Antisera raised against the second variable region of the external envelope glycoprotein of human immunodeficiency virus type 1 cross-neutralize and show an increased neutralization index when they act together with antisera to the V3 neutralization epitope

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Antibodies have been raised against a synthetic peptide (IRDKIQKENALFRNL) containing a neutralizing epitope within the second variable region of the human immunodeficiency virus type 1 (HIV-1) SF2 strain external envelope glycoprotein (gp120) and also against equivalent peptides of the HIV-1 LAI, RF and MN isolates. The resulting antisera cross-react with heterologous peptides but binding to heterologous recombinant gp120 is more restricted. Antisera to HIV-1 SF2, RF and MN are able to neutralize homologous virus. Some cross-neutralization is also observed, but a consensus peptide failed to induce neutralizing antibodies to any of the isolates studied. Antibodies to the V2 and V3 epitopes give a higher neutralization index when acting together than when the individual sera are used alone. Antibodies induced in natural infection bind to two sets of hexamers within the region encompassed by the 15-mer peptide, and the response to these can differ between infected individuals and within the same host over time.

Introduction

One of the principal problems encountered in efforts to vaccinate against human immunodeficiency virus type 1 (HIV-1) is the extreme variability of the virus. This variability exists not only between isolates of the virus taken from different individuals but also within a single host so that under conditions of natural transmission it is possible that no two inocula will be the same. Currently, successful vaccine appears to be associated with antibodies to the third variable region (V3) of the external envelope glycoprotein (gp120; Berman et al., 1990; Girard et al., 1991). If broader protection is to be obtained then other, less variable, epitopes will have to be introduced into vaccination strategies.

We have produced antisera to a complete set of overlapping synthetic peptides representing the gp120 of the HIV-1 SF2 isolate (Davis et al., 1990b). When these sera were used in radioimmunoassay (RIA) against recombinant gp120s of HIV-1 SF2 and BH10 strains, antibodies to two regions were seen to bind at high levels in the homologous system but not in the heterologous combination. These two regions correspond to the second (V2) and third (V3) variable regions. In our first attempts to set up neutralization assays with these sera we therefore used these isolate-specific antibodies, and showed that antibodies to both the V2 and V3 regions could neutralize HIV-1 SF2. Neurath et al. (1990) also showed that neutralizing antibodies could be raised against synthetic peptides representing the V2 region.

The V2 neutralizing epitope may be an important factor in developing vaccines against HIV-1 since high levels of antibodies are induced against it following injection of baboons with recombinant gp120 (Stephens et al., 1992). In addition, there is the possibility of cross-neutralization since, although the antipeptide antibodies bind in an isolate-specific manner in RIA, there is only limited variability between isolates when these are grouped geographically (Table 1). In support of this, monoclonal antibodies raised in mice against affinity-purified virion-derived HIV-1 IIIB gp120 that bind to peptides representing the V2 region do show cross-neutralization (Fung et al., 1992). However, if they are to be used as part of a vaccination strategy, it must be established that cross-neutralizing antibodies can be induced to synthetic peptides representing the V2 region.

Methods

Cells. H9 cells (Popovic et al., 1984; Mann et al., 1989) were obtained through the MRC AIDS Directed Programme. C8166 cells (Salahuddin et al., 1983) were obtained from the European Collection of Animal Cell Cultures, Porton Down, U.K. Cells were cultured in RPMI medium with 10% fetal calf serum (FCS) in roller bottles. Cultures were split every 3 to 4 days.
Table 1. Alignment within the V2 region of European/American and African strains of HIV-1

