Monoclonal Antibodies Directed against Human Immunodeficiency Virus
(HIV) gag Proteins with Specificity for Conserved Epitopes in HIV-1,
HIV-2 and Simian Immunodeficiency Virus

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SUMMARY

Monoclonal antibodies (MAbs) were raised against gag proteins of human immunodeficiency virus type 1 (HIV-1), strain HTLV-IIIB. One of 29 antibodies was specific for p17 of HIV-1. Twenty of 28 MAbs reactive with the major core protein p24 of HIV-1 showed cross-reactivity with HIV-2, and five of these also detected the corresponding antigens of simian immunodeficiency virus (SIVmac). The MAbs were reactive in several tests, i.e. ELISA, immunostaining of Western blots, immunofluorescence, alkaline phosphatase-anti-alkaline phosphatase immunocytochemistry and immunoelectron microscopy. The submembrane protein p17 was clearly localized within the virion.

Monoclonal antibodies (MAbs) may be of advantage in discriminating virus isolates on the basis of type- or subtype-specific epitopes. MAbs directed against the gag gene products of the human immunodeficiency virus (HIV-1) have been described by several groups (Veronese et al., 1985; Chassagne et al., 1986; Ferns et al., 1987). Some of these antibodies were used to differentiate between HIV-1 isolates originating in the U.K., the U.S.A., Haiti and Africa. Our aim was to develop MAbs against type-specific HIV-1 determinants as well as epitopes common to more distantly related immunodeficiency viruses, i.e. to HIV-2 and to the simian immunodeficiency virus SIVmac. Antibodies with a broad cross-reactivity may be useful in reliable screening for new isolates or to identify HIV antigens in tissues and cells suspected to be infected by HIV or a related virus.

A panel of MAbs directed against HIV-1 strain HTLV-IIIB was selected after fusion of NS-1 cells with spleen cells of a BALB/c mouse (Köhler & Milstein, 1975). The mouse had been immunized with HTLV-IIIB virions purified from supernatants of infected KE37 cells (Popovic et al., 1984a, b) by pelleting the virus through a 10% sucrose layer. Cells of one fusion were seeded into twelve 96-well microtitre plates. Identification of HIV-1-specific MAbs was achieved by ELISA with HIV-1 antigens coupled to tissue culture grade microtitre plates (Nunc). Fifty-five hybridoma cultures showed positive reactions. Twenty-nine of these with strong specific binding were subcloned three times by limiting dilution. Representative MAbs characterized in more detail are listed in Table 1.

In Western blot analysis with HTLV-IIIB antigens, one antibody (3-H-7) recognized the submembrane protein p17 and the precursor molecule p32, whereas all other MAbs reacted with the major core protein p24. Some of the anti-p24 antibodies also exhibited significant reactions with the gag precursor p55. Depending on the HIV-1 preparation and the antibody used, several minor bands of Mr between 24K and 55K were observed in addition to the main bands. In immunofluorescence and in alkaline phosphatase-anti-alkaline phosphatase (APAAP) immunocytochemistry (Cordell et al., 1984) the antibodies detected antigens in the cytoplasm of infected cells (Fig. 1a,b). Immunoprecipitation assays (Conraths et al., 1988) using partially purified HIV-1 revealed that the MAbs precipitated the same antigens as were recognized in
### Table 1. MAb reactivities

