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Specificity of Antibodies Produced against Native or Desialylated Human Immunodeficiency Virus Type 1 Recombinant gp160

ABDELAZIZ BENJOUAD,[1]† JEAN-CLAUDE GLUCKMAN,[2] LUC MONTAGNIER,[3] AND ELMOSTAFA BAHRAOUI[1]*

Centre National de la Recherche Scientifique URA 1455, Laboratoire de Biochimie, Faculté de Médecine Nord, 13326 Marseille Cedex 15,[1] Laboratoire de Biologie et Génétique des Pathologies Immunitaires, Centre National de la Recherche Scientifique URA 1463, Centre d'Étude et de Recherche Viro-Immunologiques, Hôpital de la Pitié-Salpétrière, 75651 Paris Cedex 13,[2] and Unité d'Oncologie Virale, Institut Pasteur, 75015 Paris Cedex,[3] France

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In a previous report we have shown that, in contrast to antibodies produced against native or fully deglycosylated human immunodeficiency virus type 1 (HIV-1) gp160 in rabbits, antibodies raised against desialylated HIV-1 gp160 also recognize gp140 from HIV-2 at high titers. Here, we characterize the fine specificity of these cross-reactive antibodies. Inhibition assays with a panel of synthetic peptides as competitors showed that cross-reactivity to gp140 was due to antibodies that were specific for the region encompassing HIV-1 gp41 immunodominant epitope, mimicked by peptide P39 (residues 583 to 609), the latter being able to totally inhibit the formation of complexes between radiolabeled HIV-2 gp140 and antibodies elicited by desialylated HIV-1 gp160. In addition, anti-desialylated gp160 antibodies retained on a P39 affinity column still bound HIV-2 gp140. Fine mapping has enabled us to localize the cross-reactive epitope within the N-terminal extremity of the gp41 immunodominant region. Interestingly, this cross-reactive antibody population did not recognize glycosylated or totally deglycosylated simian immunodeficiency virus gp140 despite an amino acid homology with HIV-1 within this region that is comparable to that of HIV-2. This cross-reactivity between HIV-1 and HIV-2 did not correlate with cross-neutralization. These results illustrate the influence of carbohydrate moieties on the specificity of the antibodies produced and clearly indicate that such procedures may be an efficient way to raise specific immune responses that are not type specific. Moreover, this cross-reactivity might explain the double-positive reactivity observed, in some human sera, against both HIV-1 and HIV-2 envelope antigens.

Human immunodeficiency virus (HIV) type 1 envelope glycoprotein precursor gp160 is cleaved in the Golgi apparatus by host proteases, generating two functional subunits. One is gp120, the external subunit responsible for CD4 receptor tropism (7, 21, 32), and the other is transmembrane protein gp41, which is involved in fusion between the viral envelope and host cell membranes (22). Both the external and transmembrane glycoproteins are major targets for the immune response. They are heavily glycosylated, and about half of their molecular weight is due to N-linked glycans (2, 10, 24). The role of N-linked glycans in the HIV replicative cycle has been investigated in several studies (16, 28, 29, 31), but their effect on antigenicity and immunogenicity has been less extensively studied.

To explain the attenuated immunogenicity and antigenicity of certain regions of the viral glycoproteins, it was initially proposed, since the virus uses both the glycoprotein machinery and oligosaccharide chains of the infected cells, that exposed carbohydrate chains on the surface of the protein are recognized by the host immune system as self antigens. The carbohydrate moieties may modulate the antigenic structure of the glycoprotein by masking the interaction of some regions from the immune system. For example, Skehel et al. (35) have shown that addition of a glycosylation site at positions 63 to 65 in a variant of 1968 Hong Kong influenza virus hemagglutinin abolished the mutant interaction with a monoclonal antibody specific to the parent virus. Further evidence of the role of carbohydrate moieties in modulating antigenic reactivity, obtained by using polyclonal antisera against glycosylated or deglycosylated proteins, has been reported (1, 9, 20).