<table>
<thead>
<tr>
<th>European/American strains</th>
<th>Consensus</th>
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<tbody>
<tr>
<td>CDC4</td>
<td>I R D K V Q K E Y A L F Y K L</td>
</tr>
<tr>
<td>SC</td>
<td>- S - - - - - - - - - - - - - -</td>
</tr>
<tr>
<td>LA1</td>
<td>- G - - - - - - - - - - - - - -</td>
</tr>
<tr>
<td>HXB2</td>
<td>- G - - - - - - - - - - - - - -</td>
</tr>
<tr>
<td>JH3</td>
<td>- - M - - - - - - - - - - - -</td>
</tr>
<tr>
<td>MN</td>
<td>- - - - - - - - - - - - - - - L</td>
</tr>
<tr>
<td>WMJ2</td>
<td>R - - - - - - - - - - - - - - N</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>African strains</th>
<th>Consensus</th>
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<tbody>
<tr>
<td>ZJ21</td>
<td>L - - - Q R - I - - - - - - - -</td>
</tr>
<tr>
<td>MAL</td>
<td>G S - R - - - - - - - - - - - - T N</td>
</tr>
<tr>
<td>ELI</td>
<td>L K - - K Q V - - - - - - - - R</td>
</tr>
<tr>
<td>Z2</td>
<td>V - - - - T K Q V H - - - - - - - - - - - - - -</td>
</tr>
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Viruses. The following viral isolates were obtained through the MRC AIDS Directed Programme: HIV-1/France, Pasteur/LAV-BR/83 (Barre-Sinoussi et al., 1983), recently re-titled HIV-1 LAI (Wain-Hobson et al., 1991); HIV-1/USA, NIH/HTLVIII/84 (Gallo et al., 1984); HIV-1/USA, NIH/HTLVI/84 (Papolovic et al., 1984); and HIV-1/USA, CR1/SF2 (AVR-2)/84 (Cheng-Mayer & Levy, 1988). HIV-1 SF2 was cultured in HUT78 cells and the other isolates in H9 cells. Viral stocks were prepared as 1 ml samples which were fast frozen and stored in liquid nitrogen. They were titrated in C8166 cells using c.p.e. to determine endpoints. The titres were 10^48 TCID_{so} ml for HIV-1 SF2, 10^58 TCID_{so} ml for HIV-1 LAI, 10^77 TCID_{so} ml for HIV-1 RF and 10^58 TCID_{so} ml for HIV-1 MN

Peptides. Peptide env 18, IRDKIQKENALFRNLC, representing amino acids 171 to 185 within the V2 region of the HIV-1 SF2 isolate (Davis et al., 1990b), has induced sera with high levels of neutralizing activity. The equivalent peptides for HIV-1 LAI, RF and MN are IRGKVQKEYFYKL, RRDKQKYYKFLC and IRDKMQKEYALYYKLC, respectively. A consensus peptide, IRDKQMKEALYFLKLC, represents the most common amino acids at each position amongst European and American strains of HIV-1 (Table 1). Peptide env 32, SNNNTRKSIVGPPGRAC, represents amino acids 311 to 325 within the V3 region of the HIV-1 SF2 isolate (Davis et al., 1990b). The C-terminal cysines are added so that the peptides can be conjugated to a carrier protein using 3-maleimidobenzoic acid-N-hydroxysuccinimide ester.

Injection schedules. F1 AO x LOU hybrid rats were sensitized with Bacille-Calmette-Guérin and subsequently injected with peptide conjugated to purified protein derivative according to the schedule previously described (Davis et al., 1990a).

Recombinant antigens. The following reagent was obtained through the AIDS Research and Reference Reagent Program, AIDS Program, National Institute of Allergy and Infectious Disease, NIH, Bethesda, Md., U.S.A.: glycosylated gp120 (CHO cell-derived) from HIV-1 SF2 from Dr N. L. Haigwood of Chiron Corporation (Levy et al., 1984; Sanchez-Pescador et al., 1985; Haigwood et al., 1990). The following reagents were obtained through the MRC AIDS Directed Programme: glycosylated gp120 (CHO cell-derived) from HIV-1 BH10 (Ratner et al., 1985), which has the same sequence as HIV-1 LAI in the region of the peptides used in this study, from Celtech and glycosylated gp120 of the HIV-1 MN strain produced in a baculovirus expression system by Agmed.

