Innovative, Intuitive, Flexible.
Luminex Flow Cytometry Solutions with Guava® and Amnis® Systems
Learn More >
Luminex complexity simplified.

RESEARCH ARTICLE | MARCH 01 1994

Effect of antibody valency on interaction with cell-surface expressed HIV-1 and viral neutralization.

L A Cavacini; ... et. al

J Immunol (1994) 152 (5): 2538–2545.
https://doi.org/10.4049/jimmunol.152.5.2538

Related Content

Influence of heavy chain constant regions on antigen binding and HIV-1 neutralization by a human monoclonal antibody.

J Immunol (October,1995)

An IgG human monoclonal antibody that reacts with HIV-1/GP120, inhibits virus binding to cells, and neutralizes infection.

J Immunol (June,1991)

Surfactant Protein A Binds to HIV and Inhibits Direct Infection of CD4+ Cells, but Enhances Dendritic Cell-Mediated Viral Transfer

J Immunol (July,2008)

Downloaded from http://journals.aai.org/jimmunol/article-pdf/152/5/2538/1066932/2538.pdf by guest on 30 November 2022

Effect of Antibody Valency on Interaction with Cell-Surface Expressed HIV-1 and Viral Neutralization[1]

Lisa A. Cavacini,[2] Charlotte L. Emes, Jennifer Power, Mark Duval, and Marshall R. Posner

Department of Medicine, New England Deaconess Hospital and Harvard Medical School, Boston, MA 02215

F(ab) and F(ab')$_2$ fragments of the human mAb, F105, were compared to intact IgG1 for binding to the CD4 binding site of HIV-1/gp120 on the surface of infected cells and viral neutralization. F105 IgG1 and F(ab')$_2$ bound to IIIB, MN, and RF infected cells and neutralized these strains in an identical fashion, whereas strain-specific differences were observed in F(ab) activity. Although F105 F(ab) bound with equivalent affinity to IIIB-infected cells, there was a 4- to 10-fold decrease in the neutralization of IIIB by monovalent F(ab) compared to the bivalent molecules. F105 F(ab) demonstrated both diminished binding and neutralization of the MN strain and failed to bind or neutralize the RF strain. When cooperativity of V3 loop antibody (V3ab) with F105 IgG and fragments was examined, the binding of F105 IgG and F(ab')$_2$ to IIIB-, MN-, or RF-infected cells was modestly enhanced by V3ab; viral neutralization was substantially enhanced by the combination of V3ab and F105 IgG and F(ab')$_2$. The combination of F105 F(ab) with V3ab also resulted in significant cooperative neutralization of IIIB and MN, but the lack of F105 F(ab) binding and neutralization of RF was unaltered by V3ab. These results suggest that bivalent interaction may be important in binding and neutralization of virus, and support the notion that this interaction may depend on conformational changes in oligomeric gp120 on intact virions and cell surface rather than on affinity or steric effects. *Journal of Immunology,* 1994, 152: 2538.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/152/5/2538/1066932/2538.pdf by guest on 30 November 2022

Understanding the humoral and cellular immune response to HIV-1 infection is critical to effective development of therapeutic agents and vaccines. Despite an extensive immune response to HIV infection, the disease ultimately progresses (1). Dissection of the immune response may identify the elements that contribute to protection from disease and maintenance of a healthy state. Components of the humoral immune response can be captured in the form of human monoclonal antibodies (HmAb)[3]. These captured Abs can be used in laboratory models to explore the impact of different facets of the humoral response on viral neutralization or effective immunization and define the interactions of Abs with virion components. F(ab) and F(ab')$_2$ fragments of a single mAb can be used to study the impact of valency and size-related steric effects on affinity, binding, and neutralization of HIV-1. While Abs reactive with a variety of viral proteins have been described, the humoral response to envelope Ags is associated with neutralizing Ab (2–4). Thus, the study of Abs reactive with gp41 and gp120 is most critical. We have used one HmAb, F105 (5) to examine the effect of Ab valency and size on viral binding and neutralization.

IgG molecules are composed of two identical light chains and two identical heavy chains that are folded into domains by noncovalent and covalent (disulfide bonds) interactions. Ag specificity is conferred by the interactive combination of heavy and light chains forming the variable region, and effector functions are localized in the constant regions. Digestion of IgG at the hinge region between constant regions 1 and 2 with papain results in the formation of two F(ab) fragments while digestion with pepsin results in the generation of one F(ab')$_2$ fragment. Both of these fragments retain Ag-binding capability; however, the F(ab) fragment is univalent, whereas the F(ab')$_2$ fragment is bivalent, as is the original IgG. However, in contrast to IgG, neither Ab fragment retains effector function (e.g., complement activation and Fc receptor binding) and are of smaller size, the F(ab) being the smallest Ab fragment.

The virion surface is covered with multimeric gp120 that appear as "knobs" and noncovalently interact with

Received for publication September 2, 1993. Accepted for publication December 7, 1993.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported by NIAID AI26926 and AI07387.

[2] Address correspondence and reprint requests to Dr. Lisa A. Cavacini, New England Deaconess Hospital, Research-CRI, 185 Pilgrim Road, Boston, MA 02215.

[3] Abbreviations used in this paper: HmAb, human monoclonal antibody; sCD4, recombinant soluble CD4; CD4BS, CD4 binding site of gp120; V3ab, V3 loop antibody; MN, H9/HTLV-III$_{MN}$ NIH 1984; RF, H9/HTLV-III$_{RF}$; IIIB, H9/HTLV-III$_B$ NIH 1983; HIVIG, IgG purified from HIV sera; MFI, mean fluorescent intensity.

