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Antibody to Adhesion Molecule LFA-1 Enhances Plasma Neutralization of Human Immunodeficiency Virus Type 1

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We have shown that a monoclonal antibody to the cell surface adhesion molecule LFA-1 (CD18/CD11a) enhances plasma neutralization of a laboratory isolate (HIV_{MN}) and a primary isolate (HIV_{28R}) of human immunodeficiency virus type 1. Human phytohemagglutinin blasts were infected with HIV_{MN} or HIV_{28R} in the presence of plasma pooled from HIV-positive individuals (AIDS plasma) or immunoglobulin G from AIDS plasma alone or combined with a monoclonal antibody (MAb) to LFA-1. While AIDS plasma alone at a dilution of 1:1,250 neutralized HIV_{MN} and HIV_{28R} infection by 15 and 0%, respectively, in the presence of a saturating concentration of the MAb to LFA-1 the plasma neutralized both viruses by more than 80% at this dilution. Immunoglobulin G purified from AIDS plasma, when used in combination with the MAb to LFA-1, showed the same synergistic effect in HIV neutralization as seen with the AIDS plasma and anti-LFA-1. The MAb against LFA-1 partially neutralized both viral isolates (45 to 55%) on its own. These results demonstrate significant synergy between the plasma and antibody against LFA-1 in the neutralization of HIV. The observations therefore suggest an important role for adhesion molecules in HIV infectivity and transmission. The results have implications for the recently observed host effect on HIV susceptibility to antibody neutralization.

Human immunodeficiency virus type 1 (HIV-1) is the causative agent of AIDS, a chronic immunosuppressive disease leading to severe opportunistic infections and neurological disorders (7, 21). CD4 has been shown to be the principal cell surface receptor for HIV-1 on T lymphocytes and macrophages (5, 22). CD4 is the phenotypic marker for helper T cells and plays a role in antigen recognition and presentation through its interaction with class II major histocompatibility complex (MHC) molecules on antigen-presenting cells (6, 8). Binding of CD4 to gp120, a surface glycoprotein of the virus, is thought to lead to exposure of the fusion domain of the transmembrane protein gp41 and fusion of the envelope of HIV-1 with the plasma membrane of the cell (23, 36). The events leading up to membrane fusion are still not fully understood. Moore and colleagues have reported that association of gp120 and soluble CD4 leads to exposure of the fusion domain of gp41 (25, 26). They suggest that a conformational change in the HIV envelope protein uncovers the region of gp41 necessary for fusion of the virus envelope with the host cell membrane. Therefore, an accessory molecule could, by tethering virus particles to the cell surface, facilitate binding of gp120 to CD4, thereby increasing the rate of exposure of the gp41 fusion domain and enhancing HIV infection.

Several investigators have reported that cell membrane proteins are incorporated into retroviruses (19, 20). Both class I and class II MHC molecules are incorporated into the membrane of HIV as it buds from host cells (1, 9, 10). In addition, antisera against class I and II MHC molecules have been shown to block HIV infection (10). HIV appears to acquire complement factors from host cells that affect complement-mediated virolysis (30). We have recently demonstrated the acquisition of adhesion molecules by HIV and simian immunodeficiency virus from host cells (27). One of the adhesion molecules acquired by the viruses is LFA-1. This adhesion molecule is present on the surface of all leukocytes and is involved in a wide range of lymphocyte functions involving cell-cell adhesion (34, 35). Studies in our laboratory and those of others have established an important role for this cell adhesion molecule in virus-induced syncytium formation and virus infectivity (3, 12, 15, 18, 37). The acquisition of adhesion molecules by budding viruses could provide an additional ligand-receptor interaction between cell and virus which would stabilize the interaction between CD4 and gp120. This increase in binding avidity between virus and cell could lead to a decrease in the effective rate of CD4-gp120 disassociation and to increased gp41-mediated fusion with the plasma membrane. The overall result would be a more efficient virus infection. Such a model predicts that there may be synergy in virus neutralization by antibodies to virus glycoproteins and antibodies to cell adhesion molecules.

