The neutralizing antibody response against a conserved region of human immunodeficiency virus type 1 gp41 (amino acid residues 731–752) is uniquely directed against a conformational epitope

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Amino acids 731–752 (731PRGPDRPEGIEEEGERD-RDRS752) of the transmembrane glycoprotein gp41 of human immunodeficiency virus type 1 (HIV-1) are conserved in most virus isolates and are controversially reported to be implicated in virus neutralization. The humoral response in infected patients against this region is poor and humans immunized with gp160 show high levels of antibodies against the peptide but poor neutralization titres. Nonetheless, several groups have succeeded in obtaining neutralizing antibodies against this sequence using different antigen-presenting systems. In order to identify the sequence(s) against which the neutralizing response was generated, we used the flock house virus (FHV) antigen-presenting system to analyse neutralizing antiserum from mice immunized with a cowpea mosaic virus (CPMV) chimera expressing the 731–752 sequence. Data show that the neutralizing response is uniquely directed against a conformational epitope mapping to the ERDRD portion of this sequence, although the major antibody response, which is non-linear, and is not neutralizing, is against an IEEE epitope. These results provide an explanation for the controversy regarding the immunogenicity of this region of gp41 and suggest that this conformational epitope, in the absence of the non-neutralizing epitope, should be considered for a subunit vaccine. In addition, this study highlights the usefulness of antigen-presenting systems that preserve epitope conformation in the investigation of immune responses.

Introduction

Individuals infected with human immunodeficiency virus type 1 (HIV-1) develop a neutralizing antibody response which is principally directed against epitopes of the surface glycoproteins gp120 and gp41 (Weiss, 1993; Tsoukas & Bernard, 1994). Four major seroreactivity regions that correlate closely with virus-neutralizing ability have been identified in patients' sera (Broliden et al., 1992). Two of these regions are involved in the early stages of virus entry and have been localized on gp120: the V3 loop region and the CD4 binding site (Ho et al., 1991; Weiss, 1993; Moore et al., 1994). The other two neutralizing regions are located on the transmembrane envelope glycoprotein gp41. One is found in the extracellular domain of gp41 at residues 647–671 (Muster et al., 1993; Sattentau et al., 1995). Monoclonal antibodies (MAbs) against this region may neutralize by blocking the interaction between gp41 and gp41-binding molecules on human cells (Chen & Dierich, 1996). The other (Kennedy et al., 1986) is peculiarly situated in a supposedly intravirion or intracellular location, at residues 731PRGPDRPEGIEEEGERD-RDRD (Modrow et al., 1987; Gallagher, 1992), and although weakly neutralizing MAbs have been raised to it (Dalglish et al., 1988; Vella et al., 1993), its function as a neutralization site is still the subject of controversy.

The antigenic properties of the gp41 731–752 region were first described by Kennedy et al. (1986). The antibody response in infected patients to this region is poor (Davis et al., 1990; Vella et al., 1991), but may have an uneven geographical

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distribution (Cheinsong-Popov et al., 1992). In addition, this epitope has been the subject of much controversy, regarding both its location inside the virion (Dalgleish et al., 1988; Sattentau et al., 1995; Buratti et al., 1997) and its importance for virus neutralization. In fact, although several groups have succeeded in obtaining neutralizing antibodies using different antigen-presenting systems (Evans et al., 1989; Kalyan et al., 1994; McLain et al., 1995, 1996a, b) or synthetic peptides (Chan et al., 1986), this has not been always the case (Newton et al., 1995). Moreover, Pincus et al. (1993) showed that although human vaccinees responded strongly with antibodies against gp41 731–752 region, these did not correlate with virus neutralization. Analogous results were obtained for vaccinated chimpanzees (Pincus et al., 1994), casting serious doubts on the importance of this region for virus neutralization. In addition, the isolation of a non-neutralizing MAbs from BALB/c mice immunized in the same way suggested that this epitope is functionally irrelevant (Pincus et al., 1993).

