Highly conserved epitope domain in major core protein p24 is structurally similar among human, simian and feline immunodeficiency viruses

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Linear B cell epitopes were mapped on the major core protein p24 of human immunodeficiency virus type 1 (HIV-1), simian immunodeficiency virus (SIVAGM) and feline immunodeficiency virus (FIV) using a fusion protein-based method and murine monoclonal antibodies reactive against the p24 antigens expressed on the surface of HIV-1- and FIV-infected cells. The results suggest that the sites identified here are encoded at similar positions in the three virus genomes and consist of highly conserved epitopes, which could exhibit immunodominance.

Human immunodeficiency virus type 1 (HIV-1), the causative agent of AIDS (Barré-Sinoussi et al., 1983; Popovic et al., 1984), is not easily accessible to neutralizing antibodies because of its cell-associated nature. Therefore it is generally believed that HIV-1-specific immune reactions to HIV-1-infected cells are more important than those to virions, as a host defence mechanism against infection. Immune reactions such as antibody-dependent cellular cytotoxicity (Rook et al., 1987) and cytotoxic T lymphocytes (Nixon et al., 1988) have been observed toward the conserved gag p24 antigen, although such immune reactions are directed mainly toward the hypervariable V3 domain of the env gene product gp120 (Palker et al., 1988; Takahashi et al., 1988). In addition, there exists a clear correlation between the decline of antibodies to gag p24 and p17 and the alteration of the clinical condition from the asymptomatic phase to the symptomatic phase (Weber et al., 1987). These results lead us to expect that these gag proteins are likely to play a significant role as prognostic factors for the onset of AIDS. However such studies directed to AIDS vaccine development still have great difficulties owing to the lack of good animal models.

Simian immunodeficiency virus isolated from African green monkeys (SIVAGM) also belongs to the lentivirus subfamily of the Retroviridae (Fukasawa et al., 1988). Similarly, feline immunodeficiency virus (FIV) is also a T lymphotropic lentivirus that was initially isolated in the U.S.A. from a group of cats with an immunodeficiency-like syndrome (Pederson et al., 1987). Recently, it has been shown that cell surface gag p24 antigen expression is a phenomenon observed in HIV-1, SIV and FIV using flow cytometry and MAbS against HIV-1 and FIV p24 antigens, and that two MAbS of HIV-1 p24 are cross-reactive with SIVAGM but not with FIV (Nishino et al., 1992). In the present study, epitope mapping of p24 antigens of HIV, SIVAGM and FIV was carried out using these MAbS to demonstrate similarity between the molecular structures of these p24 antigens.

The preparation of hybridoma clones (V10 and V107) producing MAbS to HIV-1 gag p24 from BALB/c mice immunized with purified HIV-1 virions has been described previously (Ikuta et al., 1989). Preparation of hybridoma clones (F17, F31 and F48) producing MAbS to FIV gag p24 from BALB/c mice immunized with the purified FIV gag precursor protein (expressed by a baculovirus system) (Morikawa et al., 1991), and the reactivity of these MAbS with the surface of FIV-infected cells have been described by Nishino et al.
Each recombinant plasmid was transformed into the E. coli EQ192 strain (Yamaguchi et al., 1991) to obtain β-gal fusion protein-expressing recombinant clones. SDS-PAGE (7.5%) and Western blotting were performed as described in our previous report (Matsuo et al., 1988).

To elucidate the molecular structure of the HIV-1 gag p24 protein expressed on the surface of the HIV-1-infected cells, we performed epitope mapping with MAbs V10 and V107 using a β-gal fusion protein-based method, since B cell epitopes have been shown to be mapped easily by this method (Yamaguchi et al., 1991) with a precision comparable to that of the Pepscan system (Giesen et al., 1984; Langedijk et al., 1990). The restriction map of a 1.18 kbp HindIII fragment, containing a part of the HIV-1 gag region, and regions fused with the lacZ gene in the pUR vectors are shown in Fig. 1 (a). The results of Western blotting using these clones are shown in Fig. 1 (b). Both MAbs V10 and V107 reacted with all clones in Fig. 1 (a) (Fig. 1, b, lanes 2, 3, 4, 6, 7 and 8). Thus the epitopes should be located in the sequence Thr240 to Ala309 of HIV-1 gag precursor p55. To map further defined epitopes, we adopted a variety of synthetic DNA fragments coding for sequences in this region to fuse with the lacZ gene. The sequence contained in each clone is shown in Fig. 1 (c) together with the reactivity of V10 and V107. Western blotting revealed that the minimum units necessary for recognition by V10 and V107 were truncated to sequences of 15 amino acids (287 to 301), QGPEKEFR-DYDVF (referred to as the V10 epitope) contained in clone p24-14, and 23 amino acids (287 to 309), QGKE-PFRDVF (referred to as the V107 epitope) contained in clone p24-16, respectively, and that the further truncated clones p24-13 and p24-15 had lost their reactivity.