In the present study we show that a monoclonal antibody (MAb) against LFA-1 is able to enhance neutralization of a laboratory isolate and of a primary isolate of HIV-1 by plasma pooled from HIV-positive individuals (AIDS plasma) and by immunoglobulin G (IgG) purified from this AIDS plasma. This antibody, which only partially neutralizes HIV_{MN} and HIV_{28R} infection by itself, appears to act in synergy with plasma from AIDS patients and with IgG purified from plasma.

MATERIALS AND METHODS

Preparation of cells. Peripheral blood mononuclear cells from healthy human volunteers were prepared by Ficoll-Hypaque (Pharmacia, Piscataway, N.J.) gradient centrifugation of blood diluted in phosphate-buffered saline. The cells were cryopreserved in 90% fetal calf serum (HyClone, Logan, Utah)–10% dimethyl sulfoxide (J. T. Baker, San Francisco, Calif.) and stored in the vapor phase of liquid nitrogen. The same batch of pooled peripheral blood mononuclear cells was used for all neutralization assays. Blasts were generated from frozen aliquots of peripheral blood mononuclear cells by culturing in RPMI 1640 (Whittaker, Walkersville, Md.) containing 10% fetal calf serum, 2 mM L-glutamine, 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), and 1 μg of phytohemagglutinin (PHA) (Burroughs Wellcome, Research Triangle Park,

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N.C.) per ml for 3 days. PHA blasts were then washed and resuspended in cRPMI (RPMI, 10% fetal calf serum, 2 mM L-glutamine, 10 mM HEPES).

Virus. (i) HIV_{MN}. Virus stocks of HIV_{MN} (obtained from Robert Gallo through the National Institutes of Health AIDS Repository) were prepared in Jurkat T cells cultured in cRPMI. The cells were pelleted by centrifugation, and the culture supernatant was filtered through a 0.45-μm-pore-size filter. The infectious virus titer of the viral stock was determined on PHA blasts and found to be 10^5 50% tissue culture infective doses ($TCID_{50}$s) ml^{-1}. This virus stock was diluted 1:100 (final, 10^3 $TCID_{50}$s ml^{-1}), and 100 μl was added to 10^5 PHA blasts in neutralization assays.

(ii) HIV_{28R}. Virus stocks of HIV_{28R} were prepared by coculturing cells from an AIDS patient (visiting the neurological clinic at Johns Hopkins Hospital, Baltimore, Md.) with PHA blasts in cRPMI and recombinant interleukin 2 (Boehringer Mannheim) (25 U/ml) for 5 weeks. Fresh recombinant interleukin 2-containing medium was added every seventh day at 25 U/ml. The viral supernatant was collected as described above, and the infectious virus titer was determined on PHA blasts ($TCID_{50} = 5 \times 10^3$ ml^{-1}). This virus stock was diluted 1:5 (final, 10^3 $TCID_{50}$s ml^{-1}), and 100 μl was added to 10^5 PHA blasts in neutralization assays.

MAbs and antisera. The MAbs used were H52 (anti-LFA-1β, CD18) (16), PLM-2 (anti-LFA-1β, CD18; recognizes a nonfunctional epitope) (17), SIM.7 (anti-CD4), and Gag.M1 (anti-HIV p24 core protein). SIM.7 and Gag.M1 MAbs were produced in our laboratory with a previously described protocol (17), using SupT1 cells and recombinant p24, respectively, as the immunogens. Immunoglobulins were purified from ascites fluid by protein G-Sepharose (Pharmacia) affinity chromatography. In neutralization assays, the MAbs were used at 20 μg/ml, which was saturating for the number of cells used in the assays. Plasma samples obtained from HIV-positive individuals at different stages of infection were obtained from Walter Royal (Department of Neurology, Johns Hopkins Medical School) and pooled for use as a positive control (AIDS plasma). IgG was purified from the pooled HIV-positive plasma by protein G-Sepharose affinity chromatography. Normal human sera were obtained from GIBCO (Grand Island, N.Y.).