We have used the fowl house virus (FHV) antigen-presenting system (Tisminetzky et al., 1994) to express parts of the above sequence for the analysis of neutralizing antisera from mice immunized with a cowpea mosaic virus (CPMV) chimera expressing the gp41 731–752 region (CPMV–HIV/1) (McLain et al., 1995, 1996a, b). Data show clearly that the neutralizing antibody response is uniquely directed against the ERDRD portion of this sequence, although the major antibody response is against IEEE, and is not neutralizing. These results provide an explanation for the controversy regarding the immunogenicity of the 731–752 region of gp41.

Methods

**Virus and antibodies.** MAbs 1575 specific to the epitope IEEE, and MAbs 1577, 1583 and 1908, all specific for the epitope ERDRD of the gp41 transmembrane protein of HIV-1, were kindly donated by C. Vella (Vella et al., 1995). Viruses used are described in the appropriate sections below.

**Immunization of mice with CPMV–HIV/1.** Adult C57/BL6 mice (H-2b; B&K International, Grinstein, Aldborough, Hull, UK) and C3H/He-mg mice (H-2k; bred in-house) were immunized at 6–8 weeks of age as described by McLain et al. (1995). Briefly, 1 vol. wt CPMV or the chimera CPMV–HIV/1 (described in McLain et al., 1995) was mixed with 4 vols aluminium hydroxide adjuvant (Inject Alum, Pierce) for 30 min at room temperature. Mice were immunized subcutaneously at two sites between the scapulae on day 0 and day 28 with 100 µg virus–adjuvant mix containing 3 µg virus. Blood was obtained from the heart 14 days after the second injection. Sera were pooled, heat-treated at 56 °C for 30 min and stored at −70 °C. All experiments followed the guidelines laid down by the UK Co-ordinating Committee for Cancer Research.

**FHV–gp41 recombinant proteins and gp41 synthetic peptide.** All the proteins used in this study have been described in detail elsewhere (Buratti et al., 1996). Briefly, we inserted a BsuI cloning site in four different positions in the FHV-RNA2 capsid protein gene (L1, L2, L3 and L2), such that any peptide expressed at these sites appeared on the outer surface of the protein. A fifth insertion position (L3) was provided by a naturally occurring BsuI site. The introduction of epitope sequences in these positions was accomplished by synthesizing sense and antisense sticky-end oligonucleotides that encoded the desired sequence. Two series of recombinant proteins were produced in this study: one containing the gp41 IEEEGERDRDR sequence (A), and the other containing a part of this sequence GERDRD (B) (Buratti et al., 1996). All these recombinant proteins were produced in *Escherichia coli* BL21 cells using a pET expression system (Novagen) and purified by electroelution from SDS–PAGE gels. The resulting proteins were named FHV-L1–A, FHV-L2–A, FHV-L3–A, FHV-L2–A, FHV-L2–A and FHV-L1–B, FHV-L2–B, FHV-L3–B, FHV-L1–B, FHV-L2–B, FHV-L3–B, FHV-L3–B. A synthetic peptide, DRPEGEEEGERDRDRSC, from the 735–752 region of HIV-1 gp41 was synthesized in a Milligen 9050 Peptide Synthesizer (Millipore).

**ELISA and Western blot assays using the CPMV–HIV/1 sera.** Protein and synthetic peptide (0.2 µg per well, in 200 mM carbonate buffer, pH 9.6) were bound to a 96-well microplate (MaxiSorp, Nunc) by incubation overnight at 4 °C. The wells were then washed three times with PBS, blocked with 5% non-fat dried milk in PBS, and incubated with MAbs or sera at room temperature for 1 h. All MAbs and serum dilutions were prepared in 2% non-fat dried milk in PBS. Synthetic peptide 731–752 in solution was also reacted with antibody for 2 h at room temperature; unbound antibody was detected by incubation with 0.1 µg per well FHV-L1–B immobilized on a microplate, also for 2 h at room temperature. After three washes with PBS, 1:2000-diluted horseradish peroxidase-conjugated goat anti-mouse IgG (P447; Dako) was applied to the wells for 1 h at room temperature. After a further three washes with PBS, colour was developed with o-phenylenediamine dihydrochloride (Sigma Fast). The reaction was stopped after 5 min by addition of 3 M sulphuric acid and the absorbance was measured at 492 nm. Western blots (HIV-1 Western blot kit; CBC) were performed according to the manufacturer’s instructions. Dilutions of MAbs and sera are given in the figure legends.