Copyright © 1994 by The American Association of Immunologists

0022-1767/94/$02.00

the transmembrane protein gp41 (6–8). The initial step in viral infection is interaction of gp120 with cell surface-expressed CD4. Studies with soluble gp120 and soluble CD4 or mAb to the CD4 binding site of gp120 have demonstrated that the regions of gp120 involved in CD4 binding are localized to several constant regions of gp120, involving discontinuous stretches of amino acids over an extended portion of the protein (9–14). In addition to viral binding, the interaction of CD4 with gp120 effects post-binding events such as membrane fusion, possibly by inducing conformational changes in the gp120/gp41 oligomer. Binding of sCD4 also induces the release of a fraction of gp120 molecules from the virion surface or envelope-expressing cells (15–18) or gp120 cleavage (19) that, in turn, may contribute to further conformational changes in envelope protein multimeric structure that activate fusogenic mechanisms (20–21). Of importance, while the binding affinity of sCD4 for soluble gp120 from primary isolates of HIV-1 is similar to laboratory isolates (22–23), affinity of sCD4 for cell surface expressed gp120 from primary isolates appears to be lower than laboratory isolates (24–25). Thus, conformational changes as assessed using soluble gp120 may not necessarily represent the changes observed on oligomeric, native gp120.

F105 (5) reacts with a discontinuous epitope in the CD4 binding site (CD4BS) (13) and overlaps epitopes of several other CD4BS HmAb and rat mAbs (26–27). Recombinant gp120 and sCD4 inhibit the binding of F105 to infected cells (5). The Ab reacts with a broad range of laboratory isolates with affinity from 9 to 90 nM and neutralizes a range of laboratory and primary isolates (28). F105 cooperates in additive or supra-additive viral neutralization in combination with V3ab, of either murine (29) or human origin (27, 30), and polyclonal HIV serum antibodies from infected patients (28, 31) and vaccine recipients (32). Analysis of the interaction of F105 IgG and fragments in viral binding and neutralization, alone and in the presence of V3ab, should provide useful information regarding the presentation of gp120 on the virion and cell surface and the impact of valency on binding to native virions on the cell surface or neutralization of free virions. This data will contribute to the design and development of therapeutic agents and effective vaccines.

Materials and Methods

Cell lines and antibodies

F105, an IgG1κ HmAb, was purified from tissue culture supernatant by protein G chromatography (Pharmacia, Piscataway, NJ). The 59.1 murine mAb, reactive with the GPGRAF portion of the V3 loop (33), was kindly provided by Repligen Corporation (Cambridge, MA) and was used as purified antibody. H9/HTLV-III_{MN} NIH 1984 (MN), H9/HTLV-III_{RF} (RF), and H9/HTLV-III_B NIH 1983 (IIIB) from Dr. Robert Gallo, and MT-2 cells from Douglas Richman were provided by the AIDS Research and Reference Reagent Program, AIDS Program, NIAID, NIH (ERC Bioservices Corp., Rockville, MD). FDA-H9 cell line was provided by Dr. Barbara Potts (Repligen). IgG was purified from pooled sera from HIV-1 patients using protein G chromatography (Pharmacia). Purified IgG was quantitated by capture ELISA and concentration determined from a standard curve of known concentrations of human IgG (Sigma, St. Louis, MO). This purified IgG is hereafter referred to as HIVIG.

Generation of antibody fragments

F105 was digested with insoluble papain (Sigma) to generate F(ab) fragments as follows. F105 was dialyzed into 0.1 M phosphate buffer, pH 7.5 containing EDTA (1.17g EDTA/liter buffer). After adding cysteine (Sigma) to a final concentration of 50 mM, the antibody was incubated with papain at a ratio of 4 U of papain/10 mg of antibody for 4 h at 37°C. Papain beads were removed by centrifugation. Undigested antibody and Fc fragments were removed using protein G chromatography (Pharmacia) with F(ab) fragments collected in the column flow-through.

$F(ab')_2$ fragments were generated by digestion of F105 with pepsin. F105 was dialyzed into 0.1 M sodium acetate buffer, pH 5 prior to the addition of insoluble pepsin (Sigma) at a ratio of 25,000 units of pepsin/10 mg of antibody. After incubating at 37°C with rotation, for 6 to 8 h, the digestion was terminated by removal of the beads by centrifugation followed by addition of 400 μl of 1 M Tris, pH 9.0/10 mg of antibody. Undigested antibody and Fc fragments were removed using protein G chromatography (Pharmacia) with $F(ab')_2$ fragments collected in the column flow-through. F105 $F(ab')_2$ fragments were further purified from the protein G column flow-through by passage over an affinity column containing a mouse anti-idiotype antibody reactive with F105 (F20924Dc1). Bound antibody was eluted using 100 mM glycine-HCl, pH 2.5, followed by neutralization with 1 M Tris-HCl, pH 8.5. Positive selection of F105 $F(ab')_2$ using an anti-idiotype column was necessary due to contamination of the $F(ab')_2$ preparation with pepsin, as evidenced by the ability of $F(ab')_2$ fragments prepared from HIV-1 seronegative human IgG to neutralize virus. In the preparation of F(ab) fragments, there was no evidence of contamination by papain.

All Ab preparations [F105 IgG, $F(ab')_2$, F(ab)] were quantitated by idiotype-specific ELISA. Plates were coated with F20924Dc1, and bound Ab was detected by biotinylated goat anti-human-κ chain (Fisher, Pittsburgh, PA) followed by HRP-labeled streptavidin (Caltag, San Francisco, CA) and *o*-phenylenediamine (Sigma) as substrate. Known concentrations of F105 were used to generate a standard curve. It was necessary to detect bound Ab with a reagent specific for the κ light chain to correctly quantitate the antibody fragments. Complete digestion and purity were confirmed using SDS-PAGE analysis under reducing and nonreducing conditions.

Flow cytometry

Direct Ab binding. F105 IgG and fragments were individually titered for reactivity with HIV-infected cell lines using biotinylated goat anti-human κ chain (Fisher) followed by FITC-conjugated streptavidin (Tago, Burlingham, CA). The use of a κ chain Ab limited the detection of bound Ab to light chains, which are equivalently expressed by $F(ab')_2$, 2 F(ab) molecules, and intact IgG, in contrast to the heavy chains that are digested to some degree in the generation of different Ab fragments. Normal human serum (NHS) diluted 1:50 was used to determine background fluorescence.

