to both control and heparitinase-treated H9h cells by 90%. This

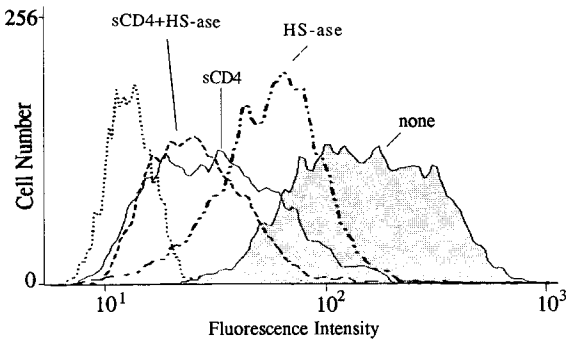


FIG. 1. Effects of heparitinase and sCD4 on binding of HIV-1_{IIIB} to H9h cells. Cells treated or not treated with heparitinase (HS-ase) were exposed to HIV-1_{IIIB} that had been incubated in the absence or presence of sCD4. Virus binding was detected by indirect immunofluorescence with human antiserum to HIV-1 and FITC-conjugated goat antibodies to human IgG. Cells were fixed with paraformaldehyde and analyzed by flow cytometry. The peak cell number of secondary antibody background fluorescence, shown by the dotted line, was half that of the other samples. The results shown here are representative of three experiments.

apparent overlap in virus binding to CD4 and HS is consistent with a fraction of HIV-1 being simultaneously bound to both CD4 and HS.

The effect of heparitinase treatment of H9h cells on early virus entry was studied with semiquantitative PCR amplification to measure the amount of initial reverse-transcribed product. Treatment of cells with heparitinase prior to the addition of virus decreased by greater than 90% the amount of initial reverse-transcribed product measured 4 h after infection (Fig. 2).

Envelope structural requirements for interactions with HS. The interaction between the HIV-1 envelope and cell surface HS was investigated with three soluble forms of recombinant HIV-1_{BH8} envelope protein: gp120, gp140 (a truncated form of gp160 without the gp41 transmembrane region), and gp140N/C (a truncated envelope without a cleavage site between gp120 and gp41). The recombinant gp140 proteins, but not gp120, form stable dimers and higher-order complexes (9). Treatment of cells with heparitinase, but not chondroitinase, reduced cell surface binding of gp140 and gp140N/C but did not inhibit gp120 binding (Fig. 3). These results are consistent with a requirement for oligomerization of the envelope protein or for interaction between gp41 and gp120 for formation of a conformationally stable HS-binding site. The binding of other soluble recombinant gp120 preparations (MN and IIIB) also showed resistance to treatment with heparitinase or soluble heparin (data not shown), indicative of HS-independent interactions with the cell surface.

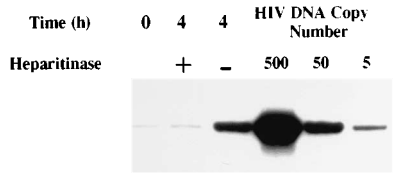


FIG. 2. Effect of heparitinase on entry of HIV-1_{IIIB} into H9h cells. Cells were treated or not treated with heparitinase prior to infection with HIV-1_{IIIB}. DNA in cell lysates prepared 0 and 4 h after infection was subjected to PCR amplification with *gag*-specific primers, and the amplified products were detected with an internal labeled primer by oligomer hybridization. Control samples of known HIV-1 DNA copy number (5 to 500) were analyzed in parallel. The results shown are representative of three experiments.