**Antibody elution from mouse sera using antigen-coated Petri dishes.** The required FHV protein in 200 mM sodium carbonate buffer (Na₂CO₃–NaHCO₃, pH 9.6) was adsorbed to a 35 mm untreated polystyrene dish (Corning) in an overnight incubation at 4 °C. There was approximately 6 µg protein per dish. Dishes were then blocked for 1 h at room temperature with 5% non-fat dried milk in PBS, and incubated with MAbs or sera at room temperature for 1 h. After washing the dish with PBS, 1:10 antiserum in 2% non-fat dried milk in PBS was added, and the dish was incubated for 1.5 h at room temperature on a horizontal shaker. The antiserum was then removed, and the dish was washed three times with PBS over a period of 15 min, placed on ice and incubated for 15 min with 250 µl cold 0.1 M citrate buffer (pH 3.0) to elute antibody. The solution was then brought to pH 7.5 with 150 µl cold 2 M phosphate buffer. In the meantime, the removed antiserum was repeatedly adsorbed in fresh protein-coated dishes. The resulting antibodies were eluted in the same volume as the starting antiserum and used in neutralization assays, ELISAs and Western blot analysis. Neutralization assays were carried out at a 1:10 final dilution; unfractionated antiserum gave 90% neutralization at a 1:30 dilution.

**Assay of HIV-1-neutralizing antibody.** Neutralizing antibody was assayed as previously described using a syncytium-inhibition assay adapted from McLain & Dinnock (1994), except that here we used a mutant of HIV-1 IIIB (S. M. Cleveland & N. J. Dimmock, unpublished data) that escaped the low level of HIV-1-neutralizing activity that is always stimulated by wt CPMV (McLain et al., 1995). Dilutions of antibody were incubated with an equal volume of 2000 syncytium forming units (s.f.u.) per ml HIV-1 IIIB for 1 h at 37 °C. Semi-confluent monolayers of C8166 cells (5 × 10⁶ cells per well) were prepared in 96-well tissue culture plates (Nunclon; Life Technologies) that had been pre-
treated with poly(γ-lysine) (Sigma). Medium was removed from the cells and each of six replicate wells was inoculated with 50 µl virus alone or virus plus antibody. Cells were then incubated for 3 days at 37 °C, and syncytia were counted with the aid of a microscope. Neutralization was calculated as the percentage reduction in s.f.u. per well due to antibody compared to a non-neutralized virus control. In addition, the extent of neutralization was determined by two other assays: inhibition of infectious progeny production and inhibition of p24 antigen production. In the former, the amount of infectious progeny virus present in clarified tissue-culture fluid (TCF) of the above cultures at 3 days after infection was determined by counting syncytia as described above, while in the latter p24 was assayed by capture with 1 µg per well sheep anti-p24 antiserum (D7320; Aalto Bioreagents) bound to wells of ELISA plates (Immulon 2; Dynatech). After washing with Tris-buffered saline, (TBS; 0.14 M NaCl, 0.02 M Tris–HCl, pH 7.6), non-specific binding sites were blocked with 3% BSA (Sigma) in TBS. TCF was treated with 1% Empigen detergent (Calbiochem) in PBS at 56 °C for 1 h, and 100 µl was then added to wells and plates were held at room temperature overnight. Recombinant p24 (MRC AIDS Reagent Project) was used as a standard when required. Wells were washed with TBS containing 0.05% Tween (TBST) and 100 µl per well biotinylated mouse anti-p24 MAb was added (1:1000 in TBST plus 0.1% BSA; MRC AIDS Reagent Project). Plates were held for another 2 h, and then wells were washed and streptavidin–alkaline phosphatase was added (Amersham). This was removed 2 h later, the wells were washed and substrate was added [1 mg ml⁻¹ p-nitrophenyl phosphate (Sigma) in 9.7% diethanolamine, 0.5 M MgCl₂, pH 9.8 (Pierce)]. Plates were incubated at 37 °C in the dark and then the absorbance was measured at 405 nm. Percentage neutralization was calculated from these data.