Interactive effects of Abs. Prior to examining the interaction between HIV Abs in binding to H9 cells chronically infected with HIV-1 by flow cytometry, F105 IgG and fragments, and 59.1 Ab were individually titered for reactivity with each HIV-infected cell line, using FITC-conjugated goat $F(ab')_2$ anti-human Ig (Tago) or FITC-conjugated goat anti-mouse IgG + IgM (Accurate Chemical and Scientific Corp., Westbury, NY), respectively. From this titration, the concentration of Ab necessary for 50% maximal binding was determined. To examine interactive effects (30), infected cells were incubated with saturating concentration of 59.1 Ab for 30 min at 4°C. After washing the cells free of unbound 59.1 Ab, cells were incubated with F105 IgG and fragments at an Ab concentration yielding half-maximal binding for 30 min at 4°C. After incubation with F105, cells were washed and incubated with appropriate FITC-conjugated goat anti-human Ig. As a control, cells were incubated in normal mouse serum (1:50) followed by FITC-conjugated goat anti-human Ig. Samples were acquired on a FACScan (Becton-Dickinson) and analyzed using LYSYS II software to determine mean fluorescent intensity (MFI). The percentage of change was determined as the difference between the MFI obtained using F105 antibody alone and the MFI detected by F105 Ab after preincubation with 59.1 Ab divided by the MFI incubated with F105 Ab alone.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/152/5/2538/1066932/2538.pdf by guest on 30 November 2022

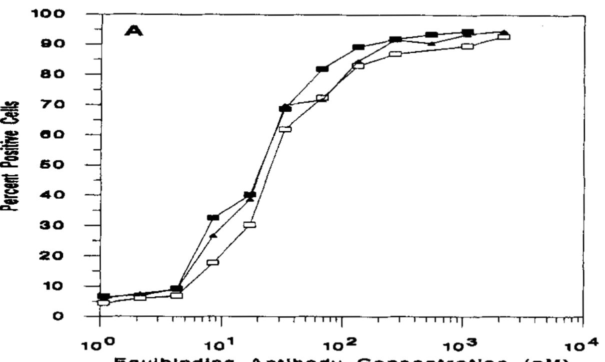
Neutralization assay

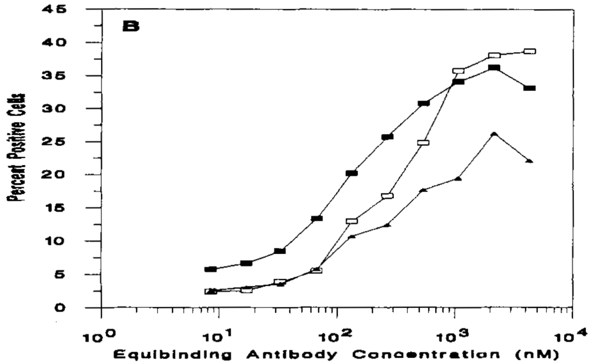
Neutralization assays for laboratory strains of HIV were performed by a slight modification of an MT-2 cell cytopathogenicity system (34) as described previously (28, 30–31). Serial dilutions of Abs were incubated with appropriately diluted viral stock capable of causing 80% to 90% cytopathic effect for 1 h at 4°C, after which MT-2 cells were added. Plates were incubated for 6 days at 37°C and viable cells measured colorimetrically using metabolic conversion of the formazan dye MTT. The antibodies were tested in fourfold dilutions with experimental points done in triplicate or quadruplicate. The neutralization activity of each Ab was titered on IIIB, MN, and RF virus. To study the interactive effects of the Abs, one Ab was maintained at a constant concentration (determined to neutralize approximately 25% of cytopathic effect) while titering the other Ab. Abs were added simultaneously.

Results

Reactivity of F105 IgG and fragments with HIV-1 infected cells

Equimolar binding of F105 IgG and fragments to native, oligomeric gp120 expressed on the surface of HIV-1 infected cells was measured by flow cytometry (Fig. 1). HIVIG was included in all experiments as a positive control and viral Ag expression varied between 30% and >90% of the cells and was dependent upon the cell line and day of experiment. The percentage of cells immunoreactive with saturating concentrations of F105 IgG were within 5% of the HIVIG control in individual experiments. F105 $F(ab')_2$ and F(ab) were appropriately diluted to achieve Ab binding site concentrations equivalent to F105 IgG (equibinding). For $F(ab')_2$, equibinding Ab concentration is equivalent to equimolar Ab concentration, whereas for F(ab), equibinding concentration is double the equimolar Ab concentration. To allow a direct comparison of Ab binding site concentrations, results are expressed as nanomolar Ab concentration adjusted for equibinding Ab concentrations. For example, to achieve equibinding concentration equivalent to 40 μg/ml of F105 IgG, 29.3 μg/ml of $F(ab')_2$ and 26.7 μg/ml of F(ab) Ab were used. This yields an adjusted Ab concentration of 266 nM for IgG, $F(ab')_2$ and F(ab), but actually represents 532 nM of F(ab). Equivalent binding to IIIB-infected cells was observed for F105 IgG and fragments. As a relative estimation of affinity for gp120 as expressed on live HIV-1-infected cells, antibody concentration required for 50% maximal binding to 10^6 infected cells is summarized in Table I. Based on this estimation, relative affinity of the IgG, $F(ab')_2$ and the F(ab) for IIIB-infected cells was comparable (57 to 69 nm). A reduction in binding of F105 F(ab) to MN-infected cells was observed while binding comparable to IgG was observed for $F(ab')_2$ on this cell line. This reduction in binding to MN resulted in a twofold decrease in relative affinity from 0.24 to 0.37 μM for IgG and $F(ab')_2$ to 0.70 μM for F105 F(ab). Minimal binding of F105 F(ab) to RF-infected cells was apparent despite comparable binding for F105 $F(ab')_2$ and intact F105 with these cells (46 to 69 nM).