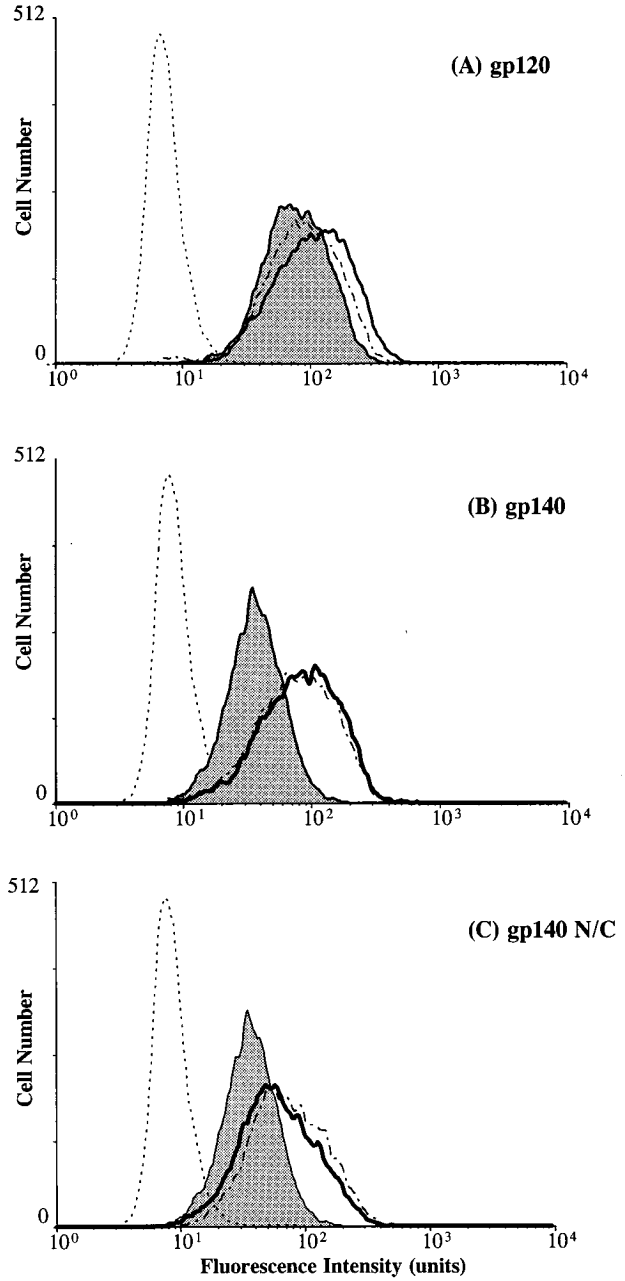


FIG. 3. Effect of heparitinase on the binding of recombinant HIV-1 envelope proteins to H9h cells. Cells were treated or not with heparitinase or chondroitinase (as a control) and then exposed to soluble recombinant gp120 (A), gp140 (B), or gp140N/C (C). The amount of cell-associated envelope was measured by indirect immunofluorescence with sheep antibodies to gp120 followed by biotinylated rabbit antibodies to sheep IgG and FITC-conjugated avidin D. Dotted line, secondary antibody control; bold line, no enzyme treatment; dotted-dash line, chondroitinase treatment; shaded area, heparitinase treatment. The results shown are representative of five separate experiments.

Interaction of gp140 with CD4 and HS. H9h cells were labeled with $Na_2{}^{35}SO_4$, incubated with recombinant gp140, lysed, and subjected to immunoprecipitation with antibodies to gp120 or CD4. SDS-PAGE and autoradiography revealed a heterogeneous population of ^{35}S-labeled molecules of a broad size range (150 to 300 kDa) centered in the range of 180 to 200 kDa that were immunoprecipitated with antibodies to both gp120 and CD4 but not with antibodies to LFA-1, a negative

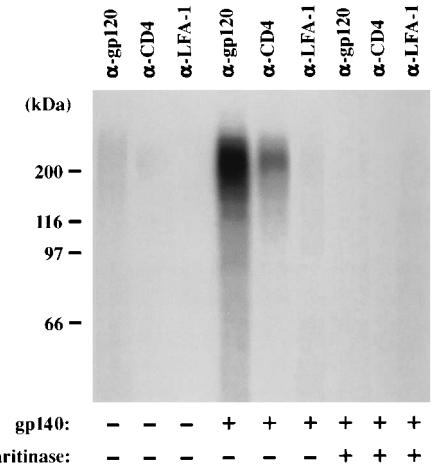


FIG. 4. Formation of a trimolecular complex of gp140, CD4, and HS in H9h cells. Cells were labeled with $Na_2{}^{35}SO_4$, incubated with soluble gp140, lysed, and subjected to immunoprecipitation with a polyclonal sheep antibody to gp120 (α-gp120) or MAbs to CD4 (α-CD4) or LFA-1 (α-LFA-1). Precipitates were incubated in the absence or presence of heparitinase and resolved by SDS-PAGE on an 8% gel, which was then subjected to autoradiography.

