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Contribution of Virion ICAM-1 to Human Immunodeficiency Virus Infectivity and Sensitivity to Neutralization

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Incorporation of the intercellular adhesion molecule ICAM-1 into human immunodeficiency virus type 1 (HIV-1) particles increased virus infectivity on peripheral blood mononuclear cells (PBMCs) by two- to sevenfold. The degree of ICAM-1-mediated enhancement was greater for viruses bearing envelope glycoproteins derived from primary HIV-1 isolates than for those bearing envelope glycoproteins from laboratory-adapted strains. Treatment of target PBMCs with an antibody against LFA-1, a principal ICAM-1 receptor, was able to nullify the ICAM-1-mediated enhancement. The incorporation of ICAM-1 rendered HIV-1 virions less susceptible to neutralization by a monoclonal antibody directed against the viral envelope glycoproteins. Surprisingly, an antibody against ICAM-1 completely neutralized infection by ICAM-1-containing viruses, reducing the efficiency of virus entry by almost 100-fold. Thus, HIV-1 neutralization by an ICAM-1-directed antibody involves more than an inhibition of the contribution of ICAM-1 to virus entry.

Human immunodeficiency virus type 1 (HIV-1) is the etiologic agent of AIDS (4, 19). HIV-1 infects $CD4^+$ lymphocytes, monocytes, and dendritic cells, eventually causing depletion of $CD4^+$ lymphocytes. The initial attachment of HIV-1 to the target cell involves an interaction between the exterior envelope glycoprotein (gp120) and the cellular receptor, CD4 (9, 27). The formation of the gp120-CD4 complex creates a high-affinity binding site for particular members of the chemokine receptor family (29, 46, 48), which serve as necessary second receptors for HIV-1 (1, 8, 10, 12, 14, 18). Ultimately, the gp41 transmembrane glycoprotein is thought to undergo conformational changes and to mediate fusion of the cellular and viral membranes (6, 28, 42).

In addition to the interactions described above, which are known to be essential for HIV-1 infection, nonessential factors may serve to enhance the infectivity of HIV-1 particles. Previous work has indicated that cell surface proteins, including those of major histocompatibility complex (MHC) class I and II, intercellular adhesion molecule-1 (ICAM-1 [CD54]), lymphocyte function antigen-1 (LFA-1 [CD11a/CD18]), and CD44 can be incorporated into HIV-1 particles (2, 7, 20, 24, 34).

Here we test the hypothesis that a virion-associated cellular adhesion protein can bind its cognate receptor on a target cell, increase the avidity of virus-cell attachment, and enhance virus entry. We also examine whether these adhesion molecule interactions influence the sensitivity of HIV-1 to neutralization by antibodies. ICAM-1 was chosen as a model protein for these studies because it is expressed on a variety of cells relevant to HIV-1 infection (15, 41), has known receptors (LFA-1 and Mac-1 [CD11b/CD18]) which are also expressed on relevant target cells (11, 26, 43), can contribute to the efficiency of HIV-1-induced syncytium formation (23, 37, 47), and has been observed to be associated with HIV-1 particles (7, 34).

We first wished to document the incorporation of ICAM-1 into HIV-1 particles produced in a genetically defined system. The determination of a physical association between a cellular protein and a retrovirus particle can be complicated by the presence of cellular debris containing the protein of interest. In practice, retrovirus preparations are invariably contaminated with cellular debris in the form of vesicles of similar size and density to bona fide virions (35). To control for such contamination, we designed two HIV-1 provirus constructs, one of which does not form particles in transfected cells. Both constructs are derived from an HXBc2 molecular provirus clone from which the *pol* gene was deleted. The first construct, ΔBP4, contains a deletion extending from the 5′ *Bcl*I site (nucleotide 2010) to the second 3′ *Pfl*MI site (nucleotide 4883). In COS-7 cells, this construct directs the synthesis of pseudovirus particles that because of the absence of the viral protease, contain an intact Pr55 Gag precursor. The second construct, ΔBP4G, is identical to ΔBP4 except for a premature termination codon in place of codon 8 of the *gag* gene and an additional frameshift in the CA coding region (13). The ΔBP4G construct is therefore unable to form virus particles.