With respect to HIV, although the precise role of HIV carbohydrate in modulating the antigenicity and immunogenicity of the envelope glycoproteins remains poorly understood, several reports have stressed the importance of carbohydrate moieties for the specificity and neutralizing activity of antibodies produced against glycosylated or deglycosylated viral proteins. For example, Muller et al. (24a) and Hansen et al. (17a) have shown that polyclonal antibodies produced against mannan or monoclonal antibodies produced against O-linked carbohydrate epitopes (Le^y, A1, and Sialyl-Tn) were able to neutralize HIV infection. Moreover, Davis et al. (7a) analyzed the reactivities of antibodies produced against an overlapping panel of synthetic peptides against both glycosylated and deglycosylated recombinant HIV-1 gp120. They showed, in agreement with the work of Neurath et al. (26a), that the presence of carbohydrate moieties could block or mask antibody binding to specific epitopes. Similar conclusions were previously reported by Alexander and Elder (1) after analysis of the reactivity of glycosylated and deglycosylated Rauscher murine leukemia virus against antibodies produced to intact Rauscher murine leukemia virus particles or purified glycoproteins. Also, it was recently shown that a carbohydrate chain of HIV-1 gp160 may modulate T-cell epitope recognition by masking the corresponding antigenic region (4).

* Corresponding author.

† Present address: Laboratoire de Biologie et Génétique des Pathologies Immunitaires, Centre National de la Recherche Scientifique URA 1463, Centre d'Étude et de Recherche Viro-Immunologiques, Hôpital de la Pitié-Salpétrière, 75651 Paris, France.

We have recently shown (3) that oligosaccharides of HIV envelope glycoprotein can affect the immunogenicity and neutralizing capacity of antibodies elicited in rabbits against mannosidase-, neuraminidase-, and endoF–*N*-glycanase-treated gp160 from HIV-1-LAV (38, 39). To do this, we injected 200 μg of native or deglycosylated gp160 in Freund's adjuvant intradermally into rabbits on day 0 and repeated this procedure with incomplete Freund's adjuvant on days 30, 60, 90, and 120. Most reactive sera were further analyzed. In contrast to antibodies produced against native or fully deglycosylated gp160, only antibodies produced against desialylated gp160 recognized gp140 from HIV-2-Rod (17) at high titers, even when the latter was fully deglycosylated (3). To carry out the fine characterization and mapping of these cross-reactive antibodies and to understand the mechanism involved, antibodies raised against desialylated gp160 were tested in a competition assay against radiolabeled HIV-2 gp140 and in a direct binding enzyme-linked immunosorbent assay (ELISA) with a panel of synthetic peptides representing different regions of gp120 and gp41.

This cross-reactivity was not directly due to anti-N-linked oligosaccharide antibodies, since the antibodies recognized both native and fully deglycosylated HIV-2 gp140 to the same extent. Sera from rabbits immunized with native or desialylated gp160, as described previously (3), were screened by ELISA against a panel of synthetic peptides mimicking variable and conserved regions of gp160. In addition to the same reactivity pattern as that obtained with anti-native gp160, anti-desialylated gp160 antibodies recognized synthetic peptide P41 (residues 252 to 266) (3). To identify the region(s) which could be responsible for cross-reactivity of anti-desialylated gp160 antibodies with HIV-2 gp140, we used a panel of synthetic peptides (3) including P22, P29, and P21, modeling the gp120 hypothetical CD4-binding site (23), the principal neutralizing epitope (15, 25, 30), and the gp41 fusogenic domain (12), respectively. Of all the synthetic peptides tested as competitors in the inhibition assay between radiolabeled HIV-2 gp140 and anti-desialylated HIV-1 gp160 antibodies, including P41, only peptide P39 (residues 583 to 609), which mimics the HIV-1 gp41 immunodominant epitope (14, 26, 33, 34, 37, 40) strongly and dose-dependently inhibited this interaction (Fig. 1). The concentrations of peptide P39 and gp160 that inhibited antibody binding to ^{125}I-gp140 by 50% ($K_{0.5}$) were about 100 and 1 nM, respectively. These $K_{0.5}$ values indicate that gp140 cross-reacting anti-desialylated gp160 antibodies recognized P39 with an affinity 100-fold lower than that obtained for native gp160. On the other hand, the fact that anti-peptide P39 antibodies were present at the same titer in sera from rabbits immunized with native (the titer varied from 0.3 $\times 10^{-3}$ to 0.6 $\times 10^{-4}$) or desialylated (the titer varied from 1.4 $\times 10^{-3}$ to 1.7 $\times 10^{-4}$) gp160 (Fig. 2) is in apparent contradiction with the selective ability of anti-desialylated gp160 to recognize HIV-2 gp140. However, this apparent contradiction could be explained by the existence of an HIV-2 gp140 cross-reactive subpopulation of anti-P39 antibodies that is highly dependent on structure modifications (modification of conformation or site demasking) and that was elicited only after enzymatic removal of sialic acids. To assess the specificity of this interaction, specific anti-P39 antibodies were isolated from anti-desialylated gp160 sera by immunoaffinity chromatography on a P39-coupled Sepharose column and were then assayed for their ability to recognize HIV-2 gp140. Peptide P39 purified antibodies bound to HIV-2 gp140 in a dose-dependent manner, in good agreement with results of the inhibition assay (Fig. 3A). This binding was specific, since it was inhibited by increasing concentrations of soluble P39 (Fig. 3B) with a $K_{0.5}$ of about 100 nM, showing that the avidity of immunopurified antibodies was similar to that found when total sera produced against desialylated gp160 were used (Fig. 1).