control (Fig. 4). Treatment of the immunoprecipitates with heparitinase resulted in degradation of the labeled bands, confirming that they were HS. The heterogeneous HS band is similar in molecular weight to the total proteoglycans from these cells (data not shown) and is consistent with the HS species analyzed by gel filtration in our previous study (35) and with HS proteoglycans described in a study on murine T cells (40). Antibodies to CD4 precipitated ^{35}S-labeled HS only in the presence of gp140, which shows that CD4 is not associated with HS before envelope binding and that the envelope complex binds directly to CD4 and HS simultaneously to form a trimolecular complex on the cell surface.

HS blocks interactions between HIV-1 envelope and antibodies directed against the V3 and C4 domains. To determine the regions on the envelope that interact with HS, we investigated the effects of soluble HS or DS on the binding of a panel of antienvelope MAbs to the surface of H9 cells chronically infected with HIV-1_{IIIB}. Infected cells were incubated with the sulfated blockers and then assayed for MAb binding by indirect immunofluorescence (Fig. 5) as previously described (35). Soluble HS, to a lesser extent than DS, blocked the binding of MAbs to the V3 domain of cell surface-expressed envelope protein. However, differences in the sensitivity of anti-V3 MAb binding to inhibition by HS were evident; for example, the binding of MAb 110.4 was more resistant to HS than the binding of MAb 0.5β, even though DS blocked the binding of both antibodies efficiently. The binding of two antibodies to the C4 domain of the envelope protein, 13H8 and G3.508 (Fig. 5), was also blocked by HS and DS, whereas the binding of MAbs to the CD4-binding site of the envelope protein (F105 and 1B1), the anti-V2 domain MAb 6E10, and the anti-gp41 MAb 50.69, as well as the binding of sCD4, was not blocked by HS (Fig. 5A). Both F105 and 1B1 were partially blocked by DS. The binding of two anti-V2 MAbs, G3.4 and G3.136, was enhanced by DS and HS treatment (Fig. 5B). The blocking of MAb binding to the V3 and C4 regions of gp120 indicates that these regions are possible sites of interaction between the envelope and HS. As a consequence of binding to HS, other regions of gp120, such as V2, may become more exposed on the envelope oligomer.