Neutralization assay. The HIV neutralization assay was performed as follows. PHA blasts were cultured at a density of 10^5 per well in 96-well round-bottom tissue culture plates (Corning Glass Works, Corning, N.Y.) in cRPMI containing interleukin 2 (25 U/ml). Fifty microliters of MAb, diluted plasma, or purified IgG from pooled HIV^+ plasma diluted in cRPMI was added to the appropriate wells. One hundred microliters of virus stock (HIV_{MN} or HIV_{28R}), diluted appropriately in cRPMI, was then added to the wells. Virus was also added to mock wells containing medium with no cells. These wells were processed identically to positive control wells and provided controls for virus input. The plates were incubated for 40 to 48 h at 37°C and 5% CO_2. The cells were washed three times with incomplete RPMI and resuspended in 200 μl of cRPMI containing recombinant interleukin 2 (25 U/ml). On day 9 from initiation of the neutralization assay, supernatants were collected and, after the addition of Triton X-100 to a final concentration of 1%, were assayed for HIV p24 core protein. Percent neutralization was calculated as $100 \times [1 - (\text{experimental p24} - \text{input p24})/(\text{positive control p24} - \text{input p24})]$, where p24 is expressed in picograms per milliliter. The input virus p24 value was usually below the limit of detection in the p24 assay. Virus stocks were diluted appropriately on the basis of $TCID_{50}$ titers so that equal input virus was used in all assays.

p24 antibody capture assay. Measurement of virus particle p24 was determined by an antibody capture assay (13). In summary, flat-bottom 96-well plates (Costar, Cambridge, Mass.) were coated with a MAb, Gag.M1, to p24 core protein. After the wells were washed and blocked with bovine serum albumin (Sigma, St. Louis, Mo.), viral supernatant lysates were added to the wells at various dilutions along with samples of known p24 concentration, in order to establish a standard curve. Following overnight incubation, the plates were washed, and biotinylated HIV^+ IgG was then added. After a 2-h incubation, the plates were washed, and streptavidin-peroxidase conjugate was added; this was followed by the addition of the substrate (240 μg of tetramethyl benzidine per ml and 0.015% H_2O_2 in 4 M sodium acetate, pH 4.0). Unknown p24 values were calculated on the basis of regression analysis of p24 standards over a linear range of 15 to 1,000 pg/ml.

RESULTS AND DISCUSSION

In order to determine if a MAb to the functional epitope of LFA can inhibit HIV infection, a neutralization assay was set up, using PHA blasts as host cells and two MAbs to LFA-1. As described in Materials and Methods, H52 and PLM-2 react with functional and nonfunctional epitopes of LFA-1, respectively (16, 17). A MAb to CD4, SIM.7, was used as a positive control for virus inhibition. As shown in Fig. 1A, H52 neutralized HIV_{MN} infection of PHA blasts by 45 to 65%, while the control MAb PLM-2 did not neutralize HIV_{MN}. Similarly, as seen in Fig. 1B, H52 neutralized infection of PHA blasts by the primary isolate HIV_{28R} by 42 to 50%. PLM-2 again failed to neutralize infection of PHA blasts in the case of HIV_{28R}. This