One hypothesis to explain the broad reactivity spectrum observed with anti-desialylated gp160 is that the antibody

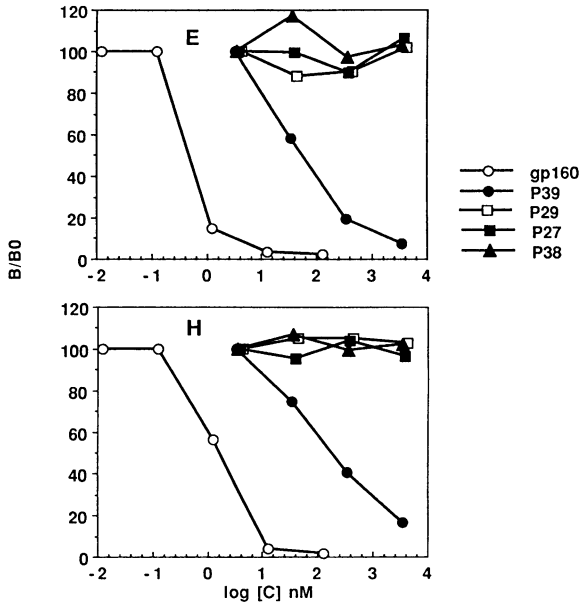

FIG. 1. Inhibition by synthetic peptides derived from gp120/gp41 of HIV-1-Lai (38, 39) of the interaction between radiolabeled HIV-2 gp140 and antibodies produced in rabbits against desialylated gp160 (E and H). All synthetic peptides tested were described previously (3). The figure shows results obtained with P27 (residues 166 to 190), P29 (residues 308 to 328), P38 (residues 561 to 586), and P39 (residues 583 to 609). Immunization procedures, products, and buffers have been described previously (3). B/B0, binding obtained in the presence of competitor divided by binding in the absence of competitor. Symbols: ○, gp160; ●, P39; □, P29; ■, P27; ▲, P38.

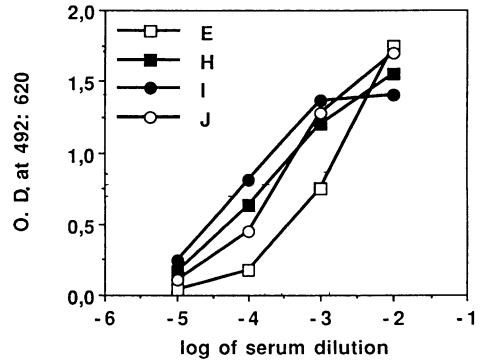

FIG. 2. ELISA detection (titration curve) of antibodies against synthetic peptide P39 in sera from rabbits immunized with native (I and J) or desialylated (E and H) gp160. Microtiter plates (96-well plates; Nunc, Roskilde, Denmark) were coated for 2 h at 37°C with 500 ng of P39 per well in 50 μl of phosphate-buffered saline (pH 7.4). Wells coated with 50 ng of gp160 or with casein were used as positive and negative controls, respectively, as described previously (3). O. D. at 492, optical density at 492 nm.