Effects of antienvelope MAbs on heparitinase-sensitive

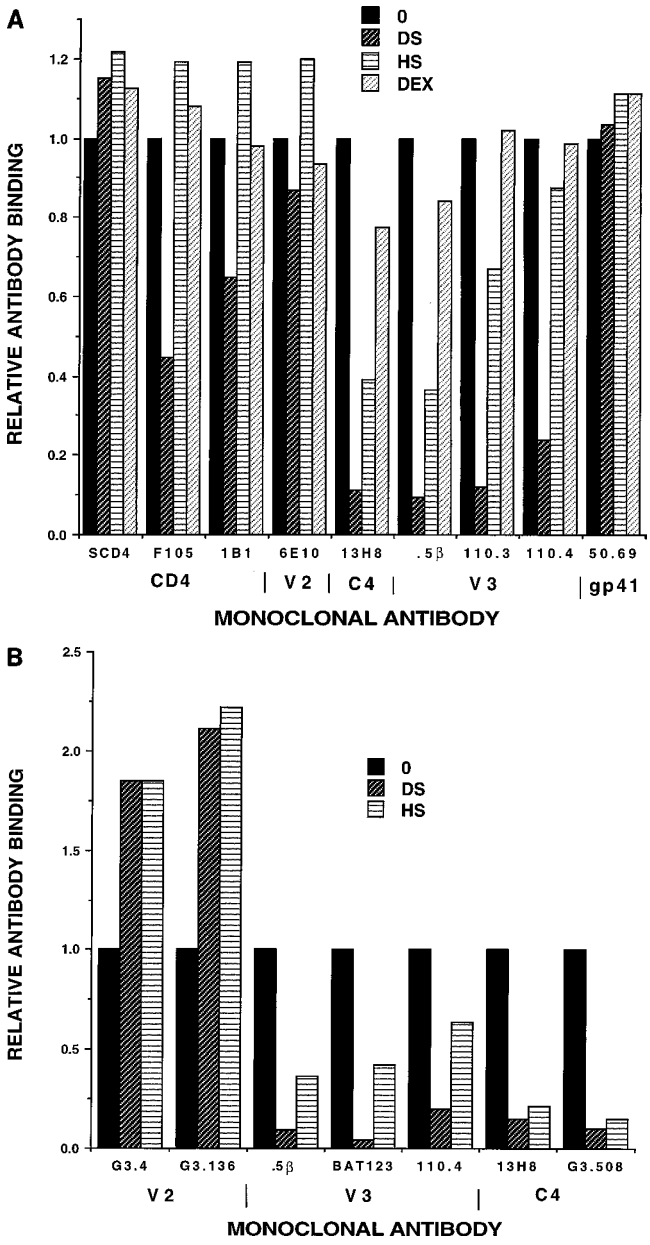


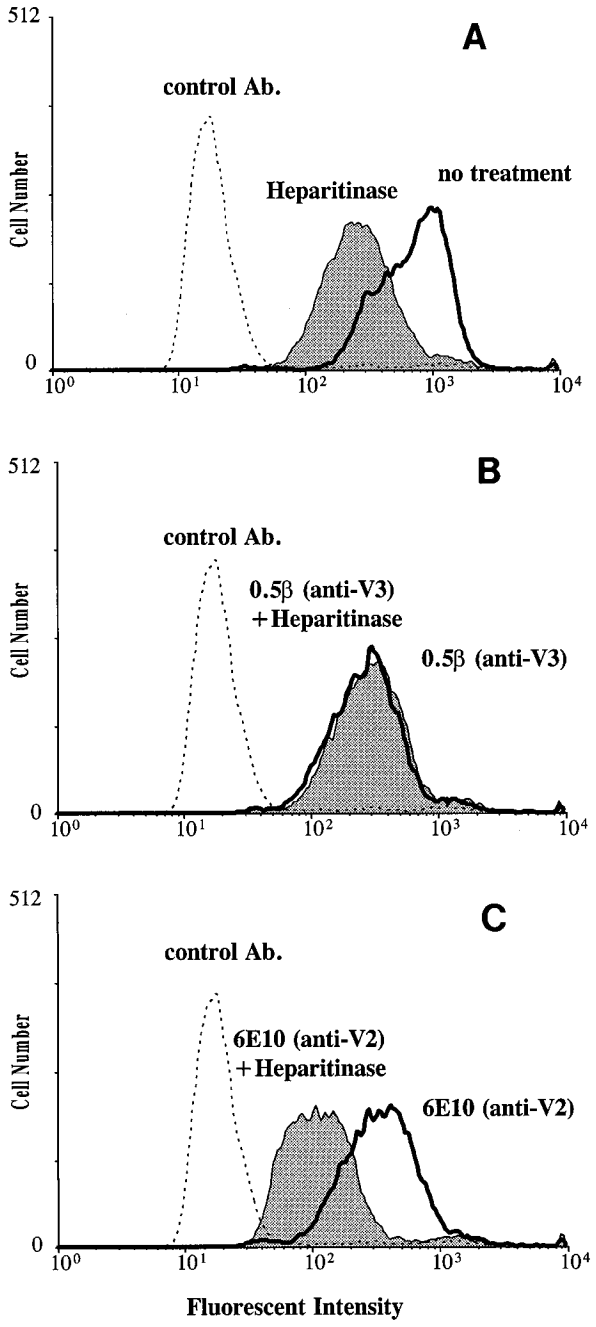
FIG. 5. Effects of DS, HS, and dextran on the binding of antienvelope MAbs to H9 cells chronically infected with HIV-1_{IIIB}. Infected cells were incubated with blockers and then with sCD4 or MAbs. MAb binding was detected with FITC-conjugated secondary antibodies; sCD4 binding was detected with phycoerythrin-conjugated OKT4. (A) The concentrations of DS and dextran (DEX) were 50 μg/ml; the HS concentration was 100 μg/ml. The HIV-specific linear mean fluorescent intensities of MAb binding were as follows: F105, 219; 1B1, 86; 6E10, 277; 13H8, 132; 0.5β, 722; 110.3, 722; 110.4, 838; 50.69, 37. (B) DS and HS were at 100 μg/ml. The intensities of MAb binding were as follows: G3.4, 10; G3.136, 16; 0.5β, 190; BAT123, 55; 110.4, 286; 13H8, 41; G3.508, 13. Envelope protein domains targeted by the MAbs are indicated (V2, V3, C4, CD4 binding, and gp41). The results of two separate experiments are shown.