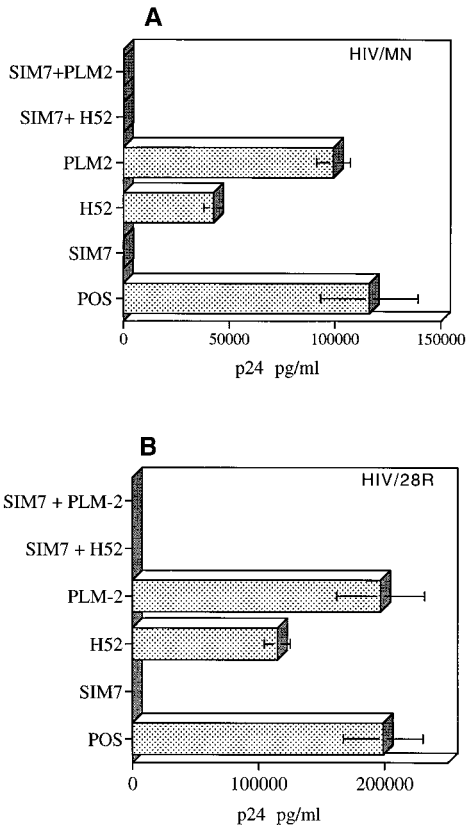

FIG. 1. Neutralization of HIV by MAbs against CD4 and LFA-1. Human PHA blasts were used in neutralization assays as described in Materials and Methods. The cells were infected with HIV_{MN} (A) or HIV_{28R} (B). The multiplicity of infection was 0.001 in the absence and in the presence of a MAb to CD4 (SIM.7) and/or MAbs to LFA-1 (H52, PLM-2) (20 μg/ml). PLM-2 is an anti-CD18 antibody which recognizes a nonfunctional epitope of CD18. The data shown are representative of four experiments. POS, cells and virus with no inhibitor added.

range of inhibition for H52 was observed in several experiments for both laboratory and primary isolates and indicated a significant role for the adhesion molecule in HIV infection. The anti-CD4 MAb SIM.7 completely inhibited infection by both laboratory and primary virus strains. Virus infection was also completely inhibited by the combination of anti-CD4 and anti-LFA-1 MAbs. Neither H52 nor SIM.7 MAb had any toxic effect on cells, as determined by a formazan dye cell viability assay (data not shown). The H52 MAb inhibits activation of T cells by PHA but has no effect on cell proliferation after the cells have already been activated (18a).

The capacity of AIDS plasma to neutralize HIV_{MN} and HIV_{28R} infection of PHA blasts was determined by performing neutralization assays as described in Materials and Methods. Anti-LFA-1 MAb H52 was added to AIDS plasma to determine if the combination of H52 and AIDS plasma would result in greater neutralization of virus than that seen with either treatment alone. PLM-2, which recognizes a nonfunctional epitope on LFA-1, was used to control for the presence of MAb. Since our goal was to determine if plasma neutralization was more efficient in the absence of LFA-1 function, we used the anti-LFA-1 MAb at a constant saturating concentration of 20 μg/ml. The anti-LFA-1 MAb inhibited HIV_{MN} and HIV_{28R} infection by 60 and 42% respectively (Fig. 2). As shown in Figure 2A, pooled AIDS plasma exhibited a plateau level of