Results

Reactivities of CPMV–HIV/1 antiserum with the FHV recombinant proteins

The ELISA reactivities of sera from mice immunized with CPMV–HIV/1 and wt CPMV were compared with those observed for MAbs directed against the IEEE and ERDRD portions of the 735–752 sequence of gp41 (MAb 1575 and MAb 1908, respectively; Vella et al., 1993). In the solid phase we immobilized a full panel of FHV recombinant proteins carrying either the full epitope IEEEGGERDRD or just the GERDRDR sequence. Fig. 1(a) shows that the antiserum from the CPMV–HIV/1-immunized mice reacted with all the FHV recombinant proteins and with the immobilized synthetic peptide, but not with the wt FHV protein (FHV-wt). The specificity of the reactivity is also evident in Fig. 1(b), where we show that antiserum from mice immunized with wt CPMV virus did not react with any of the FHV recombinant proteins or the immobilized synthetic peptide, but not with the wt FHV protein (FHV-wt). The specificity of the reactivity is also evident in Fig. 1(b), where we show that antiserum from mice immunized with wt CPMV virus did not react with any of the FHV recombinant proteins or the immobilized synthetic peptide. Interestingly, the reactivity profile in Fig. 1(a) was qualitatively similar to that obtained with MAb 1908 (Fig. 1d), with the difference that MAb 1908 did not react with the immobilized gp41 synthetic peptide. On the other hand, the reactivity against the immobilized synthetic peptide (Fig. 1c) was characteristic of MAbs directed specifically against the IEEE sequence, such as MAb 1575 (Buratti et al., 1996). These results suggest that antiserum from mice immunized with CPMV–HIV/1 contains antibodies against both the ERDRD and IEEE epitopes.

Purification of antibodies from CPMV–HIV/1 antiserum by adsorption and elution from FHV recombinant proteins

The different populations of antibodies contained in the CPMV–HIV/1 antiserum were fractionated using an
neutralize the virus (Table 1, procedure C). Finally, the control ERDRD sequence, the eluted antibodies were not able to gp41 735–752 synthetic peptide that also contained the from the antiserum (Table 1, procedure B). Interestingly, when contained only the ERDRD epitope. As expected, incubation with FHV wt protein did not remove any neutralizing ability is in keeping with our previous observations that the adsorption with procedure A. Finally, procedure C shows that the adsorption procedure similar to that described by McLain et al. (1996a). CPMV–HIV/1 antiserum was successively incubated on Petri dishes coated with different antigens (FHV-L1–B, FHV-L2–A, the gp41 peptide and FHV-wt). After a 1 h incubation antibodies attached to the solid phase of each Petri dish were eluted and tested for neutralizing activity (Table 1 and Fig. 2) and by ELISA (Fig. 3). Table 1 shows that in all elutions performed with the CPMV–HIV/1 antiserum (procedures A, B and C), neutralizing antibodies could only be isolated from the FHV-L1–B-coated dish that contained only the ERDRD epitope. As expected, incubation with FHV wt protein did not remove any neutralizing ability from the antiserum (Table 1, procedure B). Interestingly, when as a first step the antiserum was adsorbed onto the immobilized gp41 735–752 synthetic peptide that also contained the ERDRD sequence, the eluted antibodies were not able to neutralize the virus (Table 1, procedure C). Finally, the control wt CPMV antiserum failed to exhibit any neutralization with elutions from FHV-L1–B and FHV-L2–A (Table 1, procedure D). The neutralization assay was repeated in formats that measured the inhibition of p24 antigen production or of infectious progeny virus production, to check that we were not preventing the appearance of syncytia without inhibiting virus multiplication. Fig. 2 shows that a similar degree of inhibition was achieved by antibody eluted from FHV-L1–B with each of the neutralization assays. The MAbs 1575 (IEEE-specific) and 1577 (ERDRD-specific) were used to control for specificity of the binding reactions. MAb 1575 bound well to immobilized peptide and MAb 1577 bound well to FHV-L1–B, with no cross-reactivity (data not shown).

Fig. 2 shows that a similar degree of inhibition was achieved by antibody eluted from FHV-L1–B with each of the neutralization assays. The MAbs 1575 (IEEE-specific) and 1577 (ERDRD-specific) were used to control for specificity of the binding reactions. MAb 1575 bound well to immobilized peptide and MAb 1577 bound well to FHV-L1–B, with no cross-reactivity (data not shown).