binding of gp140 to H9h cells. We investigated the effects of both prior treatment of H9h cells with heparitinase and prior incubation of recombinant gp140 with various antienvelope MAbs on the binding of gp140 to the surface of H9h cells. If a MAb blocks the binding of gp140 to the cell surface by binding to the site on the envelope that interacts with cell surface HS, then pretreatment of the cell with heparitinase should not result in further inhibition of envelope binding. Further inhibition of envelope binding by heparitinase treatment would indicate that the MAb binds to a site on the envelope spatially independent of the HS-binding site. Figures 6A to C presents the cytometric profiles for envelope binding to the H9h cells in the presence of antibodies and heparitinase for one representative experiment. Figure 6A shows the total binding in the absence of blockers (no treatment) and the reduced binding caused by heparitinase alone. In comparison with the controls without MAbs (Fig. 6A), Fig. 6B shows that anti-V3 MAb 0.5β alone reduces envelope binding and appears to bind to a site on gp140 that completely overlaps with the HS-binding site, because the extent of inhibition observed with 0.5β was not increased by pretreatment of cells with heparitinase. In contrast, the pattern of inhibition observed with the anti-V2 domain MAb 6E10 indicates that the 6E10-binding site on gp140 does not overlap with the HS-binding site because the extent of inhibition observed with both 6E10 and heparitinase pretreatment exceeded that apparent with 6E10 alone (Fig. 6C). Figure 6D shows another experiment testing the combined effects of a number of MAbs and heparitinase. Two patterns were observed with anti-V3 MAbs: the binding sites of 0.5β, 10D8, and 11G5 showed considerable overlap with the HS-binding site, whereas the binding sites of 110.4 and 9284 had less overlap with the HS-binding site (Fig. 6D). The C4 epitope defined by MAb 13H8 also appeared to overlap only partially with the HS-binding site, even though the binding of this MAb to the envelope expressed on H9 cells was sensitive to soluble HS (Fig. 5). Other sites in V2 (G3.4) and C4 (5B3) showed minimal overlap with the HS-binding site. These results demonstrate that oligomeric envelope binding to the T cell is dependent on multiple envelope sites including CD4, V2, V3, and C4. Of these sites, the V3 domain of the envelope protein is the predominant site of interaction with cell surface HS. The binding of gp140 was completely inhibited by prior incubation with sCD4 in cells treated or not treated with heparitinase.

DISCUSSION

The present data extend our original observation on the role of HS in mediating HIV-1 binding and entry into $CD4^+$ T-cell lines. With the use of an H9 clone (H9h), selected on the basis of its high level of CD4 expression, we observed that the majority of HIV-1 and envelope binding is dependent on cell surface CD4, as shown by blocking assays with sCD4. Heparitinase treatment of H9h cells inhibited HIV-1 binding by about 50%, suggesting that a significant fraction of the virus is attached to the cell surface through both CD4 and HS. Heparitinase treatment also greatly reduced early viral entry into the cells measured 4 h after infection, supporting a role for HS in early cell surface attachment and infection events that precede first-strand DNA synthesis. Further studies are required to determine the possible role of HS in postbinding steps.

Experiments in which HIV-1 binding was modeled with recombinant envelope proteins revealed that the binding of gp140 and gp140N/C, which are capable of oligomerization, showed a greater dependence on HS than the binding of monomeric gp120 to the cell surface. Other soluble recombinant gp120 proteins, including both IIIB and MN types (data not shown), showed a heparitinase-insensitive binding pattern similar to that of vaccinia virus-expressed HIV-1_{BH8} gp120. These observations are consistent with previous results showing that DS did not prevent the binding of recombinant gp120 to soluble or cell-associated CD4 but markedly inhibited virus attachment to the cell surface (4). Thus, the conformation or structure of the envelope appears important for the formation