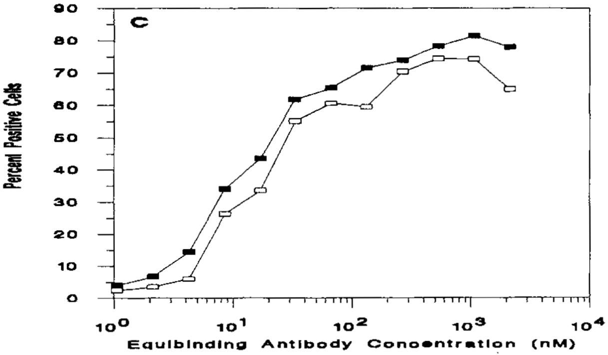


FIGURE 1. Binding of intact F105 and fragments to HIV-1 infected cells. Cells (1 × 10^6) infected with IIIB (*A*), MN (*B*) or RF (*C*) were incubated with titering quantities of F105 IgG (■), $F(ab')_2$ (□), and F(ab) (▲). Cells were washed and incubated with biotinylated goat anti-human κ chain followed by FITC-conjugated streptavidin. The percentage of positive cells was determined after sample acquisition using a marker set at 5% to 7% on cells incubated with normal human IgG followed by biotinylated goat anti-human κ chain and FITC-conjugated Streptavidin. The percentage of positive cells is plotted against Ab concentration (nM) necessary for equivalent number of Ag binding sites. Results are representative of four experiments.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/152/5/2538/1066932/2538.pdf by guest on 30 November 2022

Table I. *Relative estimation of functional antibody affinity*[a]

	Antibody Concentration (μg/ml) for 50% binding[b]		
	IIIB	MN	RF
F105 IgG	10.5 (69 nM)	36.2 (242 nM)	6.9 (46 nM)
F105 F(ab')$_2$	7.3 (66 nM)	40.4 (368 nM)	7.7 (69 nM)
F105 F(ab)[c]	5.7 (57 nM)	69.9 (699 nM)	NR[d]

[a] Reactivity of serial dilutions of F105 IgG, F(ab')$_2$, and F(ab) with HIV-1 infected cells (1 × 10^6/sample) was determined by flow cytometry. F105 IgG and fragments were all analyzed on the same day using the same population of HIV-1 infected cells to control for variation in viral expression that occurs in culture.
[b] Antibody concentrations (μg/ml) required for 50% binding using a total of 10 to 12 antibody concentrations to generate binding curves to achieve antibody saturation and background fluorescence. The numbers in parentheses represent molar antibody concentration for an equivalent number of antibody binding sites.
[c] To achieve equivalent binding sites for F(ab) fragments, twice the equimolar concentration of IgG and F(ab')$_2$ were used in these studies.
[d] NR = Not reactive.

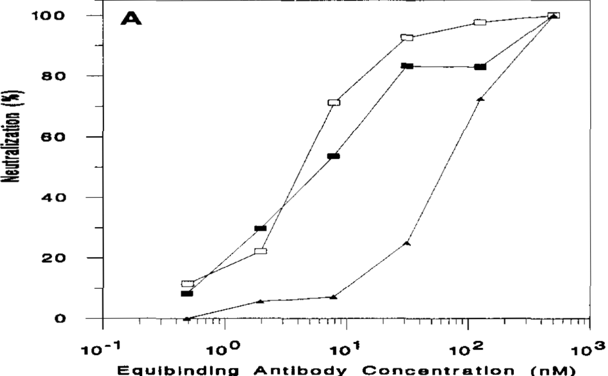
Neutralization of HIV-1 by F105 IgG and fragments

Neutralization of cell-free HIV-1 by F105 IgG and fragments was measured in a MT-2 cytopathogenicity assay (Fig. 2). Despite equimolar binding of F105 IgG, F(ab')$_2$ and F(ab) to IIIB-infected cells, and comparable neutralization for IgG and F(ab')$_2$, at low antibody concentrations there is a 4- to 10-fold reduction in neutralization by F105 F(ab). This decrease in F(ab) neutralization of IIIB is in stark contrast to equivalent binding of F(ab) to native Ag on the surface of infected cells and was consistent in more than six individual experiments using three preparations of viral stock. While neutralization of MN by F105 IgG and F(ab')$_2$ were equivalent, at the highest concentration tested there was a fivefold reduction in neutralization by F105 F(ab). Only modest neutralization of RF is observed for high concentrations of F105 IgG and F(ab')$_2$ with no neutralization observed for F105 F(ab) (data not shown).

Effect of 59.1 on binding of F105 IgG and fragments to HIV-1-infected cells and neutralization of cell-free virus

The effect of an Ab reactive with the V3 loop of gp120, 59.1, on binding of F105 IgG and fragments to cells infected with HIV-1 was measured by flow cytometry (Table II). IIIB-, MN-, and RF-infected cells were preincubated with 59.1 Ab using saturating antibody concentrations. After removing unbound 59.1 Ab by washing, cells were incubated with F105 IgG, F(ab')$_2$, and F(ab) Ab followed by fluoresceinated goat anti-human Ig, and the percentage of change was calculated based on MFI. The binding of F105 IgG, F(ab')$_2$, and F(ab) to HIV-1 infected cells (IIIB, MN, RF) was marginally increased by preincubation of the cells with 59.1 Ab.

The effect of V3ab on neutralization of cell-free virus by F105 IgG and fragments was measured by MT-2 cytopathogenicity. For these experiments, virus was preincubated with titering concentrations of F105 IgG and fragments and a constant concentration of 59.1 shown previously to neutralize 25% to 30% of cytopathic effect. The V3ab 59.1 consistently enhanced neutralization of IIIB (Fig. 3) and MN (Fig. 4) by F105 IgG, F(ab')$_2$, and F(ab). At low limiting concentrations of F105 IgG and fragments, this effect was substantially supra-additive. Supra-additive neutralization was most dramatic for F105 F(ab), which alone displays diminished neutralization of



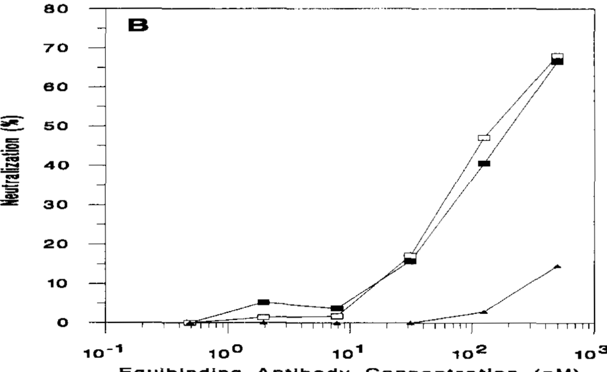


FIGURE 2. Neutralization of HIV-1 by F105 IgG and fragments. IIIB virus (1100 $TCID_{50}$/ml) or MN virus (820 $TCID_{50}$/ml) was preincubated for 1 h with titering quantities of F105 IgG (■), F(ab')$_2$ (□), and F(ab) (▲) prior to addition of MT-2 cells. After 6 days, viability was determined using MTT dye. The percentage of neutralization is plotted against Ab concentration (*nM*) necessary for equivalent number of Ag binding sites. Results are representative of three experiments. IIIB was neutralized 57% and MN 70% by HIVIG at a concentration of 100 μg.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/152/5/2538/1066932/2538.pdf by guest on 30 November 2022