The ΔBP4 and ΔBP4G plasmids were transfected into COS-7 cells along with a full-length ICAM-1 expression plasmid, p245 (provided by G. Freeman), by the DEAE-dextran technique. At 48 h after transfection, the cells were metabolically labeled with 50 μCi of [^{35}S]cysteine and [^{35}S]methionine per ml. Supernatants were harvested 12 to 16 h later and cleared by low-speed (1,000 × *g*) centrifugation for 10 min at 4°C. The supernatants were then passed through a 0.45-μm-pore-size filter, layered on a cushion of 20% sucrose in phosphate-buffered saline, and pelleted with an SW28 swinging bucket rotor (Beckman, Fullerton, Calif.) at 27,000 rpm for 2 h at 4°C. Pellets were resuspended in Nonidet P-40 (NP40) buffer (0.5 M NaCl, 0.5% NP40, 10 mM Tris [pH 7.5]), and aliquots were immunoprecipitated overnight by the addition of an anti-ICAM-1 monoclonal antibody, 15.2 (Boehringer Mannheim, Indianapolis, Ind.), and protein G-Sepharose (Pharmacia, Piscataway, N.J.). Immunoprecipitates were washed in NP40 buffer and run on a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel (10% polyacrylamide). As shown in Fig. 1, ICAM-1 was present in the pelletable fraction from supernatants of ΔBP4G-transfected cells. This result was

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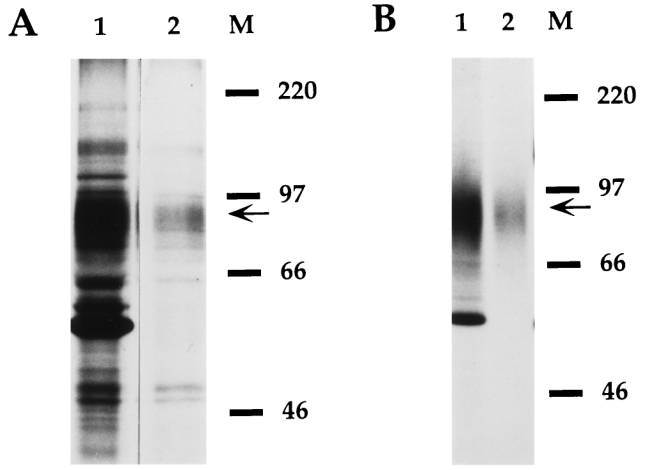

FIG. 1. Proteins associated with transfected cell supernatants. (A) COS-7 cells were transfected with the ΔBP4 (lane 1) or ΔBP4G (lane 2) plasmids and metabolically labeled for 12 to 16 h. The labeled supernatants were sedimented through a sucrose cushion, lysed, and then run directly on an SDS-PAGE gel. Arrows indicate the position of ICAM-1. (B) Sucrose-purified lysates in panel A were precipitated with an anti-ICAM-1 antibody (15.2; Boehringer Mannheim). M, molecular mass markers (kilodaltons).

expected, since some cellular vesicles typically pellet under these conditions (35). The amount of ICAM-1 precipitated from the supernatants derived from the ΔBP4-transfected cells was reproducibly higher than that seen for the ΔBP4G-transfected cells. This result suggests that a substantial fraction of the pelletable ICAM-1 in the supernatants of ΔBP4-transfected cells is virion associated.

Next, using an *env* complementation assay that allows measurement of a single round of HIV-1 infection (22), we sought to determine if the presence of ICAM-1 in the virus-producing cell influenced virus infectivity. Briefly, an HIV-1 (HXBc2) provirus containing a deletion in the *env* gene and a bacterial chloramphenicol acetyltransferase (CAT) gene in place of the *nef* gene (pHXBΔenv CAT) was cotransfected into COS-7 cells with one of several envelope glycoprotein expressor plasmids (pSVIIIenv) in the presence or absence of the plasmid encoding ICAM-1. Viruses were harvested 60 h after transfection, normalized by reverse transcriptase activity, and used to infect phytohemagglutinin (PHA)–interleukin-2 (IL-2)-stimulated peripheral blood mononuclear cells (PBMCs). PBMCs had been purified from whole blood by centrifugation over Ficoll (Pharmacia), and then were washed and resuspended in RPMI medium–10% fetal bovine serum–penicillin–streptomycin with 1 μg of PHA (Murex, Dartford, United Kingdom) per ml. After 48 to 72 h, either recombinant human IL-2 (20 U/ml; Becton Dickinson, Bedford, Mass.) or human T-STIM (5% final concentration; Becton Dickinson) was added to the cultures. Infections were initiated 18 to 30 h after the addition of IL-2 or T-STIM. Virus-containing supernatants were added to 1.5×10^6 stimulated PBMCs in complete medium plus 20 U of IL-2 per ml or 5% human T-STIM in 24-well plates. After 60 h at 37°C, PBMCs were harvested, lysed, and assayed for CAT enzyme activity. Figure 2 demonstrates that the presence of ICAM-1 in the virus-producing cells enhanced the infectivity of viruses bearing envelope glycoproteins derived from either laboratory-adapted (HXBc2, MN) or primary (YU2, ADA) HIV-1 isolates and that this enhancement was significantly greater for viruses bearing primary envelope proteins. The presence of ICAM-1 in the producer cell also enhanced the infectivity of HIV-1 pseudotyped with the envelope glycoprotein from the amphotropic murine leukemia virus 4070 A (36). Interestingly, in the absence of envelope glycoproteins, the presence of ICAM-1 in the virus-producing cells resulted in reproducibly less CAT activity in the target PBMCs than was seen in the absence of ICAM-1. Perhaps the presence of ICAM-1 on noninfectious virus particles facilitates their endocytosis and destruction by the PBMCs.