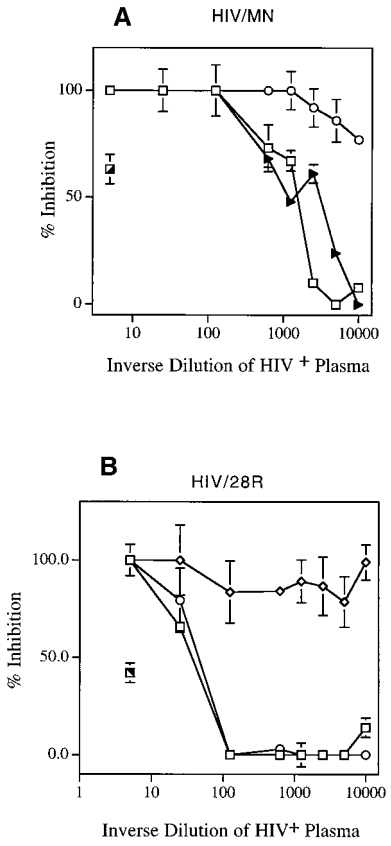

FIG. 2. Synergistic neutralization of pooled AIDS plasma and MAbs against LFA-1. Neutralization assays were carried out with human PHA blasts as described in Materials and Methods. The cells were infected with HIV_{MN} and HIV_{28R} in the presence of dilutions of pooled AIDS plasma alone or in combination with MAbs to LFA-1 (H52 and PLM-2; 20 μg/ml). Data shown are values for percent inhibition of p24 production relative to controls with no plasma or MAb. p24 values in the absence of plasma or MAb were 142,800 and 230,000 pg/ml for HIV_{MN} and HIV_{28R}, respectively. The data shown are representative of three experiments. (A) ◪, H52 (20 μg/ml); □, HIV^+ plasma; ○, HIV^+ plasma + H52; ▶, HIV^+ plasma + PLM2. (B) ◥, H52 (20 μg/ml); □, HIV^+ plasma; ◇, HIV^+ plasma + H52; ○, HIV^+ plasma + PLM2.

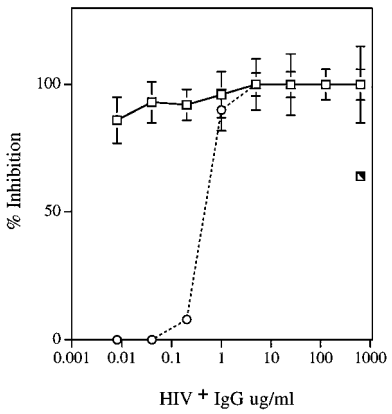

FIG. 3. Synergistic neutralization of HIV_{28R} by HIV^+ IgG and a MAb against LFA-1. Human PHA blasts were infected with HIV_{28R} in the presence of dilutions of HIV^+ IgG alone or in combination with a MAb to LFA-1 (H52 at 20 μg/ml). Data shown are values for percent inhibition of p24 production relative to controls with no inhibitor added (195,600 pg/ml). Data shown are representative of two experiments. ◥, H52 (20 μg/ml); ○, HIV^+ IgG; □, HIV^+ IgG + H52.

inhibition of 100% out to a dilution of 1:125 for the laboratory isolate HIV_{MN}. The inhibition dropped off sharply beyond this dilution, and no neutralization was seen at dilutions greater than 1:1,250. In contrast, when the same dilutions of pooled AIDS plasma were used in combination with the anti-LFA-1 MAb at 20 μg/ml, complete inhibition was observed out to a dilution of 1:1,250. The inhibition by the combination of plasma and anti-LFA-1 MAb dropped off very gradually, with 77% neutralization seen at the highest dilution of 1:10,000. Figure 2B shows results obtained for neutralization of the primary isolate. Pooled AIDS plasma exhibited 100% inhibition only at the lowest dilution of 1:5 for HIV_{28R}, confirming previous observations of higher resistance to serum neutralization of primary HIV isolates. The inhibition dropped off sharply beyond this dilution, and no neutralization was seen at dilutions greater than 1:125. As seen for the laboratory isolate, when the same dilutions of pooled AIDS plasma were used in combination with the anti-LFA-1 MAb, complete inhibition was observed out to a dilution of 1:125, with a very gradual decrease to 85% neutralization at 1:10,000. The control antibody showed only a marginal effect on neutralization by the pooled plasma of either isolate. Normal human serum had a minimal effect on virus infection, and its neutralization of HIV in combination with H52 was no greater than that for H52 alone (data not shown). These results suggested a role for LFA-1 in HIV infection, as indicated by the ability of the anti-LFA-1 MAb to significantly potentiate virus neutralization by dilutions of AIDS plasma which exhibited no neutralization alone.

