Fine Serotyping of Human Immunodeficiency Virus Serotype 1 (HIV-1) and HIV-2 Infections by Using Synthetic Oligopeptides Representing an Immunodominant Domain of HIV-1 and HIV-2/Simian Immunodeficiency Virus

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Received 5 February 1991/Accepted 12 April 1991

In this study, enzyme immunoassays for detection of type-specific antibodies to human immunodeficiency viruses (HIV) were developed by using short peptides corresponding to sequences located within the immunodominant domain of the transmembrane glycoproteins of both HIV-1 and HIV-2/simian immunodeficiency virus (SIV). The assays were highly sensitive with currently available sera from various geographical areas. Furthermore, they appeared to be more specific in HIV serotyping than the Western blot (immunoblot) assay, since all of the sera were clearly discriminated as one or the other type. It was also shown that in contrast to HIV-1, the C-terminal cysteine residue (amino acid 620, SIV from captive macaques, Mm142 strain) of the HIV-2-SIV peptide is not necessary for recognition of the peptide by antibody to HIV-2.

Materials and Methods

Peptides. Peptides corresponding to sequences located in the immunodominant domain of the TGP of HIV-1 and SIVmac were synthesized to be used as antigen sources in solid-phase enzyme immunoassays. Two peptides corresponding to sequences of HIV-1 (amino acids 594 to 603 and 590 to 607 of the envelope polyprotein, HXB2 clone, human T-cell lymphotropic virus type IIIB strain [16]) and one peptide corresponding to a sequence of SIVmac (amino acids 610 to 619 of the envelope polyprotein, Mm142 strain [5]) were prepared. The sequences are shown in Fig. 1. Peptides HIV-1-TMSP10 and SIV-TMSP10 were made by the solid-phase procedure developed by Merrifield (14), on an automated peptide synthesizer (Applied Biosystems 431A) with tert-butyloxycarbonyl-protected amino acids and phenylacetylmethyl resin. After synthesis, the resin support and the side chain-protecting groups were removed with the low-high trifluoromethanesulfonic acid procedure (18) by using p-cresol, dimethylsulfide, and dithiothreitol as scavengers. After synthesis, the peptides were purified by reverse-phase chromatography using C8 columns (Aquapore octyl, 100 by 10 mm, 20 μm; Applied Biosystems). As shown in Fig. 1, five lysine residues were added at the N-terminal extremities of HIV-1-TMSP10 and SIV-TMSP10 during synthesis to improve both binding of the peptides on the solid phase and accessibility of the epitope to the antibodies. Previous data obtained in our laboratory indicated that use of peptides HIV-1-TMSP10 and SIV-TMSP10 without N-terminal lysine residues both extensively reduced binding of specific antibodies to the peptides and increased nonspecific binding of normal sera. Peptide HIV-1-TMSP18 was synthesized at Appligene (Illkirch, France) by the tert-butyloxycarbonyl procedure.

Peptide enzyme-linked immunosorbent assays. Peptide HIV-1-TMSP18 was coated onto wells of polystyrene microtiter plates (Falcon) at 5 μg/ml in 0.05 M bicarbonate buffer,
HIV-1-TMSP10  KKKKGIWGCSGKLI  594-603  HTLV-III
HIV-1-TMSP18  DQLLGIWGCSGKLICTTA  590-607  HTLV-III
SIV-TMSP10  KKKKNWAGCAXFQY  610-619  SIVmac Mm142

FIG. 1. Sequences of synthetic peptides derived from the TGP s of HIV-1 and SIV. The amino acid (AA) position numbers for HIV-1 are based on the sequence published by Ratner et al. (16), and those for SIV are based on the sequence published by Chakrabarti et al. (5). Boldface letters correspond to residues present in the virus TGP s. Plain letters (K) correspond to the lysine residues added during synthesis. HTLV-III B, human T-cell lymphotropic virus type III B.