Table II. *Effect of 59.1 on F105 binding to HIV-1 infected cells*[a]

	Percentage of Change[b]		
	IIIB	MN	RF
F105 IgG	14.8 ± 1.3	8.5 ± 1.5	28.6 ± 6.7
F105 $F(ab')_2$	27.6 ± 4.7	10.2 ± 1.8	10.6 ± 4.1
F105 F(ab)	23.9 ± 10.7	15.9 ± 3.4	8.5 ± 3.1

[a] Reactivity of subsaturating concentrations of F105 IgG or fragments after preincubation of HIV-1 infected cells (1×10^6/sample) with saturating concentrations of 59.1 antibody (12.7 μg/ml for IIIB, 25 μg/ml for MN and RF) was determined by flow cytometry to determine MFI.

[b] The percentage of change was determined as the difference in MFI observed with F105 antibody alone and F105 antibody following preincubation with 59.1 antibody divided by the MFI with F105 antibody alone. Results represent the average ± SE for three experiments.

IIIB. The combination of F105 F(ab) and 59.1 Ab neutralized IIIB as well as the combination of F105 IgG or $F(ab')_2$ and 59.1 Ab. Neutralization of MN by F105 F(ab) and 59.1 Ab was enhanced sixfold over either Ab alone and the neutralization by the combination of F105 F(ab) and 59.1 was equivalent to the neutralization observed for F105 IgG alone.

Discussion

In these studies, a HmAb, F105, reactive with a discontinuous epitope encompassing the CD4 binding site of gp120 was used to characterize the effect of Ab valency and size on binding to HIV-1 infected cells and neutralization of cell-free virus. F105 is broadly reactive with diverse strains of HIV-1-infected cells (5, 28) and neutralizes selected laboratory and primary isolates (28, 31). The epitope recognized by F105 has been shown to lie within the binding site of sCD4 and overlaps the epitopes of several other HmAb and rat mAbs reactive with the CD4BS (13, 35). Thus, F105 is an excellent model to study the interaction of Ab and gp120 CD4BS for effective binding and neutralization. Additionally, the effect of V3ab on this interaction and subsequent conformation changes of gp120 CD4BS were examined as the potential importance of synergistic neutralization by the interactive effect of V3ab and Ab to the CD4BS has become increasingly evident (26–30, 36, 37).

Infection of T cells with HIV-1 is initiated by binding of gp120 to cell-surface expressed CD4. A series of events are initiated by the association of viral gp120 and CD4, resulting in pH independent fusion of viral and cell membranes. A number of fusion models have been proposed for the interaction of viral and cellular components in the fusion process (20, 21). Using sCD4 to assess the effect of CD4 binding on virus, conformational changes in the structure of gp120 and gp41 have been observed upon interaction of viral gp120 with CD4. There is increased exposure of the V3 domain and gp41 epitopes following sCD4 binding to gp120 or HIV-1 envelope-expressing cells (38). Shedding of gp120 from the virion or cell surface may or may not accompany the conformational changes associated with the interaction of gp120 with CD4 (15, 25, 38–40). It is postulated that the conformational changes resulting from interaction of virion expressed gp120 with soluble and cell surface-expressed CD4 exposes the fusogenic domain of gp41, which is necessary for virus-cell fusion to occur (40). While these studies have provided significant information on the interaction of viral gp120 and CD4, they are limited by the use of soluble CD4, and, in some cases, isolated gp120 or vaccinia expressed envelope protein that may not adequately represent the presentation of envelope glycoproteins in native, oligomeric form. In addition to potential differences in the molecular events following the interaction of oligomeric envelope glycoproteins with cell surface-expressed CD4 rather than soluble CD4, other cellular proteins potentially may be involved in the interaction of envelope glycoproteins.

From the studies presented here, it is clear that the binding of Ab to free gp120 is different from that of binding to cell-associated or virion-associated gp120. Moreover, Ab binding does not necessarily predict effective neutralization, as shown here, and Ab valency has influential effects on this activity and may differentiate between cell-associated and cell-free virus. Whereas univalent Ab (F(ab)) will often effectively bind to HIV-1 infected cells, bivalent Ab (IgG and $F(ab')_2$) was critical for effective neutralization of cell-free virus. This differential effect of Ab valency on binding or viral neutralization is not a function of Ab affinity or steric effects. For example, similar affinities of univalent and bivalent Ab for cell surface-expressed virions of the IIIB isolate was observed; however, there was a decrease in viral neutralization by univalent Ab. These differences in binding and viral neutralization may be more dependent on differences in oligomeric gp120 conformation and the effects induced by bivalent Abs vs univalent F(ab). This notion is supported by the enhanced viral neutralization that occurs with the combination of either univalent or bivalent F105 Ab with Ab to the V3 loop (59.1), which can induce conformational changes in gp120 (27). Consistent with the ability of univalent F105 Ab to sometimes bind as effectively as bivalent Ab, the effect of V3ab on the binding of F105 IgG, $F(ab')_2$ and F(ab) did not necessarily predict the cooperativity that was

Downloaded from http://journals.aai.org/jimmunol/article-pdf/152/5/2538/1066932/2538.pdf by guest on 30 November 2022