In order to confirm that the MAb to LFA-1 was enhancing HIV neutralization by antibodies to HIV and not other plasma components, IgG purified from the AIDS plasma was used in a neutralization assay at various dilutions in the presence or in the absence of the anti-LFA-1 MAb. As shown in Fig. 3, HIV^+ IgG completely neutralized the primary isolate HIV_{28R} at concentrations of 5 μg/ml and greater. Little or no inhibition of HIV_{28R} by HIV^+ IgG was seen at concentrations of less than 1 μg/ml. When the anti-LFA-1 MAb was used in combination with the HIV^+ IgG, near complete neutralization was observed at all IgG concentrations tested, with 85% inhibition seen at the lowest concentration of HIV^+ IgG used, 8 ng/ml. These results confirmed that the synergy in virus neutralization occurred between the LFA-1 antibody and antibodies against HIV present in the AIDS plasma.

We have shown that a pool of AIDS plasma is able to neutralize HIV_{MN} and HIV_{28R} only at low dilutions. However, when an antibody to LFA-1 was combined with the AIDS plasma, neutralization of HIV was observed even at very high plasma dilutions. Furthermore, this effect was confirmed with IgG purified from the AIDS plasma. The synergy observed, between anti-HIV antibodies in AIDS plasma and anti-LFA-1 antibody in HIV neutralization, further implicates adhesion molecules in the biology of HIV.

The exact mechanism by which anti-LFA-1 antibody potentiates virus neutralization by AIDS plasma remains to be determined. We propose that adhesion molecules acquired by virus provide an accessory interaction between the virus and cell, thereby increasing the overall binding avidity. Blocking the adhesion molecules and thereby reducing the binding avidity would, therefore, increase virus sensitivity to neutralization by anti-HIV antibodies. We have previously shown that membrane proteins, including major adhesion molecules (LFA-1, CD44), CD63, CD43, CD8, and MHC class I and II are incorporated into HIV and simian immunodeficiency virus (27). In

addition, Arthur and colleagues have reported that HIV acquires MHC class I and II molecules upon budding from cells (1). Moreover, they showed that antibodies to these molecules were able to inhibit virus infection. Others have demonstrated that CD55 and CD59 acquired by HIV from host cells render the virus susceptible to lysis by complement in the presence of anti-CD55 and anti-CD59 antibodies, while virus grown in cells not expressing these surface molecules was insensitive to complement lysis. Using the CD44 hyaluronan receptor as a model system, we have now demonstrated for the first time that adhesion molecules on HIV are functional (14). Therefore, the observed enhancement of neutralization demonstrated by LFA-1 antibody in the present studies may be due to the involvement of cell adhesion molecules in virus entry, whereby blocking binding between LFA-1 on the membrane of the virus and intercellular adhesion molecule 1 (ICAM-1) on the PHA blasts (or vice versa) significantly lowers the avidity of virus binding. The lower avidity would result in more efficient neutralization by the anti-HIV IgG.

The H52 MAb alone inhibited infection by HIV produced in PHA blasts and in Jurkat cells to about the same extent, even though PHA blasts express three to five times as much LFA-1 as do Jurkat cells (18a). However, HIV produced in Jurkat cells does carry LFA-1 (27), and results of recent studies show that capture of HIV from Jurkat cells with anti-LFA-1 MAb was as efficient (relative to control antibodies) as capture of HIV from PHA blasts (18a). This result indicates that LFA-1 on HIV particles may be enriched relative to some host cells and that HIV produced in Jurkat cells, like HIV from PHA blasts, may be subject to neutralization by direct binding of anti-LFA-1 MAb to the virus particles. In the case of virus produced in PHA blasts, the virus could acquire either ICAM-1 or LFA-1 making it possible for the interaction to be through ICAM-1 on the virus binding to LFA-1 on the cells.