Solid-phase radioimmunoassay. Peptides and recombinant proteins were used as the solid phase in a radioimmunoassay as previously described (Davis et al., 1990a, b).

Sera. Blood was obtained from 13 HIV-1 seropositive patients attending a clinic in Cambridge. Ten were in Centers for Disease Control Stage II, two were in Stage III and a further one was in Stage IV. Blood was also obtained from one Stage II and seven Stage IV patients attending a clinic in Edinburgh. The blood was allowed to clot and then centrifuged. The serum was removed and heat-inactivated at 56 °C for 30 min and treated with Triton X-100 to give a final concentration of 1% (v/v).

Epitope mapping with absorption/elution/re-absorption/amplification assay. Hexamers were synthesized on polyacrylic acid-coated polyethylene rods by the FMOC–polyamide mode of synthesis as previously described (Davis et al., 1990b). These peptides represented amino acids 161 to 185 of the HIV-1 SF2 isolate. They were incubated overnight with a 1:50 dilution of serum in PBS with 0.05% Tween 20, washed in diluent and incubated in Universal buffer (1 M-citric acid, 1 M-phosphoric acid, 1 M-sodium hydroxide, 0.1 M-hydrochloric acid and 50 mM-orthoboric acid, pH 2.5) for 10 min at room temperature. Antibody bound to the pins is thus eluted into the wells of a 96-well plate. Sufficient 0.1 M-sodium hydroxide was then added to each well so as to return the pH to 7, and was followed by PBS containing 0.05% Tween 20 and 2% BSA to minimize antibody binding to the microtitre plate. The peptides on their rods were cleaned by sonication as previously described (Davis et al., 1990b). The clean peptides were then re-incubated in their corresponding wells at 4 °C overnight, washed and transferred to wells containing alkaline phosphatase-conjugated anti-human immunoglobulin before incubating at room temperature for 90 min on a rocking table. The binding of enzyme-labelled antibody to the rods was detected using the substrate and amplification system provided with AMPAK (Novo Biolabs) according to the manufacturer’s directions. Colour development was monitored visually and the reaction was stopped to give optimal discrimination between positive and negative wells while retaining low backgrounds.

Neutralization assay. Sera were heat-inactivated at 56 °C for 30 min. Virus was diluted in 400 μl of RPMI medium with 10% FCS to give samples of 500 TCID_{so} and incubated with a 1:5 dilution of rat serum at 37 °C in 7% CO₂. After 24 h, four samples of 100 μl of each virus-serum/dilution combination were dispensed into 24-well tissue culture plates. C8166 cells were added to each well to give 2 x 10⁴ cells in 1 ml of medium per well. A further 1 ml of medium was added after 3 days and 1 ml of medium was replaced twice weekly. The cell cultures were examined for c.p.e. and 1 ml of supernatant was harvested into Triton X-100 to give a final concentration of 1% when control cultures, in which virus had been incubated with normal rat serum, showed extensive c.p.e. The reverse transcriptase and gag p24 protein content of the supernatants were determined by radioimmunoassay.

Reverse transcriptase assay. An immunoradiometric assay has been set up using pairs of monoclonal antibodies to HIV-1 LAI reverse transcriptase (Tisdale et al., 1988). In the most sensitive combination, monoclonal antibody 8 was diluted in PBS to give 10 μg/ml and 50 μl was dispensed into wells on a 96-well Falcon microtest flexible plate. The plates were then covered and incubated overnight at 4 °C in a water-saturated atmosphere. Plates were washed three times in PBS with 0.05% Tween 20 and 10 mM-azide. They could then be stored, covered, in a dry atmosphere at 4 °C, if the wells were rinsed in 1% sucrose in distilled water with 0.1% azide and dried at 37 °C so as to leave a thin sugar coating over the solid-phase antibody. Stored plates were rinsed in PBS before use in an assay. Supernatants were added to the wells and incubated at 4 °C overnight. Wells were again washed and incubated with 50 μl monoclonal antibody 6 which had been radiolabelled with 125I to give 10 μCl/μg using Iodo-gen. After further
HIV-1 V2 neutralization epitope