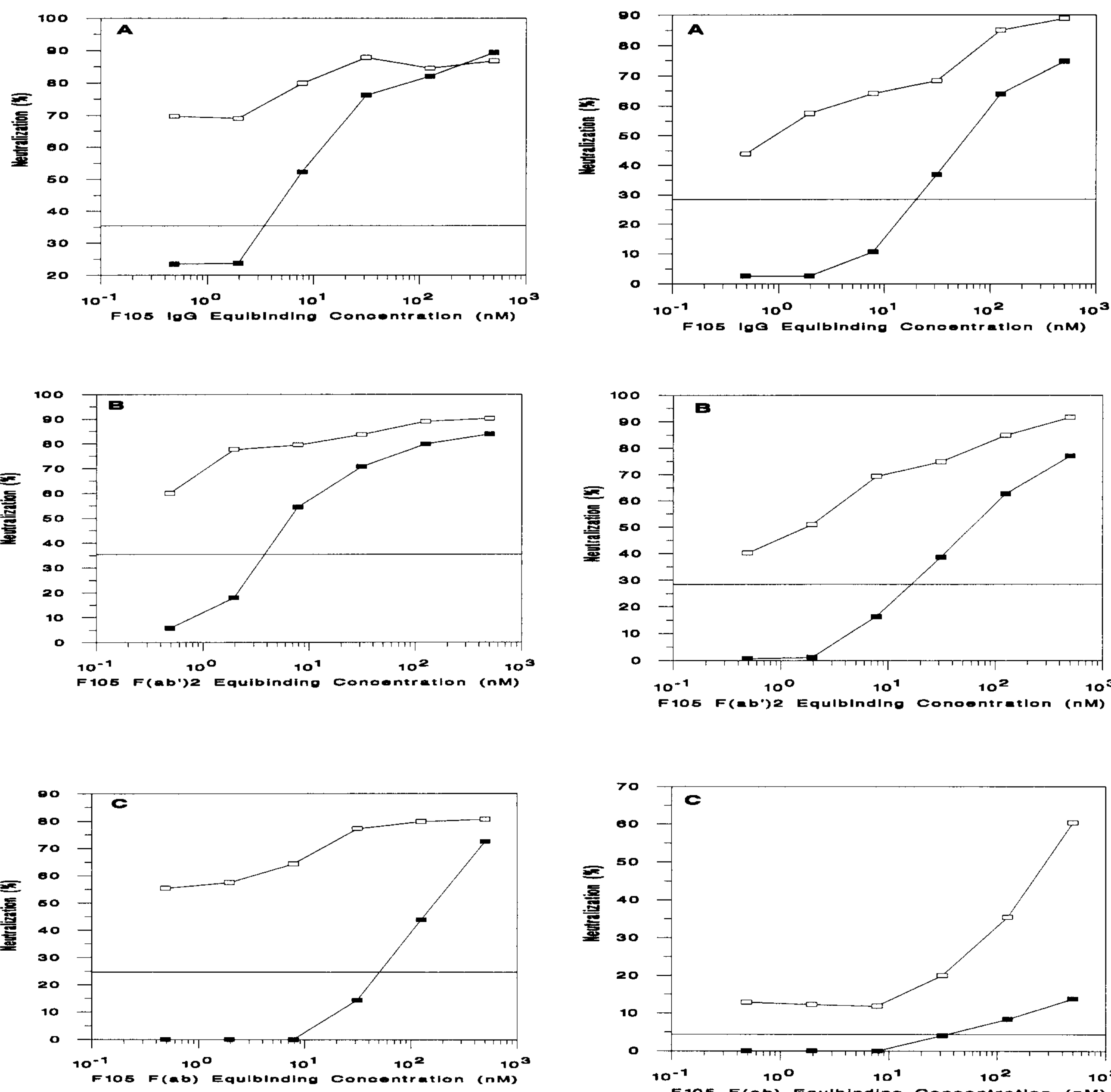


FIGURE 3. Enhanced neutralization of IIIB by F105 IgG or fragments and V3 loop Ab. Serial fourfold dilutions of forms of F105 (■), dilutions of F105 or fragment with V3ab (59.1) at a constant concentration of 5 μg/ml (□), and 59.1 alone (——) were tested for neutralization of IIIB (1100 $TCID_{50}$/ml) in a MT-2 cytopathogenicity assay. Depicted in (*A*) are results obtained with F105 IgG, in (*B*) results for F105 $F(ab')_2$, and in (*C*) results for F105 F(ab). The percentage of neutralization is plotted against Ab concentration (nM) necessary for equivalent number of Ag binding sites. Results are representative of three experiments.

FIGURE 4. Enhanced neutralization of MN by F105 IgG and fragments and V3 loop Ab. Serial fourfold dilutions of F105 (■), dilutions of F105 with V3ab (59.1) at a constant concentration of 25 μg/ml (□) and 59.1 alone (——) were tested for neutralization of MN (820 $TCID_{50}$/ml) in a MT-2 cytopathogenicity assay. Depicted in (*A*) are results obtained with F105 IgG, in (*B*) results for F105 $F(ab')_2$ and in (*C*) results for F105 F(ab). The percentage of neutralization is plotted against Ab concentration (nM) necessary for equivalent number of Ag binding sites. Results are representative of three experiments.

observed in viral neutralization. These results are consistent with the importance of a conformational change induced in gp120 oligomer for effective viral neutralization.

The use of the F105 HMab as a prototype CD4BS antibody to analyze the interaction of this class of antibody with viral Ags is supported by the fact that other human

Downloaded from http://journals.aai.org/jimmunol/article-pdf/152/5/2538/1066932/2538.pdf by guest on 30 November 2022

and rat mAbs to discontinuous epitopes within the CD4 binding site have been shown to encompass overlapping, but distinct, components of this extensive, functionally defined region of gp120 (26, 27). While a systemic comparative analysis of these Abs has not been performed, CD4BS Abs exhibit some cross-reactivity and neutralize a variable spectrum of strains with similar efficiency to one another (27, 28, 35, 41). Further characterization of the critical interaction of univalent and bivalent Abs with the CD4 binding site and other regions of gp120 in the context of infected cells and cell-free virions should provide useful information on the presentation of oligomeric gp120. The effect of univalent and bivalent Abs on binding and neutralization of primary isolates remains to be determined. Additionally, it is possible that the particular cell infected with HIV-1 may play an important role in the Ag-Ab interaction. The results described in this study and further characterization of this interaction should contribute to the design of effective immunotherapeutics and vaccines.