We and others have previously demonstrated a role for LFA-1 in HIV-induced syncytium formation and infection (15, 18). gp160-transfected cells expressing greater amounts of ICAM-1 are less sensitive to inhibition of syncytium formation by anti-gp120 MAb and sCD4 (2). Results of other studies suggested that antibodies to LFA-1 block HIV infection at a level other than that of inhibition of cell-to-cell transmission of virus (28, 29). A MAb against ICAM-3 has been shown to inhibit HIV entry but not syncytium formation between CEM-X174/$_{LAI}$ and SUPT-1 cells (33). These observations clearly indicate that cell adhesion molecules, LFA-1 in particular, play an important role in HIV biology. The molecule appears to play a role in the binding and entry of the virus as well as in HIV-mediated cell-cell fusion and syncytium formation, the latter possibility implying a potentially important role for LFA-1 in cell-to-cell transmission of HIV. Therefore it is possible that antibodies to LFA-1 and gp120 show synergy in HIV neutralization, because together they efficiently inhibit attachment and entry of free virus as well as any subsequent transmission of virus by the cell-to-cell route. Our data do not definitively show a direct role for adhesion molecules on the virus particles. The observed effect could be explained by inhibition of cell-to-cell transmission by binding of the anti-LFA-1 MAb to the cells alone. The relative importance of these two routes of infection and that of adhesion molecules on virus particles are the subjects of ongoing studies in our laboratory.

The number of host cell surface molecules acquired by a virus as it buds from a cell would presumably be determined by the state of the cell at the time. Therefore, a virus budding from an activated normal CD4$^+$ T cell might acquire a greater number of adhesion molecules than one budding from cell lines, since activated T cells generally express much higher levels of adhesion molecules. This is especially true of PHA blasts, which are commonly used to passage primary HIV isolates. Differential acquisition of adhesion molecules by HIV may result in differential sensitivity to serum neutralization. Indeed, Sawyer and colleagues have reported differential neutralization of HIV by antibodies to gp120 and CD4, dependent on the type of cell from which the virus buds (31). We also have data showing that passage of HIV through different cell types alters sensitivity to neutralization (11a). Interestingly, it has been reported that HIV-1 primarily infects CD4$^+$ memory T cells, which are characterized by high expression of adhesion molecules (32). Several published studies have shown that soluble CD4 and sera from gp120 vaccinees were unable to neutralize primary isolates as efficiently as laboratory isolates (4, 11, 24, 32). This observation may be explained by the binding of adhesion molecules on HIV acquired from PHA blasts to counterreceptors on target cells and the resulting increase in the overall binding avidity of the virus to the cells. The result of this phenomenon would be decreased sensitivity to serum neutralization, especially where neutralization is primarily through blockade of CD4-gp120 interaction.

It has been our hypothesis for some time that adhesion molecules on virus-susceptible cells and the virus particles themselves play an important role in virus infection (13, 18, 27). On the basis of the work presented in this report, our previous data, and the studies by others cited above, we propose a two-step model of HIV infection. In the first step, HIV binds to target cells through interaction of gp120 with CD4 or through the binding of adhesion molecules on the virus to counterreceptors on cells. When CD4 expression on cells or gp120 expression on the virus is very low, acquired adhesion molecules would constitute the primary mode of virus adsorption to cells. The second step occurs when fusion between the virus membrane and cell membrane is triggered. This would presumably be mediated by the fusion domain in the gp41 molecule or by some other as yet undetermined mechanism. It should be noted that any adhesion molecule that could be acquired by HIV could potentially serve as a virus attachment protein if the molecule retains its function. This implies that HIV has the ability to bind to any cell type bearing counterreceptors for its acquired functional adhesion molecules. Adhesion molecules on HIV may in fact explain infection of CD4-negative cells by the virus. As discussed above, the two-step model of HIV infection in the context of acquired adhesion molecules may explain synergy between anti-LFA-1 MAb and AIDS plasma in neutralization of HIV. The published observations indicating poor serum neutralization of primary HIV isolates produced in activated T cells expressing high levels of adhesion molecules versus that of viruses produced in cell lines are also accommodated by this model.

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