<table>
<thead>
<tr>
<th>MAb</th>
<th>IgG*</th>
<th>ELISA†</th>
<th>Western blot†</th>
<th>Immunofluorescence‡</th>
<th>Immunoprecipitation§</th>
<th>APAAP∥</th>
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<tr>
<td></td>
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<td>HIV-1</td>
<td>HIV-2</td>
<td>SIV</td>
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<td>7-A-8</td>
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<tr>
<td>8-D-2</td>
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<tr>
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<td>3-B-7</td>
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* Mouse immunoglobulin subclass.
† HTLV-IIIB grown in KE37 cells, LAV-2 grown in CEM cells and SIVmac grown in H9 cells were used as antigens.
‡ HTLV-IIIB-infected H9 cells and LAV-2-infected CEM cells were used.
§ Procedure according to Conraths et al. (1988). HTLV-IIIB and LAV-2 were used as antigens. Proteins were separated by SDS-PAGE. Staining was done with HIV-positive human sera and anti-human IgG coupled to alkaline phosphatase.
∥ HTLV-IIIB-infected H9 cells were attached to slides using a Shandon Cytospin.
¶ + +, strong reaction (in ELISA > 0.4); +, positive reaction (ELISA > 0.2); (+), weak positive reaction; -, negative reaction; NT, not tested.
Fig. 1. Immunocytochemistry of HTLV-IIIB-infected H9 cells. (a) Immunofluorescence using anti-p17 MAb 3-H-7 (hybridoma culture supernatant, concentrated tenfold by ammonium sulphate precipitation) and fluorescein-labelled anti-mouse IgG (1:20, Dianova, Hamburg, F.R.G.). (b) APAAP using anti-p24 MAb 6-D-12 (ascites at a 1:1000 dilution) in the APAAP technique (Dianova; Cordell et al., 1984). Bar marker represents 10 μm.

immunoblotting (not shown). In immunoelectron microscopy (IEM) the incubation of ultrathin cryostat sections with anti-p17 and anti-p24 MAbs led to a labelling of internal viral components (Fig. 2a, b, c) thus corroborating earlier observations on the localization of the structural proteins (Gelderblom et al., 1987, 1988). In pre-embedding IEM, a technique allowing the exclusive detection of external antigens, no labelling was observed with these antibodies (data not shown).

Of special interest was the identification of HIV-1-specific MAbs cross-reacting also with HIV-2, strains LAV-2_{ro} and SBL 6669 (Clavel et al., 1986; Albert et al., 1987), and with SIV_{mac} (Daniel et al., 1985). Four different reaction patterns were observed with regard to reactivity in ELISA and immunoblotting using antigens derived from partially purified HTLV-IIIB, LAV-2, and SIV_{mac} (Table 1). While the p17-specific antibody detected exclusively p17 of HIV-1, 20 of 28 p24-specific MAbs reacted in immunoblotting with the corresponding core protein of HIV-2 (p26), and five of these also recognized the analogous SIV_{mac} antigen (p28). All HIV-1-specific antibodies reactive with the simian virus cross-reacted with HIV-2; however, not all MAbs cross-reacting with HIV-2 recognized SIV_{mac}. These results were predictable from the sequence homologies of HIV-1, LAV-2 and SIV_{mac} (Wain-Hobson et al., 1985; Ratner et al., 1985; Guyader et al., 1987; Chakrabarti et al., 1987). The characterization of the respective epitopes...
using oligopeptides is now in progress. Representative reaction patterns of some of the MAbs are shown in Fig. 3.

The major core proteins of the three viruses evidently share common epitopes. Cross-reacting antibodies directed against such conserved epitopes may help to screen efficiently in diagnostic assays for new HIV isolates and variants which can be typed subsequently by immunological and molecular biological techniques. The applicability of these antibodies in a variety of test systems offers the possibility of differentiating between isolates belonging to the same virus type (Ferns et al., 1987) and between different HIVs, i.e. between HIV-1, HIV-2 and possibly between further strains.
Fig. 3. Western blot analysis using three virus preparations. Lanes 1; HTLV-IIIB; lanes 2, LAV-2; lanes 3, SIVmac. All five MAbs stained p24 of HTLV-IIIB. 11-C-5 shows a positive reaction only with HTLV-IIIB p24 and the precursor p55. 6-E-7 reacted with p24 of HTLV-IIIB, the p26 of LAV-2, and weakly with the p28 of SIVmac. The three other MAbs (1-B-7, 10-E-7, 12-B-4) detected the major core protein of all three viruses. Some of the MAbs reacted with a protein of Mr, 22K in addition to p26 of LAV-2. This protein might represent a modified protein or a cleavage product. Mr markers (× 10^-3) are indicated on left.

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REFERENCES


Short communication


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