Acknowledgments

The authors thank Drs. Randal Byrn and Barbara Potts for their helpful discussion and critical review of the manuscripts, Frances Desharnais for technical assistance, and Joanne Arruda for assistance in the preparation of the manuscript.

References

1. Pantaleo, G., C. Graziosi, and A. S. Fauci. 1993. New concepts in the immunopathogenesis of human immunodeficiency virus infection. *N. Engl. J. Med. 328:327.*
2. Steimer, K. S., P. J. Klasse, and J. A. McKeating. 1991. HIV-1 neutralization directed to epitopes other than linear V3 determinants. *AIDS 5 (Suppl. 2):S135.*
3. Nara, P. L., R. R. Garrity, and J. Goudsmit. 1991. Neutralization of HIV-1: a paradox of humoral proportions. *FASEB J. 5:2437.*
4. Moore, J. P., and D. D. Ho. 1993. Antibodies to discontinuous or conformationally sensitive epitopes on the gp120 glycoprotein of human immunodeficiency virus type 1 are highly prevalent in sera of infected humans. *J. Virol. 67:863.*
5. Posner, M. R., T. Hideshima, T. Cannon, M. Mukherjee, K. H. Mayer, and R. A. Byrn. 1991. An IgG human monoclonal antibody that reacts with HIV-1/gp120 inhibits virus binding to cells, and neutralizes infection. *J. Immunol. 146:4325.*
6. Allan, J. S., J. E. Coigan, F. Barin, M. F. McLane, J. G. Sodroski, C. A. Rosen, W. A. Haseltine, T. H. Lee, and M. Essex. 1985. Major glycoprotein antigens that induce antibodies in AIDS patients are encoded by HTLV-III. *Science 228:1091.*
7. Gelderblom, H. R., E. H. Hausmann, M. Ozel, G. Pauli, and M. A. Koch. 1987. Fine structure of human immunodeficiency virus (HIV) and immunolocalization of structural proteins. *Virology 156:171.*
8. Robey, W. G., B. Safai, S. Oroszlan, L. O. Arthur, M. A. Gonda, R. C. Gallo, and P. J. Fischinger. 1985. Characterization of envelope and core structural gene products of HTLV-III with sera from AIDS patients. *Science 228:593.*
9. Capon, D. J., and R. H. R. Ward. 1991. The CD4-gp120 interaction and AIDS pathogenesis. *Annu. Rev. Immunol. 9:649.*
10. McKeating, J. A., M. Thali, C. Furman, S. Karwowska, M. K. Gorny, J. Cordell, S. Zolla-Pazner, J. Sodroski, and R. A. Weiss. 1992. Amino acid residues of the human immunodeficiency virus type I gp120 critical for the binding of rat and human neutralizing antibodies that block the gp120-sCD4 interaction. *Virology 190:134.*
11. Olshevsky, V., M. Helbeth, C. Furman, J. Li, W. Haseltine, and J. Sodroski. 1990. Identification of individual human immunodeficiency virus type 1 gp120 amino acids important for CD4 binding. *J. Virol. 64:5701.*
12. Thali, M., U. Olshevsky, C. Furman, D. Gabuzda, J. Li, and J. Sodroski. 1991. Effects of changes in gp120-CD4 binding affinity on human immunodeficiency virus type 1 envelope glycoprotein function and soluble CD4 sensitivity. *J. Virol. 65:5007.*
13. Thali, M., U. Olshevsky, C. Furman, D. Gabuzda, M. Posner, and J. Sodroski. 1991. Characterization of a discontinuous HIV-1 gp120 epitope recognized by a broadly reactive neutralizing human monoclonal antibody. *J. Virol. 65:6188.*
14. McKeating, J. A., J. P. Moore, M. Ferguson, H. S. Marsden, S. Graham, J. W. Almond, D. J. Evans, and R. A. Weiss. 1992. Monoclonal antibodies to the C4 region of human immunodeficiency virus type 1 gp120: use in topological analysis of a CD4 binding site. *AIDS Res. Hum. Retro. 8:451.*
15. Moore, J. P., J. A. McKeating, R. A. Weiss, and Q. J. Sattentau. 1990. Dissociation of gp120 from HIV-1 virions induced by soluble CD4. *Science 250:1139.*
16. Moore, J. P., J. A. McKeating, W. A. Norton, and Q. J. Sattentau. 1991. Direct measurement of soluble CD4 binding to human immunodeficiency virus type 1 virions: gp120 dissociation and its implications for virus-cell binding and fusion reactions and their neutralization by soluble CD4. *J. Virol. 65:1133.*
17. Hart, T. K., R. Kirsh, H. Ellens, R. W. Sweet, D. M. Lambert, S. R. Petteway, J. Leary, and P. Bugelski. 1991. CD4-HIV-1 interactions: binding of soluble CD4 (sT4) to HIV-1 and HIV-1 infected cells induces shedding of envelope gp120. *Proc. Natl. Acad. Sci. USA 88:2189.*
18. Berger, E. A., J. R. Sisler, and P. L. Earl. 1992. Human immunodeficiency virus type 1 envelope glycoprotein molecules containing membrane fusion impairing mutations in the V3 region efficiently undergo soluble CD4-stimulated gp120 release. *J. Virol. 66:6208.*
19. Werner, A., and J. A. Levy. 1993. Human immunodeficiency virus type 1 envelope gp120 is cleaved after incubation with recombinant soluble CD4. *J. Virol. 67:2566.*
20. Eiden, L. E., and J. D. Lifson. 1992. HIV interactions with CD4: a continuum of conformations and consequences. *Immunol. Today 13: 201.*
21. Allan, J. S. 1992. Receptor-mediated activation in HIV/SIV viral entry. *AIDS Res. Rev. 2:133.*
22. Turner, S., R. Tizard, J. DeMarinis, R. B. Pepinsky, J. Zullo, R. Schooley, and R. Fisher. 1992. Resistance of primary isolates of human immunodeficiency virus type 1 to neutralization by soluble CD4 is not due to lower affinity with the viral envelope glycoprotein gp120. *Proc. Natl. Acad. Sci. USA 89:1335.*
23. Brighty, D. W., M. Rosenberg, I. S. Y. Chen, and M. Ivey-Hoyle. 1992. Envelope proteins from clinical isolates of human immunodeficiency virus type 1 that are refractory to neutralization by soluble CD4 possess high affinity for the CD4 receptor. *Proc. Natl. Acad. Sci. USA 88:7802.*
24. Ashkenazi, A., D. H. Smith, S. A. Marsters, L. Riddle, T. J. Gregory, D. D. Ho, and D. J. Capon. 1992. Resistance of primary isolates of human immunodeficiency virus type 1 to soluble CD4 is independent of CD4-rgp120 binding affinity. *Proc. Natl. Acad. Sci. USA 88:7056.*
25. Moore, J. P., J. A. McKeating, Y. Huang, A. Ashkenazi, and D. D. Ho. 1992. Virions of primary human immunodeficiency virus type 1 isolates resistant to soluble CD4 (sCD4) neutralization differ in sCD4 binding and glycoprotein gp120 retention from sCD4-sensitive isolates. *J. Virol. 66:235.*
26. Thali, M., C. Furman, B. Wahren, M. Posner, D. D. Ho, J. Robinson, and J. Sodroski. 1992. Cooperativity of neutralizing antibodies directed against the V3 and CD4 binding regions of the human immunodeficiency virus gp120 envelope glycoprotein. *J. Acquir. Immun. Defic. Syndr. 5:591.*
27. Wyatt, R., M. Thali, S. Tilley, A. Pinter, M. Posner, D. Ho, J. Robinson, and J. Sodroski. 1992. Relationship of the human immunodeficiency virus type 1 gp120 third variable loop to a component of the CD4 binding site in the fourth conserved region. *J. Virol. 66:6997.*