Fig. 1. Activity of 1:500 dilution of rat antisera raised against V2 peptides of HIV-1 SF2 (a, f, k, p, u), HIV-1 LAI (b, g, l, q, v), HIV-1 RF (c, h, m, r, w), HIV-1 MN (d, i, n, s, x), and a consensus sequence derived from European and American strains (e, j, o, t, y) against solid-phase peptides HIV-1 SF2 (a to e), HIV-1 LAI (f to j), HIV-1 RF (k to o), HIV-1 MN (p to t) and consensus (u to y). Histograms of antibody determination by radioimmunoassay are displayed for individual rat sera.

Peptide washing, individual wells were removed with a hot wire and residual radioactivity was determined. Aliquots of recombinant reverse transcriptase were stored in liquid nitrogen and used to prepare standard curves.

Gag p24 assay. A similar assay was set up using a modified version of the gag p24 assay described by Moore et al. (1990) and McKeating et al. (1991) in which sheep polyclonal antisera raised against gag peptides (Aalto Bioreagents) are used as the solid phase and monoclonal antibody EH12EI (Spence et al., 1989) is used to detect captured gag p24 antigen. Bound monoclonal antibody is detected using 125I-radiolabelled anti-mouse IgG. Samples of HIV-1 supernatant were stored in liquid nitrogen and used to prepare standard curves.

Results

Cross-reactivity of anti-peptide antibodies against peptides

Antibodies were raised against the HIV-1 SF2, HIV-1 LAI, HIV-1 MN and consensus peptides at high titres (Fig. 1). These antibodies also showed high levels of activity against some heterologous peptides. Thus, antibodies to the HIV-1 SF2 and consensus peptides bound to the HIV-1 SF2, LAI, MN and consensus peptides at high titres. Similarly, antibodies to the HIV-1 MN peptide showed activity against the HIV-1 SF2 and consensus peptides. Antibodies to the HIV-1 LAI peptide showed low levels of binding to the HIV-1 MN and consensus peptides. Only low levels of antibodies to the HIV-1 RF peptide were raised.

Cross-reactivity of anti-peptide antibodies against recombinant proteins

Anti-peptide antibodies to HIV-1 SF2, LAI and MN bound to their homologous recombinant gp120 at high titres but antibodies raised against HIV-1 RF showed only low levels of binding. Antibodies to the consensus peptide recognized recombinant gp120 from HIV-1 LAI and MN but not from HIV-1 SF2 (Fig. 2). The titre of HIV-1 SF2 antibodies to homologous glycoprotein (1:2500) was fivefold greater than the titre of HIV-1 LAI antibodies to BH10 (data not shown).

Reverse transcriptase assay

Preliminary experiments indicated that the solid phase should be prepared with antibody diluted in PBS rather than bicarbonate buffer and that the detecting antibody should be freshly radiolabelled to yield at least 10 μCi/μg. Delays between radiolabelling and the use of the antibody led to a considerable reduction in the
sensitivity of the assay. The most sensitive combination of antibodies tested was monoclonal antibody 8 as the solid phase and monoclonal antibody 6 as the detecting antibody. There was a progressive increase in the concentration of recombinant reverse transcriptase required to produce standard curves, probably indicating that this material was unstable even when stored in liquid nitrogen. Results are therefore recorded as the amount of radiolabel bound in assays of supernatants from HIV-1-infected cultures.

Neutralization with anti-peptide antibodies

Sera were screened for neutralization activity at a 1:100 dilution. Only HIV-1 SF2 antibodies neutralized homologous virus. Neutralization was detected by reduction in both gag and reverse transcriptase protein levels in cell culture supernatants. Antibodies to the other virus strains failed to neutralize their homologous virus. The reverse transcriptase protein could only be detected in supernatants of HIV-1 LAI and not with HIV-1 RF nor HIV-1 MN cultures (data not shown). Subsequent neutralization assays were performed using the gag assay.