Table 1. Cloning vectors used for construction of p24–β-gal fusion protein expression vectors

<table>
<thead>
<tr>
<th>Clone</th>
<th>Vector/Cloning site(s)</th>
</tr>
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<tbody>
<tr>
<td>p24F195</td>
<td>pUR278/BamHI</td>
</tr>
<tr>
<td>p24F255</td>
<td>pUR288/BamHI</td>
</tr>
<tr>
<td>p24F148</td>
<td>pUR278S/SmaI</td>
</tr>
<tr>
<td>p24Fkpn2*</td>
<td>pUR289S/SmaI</td>
</tr>
<tr>
<td>p24F42</td>
<td>pUR291/BamHI-PstI</td>
</tr>
<tr>
<td>p24F130*</td>
<td>pUR292/PstI-HindIII</td>
</tr>
<tr>
<td>p24Fkpn1*</td>
<td>pUR292/BamHI-PstI</td>
</tr>
<tr>
<td>p24F228</td>
<td>pUR288/XbaI-HindIII</td>
</tr>
<tr>
<td>p24F1</td>
<td>pUR292/BamHI-PstI†</td>
</tr>
<tr>
<td>p24F4</td>
<td>pUR292/BamHI-SalI†</td>
</tr>
<tr>
<td>p24F2</td>
<td>pUR292/BamHI-SalI†</td>
</tr>
<tr>
<td>p24F12</td>
<td>pUR292/BamHI-SalI†</td>
</tr>
<tr>
<td>p24F16</td>
<td>pUR292/BamHI-SalI†</td>
</tr>
<tr>
<td>p24S14</td>
<td>pUR292/BamHI-SalI†</td>
</tr>
<tr>
<td>p24S16</td>
<td>pUR292/BamHI-SalI†</td>
</tr>
</tbody>
</table>

* Construction procedure is described in the text.
† Vectors used for synthetic DNA fragments.

(1992). The infectious DNA clones pNL432 (Adachi et al., 1986) and pFTM191 (Miyazawa et al., 1991) were used as respective gag gene sources of HIV-1 and FIV. Oligonucleotides coding for gag p24 sequences from the HTLV-IIIIB strain (Ratner et al., 1985) and the FIV Petaluma strain (Talbott et al., 1989) were designed based on the optimum codons in bacterial genes and synthesized as described previously (Matsuo et al., 1988). The in-frame pUR (Rüther & Müller-Hill, 1983) and pURS (Yamaguchi et al., 1991) vectors capable of expressing β-galactosidase (β-gal) fusion proteins in Escherichia coli were used to subclone DNA fragments. The cloning vectors used for constructing various recombinant clones shown in Fig. 1 to 4 are summarized in Table 1. For clone p24Fkpnl, a 150 bp KpnI-EcoRI fragment contained in p24F255 was converted to a SmaI-HindIII fragment using the polylinker sites of pUC18 and then cloned into the SmaI–HindIII sites of pUR289S. Clone p24Fkpnl2 was obtained by religation of a KpnI–SalI-digested and blunt-ended p24F255 clone. For clone p24F150, a 150 bp BalI–SalI fragment was blunt-ended and cloned into the SmaI site of pUR278S. Each recombinant plasmid was transformed into the E. coli EQ192 strain (Yamaguchi et al., 1991) to obtain β-gal fusion protein-expressing recombinant clones. SDS-PAGE (7.5%) and Western blotting were performed as described in our previous report (Matsuo et al., 1988).

For clone p24F150, a 150 bp BalI–SalI fragment was blunt-ended and cloned into the SmaI site of pUR278S. The cloning vectors used for constructing various recombinant clones shown in Fig. 1 to 4 are summarized in Table 1. For clone p24Fkpnl, a 150 bp KpnI–EcoRI fragment contained in p24F255 was converted to a SmaI–HindIII fragment using the polylinker sites of pUC18 and then cloned into the SmaI–HindIII sites of pUR289S. Clone p24Fkpnl2 was obtained by religation of a KpnI–SalI-digested and blunt-ended p24F255 clone. For clone p24F150, a 150 bp BalI–SalI fragment was blunt-ended and cloned into the SmaI site of pUR278S. Each recombinant plasmid was transformed into the E.
mouse immunized with the FIV precursor gag protein (Fig. 2d), two recombinant clones containing the epitope (p24F255 and p24F2) possessed specific reactivity and the other clones did not. Surprisingly, the location of the epitope was consistent with that of the V10 epitope in the range of 15 amino acids on the amino acid sequence alignment shown in Fig. 3.

