Characterization of monoclonal antibodies that distinguish simian immunodeficiency virus isolates from each other and from human immunodeficiency virus types 1 and 2

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Two monoclonal antibodies (MAbs) against p27 and one against p17 of simian immunodeficiency virus (SIV) from rhesus macaques were produced and characterized by reacting with disrupted, viral antigens on immunoblots. Human immunodeficiency virus type 1 (HIV-1), HIV-2 and SIV isolates from sooty mangabey, stump-tailed macaque, rhesus macaque and African green monkey (SIVsM, SIVstM, SIVMAC and SIVAGM) were used for comparative analysis. The p27 monoclonal antibodies HE³ and FA² reacted with SIVMAC and SIVsM, but not with HIV-1, HIV-2, SIVSM and SIVAGM. The p17 monoclonal antibodies reacted with SIVMAC and SIVsM, but not HIV-1, HIV-2, SIVSM and SIVAGM. The differential reactivity of these monoclonal antibodies indicated that common conserved antigenic epitopes are shared between SIVMAC and SIVsM with respect to p27 MAbs and between SIVMAC and SIVstM with respect to p17. Since these MAbs reacted differently with the SIV isolates, they are useful reagents for comparative pathogenesis studies for differentiating SIV isolates.

Retroviruses closely related to human immunodeficiency viruses (HIV) have been isolated from several species of Old World non-human primates. These primate retroviruses, called simian immunodeficiency viruses (SIVs), were isolated from rhesus macaques Macaca mulatta (SIVMAC) (Daniel et al., 1985), sooty mangabeyes Cercocebus atys (SIVsM) (Fultz et al., 1986; Lowenstein et al., 1986; Murphey-Corb et al., 1986), African green monkeys Cercopithecus aethiops (SIVAGM) (Ohta et al., 1988), a cynomolgus macaque Macaca fascicularis (Daniel et al., 1988a), a stump-tailed macaque Macaca arctoides (SIVstM) (Lowenstein et al., 1988), and a pigtailed macaque Macaca nemestrina (SIVMm) (Benveniste et al., 1988). These primate retroviruses are lentiviruses and share morphological and antigenic characteristics with HIV (Schneider & Hunsmann, 1988). SIVMAC, SIVsM, SIVSM and SIVMm induce an AIDS-like disease in rhesus monkeys (Daniel et al., 1985; Murphey-Corb et al., 1986; Lowenstein et al., 1988; Benveniste et al., 1988). However, the pathogenicity of SIVSM and SIVAGM in their natural African primate hosts has not been documented (Fultz et al., 1986; Ohta et al., 1988). As SIV infection of rhesus macaques provides a useful animal model for HIV pathogenesis, the characterization of the antigenic relationships among HIV and SIV isolates will help in the understanding of the history and evolution of these primate lentiviruses. Consequently, monoclonal antibodies (MAbs) against SIVMAC were generated and used to study the conservation of antigenic determinants.

Cell-free uncloned SIVMAC (Daniel et al., 1985) infected supernatant fluids produced in HUT-78 T cells were centrifuged at 10000 g for 10 min at 4 °C to remove cellular debris. The supernatant fluids were centrifuged at 100000 g for 1 h at 4 °C to pellet the virus, which was then resuspended with phosphate-buffered saline (PBS) at 100-fold concentration. An equal volume of the pelleted virus was mixed with complete Freund's adjuvant using Luer-lock syringes. Ten-week-old female BALB/c mice were immunized subcutaneously with 15 µg of the SIVMAC, followed by two additional immunizations in incomplete Freund's adjuvant using Luer-lock syringes. Ten-week-old female BALB/c mice were immunized subcutaneously with 15 µg of the SIVMAC, followed by two additional immunizations in incomplete Freund's adjuvant. Seropositive immune mice were given a final boost of 30 µg of SIVMAC in PBS subcutaneously and intraperitoneally 3 days before fusion with P3-X63-Ag8.653 myeloma cells, using 50% polyethylene glycol. Hybridomas were selected in HAT medium and cloned by the limiting dilution method. Three MAbs were produced and were designated FA², HE³ and HD². MAbs FA² and HE³ reacted with
homologous SIV<sub>MAC</sub> protein with an $M_r$ of 27K, whereas MAb HD<sub>5</sub> reacted with a 17K protein. The major core protein, p27, is made by post-translational cleavage of a precursor protein, designated Pr55 (Henderson et al., 1988). Both p27 MAbs reacted weakly with a 55K polypeptide, which may be an intermediate in the post-translational processing of SIV gag proteins (Henderson et al., 1988). The p17 detected by MAb HD<sub>5</sub> is likely to be the p16 gag-encoded protein of SIV (Henderson et al., 1988) and showed no reactivity with the gag precursor. MAbs HE<sub>3</sub> and HD<sub>5</sub> belong to IgG2a isotype and FA<sub>2</sub> is IgG2b, as determined by the Mouse Typer kit (Bio-Rad).

To characterize further the immune reactivity of these MAbs, they were reacted with sucrose gradient-purified lentivirus proteins on immunoblots (Fig. 1). None of the MAbs reacted with HIV-1, HIV-2 or cloned (Li et al., 1989) and uncloned (Daniel et al., 1988b) SIV<sub>AGM</sub> antigens. All MAbs reacted with both cloned and uncloned SIV<sub>MAC</sub> (Fig. 1 and 2). Both p27 MAbs reacted with SIV<sub>SM</sub>, but no reactivity was detected with MAb HD<sub>5</sub> (Fig. 1, lane 9), although MAb HD<sub>5</sub> reacted with SIV<sub>SM</sub> (Fig. 1, lane 10). This was in contrast to previous findings with broadly polyclonal antibody from rhesus macaques, sooty mangabeys, mandrills or talapoins (Kanki et al., 1985; Lowenstine et al., 1986) and infected African green monkeys (Ohta et al., 1988), which cross-reacted with the major core protein (p24) of HIV-1. In other studies, MAbs to HIV-2 p24 cross-reacted with HIV-1, SIV<sub>MAC</sub>, SIV<sub>AGM</sub> and SIV<sub>SM</sub> (Minassian et al., 1988; Niedrig et al., 1988). The SIV<sub>MAC</sub> MAbs described here are much more selective in their reactivity, especially with respect to HIV-1 or HIV-2.

Table 1. Reactivity of MAbs with HIV-1, HIV-2 and different SIV isolates

<table>
<thead>
<tr>
<th>Virus isolate</th>
<th>HE&lt;sub&gt;3&lt;/sub&gt; (p27)</th>
<th>FA&lt;sub&gt;2&lt;/sub&gt; (p27)</th>
<th>HD&lt;sub&gt;5&lt;/sub&gt; (p17)</th>
</tr>
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<tr>
<td>SIV&lt;sub&gt;MAC&lt;/sub&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>SIV&lt;sub&gt;SM&lt;/sub&gt;</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>SIV&lt;sub&gt;AGM&lt;/sub&gt;</td>
<td>-</td>
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<td>+</td>
</tr>
<tr>
<td>HIV-1</td>
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<tr>
<td>HIV-2</td>
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(Kanki et al., 1985; Lowenstine et al., 1986)
In summary (Table 1), using the HD₅ (p17) and HE₃ (p27) MAbs together, SIV₃MAC, SIV₃M, SIV₃M and SIV₃AGM can be distinguished from each other in an immunoblot assay. Furthermore these MAbs do not react with HIV and thus could be used in epidemiological studies to differentiate SIV from HIV infections in infected animals and humans. These monoclonal antibodies are available to investigators through the NIH AIDS Research and Reference Reagent Program.

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