Further experiments, using a 1:5 dilution of serum, showed that HIV-1 MN and HIV-1 SF2 neutralized their homologous virus, reducing the concentration of gag in their cell culture supernatants by greater than 10-fold. HIV-1 RF showed a reduction of greater than 50% gag concentration in neutralization assays but HIV-1 LAI was not neutralized by antisera to homologous peptide. HIV-1 LAI was neutralized, with greater than 10-fold reduction in gag protein, by antibodies to HIV-1 SF2 peptide. However, if this criterion is relaxed, so that a 50% reduction is accepted as the cutoff, antisera to HIV-1 SF2 also neutralized HIV-1 RF, antisera to HIV-1 MN
neutralized HIV-1 SF2 and antiserum raised against HIV-1 RF neutralized HIV-1 LAI and MN. Antiserum to the consensus peptide failed to neutralize any virus isolates (Fig. 3).

At antisera dilutions of 1:20, HIV-1 SF2 and MN were neutralized, but no heterologous neutralization was observed (data not shown).

Neutralization with a combination of V2 and V3 anti-peptide antibodies

Anti-peptide antibodies to the V2 and V3 regions of HIV-1 SF2 neutralize the virus. When virus is incubated with both antibodies concurrently, while retaining the same overall serum level, increased neutralization is observed (Fig. 4). This experiment was repeated three times with similar results.

Hexamers that bind antibody from HIV-1-infected patients' sera

Hexamer ENALFR bound the highest level of antibodies from the sera of patients attending a clinic in Cambridge. Minor peaks of antibody binding were observed with hexamers KIQKEN, TSIRDK, ITTSIR and NCSFNI. The hexamers within the amino acid sequence TSIRDKIQ bound the highest level of antibodies in sera of patients attending a clinic in Edinburgh with peptide ENALFR representing a secondary peak (Fig. 5). Selected sera gave similar results in replicate assays.

Fig. 4. Neutralizing activity of 1:100 dilution of normal rat serum (■) and rat antiserum raised against HIV-1 SF2 V2 (□) or V3 (△) regions or a mixture of 1:200 dilutions of both sera (○) against their homologous virus. Results of radioimmunoassays for gag p24 antigen (a) and reverse transcriptase (b) for the supernatants of C8166 cultures infected with dilutions of virus.

Fig. 5. HIV-1 gp120 V2 hexamer peptides recognized by sera from eight HIV-1 seropositive individuals from an Edinburgh clinic (a) and 13 from a Cambridge clinic (b). Cumulative totals of antibody determinations by ELISA are displayed.
Changes in epitopes recognized by patients’ sera over time

Changes in the hexamers recognized by sera taken from the same patient over time were noted. Thus, the three hexamers showing the highest binding of antibodies from the initial serum of patient A were ENALFR, KIQKEN and SIRDKI (Fig. 6a). After 38 months the epitopes recognized by this patient’s serum were further towards the N terminus: NCSFN1 and the hexamers in the FNITTSIR region (Fig. 6b). In patient B a smaller shift was observed towards the C terminus. The highest binding hexamers in the initial serum were TSIRDK and NITTSI (Fig. 6c). Thirty-eight months later the antibodies bound to SIRDK, TSIRDK and IDKIQ (Fig. 6d). In patient C the three hexamers showing the highest level of antibody binding to the initial serum were ENALFR, ALFRNL and KIQKEN (Fig. 6e). After 41 months the ENALFR still bound the highest level of antibody but the secondary peaks had shifted towards the N terminus: FNITTS and TTSIRDK (Fig. 6f).