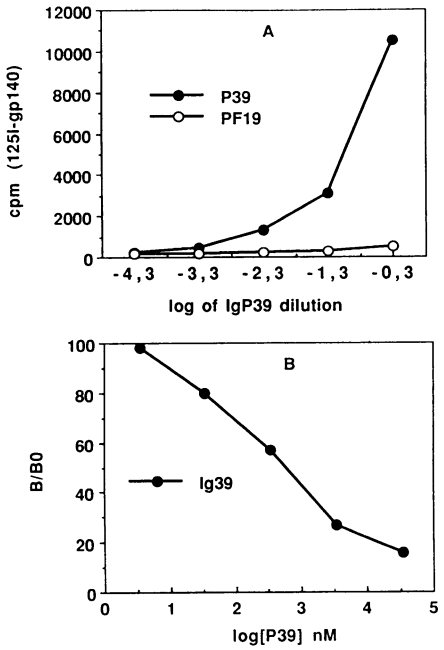

FIG. 3. (A) Binding of radiolabeled HIV-2 gp140 to P39-column affinity-immunopurified antibodies (IgP39) from rabbits immunized with desialylated gp160. A 1-mg portion of peptide P39 (residues 583 to 610) was coupled to 2 g of activated cyanogen bromide–Sepharose-CL4B as specified by the manufacturer (Pharmacia). Similarly, peptide PF19 derived from HIV-2 nef protein (residues 125 to 154) coupled with Sepharose was used for an irrelevant control affinity column. (B) Specificity and avidity of immunopurified anti-P39 antibodies as determined in a competition assay for antibody binding between different concentrations of peptide P39 and radiolabeled HIV-2 gp140. B/B0, binding obtained in the presence of competitor divided by binding in the absence of competitor.

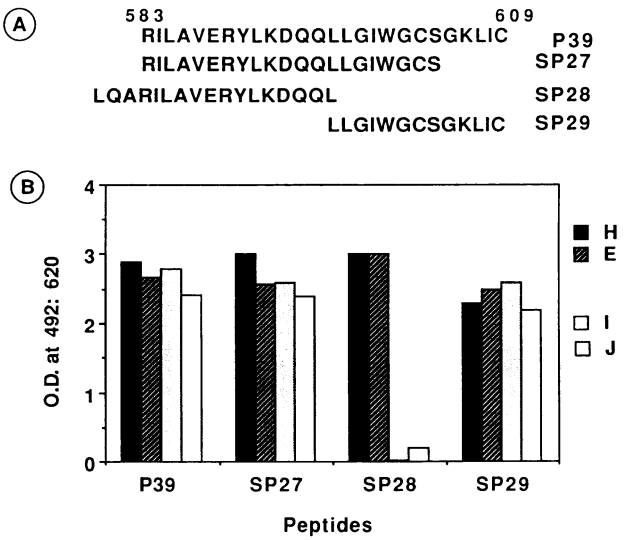

FIG. 4. (A) Amino acid sequence of synthetic peptides (SP27, SP28, and SP29) overlapping peptide P39 mimicking the gp41 immunodominant epitope. (B) ELISA reactivity of antibodies produced against native (I and J) or desialylated (E and H) gp160, with synthetic peptides (SP27, SP28, and SP29) spanning the gp41 immunodominant epitope mimicked by peptide P39. Procedures are indicated in reference 3 and the legend to Fig. 2. O.D. at 492, optical density at 492 nm.

population responsible for cross-reactivity is present only in sera from rabbits immunized with desialylated gp160. To localize the minimal sequence responsible for this cross-reactivity, three synthetic peptides overlapping the region mimicked by peptide P39 (Fig. 4A) were tested by an ELISA against anti-native and anti-desialylated gp160 antibodies. Both anti-native and anti-desialylated gp160 antibodies recognized SP27 (residues 584 to 604), which is located in the N-terminal and central regions of peptide P39, and SP29 (residues 597 to 609), which is located in the C-terminal half of P39 (Fig. 4B) and corresponds to the transmembrane gp41 immunodominant epitope as described by Gnann et al. (14). Peptide SP28 (residues 581 to 597), which is located in the N-terminal region of P39, was recognized only by antibodies produced against desialylated gp160. In addition, these peptides were tested for their ability to inhibit the interaction between anti-desialylated gp160 antibodies and HIV-2 ^{125}I-gp140. Peptide SP27 competed with ^{125}I-gp140 for antibody binding with the same avidity as peptide P39 did ($K_{0.5}$ = 100 nM), whereas only partial inhibition was obtained with peptide SP28 at 10^{-5} M (Fig. 5). This partial inhibition by peptide SP28 indicates the low affinity of the cross-reactive antibody population with the peptide and may be explained by the fact that the peptide does not possess the "good" conformation or that it represents only part of the antigenic determinant that is complementary to the antibody-binding site.