Table 1. Neutralization activity recovered from antiserum to CPMV–HIV/1 (procedures A to C) and antiserum to wt CPMV (procedure D) after cycles of adsorption to and elution from antigen-coated Petri dishes

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Successive adsorption of antiserum to:</th>
<th>Percentage neutralization by antibody eluted from:*</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>FHV-L1–B → FHV-L2–A</td>
<td>NA NA 74·4 3·8</td>
</tr>
<tr>
<td>B</td>
<td>FHV-wt → FHV-L1–B → FHV-L2–A</td>
<td>4·5 NA 71·9 0</td>
</tr>
<tr>
<td>C</td>
<td>Peptide → FHV-L1–B → FHV-L2–A</td>
<td>NA 0 68·8 0</td>
</tr>
<tr>
<td>D</td>
<td>FHV-L1–B → FHV-L2–A</td>
<td>NA NA 0 0</td>
</tr>
</tbody>
</table>

* Antigens coating the Petri dish. Neutralization was assayed by syncytium inhibition. Peptide, synthetic peptide covering residues 735–752 of gp41; NA, not applicable.
Fig. 3. ELISA reactivities of antibodies eluted from the multiple-step adsorption/elution procedures A, B and C described in Table 1. The ELISA reactivity of each eluted antibody fraction was measured using the FHV-L2–A protein. Reactivity cut-off values were determined using wt FHV protein. Procedure A shows the reactivity pattern recovered when CPMV–HIV/1 antiserum (1:10) was successively adsorbed to FHV-L1–B and then FHV-L2–A, and then eluted from each. Procedure B shows the reactivity recovered with CPMV–HIV/1 antiserum successively adsorbed to wt FHV, FHV-L1–B and then FHV-L2–A, and then eluted from each. Procedure C shows the reactivity recovered with CPMV–HIV/1 antiserum successively adsorbed to gp41 peptide, FHV-L1–B and then FHV-L2–A, and then eluted from each. The bottom graph shows the neutralizing activity (syncytium inhibition) recovered from each eluted antibody fraction. NA, Not applicable (as this step was not performed).

HIV-1 epitopes has already been noted for other conformational epitopes of HIV-1, such as the V3 loop region (Moore, 1993; Moore et al., 1994b). However, the same peptide in solution did react with ERDRD-specific affinity-purified antibody, although saturation was not achieved even with 300 µg of peptide, a molar excess of 5 x 10^5 (Fig. 4).

Antibodies that bind to the gp41 731–752 peptide and to FHV-L2–A, which both contain the IEEEGGERDRDRS sequence, clearly have different conformational requirements compared with antibodies that bind to the ERDRD-expressing FHV-L1–B. The former are not neutralizing and are probably directed against the IEEE sequence. Moreover, the immobilized peptide bound antibodies with low efficiency (probably low-affinity), as evidenced by the fact that antibodies remained to bind the FHV-L2–A in the third adsorption/elution step (Fig. 3, procedure C). This is even more significant as equal weights of protein and synthetic peptide were coated on the Petri dish, which represents an approximately 20-fold excess in the number of synthetic peptide sequences.

It is important to note that in all experiments no residual neutralization was observed when we analysed antisera after the last adsorption step (data not shown). This means that neutralization was uniquely confined to a conformational epitope mapping to the ERDRD sequence, and that no neutralizing antibodies against other conformational or linear epitopes were present.

Reactivities of adsorbed antibodies with HIV-1 Western blot strips

Antibodies eluted from the CPMV–HIV/1 antiserum adsorbed to FHV-L1–B- and FHV-L2–A-coated Petri dishes were analysed by Western blotting. MAbs specific against ERDRD (Mab 1908) and IEEE (Mab 1575) were used as controls (Fig. 5). Interestingly, both Mab 1908 (lane 1) and anti-CPMV–HIV/1 antibodies that eluted from FHV-L1–B (lane 2) were unable to recognize any protein, showing the high conformational requirements for the display of this sequence. On the other hand, the anti-CPMV–HIV/1 antibodies eluted from both FHV-L2–A (lane 3) and MAb 1575 (lane 5) recognized both gp41 and gp160. MAb 1575 also reacts with HIV-1 p17 (Fig. 5, lane 5) due to the presence of an IEEE sequence in this protein (Buratti et al., 1997). Reactivity of the CPMV–HIV/1 antiserum is shown in lane 4.
neutralization escape mutants and determining whether there is any change in the ERDRD sequence of gp41.