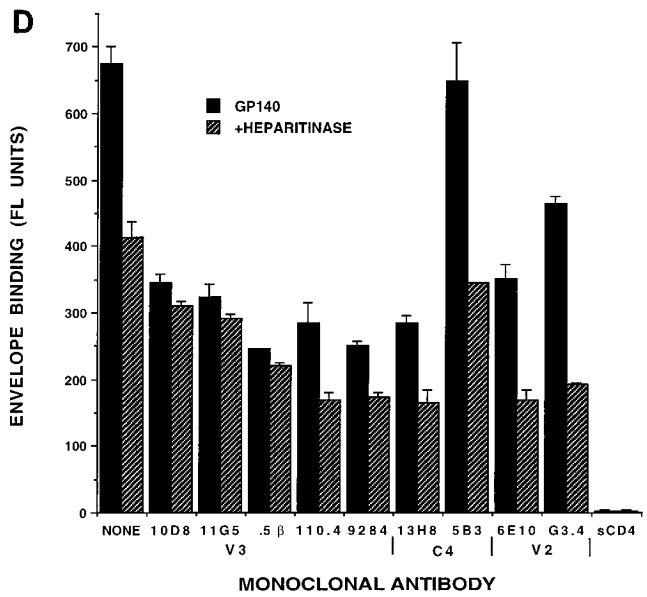


FIG. 6. Effects of heparitinase and antienvelope MAbs on gp140 binding to H9h cells. Cells were incubated in the absence or presence of heparitinase and then exposed to gp140 that had been previously incubated in the absence or presence of antienvelope MAbs or sCD4. Bound gp140 was detected with human antiserum to HIV-1 and FITC-conjugated goat antibodies to human IgG and was quantitated by flow cytometry. Flow cytometric profiles for one experiment are shown in panels A to C for the effects of no MAb (A), MAb 0.5β (anti-V3) (B), or MAb 6E10 (anti-V2) (C) with heparitinase-treated (shaded area) or untreated (bold line) cells. Dotted line, secondary antibody background fluorescence. (D) Summary of results with a panel of MAbs and sCD4. Binding is expressed as the linear HIV-1-specific mean fluorescence (FL) of duplicate samples. Each error bar indicates the range of values obtained from one experiment performed in duplicate. These results are representative of three separate experiments.

of a stable HS-binding site, with oligomerization possibly a key requirement. Envelope binding to HS does not require gp120-gp41 cleavage, which is required for membrane fusion and infection (19, 25). Thus, the HS-binding step apparently occurs before membrane fusion. We showed that HS was immunoprecipitated by antibodies to either gp120 or CD4 from cells that had been preincubated with gp140 (Fig. 4) but not from gp120-treated cells (data not shown). This finding indicates that oligomeric envelope can bind to both CD4 and HS on the cell surface to form a trimolecular complex and that HS is not stably associated with CD4 in the absence of envelope. This result demonstrates interactions of recombinant envelope with CD4 and HS and supports the conclusion that HIV can bind simultaneously to CD4 and HS.

Experiments designed to map the HS-binding site on the HIV-1 envelope revealed that the binding of anti-V3 and anti-C4 MAbs to membrane-bound envelope was inhibited by HS while antibodies to other epitopes were not inhibited. However, the extent of inhibition of anti-V3 MAb binding observed with HS was less than that apparent with DS, suggesting that the HS-binding site does not completely overlap the DS-sensitive V3 site. These results are consistent with direct HS binding to the envelope, similar to our previous finding that DS blocks anti-V3 MAb binding (4), and with a report showing that heparin, which is structurally related to HS, binds to V3 peptide and to recombinant envelope (2). Both V3 and C4 domains are positively charged regions of gp120 (4), which is compatible with an ionic interaction between these domains and negatively charged sulfated molecules. Portions of the V3 and C4 domains may juxtapose each other in the native molecule (31), and therefore these regions together may constitute a single positively charged HS-binding site. However, other mechanisms could be involved in soluble HS blocking of MAb binding to envelope; such mechanisms may include the induction of conformational changes or steric blocking from sites adjacent to the MAb epitope.