Downloaded from http://journals.aai.org/jimmunol/article-pdf/152/5/2538/1066932/2538.pdf by guest on 30 November 2022

28. Posner, M. R., L. A. Cavacini, C. L. Emes, J. Power, and R. Byrn. Neutralization of HIV-1 by F105, a human monoclonal antibody to the CD4 binding site of gp120. *J. Acquir. Immun. Defic. Syndr. 6:7.*
29. Potts, B. J., K. Field, Y. Wu, M. Posner, L. Cavacini, M. White-Scharf, and W. Herlihy. 1993. Synergistic inhibition of HIV-1 by sCD4 and V3 directed monoclonal antibodies. *Virology 197:415.*
30. Cavacini, L. A., C. Emes, J. Power, M. Gorny, S. Zolla-Pazner, and M. R. Posner. 1993. Human monoclonal antibodies to the V3 loop of gp120 mediate variable and distinct effects on binding and viral neutralization by a human monoclonal antibody to the CD4 binding site. *J. Acquir. Immun. Defic. Syndr. 6:353.*
31. Cavacini, L., C. Emes, J. Power, R. Goldstein, K. Mayer, and M. Posner. 1993. Loss of serum reactivity with the F105 epitope in HIV-1 infected patients: association with prognosis and viral neutralization. *J. Acquir. Immun. Defic. Syndr. 6:1093.*
32. Montefiori, D. C., B. S. Graham, J. Zhou, R. Bucco, D. H. Schwartz, L. A. Cavacini, M. R. Posner, and the NIH-NIAID Vaccine Clinical Trial Network. 1993. V3-specific neutralizing antibodies in sera from HIV-1 gp160 immunized volunteers block virus attachment and act synergistically with human monoclonal antibody to the conformation-dependent CD4 binding site of gp120. *J. Clin. Invest. 26: 231.*
33. White-Scharf, M. E., B. J. Potts, L. M. Smith, K. A. Sokolowski, J. R. Rusche, and S. Silver. 1993. Broadly neutralizing monoclonal antibodies to the V3 region of HIV-1 can be elicited by peptide immunization. *Virology 192:197.*
34. Robertson, G. A., B. M. Kostek, W. A. Schleif, J. A. Lewis, and A. E. Emini. 1988. A microtiter cell culture assay for the determination of anti-human immunodeficiency virus neutralizing activity. *J. Virol. Methods 20:195.*
35. Thali, M., C. Furman, D. D. Ho, J. Robinson, S. Tilley, A. Pinter, and J. Sodroski. 1992. Discontinuous, conserved neutralization epitopes overlapping the CD4 binding region of the HIV-1 gp120 envelope glycoprotein. *J. Virol. 66:5635.*
36. Buchbinder, A., S. Karwowska, M. K. Gorny, S. T. Burda, and S. Zolla-Pazner. 1992. Synergy between human monoclonal antibodies to HIV extends their effective biologic activity against homologous and divergent strains. *AIDS Res. Hum. Retro. 8:425.*
37. Tilley, S. A., W. J. Honnen, M. E. Racho, T-C. Chou, and A. Pinter. 1992. Synergistic neutralization of HIV-1 by human monoclonal antibodies against the V3 loop and the CD4-binding site of gp120. *AIDS Res. Hum. Retro. 8:461.*
38. Sattentau, Q. J., and J. P. Moore. 1991. Conformational changes induced in the human immunodeficiency virus envelope glycoprotein by soluble CD4 binding. *J. Exp. Med. 174:407.*
39. Thali, M., C. Furman, E. Helseth, H. Repke, and J. Sodroski. 1992. Lack of correlation between soluble CD4-induced shedding of the human immunodeficiency virus type 1 exterior envelope glycoprotein and subsequent membrane fusion events. *J. Virol. 66:5516.*
40. Orloff, S. L., M. S. Kennedy, A. A. Belperron, P. J. Maddon, and J. S. McDougal. 1993. Two mechanisms of soluble CD4 (sCD4)-mediated inhibition of human immunodeficiency virus type 1 (HIV-1) infectivity and their relation to primary HIV-1 isolates with reduced sensitivity to sCD4. *J. Virol. 67:1461.*
41. Ho, D. D., J. A. McKeating, X. L. Li, T. Moudgil, E. S. Daar, N. Sun, and J. E. Robinson. 1991. Conformational epitopes on gp120 important in CD4 binding and human immunodeficiency virus type I neutralization identified by a human monoclonal antibody. *J. Virol. 65:489–493.*

Downloaded from http://journals.aai.org/jimmunol/article-pdf/152/5/2538/1066932/2538.pdf by guest on 30 November 2022