Thus, epitope mapping showed that all MAbs used in this study recognized highly conserved regions. For the purpose of understanding both the antigenic similarity and diversity of the p24 protein, it was worth testing the cross-reactivity of each MAb between sequence of HIV-1, SIV<sub>AGM</sub> (Fukasawa et al., 1988) and FIV corresponding to the identified epitope. Fig. 4(a) presents the extended amino acid sequence alignment of the epitope region between the three species. We constructed recombinant clones possessing SIV<sub>AGM</sub> synthetic DNA fragments. Western blotting showed that MAbs V10 and V107 cross-reacted with the corresponding SIV<sub>AGM</sub>
clones p24S14 and p24S16, respectively, but V10 did not cross-react with the FIV clone p24F2 (Fig. 4b). Similarly, the FIV MAb F17 did not cross-react with the HIV-1 and SIVAGM clones (Fig. 4b). These results are consistent with our previous observation (Nishino et al., 1992).

In this communication, the HIV-1 gag p24 epitope region expressed on the cell surface (Ikuta et al., 1989) was clearly identified. Although the V10 and V107 epitopes were close to each other, V107 recognized a slightly larger region than V10 (see Fig. 1c, clones p24-14 and p24-16). This region almost overlaps with the epitope identified with MAbs by two other groups (Carpio et al., 1991; Niedrig et al., 1991). Recently, Niedrig et al. (1991) reported the characterization of 27 MAbs directed against p24 proteins of HIV-1 and HIV-2. Among them, eight MAbs reacted with a 30 amino acid peptide containing the sequence of the V10 epitope. This indicates that the site could be one of the immunodominant epitopes of this core protein. Inhibition experiments using human sera by Carpio et al. (1991) showed that this region is also immunogenic in humans. Moreover both MAbs are presumed to be broadly cross-reactive with various HIV isolates and also with SIVAGM, suggesting applicability to the detection of the p24 antigen in the sera of HIV-1-infected patients.

In a similar manner, an FIV gag p24 epitope expressed on the surface of FIV-infected cells (Nishino et al., 1992) was successfully identified. Three MAbs recognized the same antigenic site and no MAb reactive with another site was obtained. In addition, the polyclonal mouse antiserum had no significant reactivity with another region from the epitope (Fig. 2d). These results suggest that the site could be an immunodominant epitope in mice. Recent studies have revealed that the major neutralization antigenic sites of foot-and-mouth disease virus (Francis et al., 1987) and poliovirus (Leclerc et al., 1991) contain both B and helper T cell (Th) epitopes. These reports suggest that the immunodominance of a B cell epitope depends on the existence of a strong T cell epitope in the vicinity. In the case of the FIV epitope, a T cell epitope was predicted by computer analysis to exist adjacent to the identified B cell epitope as shown in Fig. 2(c). The sequence contains a Rothbard motif (RLFA, amino acids 294 to 297) which has been found often in a variety of known T cell epitopes (Rothbard & Taylor, 1988). This putative T cell epitope remains to be identified and characterized.

Most interestingly, the location of the FIV epitope was consistent with that of the V10 epitope of HIV-1 in the range of 15 amino acids on the amino acid sequence alignment in spite of there being no cross-reactivity with V10 (Fig. 4b). In the p24 C-terminal region, the amino acid sequence alignment clearly indicates considerable homology not only between HIV-1 and SIVAGM, but also between HIV-1 and FIV (Fig. 3), implying that the region might be important for this protein to exhibit its essential function. In the epitope regions consisting of 15 amino acids, although one amino acid change between HIV-1 and SIVAGM, and nine changes between HIV-1 and FIV were observed, most of these changes involved amino acids with similar properties as shown in Fig. 4(a). This indicates that the sequence of this region has been conserved in the presence of selective pressure. Consequently, the conserved sequence might retain the immunogenic structure of this site including hydrophili-
city, turn-and-loop structure, and possibly the relative position of the Th cell epitope.

Argos (1989) proposed a structural model of p24 whose validity was supported by Langedijk et al. (1990). The epitopes identified here as those exposed on the surface of infected cells were located at the C-terminal end of the so-called puff region on the model, which had high turn-and-coil propensity. The first nine amino acids (QGPKERPFRD) of the epitope were predicted to be the most preferred antigenic site on p24 (Argos, 1989). A similar site was suggested to be a good candidate for an AIDS vaccine since it was predicted to contain both B and T cell epitopes (Coates et al., 1987), and was experimentally identified later as a Th cell epitope (Schrier et al., 1989). In addition, the FIV cell surface gag p24 epitope region identified here also contains a structure similar to the HIV-1 epitope region. Since FIV infection of cats induces symptoms to those in HIV-1-infected individuals, investigation of immune responses to our epitope region in cats would help evaluate the applicability of the p24 antigen as a target for an AIDS vaccine.

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References


Short communication


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