Discussion

For purposes of vaccination, sterile immunity to HIV-1 requires prevention of infection following exposure to virus inocula which may be extremely variable. This variation comes from three sources. The virus will show polymorphism both within and between inocula. The problem may be further aggravated by uncertainties in the number of infectious doses in the inoculum. The viral content of seminal (Zagury et al., 1984; Ho et al., 1984; Levy et al., 1984; Krieger et al., 1991) or cervical fluids (Wofsy et al., 1986; Vogt et al., 1986) of infected individuals is very low even in terms of tissue culture infectious doses, and although a single TCID_{so} can infect a chimpanzee by the intravenous route, the number of such doses required to infect through mucosal surfaces may be very much higher, if studies with simian immunodeficiency virus (SIV) are relevant. Thus, there is a 1:1500 (Cranage et al., 1992) or a 1:10000 (Sutjipto et al., 1990) ratio of infectivity between the intravenous and mucosal routes of SIV infection in rhesus macaques. In support of this interpretation, HIV shows low transmission rates by the genital route (Holmberg et al., 1989; Blattner, 1991). These observations may reflect a very low content of infectious doses in any single virus inoculum. Alternatively, they may mean that the viral content reaches infectious levels only intermittently. The combination of a small inoculum from a highly diverse source may mean that each inoculum is unique. Under these circumstances, successful protection may require a broad immunity which is not necessarily at a high level. However, the results of the present study indicate that a broadly based immunity requires high titres of antibody.

Vaccination could be achieved by inducing high titres of neutralizing antibodies through priming individuals with recombinant proteins (or inactivated virus) and boosting the response to relevant epitopes with the corresponding peptides (Girard et al., 1991). Such a strategy requires that the neutralizing epitopes stimulate an immune response following injection of the viral envelope glycoprotein. This occurs with HIV-1 SF2 since antibodies to the peptides env 18 and 32, used in the present study, were induced at high levels in baboons injected with recombinant gp120 (Stephens et al., 1992). However, this is not the case following infection. Although most HIV-1 SF2-infected chimpanzees produced antibodies to the V3 epitope, antibodies to env 18 were produced at high levels in only a single animal that was infected with virus derived from a molecular clone. There appear to be two hexamers recognized within the amino acids encompassed by the env 18 peptide: IDKIQ and ENALFR (Fig. 5; Davis et al., 1990b; Stephens et al., 1992). However, these do not appear to induce high levels of antibodies in infected humans. Over time, the immune response seems to be drawn away from these epitopes towards an adjacent epitope that is included in peptide env 17. This has a glycosylation site which is not present in all isolates of the virus. Also, antibodies to this peptide are present in uninfected individuals (Davis et al., 1990b) which may indicate that some people have been primed to respond to this epitope.

Fig. 6. HIV-1 gp120 V2 hexamer peptides recognized by sera from three HIV-1 seropositive individuals from a Cambridge clinic. Sera were obtained from each individual in paired samples taken either 38 months apart (a, b and c, d) or 41 months apart (e, f).
by cross-reacting proteins. It is possible that the presence of a carbohydrate molecule at this site and previous exposure to a similar epitope influence how the immune system responds to the V2 region.

The extent of a serum's ability to neutralize a polymorphic mixture of viruses is better reflected in its neutralization index rather than its titre against a low dose of virus, which may be more uniform because it consists principally of the majority component. Our hypothesis is that the presence of antibodies to two major, independent epitopes within the vaccine will give better protection. Thus, if the proportion of viruses within an inoculum which can resist neutralization by antibodies to the V3 region is \( x \) and that for a second epitope is \( y \) then the proportion able to resist both sets of antibodies is the product of \( x \) and \( y \). In the current study, a mixture of antibodies to the V2 and V3 neutralizing epitopes led to an increase in the neutralization index in comparison with the sera used individually. This observation is interpreted as reflecting the presence of a mixture of virus particles which vary in their sensitivity to neutralization at the two epitopes. Some virus will be able to resist neutralization by antibodies to the V3 epitope and some will be able to resist V2 antibodies but fewer viruses will be able to withstand a combination of both than can resist the individual antisera. An alternative explanation is that one antibody produces a change in the virus particle such that a second antibody can bind more easily (McCullough, 1986).