for 20 h at 37°C (200 μl per well). Peptides HIV-1-TMSP10 and SIV-TMSP10 were coated at 1.25 μg/ml (200 μl per well). After two washings in phosphate-buffered saline (PBS) containing 0.3% Tween 20 (PBS/TW), the unoccupied sites of the wells were saturated with PBS containing 2% newborn calf serum for 45 min at 37°C. One hundred-microliter serum samples diluted 1:10 in PBS/TW containing 10% newborn calf serum were incubated for 30 min at room temperature. After five washings in PBS/TW, 100 μl of peroxidase-conjugated goat F(ab')2 anti-human immunoglobulin (TAGO, Burlingame, Calif.) diluted 1:10,000 in PBS/TW-newborn calf serum was incubated for 30 min at room temperature. After five washings in PBS/TW, the reaction was revealed by incubating a mixture of hydrogen peroxide--o-phenylenediamine for 10 min at room temperature. Color development was stopped with 2 N H2SO4, and the A492 was read. The cutoff value of every assay was calculated by using the mean optical density (OD) obtained with 105 HIV-negative sera. The mean values were 0.053, 0.052, and 0.068 for HIV-1-TMSP18, HIV-1-TMSP10, and SIV-TMSP10, respectively. All of the negative sera tested gave ODs below these mean values plus 4 standard deviations, except for one serum in the HIV-1-TMSP18 and the SIV-TMSP10 assays and two sera in the HIV-1-TMSP10 assay. The cutoff values were therefore estimated as the mean OD of the negative sera plus 4 standard deviations for every assay, i.e., 0.359, 0.363, and 0.378 for the HIV-1-TMSP18, HIV-1-TMSP10, and SIV-TMSP10 assays, respectively. The same negative control, an HIV-1 positive control, and an HIV-2 positive control were included in duplicate in every run that was then performed. The negative control serum gave ODs of 0.040 to 0.090 in different runs. Therefore, we estimated that the cutoff value could be calculated in each run as 0.300 plus the mean OD of the negative control.

Sera. A total of 235 serum samples were tested for the presence of antibody to the three peptides. The serotype specificities of the sera were previously assessed by Western blot analysis by using a procedure already described (4, 13). Sera that were reactive to the envelope glycoproteins of HIV-1 were defined as HIV-1 positive. Sera that were reactive to the envelope glycoprotein of HIV-2 were defined as HIV-2 positive. Sera that were reactive to the envelope glycoproteins of both HIV-1 and HIV-2 were defined as doubly reactive (Fig. 2). One hundred seventy-nine HIV-1-positive serum samples were collected from 71, 62, and 46 patients originating from France, Burundi (Central Africa) (1), and the Ivory Coast (West Africa) (7), respectively. Forty-six serum samples were collected from HIV-2-in-
absorbance values obtained in the HIV-1-TMSP18 and SIV-TMSP10 assays with 179 HIV-1-positive sera, 46 HIV-2-positive sera, 10 doubly reactive sera, and 105 negative sera is shown in Fig. 3. All of the HIV-1-positive and HIV-2-positive sera except one gave absorbance values clearly above the cutoff. Only two sera positive for antibody to HIV-1 (1.1%) cross-reacted with the SIV peptide. These two specimens, from patients from the Ivory Coast, gave high absorbance values in the HIV-1-TMSP18 assay and low signals (0.426 and 0.393) in the heterologous SIV-TMSP10 assay. Only one serum positive for antibody to HIV-2 cross-reacted with the HIV-1 peptide. This serum, which was also weakly positive for antibody to HIV-1 glycoproteins gp160 and gp41 by Western blot, was highly positive (OD, 1.638) in the SIV-TMSP10 assay, whereas it reacted weaker (OD, 0.583) in the HIV-1-TMSP18 assay.

Ten doubly reactive sera were tested with the HIV-1-TMSP18 and SIV-TMSP10 assays. One serum (Fig. 2, sample J) was reactive only with the SIV-TMSP10 peptide (Fig. 3C). The nine remaining sera reacted with both HIV-1-TMSP18 and SIV-TMSP10, with similar absorbance values (Fig. 3C). The specificity of these doubly reactive sera was further assessed by using the inhibition assay. The results are summarized in Fig. 4. Figure 4A shows the pattern of reactivity to HIV-1-TMSP18 and SIV-TMSP10 of a specimen positive for antibody to HIV-2, in the presence of either soluble HIV-1-TMSP18 or SIV-TMSP10. The decrease of binding of HIV-2 antibody to SIV-TMSP10 was directly related to the increase of soluble SIV-TMSP10 peptide in the reaction wells, whereas the increase of soluble HIV-1-TMSP18 peptide had no effect on the binding of antibody to HIV-2 to solid-phase SIV-TMSP10. Figure 4C and D summarizes the two kinds of patterns observed with doubly reactive sera in the inhibition assay. The sample corresponding to Fig. 4D contained antibodies whose binding was neutralized in both the HIV-1-TMSP18 and SIV-TMSP10 assays only by the homologous peptide, as if the patterns in 4A and B had been superposed. This kind of inhibition pattern might argue for the presence of two distinct populations of antibodies following dual infection by HIV-1 and HIV-2. Among the nine sera that reacted with both HIV-1-TMSP18 and SIV-TMSP10 seven gave such an inhibition pattern. The remaining two sera gave inhibition patterns like that in Fig. 4C. The sample corresponding to Fig. 4C contained antibodies that were neutralized by soluble HIV-1-TMSP18 peptide in the HIV-1-TMSP18 assay like a single HIV-1-positive serum. In contrast, the binding of antibodies present in this sample to SIV-TMSP10 was nonspecifically inhibited by both homologous and heterologous peptides, even at a low concentration. This kind of data might argue for the presence of antibody to HIV-1 that strongly cross-reacted with HIV-2. These two sera could have been collected from HIV-1-infected patients and cross-reacted strongly with HIV-2 envelope sequences.