Our results suggest an explanation of the data of Pincus et al. (1993), who found that the sera of human volunteers immunized with gp160 reacted very well against a synthetic gp41 peptide that contained the IEEE region, and yet had very little neutralizing activity. In addition, Pincus et al. (1993) isolated a MAb, B8, from mice immunized with this region, which mapped to a peptide containing the IEEE sequence and had no neutralizing ability. These data can be reconciled by our results, which show that the response against the IEEE sequence is not responsible for the neutralizing activity observed in the sera of immunized animals. Although an alternative explanation is that the CPMV–HIV/1 chimera presents the neutralizing conformation of the ERDRD sequence but not that of the IEEE sequence to the immune system, we believe that this is unlikely since previous work has demonstrated that the IEEE epitope is not conformation-dependent (Buratti et al., 1996).

Our data are at odds with those of Vella et al. (1993), who reported that MAbs to the IEEE sequence of gp41 were neutralizing. Although Buratti et al. (1997) have reported an alternative target for these MAbs in HIV-1 p17, the large amount of anti-IEEE antibodies induced by the CPMV–HIV/1 was not neutralizing. However, the MAbs described by Vella et al. (1993) all had surprisingly low neutralizing titres (< 1:100) for ascitic fluids, and it is possible that they contained a non-specific neutralizing activity in addition to the specific non-neutralizing antibody. In fact, we have failed to detect any neutralizing activity in the high titre ERDRD-specific MAbs 1583 (analogous to 1908) and 1577 that we purified from the supernatants of Vella’s hybridomas. In addition, we found no neutralizing activity in high titre MAb 1575 ascitic fluid (against IEEE), even at dilutions of 1:10 (data not shown).

The proximity of the two epitopes on gp41, separated by just two glycine residues, the smallest of the amino acids, suggests that there may be reciprocal interference between their cognate antibodies, both immunogenically and antigenically. The IEEE epitope appears dominant and under immunogenicity question, it would be interesting to prepare a version of the CPMV–HIV/1 chimera from which the IEEE sequence has been deleted, to determine whether this is more effective in stimulating high titres of neutralizing antibody. Antigenic interference is dependent on the orientation of the antibodies that bind to IEEE and ERDRD. If they both bind in the same orientation, their footprints (Amit et al., 1986; Wilson & Stanfield, 1993) might well overlap and there would be interference. On the other hand, if they bound on opposite sides of the peptide there could be little interference, as shown for two MAbs to the haemagglutinin of influenza virus that bound simultaneously to epitopes that were separated by just
three amino acid residues (Jackson et al., 1988). Clearly, further experimentation using the ERDRD epitope holds great promise for a deeper understanding of the virus life-cycle, and in particular an explanation of how HIV-1 can be neutralized via an epitope that is supposedly inside the virus particle. The explanation we favour is that the neutralizing anti-ERDRD antibodies react with the Kennedy peptide (rather than another epitope), which is in fact exposed on the surface of the virion. Evidence to support this contention will be presented in a future publication (S. M. Cleveland and others, unpublished data). The inability of recombinant gp160 to induce neutralizing responses against this region (Pincus et al., 1993) is consistent with our observation that recombinant gp160 does not bind neutralizing anti-ERDRD antibody (S. M. Cleveland & N. Dimmock, unpublished data), presumably because it lacks the required conformation. These data underline the conclusion that antigen-presenting systems may be particularly useful in circumventing such problems and in developing efficient subunit vaccines.

In conclusion, this study confirms the presence of a conformation-dependent epitope in the gp41 731–752 peptide whose expression may be in competition with the adjacent non-neutralizing linear epitope IEEE. It also highlights the need for the development of systems that can present conformational epitopes, both for the induction of neutralizing antibodies and the analysis of the antibody response.

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References


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