The binding of anti-V2 MAbs to membrane-bound envelope was enhanced by both soluble HS and DS. This observation suggests that interactions with HS induces a conformational change in the envelope that increases the exposure of the V2 region. A recent study suggests that in the native envelope, the V2 region may be near the C4 region, close to Ile-418 (11). V2 epitope exposure is also affected by CD4 binding and by antibodies that bind near the CD4 site (30, 42) in a manner com-

parable to V3 epitope exposure (28, 41). DS, previously shown to interact with the V3 region of gp120, destabilizes gp120-gp41 and induces shedding of gp120 (3) from the surface of infected cells, analogous to sCD4-induced shedding (3, 22, 29). Similar to DS, HS did not block sCD4 binding to HIV-1-infected H9 cells. Together, the data support an active role for HS binding in the infection process, given that both the V2 and V3 regions are important for infection, tropism, and neutralization sensitivity (7, 13, 15, 16, 20, 21, 24, 44).

Direct mapping of cell surface HS-binding activity to the V3 region of the envelope was demonstrated by measurement of the effects of heparitinase and antienvelope MAbs on the binding of gp140 to the surface of H9h cells. Oligomeric envelope binding was completely inhibited by sCD4 and partially inhibited by antibodies covering epitopes in V2, V3, and C4. MAbs to the V3 region exhibited the greatest degree of overlap with heparitinase in their ability to block soluble gp140 binding to the T-cell surface. The data show that although soluble HS was capable of blocking both V3 and C4 sites (Fig. 5), the V3 site was the predominant site mediating interactions with cell surface HS. These experiments illustrate that envelope sites differ in their capacity to be blocked by soluble HS and to interact with cell surface HS together with CD4. Epitopes that potentially can interact with HS, such as the C4 site, can be covered with antibody yet leave other envelope sites (V3) accessible to interact with HS.

The anti-V3 MAbs for which the binding to infected H9 cells was most sensitive to soluble HS showed the highest degree of overlap with heparitinase in blocking the binding of soluble envelope to the surface of H9h cells. For example, MAb 0.5β was more sensitive than 110.4 to the blocking effect of HS (Fig. 5) and also showed more overlap than 110.4 with heparitinase in preventing envelope binding (Fig. 6). These two anti-V3 MAbs exhibit a significant overlap in peptide mapping (8, 26), yet they apparently differ in the ability to interact with HS. The observation that the arginine residue in the V3 peptide Gly-Pro-Gly-Arg-Ala-Phe is required for 0.5β binding, but can be substituted by a variety of amino acids without affecting 110.4 MAb binding, suggests that this residue may play a role in HS interactions. The differences in HS sensitivity observed with the various anti-V3 MAbs indicate that HS may bind to but not completely occlude the V3 region and thus leave an adjacent V3 epitope available for binding. Our results are consistent with the possibility that the V3 region interacts with additional molecules, such as glycoproteins, proteoglycans, or glycolipids, before viral fusion with the cell membrane. The observation that not all strain-specific anti-V3 MAbs inhibit binding and neutralize infection to the same extent (26) might be explained by multiple V3 epitopes, each specific for different cellular components, that contribute to HIV-1 binding and infection. One important finding reported here is that the V3 region is involved in virus attachment to the cell surface through HS and possibly other ligand interactions. This function is a property of the oligomeric form of the envelope, which may be more representative of virus binding than monomeric envelope forms. Oligomeric requirements may explain why V3 interactions have not been observed in other studies on gp120 binding (43).

Our data are consistent with initial virus interactions with the cell surface occurring through either HS or CD4. Binding through HS would localize virus to the membrane and thus increase the frequency of a secondary interaction with CD4. Virus-cell interactions would be stabilized by formation of a trimolecular complex via two different binding sites on the same envelope spike. Our mapping studies indicate that the envelope HS-binding site is composed of positively charged residues in both the V3 and C4 regions. It is possible that linear HS chains (33, 49) interact with colocalized positively charged residues of the V3 and C4 domains of two oligomerized envelope subunits. HS binding may alter these associations, thus exposing other regions of the envelope required for additional interactions with the cell membrane that result in membrane fusion.

These studies were performed with the prototypic laboratory isolate HIV-1_{IIIB}, which has syncytium-inducing properties and contains a predominant positively charged V3 region. Primary, monocytotropic strains of virus show differences in the amino acid sequence of the V3 domain that 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. The interaction of HS with other strains of HIV-1 and elucidation of the molecular mechanisms by which HS and other molecules interact with the HIV-1 envelope during virus binding and entry require further study.

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