Tilley et al. (1992) showed that neutralizing antibodies binding to the V3 region of gp120 were able to produce a change in the CD4 binding region which led to an increased capability in second neutralizing monoclonal antibody binding and synergistic neutralization. However, Thali et al. (1992) found that there was not necessarily a correlation between synergy of antibody binding and cooperativity in neutralization.

The HIV-1 SF2 isolate was neutralized by a 1:100 dilution of homologous antiserum whereas the maximum dilution that could still neutralize any of the other strains was 1:20. This observation may reflect the previously reported sensitivity to neutralization of the HIV-1 SF2 strain (Weiss et al., 1986). These studies used vesicular stomatitis virus (VSV) pseudotypes for neutralization and showed HIV-1 SF2 to be 125 to 625 times more susceptible to neutralization than HIV-1 (HTLV IIIb), one to 625 times more susceptible than HIV-1 MN and five to 625 times more than HIV-1 RF. More recently, these results have been confirmed using the virus isolates themselves (McKeating et al., 1991).

Although some other workers have confirmed that HIV-1 SF2 is more susceptible than other isolates, none has shown such a wide disparity. Thus, Harada et al. (1987) showed HIV-1 SF2 to be 2-16 times more susceptible than HIV-1 (HTLV IIIb) and nine times more susceptible than HIV-1 LAV, Ho et al. (1988) showed it to be 1:25 to two times more susceptible than HIV-1 (HTLV IIIb) and 1:39 to two times more susceptible than HIV-1 RF, and Steimer et al. (1988) again showed it to be 10 times more susceptible than HIV-1 LAV. However, Cheng-Mayer et al. (1988) showed HIV-1 SF2 to have a level of neutralization similar to that of other isolates from the San Francisco area.

The disparity in the present study may be due to differences in the titre of the antisera, since the HIV-1 SF2 antiserum showed a fivefold higher titre in radio-immunoassay with solid-phase homologous recombinant gp120 than the antiserum to HIV-1 LAI. Also, the immune response to the epitopes within the env 18 peptide may be distorted since one of the epitopes contains two tyrosines (tyrosine can be an immunodominant residue, for example see Gill et al., 1963) in all of the strains other than HIV-1 SF2. An alternative explanation for any difference in neutralization sensitivity is based on the observation that the V2 neutralizing epitope, like the V3/GPGR epitope, is free of glycosylation sites, but is flanked by regions where the number and position of carbohydrates are variable between strains. The carbohydrate molecules on these flanking regions may shield the neutralizing epitope that lies between them. The flanking regions for the V2 epitope are the V1 region and the C-terminal portion of the V2 region. The V1 and V2 regions differ in length, both within and between isolates, but their relative positions are constrained since they form nested loops with their inter-cysteine bonds using residues which fall within five amino acids (residues 133 and 138 for HIV-1 SF2) of each other (Leonard et al., 1990). Thus, the exposure of the HIV-1 SF2 V2 neutralization epitope may be increased, since this isolate has a longer V2 loop in association with a shorter V1 loop, relative to HIV-1 LAI and MN. Clearly, on a similar basis, a comparison of the relative loop lengths for HIV-1 RF would indicate that this isolate should be as sensitive to neutralization as HIV-1 SF2. In the current study, this was not the case. However, only low levels of antibodies were detected using radioimmunoassays with HIV-1 RF peptide as the solid phase. The reason for this is unknown, but is not related to the solubility of the peptide, since aqueous solutions of all the peptides gave similar absorbance values by u.v.-spectrography between 200 and 300 nm.

In conclusion, the V2 region of the HIV-1 external envelope glycoprotein contains a neutralizing epitope which is isolate specific, but also has a low level of cross-reactivity. This confirms the work of Fung et al. (1992). This cross-reaction is most evident using peptides as antigens and becomes less obvious with recombinant
proteins. A peptide with the consensus sequence does not induce cross-neutralizing antibodies.

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References


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