The observed enhancement might be mediated by an interaction between virion-associated ICAM-1 and a cognate receptor(s) on PBMCs. We sought to determine if an antibody against CD11a, a component of the LFA-1 receptor, could block the effects of ICAM-1 on viral infectivity. Titrated concentrations of mouse anti-CD11a (clone 25.3.1; Immunotech, Westbrook, Maine) were incubated with 1.5×10^6 PHA–IL-2-stimulated PBMCs for 1 h at 37°C. Recombinant viruses bearing the HIV-1 ADA envelope glycoproteins were added to the treated cells, and the efficiency of infection was assayed as described above. In a representative experiment (Fig. 3), the anti-CD11a monoclonal antibody reduced the ICAM-1-mediated enhancement of virus infection in a concentration-dependent manner. The entry of a recombinant HIV-1 produced in the absence of ICAM-1 was not affected by the anti-CD11a antibody. These results suggest that ICAM-1 enhances

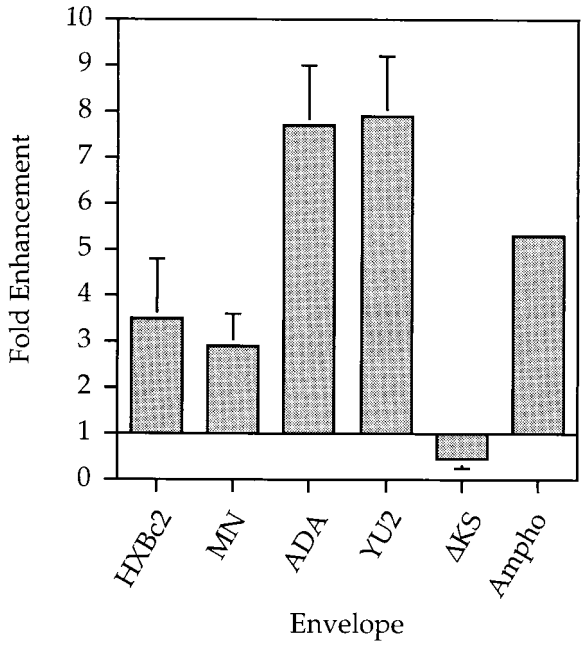

FIG. 2. Effect of ICAM-1 on infectivity of HIV-1 viruses bearing laboratory-adapted or primary HIV-1 envelope glycoproteins. PHA–IL-2-stimulated PBMCs were incubated with recombinant CAT gene-containing viruses bearing laboratory-adapted (HXBc2, MN) or primary (YU2, ADA) HIV-1 envelope glycoproteins with or without ICAM-1, and the efficiency of infection was assayed by measurement of CAT activity. Parallel experiments were conducted with recombinant CAT gene-containing viruses containing the amphotropic murine leukemia virus envelope glycoproteins (Ampho). The fold enhancement of infection attributed to the presence of ICAM-1 represents the efficiency of the ICAM-1$^+$ virus entry compared with that of the ICAM-1$^-$ virus. The values shown represent the average of results from three or more experiments conducted with PBMCs from separate donors and at least two different virus stocks. The ΔKS plasmid contains a deletion extending from a *Kpn*I site (nucleotide 5929) to a *Stu*I site (nucleotide 6412) and encodes a truncated HIV-1 envelope glycoprotein that is not incorporated into HIV particles. The average levels of CAT activity (percentage of acetylation) for a typical experiment were as follows: HXBc2/ICAM-1$^-$, 22%; HXBc2/ICAM-1$^+$, 77%; MN/ICAM-1$^-$, 5%; MN/ICAM-1$^+$, 14%; ADA/ICAM-1$^-$, 8%; ADA/ICAM-1$^+$, 63%; YU2/ICAM-1$^-$, 3%; YU2/ICAM-1$^+$, 21%; Ampho/ICAM-1$^-$, 7%; Ampho/ICAM-1$^+$, 39%; ΔKS/ICAM-1$^-$, 1.77%; ΔKS/ICAM-1$^+$, 0.87%.