DISCUSSION

Gnann et al. (8) have demonstrated that the essential epitope for immune recognition of the TGP of HIV-1 is a
seven-amino-acid sequence containing two cysteine residues (amino acids 603 to 609, lymphadenopathy-associated virus strain Bru [20]). Moreover, it appeared that the presence of both cysteine residues was essential for the antigenic conformation of the epitope via formation of disulfide bonds. In the present study, we confirm the usefulness of the two cysteine residues in the sequence of the HIV-1 peptide, since 100% of the HIV-1-positive sera bound to HIV-1-TMSP18 (DQLLG1WGCSGKLICTTA), whereas only 51% of them reacted with a shorter peptide, HIV-1-TMSP10 (kkkkkGI WGCSSLK), which did not include the C-terminal cysteine residue. Gnann et al. have synthesized a peptide whose C-terminal cysteine was substituted with a serine residue (LGLWGCSKLIS) and showed that only 9% of HIV-1-positive sera reacted with this peptide. We also found that removal of the C-terminal cysteine dramatically reduced the antigenicity of this HIV-1 epitope, although approximately half of our HIV-1 positive sera were able to bind the peptide lacking this cysteine. This difference might be due to the presence in our peptide of the N-terminal lysine residues that would increase both accessibility to antibodies and binding of peptides to the solid phase.

In contrast to the HIV-1 data, our results clearly indicate that the C-terminal cysteine is not essential for the antigenic conformation of the SIV peptide, since 100% of the HIV-2-positive sera bound to SIV-TMSP10. Previous data (not shown) indicated that approximately 80% of HIV-2-positive sera bound to lysine-free SIV-TMSP10. This means not only that good reactivity with the SIV peptide is due to the five N-terminal lysine residues but also that this chemical modification greatly enhances binding of the antibodies.

Our data show that the immunodominant epitope located on the TGPs of HIV-1 and HIV-2-SIVpac do not involve exactly the same conserved amino acids, especially the C-terminal cysteine residue. Although an important conformational conservation between the TGps of the two virus types has been reported (9), minor amino acid changes would be sufficient to shift the position of the epitopes slightly. For instance, there are three cysteine residues in the TGP of HIV-1 instead of the four cysteine residues in the TGP of HIV-2. This difference could explain the modification of the conformation and therefore the immune recognition of the TGP during natural infection.

The immunoassays using HIV-1-TMSP18 and SIV-TMSP10 were able to discriminate clearly between HIV-1 and HIV-2 infections at least as efficiently as the Western blot assay. Immunoassays using such short peptides, in which the conserved residues located on both sides of the antibody-binding site are not included, could abolish almost all of the envelope cross-reactivities observed in Western blot assays (19). Our study also shows that HIV-1-TMSP18 and SIV-TMSP10 assays can be more informative than Western blotting with doubly reactive sera.

Most of the countries in which HIV-1 and HIV-2 are both prevalent are economically poor. In such countries, a sensitive assay able to detect both viruses is essential for screening purposes. Peptide enzyme immunoassays such as the prototypes discussed in the present study are alternative procedures that must be evaluated prospectively for confirmation and serotyping. They may offer diagnostic algorithms which could limit the use of expensive Western blots.

ACKNOWLEDGMENTS

This work was supported by grants from the Agence National de Recherche sur le SIDA (ANRS) and the EEC and in part by US DAMD 1787C 7072. A.B. and B.J. were supported by doctoral fellowships from the ANRS and the Foundation Mérieux, respectively.

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