To further characterize this cross-reactivity, sera from rabbits immunized with native or desialylated gp160 were assayed for their reactivity against the envelope glycoprotein gp140 of simian immunodeficiency virus, a lentivirus closely related to HIV-2 (6, 8, 11). Simian immunodeficiency virus

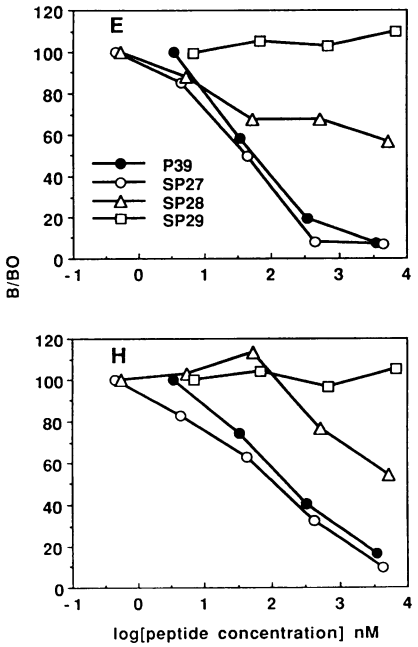

FIG. 5. Inhibition, by synthetic peptides spanning the gp41 immunodominant epitope, of the interaction between radiolabeled HIV-2 gp140 and rabbit antibodies against desialylated gp160 (E and H). B/B0, binding obtained in the presence of competitor divided by binding in the absence of competitor.

gp140 and HIV-2 gp140 share 84% amino acid homology and 46 to 50% with HIV-1 gp160 in the region mimicked by peptide P39. Despite these similarities between simian immunodeficiency virus gp140 and HIV-2 gp140 within this region, however, no cross-reactivity was observed between SIV gp140 and anti-desialylated gp160 or P39-immunopurified antibodies (data not shown). To determine whether carbohydrates could block antibody binding, we fully deglycosylated simian immunodeficiency virus gp140 and tested it for its ability to interact with anti-desialylated gp160 antibodies. Here again, despite glycan removal, no binding was detected, thus excluding any influence of carbohydrate chains on epitope accessibility (data not shown).

In addition, and in contrast to the capacity of anti-native and anti-desialylated gp160 to neutralize HIV-1 infection (3), no neutralizing activity toward HIV-2 infection was observed with anti-desialylated gp160 or with anti-native gp160 antibodies (data not shown).

Concerning the role of sialic acid on the viral surface, Huso et al. (19) have shown, in agreement with our results, that removal by neuraminidase of sialic acid from the surface of caprine arthritis-encephalitis virus enhanced the kinetics of virus neutralization by specific antibodies produced in goats. Not only were the desialylated virus particles more sensitive to proteolysis by proteinase K, in contrast to native caprine arthritis-encephalitis virus, but also these results suggest a critical role of sialic acid: it masks both antibody and proteinase K binding to the neutralizing epitope and cleavage site, respectively. In addition, the high sialic acid substitution of caprine arthritis-encephalitis virus may be responsible for its poor capacity to induce neutralizing antibodies relative to the closely related visna virus, which is apparently less sialylated than caprine arthritis-encephalitis virus and more efficient for eliciting neutralizing antibodies (19). Similarly, Smiley and Friedman (36) have shown that the binding activity of component C3b of the complement system is enhanced after neuraminidase treatment of herpes simplex virus gC-1 protein.

Most importantly, the fact that anti-desialylated HIV-1 gp160 cross-reacted with HIV-2 gp140 may explain the presence of antibodies reactive with both HIV-1 and HIV-2 in some human sera (5, 13, 18, 27), e.g., those produced against desialylated or abnormally glycosylated forms of HIV-1 envelope glycoproteins in the course of HIV-1 infection. This possibility, in addition to others based on conformational epitopes as recently described by Oldstone et al. (27), must be taken into account before making any conclusions about double infection.

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