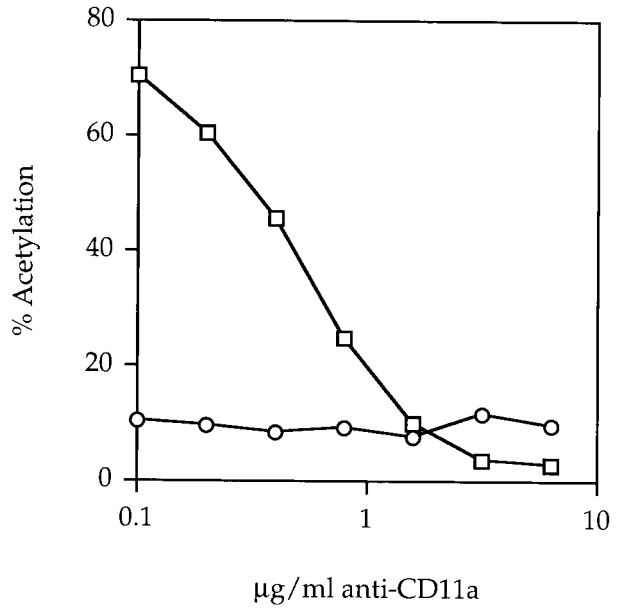

FIG. 3. Effect of a CD11a-directed monoclonal antibody on ICAM-1-mediated enhancement of HIV-1 infectivity. PHA–IL-2-stimulated PBMCs were incubated with the indicated concentrations of anti-CD11a antibody (25.3.1; Immunotech) for 1 h at 37°C. Recombinant CAT gene-containing viruses bearing the ADA-derived HIV-1 envelope glycoproteins with (□) or without (○) ICAM-1 (10,000 cpm of reverse transcriptase activity) were added to the cultures, and the efficiency of infection was assessed by measurement of CAT activity.

HIV-1 infectivity through an interaction with LFA-1 on target PBMCs.

Since ICAM-1 is present on virus particles and appears to bind at least one receptor on PBMCs, ICAM-1^+ virions might be less dependent upon gp120-CD4 interactions for cellular attachment. Therefore, gp120-directed antibodies that interfere with CD4 binding could be less effective at neutralizing an ICAM-1-containing virus compared with a virus lacking ICAM-1. To test this, we incubated different concentrations of the F105 monoclonal antibody (40) with recombinant CAT viruses containing the HXBc2 envelope glycoproteins with or without ICAM-1. F105 is a human anti-gp120 neutralizing monoclonal antibody that recognizes an epitope overlapping the CD4 binding site of gp120 (40). As a result of their greater infectivity, ICAM-1^+ virions were more resistant to neutralization than the ICAM-1^- control virus (Fig. 4A), although the intrinsic neutralizability of viruses by the F105 antibody was not significantly altered by the presence of ICAM-1 (Fig. 4B).

To determine if an antibody to ICAM-1 could neutralize HIV-1, HXBc2-enveloped viruses bearing or lacking ICAM-1 were normalized based upon reverse transcriptase levels and then incubated with different concentrations of mouse anti-ICAM-1 antibody (clone 15.2; Boehringer Mannheim, Indianapolis, Ind.) for 1.5 h at 37°C. PBMCs were then infected and assayed as described above. While viruses bearing ICAM-1 were completely neutralized by the monoclonal antibody, a control virus lacking ICAM-1 was fully infectious, even at the highest concentrations of anti-ICAM-1 (Fig. 5).

Current models of HIV-1 entry predict the formation of a trimolecular complex between HIV-1 gp120, CD4, and a particular member of the chemokine receptor family as a precursor to virus-cell fusion (29, 46, 48). Nonessential factors that facilitate or stabilize the formation of this complex may thus enhance virus entry. Though an ICAM-1–LFA-1 interaction is nonessential for HIV infection (37), LFA-1 has been implicated as a functionally important molecule for the development of syncytia in cultures of HIV-infected cells (23, 47). Recently, an anti-LFA-1 antibody has been shown to synergize with AIDS patient sera in the neutralization of HIV replication (21). Synthetic peptide analogs of ICAM-1 were demonstrated to inhibit HIV replication in MT2 cells (17). Finally, an antibody against LFA-1, but not against ICAM-1, inhibited HIV-1 replication in monocyte cultures (25). These results, while implicating LFA-1–ICAM-1 interactions in HIV-1 spread, were not designed to examine the contribution of these interactions to the efficiency of a single round of virus entry.

Here, we confirm that the cellular adhesion protein ICAM-1 can be incorporated into HIV-1 particles. Several other cellular proteins have been detected in HIV-1 and other retrovirus particles, but mechanisms for virion incorporation of host cell

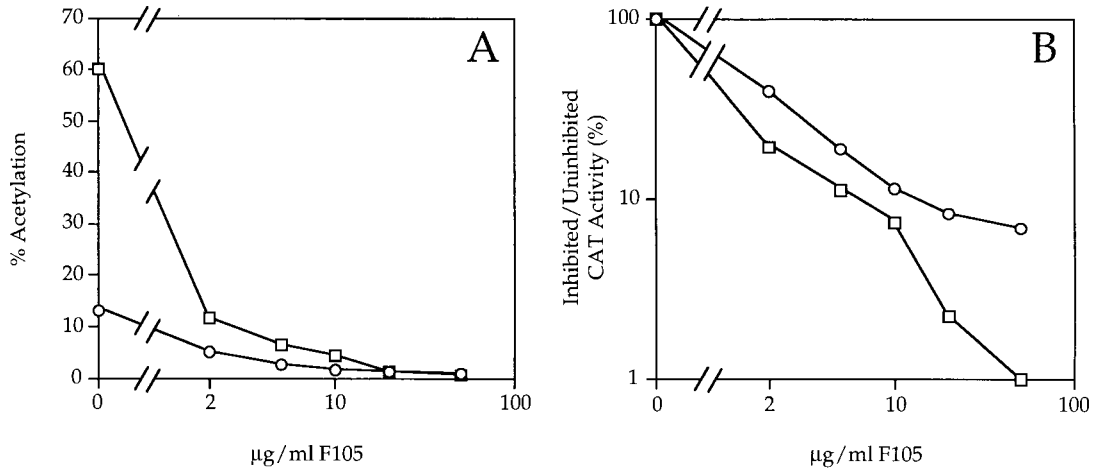

FIG. 4. Neutralization of HIV-1, produced in the presence or absence of ICAM-1, by an anti-gp120 monoclonal antibody. Recombinant CAT gene-containing viruses bearing the HXBc2-derived HIV-1 envelope glycoproteins with (□) or without (○) ICAM-1 (40,000 cpm of reverse transcriptase activity) were incubated with the indicated concentrations of F105 for 1.5 h at 37°C. Infection of PHA–IL-2-stimulated PBMCs was performed, and the efficiency of infection was measured by quantitation of CAT activity (Fig. 4A). In Fig. 4B, the percentage of CAT activity in the target cells observed in the presence of a given concentration of the F105 antibody, relative to the CAT activity observed in the absence of antibody, is shown.

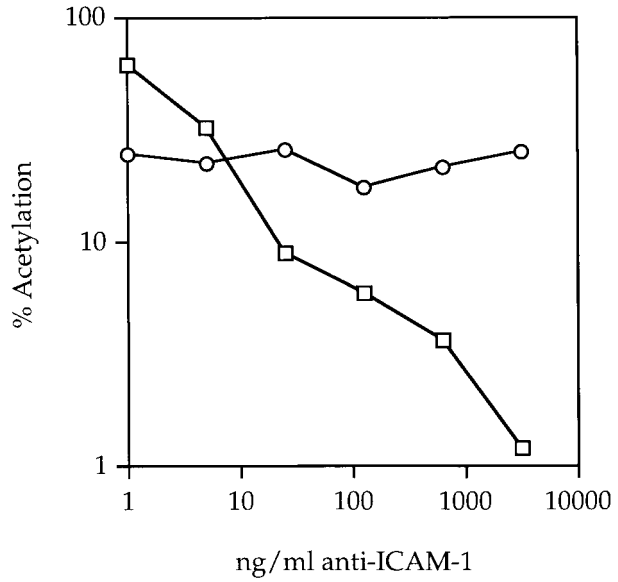

FIG. 5. Neutralization of HIV-1, produced in the presence or absence of ICAM-1, by an anti-ICAM-1 monoclonal antibody. Recombinant CAT viruses bearing the HXBc2-derived HIV-1 envelope glycoproteins with (□) or without (○) ICAM-1 (10,000 cpm of reverse transcriptase activity) were incubated with the indicated concentrations of anti-ICAM-1 antibody (15.2) for 1.5 h at 37°C. Infection of PHA–IL-2-stimulated PBMCs was performed and assayed as described in the text.

surface proteins have not been determined. It is possible that cellular glycoprotein incorporation represents a random sampling of the proteins expressed at the cell surface at the time of budding, but it is more likely that some level of selection takes place (2, 24, 34). Structural aspects of cellular glycoproteins that determine virion incorporation could include the length of the cytoplasmic tail or the nature of the transmembrane domain. Additionally, ICAM-1 and other cell surface proteins might localize to preferred sites for HIV-1 budding (16, 30, 38).

The presence of ICAM-1 in the virus-producing cell and on the virion correlated with enhanced virus infectivity. The effect was more pronounced for viruses bearing envelope glycoproteins derived from primary HIV-1 isolates than for viruses with envelope glycoproteins derived from laboratory-adapted strains. ICAM-1-mediated enhancement of HIV-1 entry may thus be of greater importance for primary viruses, which have envelope glycoprotein oligomers that exhibit a lower affinity for CD4 (33). Our data suggest that ICAM-1 on virus particles functions in a manner similar to that seen in cell-cell interactions, since the effect was eliminated by an anti-CD11a monoclonal antibody, 25.3.1, which is known to block LFA-1–ICAM-1 binding and which exhibits inhibitory activity in LFA-1-dependent functional assays (39).

HIV-1 neutralization by the monoclonal antibody F105 was less efficient for ICAM$^+$ viruses than for viruses lacking ICAM-1. Our results are consistent with those of Berman et al., who found that syncytium formation was refractory to blocking with envelope glycoprotein-directed monoclonal antibodies when cells were transfected with ICAM-1 (5). At higher concentrations of F105, ICAM-1$^+$ viruses were completely neutralized, suggesting that gp120 interactions affected by F105 remain indispensable to HIV-1 entry. Indeed, only the level of virus infectivity (Fig. 4A) and not the intrinsic neutralizability with F105 (Fig. 4B) was altered by the presence of ICAM-1 on virions. Nonetheless, the improved replicative efficiency of ICAM-1-containing viruses practically reduces the efficacy of gp120-directed antibodies and thus may contribute to the ability of HIV-1 to evade the immune system.

Treatment of viruses generated in the presence or absence of ICAM-1 with a monoclonal antibody against ICAM-1 (15.2) resulted in the complete inhibition of ICAM-1$^+$ virus entry, while ICAM-1$^-$ virions remained fully infectious at all antibody concentrations tested. It is intriguing that an antibody against a cellular protein could efficiently neutralize HIV-1. Previous studies have shown that immunization of monkeys with human MHC class II proteins provided protection from challenge with simian immunodeficiency virus grown in human cells (3, 44). Stott et al. demonstrated that this protection could be passively transferred to naive animals by inoculation with sera from monkeys immunized with murine L cells expressing HLA-DR4 (45). Our results demonstrate that cellular proteins on the virion surface other than MHC class II molecules can serve as efficient neutralization targets. Furthermore, our data indicate that sufficient concentrations of bound antibody on a virus can effectively decrease infectivity by mechanisms that extend beyond mere inhibition of the contribution of the targeted molecule to virus entry. Anti-ICAM-1 antibodies might sterically inhibit HIV-1 envelope glycoprotein function. This could be particularly relevant if the concentration of ICAM-1 on the virus surface is greater than the concentration of the envelope glycoproteins. In these experiments, at least two factors could contribute to an excess of ICAM-1 on the virus surface. First, viruses were produced in transient transfections in which the cell surface level of ICAM-1 was likely to be very high. Second, incorporation of ICAM-1, unlike incorporation of the HIV-1 envelope glycoproteins, might not be constrained by interactions with viral matrix protein (13, 49). Alternatively, the anti-ICAM-1 monoclonal antibody (mouse immunoglobulin G1) could induce the formation of noninfectious virus aggregates by cross-linking ICAM-1 on multiple virions. Finally, although unlikely to be operative in our system, complement-mediated virolysis has been proposed as a mechanism of virus neutralization for antibodies directed against host cell proteins (31, 32).

Further studies may clarify our understanding of the role of host cell proteins in HIV-1 entry and suggest new avenues for intervention.

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