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Characterization of Murine Monoclonal Antibodies Directed against the Core Proteins of Human Immunodeficiency Virus Types 1 and 2

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Monoclonal antibodies (MAbs) raised against the core proteins of human immunodeficiency virus type 1 (HIV-1; laboratory strain HTLV-IIIB) and HIV-2 (strain ROD) were investigated in a variety of tests, e.g., enzyme-linked immunosorbent assay (ELISA), immunostaining of Western immunoblots, immunofluorescence, immunoprecipitation, and alkaline phosphatase anti-alkaline phosphatase assay. The MAbs were grouped according to their cross-reactions. Seven HIV-1-specific MAbs reacted exclusively with HIV-1, and five showed cross-reactivity with HIV-2 and simian immunodeficiency virus of macaques in ELISA. Four of the 15 MAbs against HIV-2 reacted only with the HIV-2 protein p26. Six showed cross-reactivity with HIV-1, and five showed a broad reaction with all three viruses. Overlapping 30-amino-acid-long peptides derived from the p24 protein sequence of HIV-1 were used in an epitope-mapping system. Three different immunogenic regions (A, B, and C) could be defined. Specific regions where anti-HIV-1 and -HIV-2 MAbs cross-reacted were mapped with shorter oligopeptides.

The internal viral structural protein p24 (core antigen [CA] [15]) of human immunodeficiency virus type 1 (HIV-1) becomes endoproteolytically processed from the *gag* precursor and represents the major component of the HIV particle (8). Decreasing anti-p24 antibody titers as well as p24 antigenemia are considered prognostic markers for the disease (13). Monoclonal antibodies (MAbs) may be of advantage in detecting and typing antigens in tissues and cells suspected to be infected by HIV or related viruses (2, 7). A variety of MAbs have been raised against the core protein of HIV-1 (5, 9, 22). Some of these as well as human sera were further characterized by epitope mapping with peptides (6, 9, 21) or recombinant p24 proteins expressed in *Escherichia coli* (25). Up to now only a few MAbs directed against HIV-2 and simian immunodeficiency virus of macaques (SIV_{mac}) have been described (19, 27), but none of them were further characterized with respect to the recognition sequence.

We selected 12 MAbs (Table 1) directed against the CA of the HIV-1 (CA-1) laboratory strain HTLV-IIIB after fusion of SP2/0 cells with spleen cells of BALB/c mice immunized with recombinant p24–β-Gal fusion protein (12). The recombinant protein encoded by pCo1 consisted of the carboxy-terminal 11 amino acids of p17 and the entire p24 and p15 sequence and resembled an amino-terminal-deleted core precursor protein p55 from Asp-121 to Glu-512 (β-Gal_{1-375}::HIV-1 $gag_{121-512}$) (1).

Fifteen MAbs directed against HIV-2 were selected with a HIV-2 enzyme-linked immunosorbent assay (ELISA) after a two-step immunization procedure using purified HIV-2 followed by HIV-1 *gag* fusion proteins to increase the frequency of MAbs with cross-reactivities with CA-1 and CA-2 (Table 1). The specificity of the MAbs was achieved by ELISA with complete HIV-1 or HIV-2 virus. All MAbs were subcloned three times by limited dilution before being considered monoclonal.

Depending on the test system used (Table 1) the MAbs showed various degrees of cross-reaction, reflecting the conservation and availability of the respective epitopes in the different antigen preparations. By using purified virus in an ELISA, 7 of the 12 HIV-1-specific MAbs exclusively reacted with HIV-1, and 5 MAbs cross-reacted with HIV-2 and SIV_{mac}, with the exception of 1 MAb (113/038) cross-reacting only with HIV-2.

The anti-HIV-2 MAbs showed three different reaction patterns (Table 1): group-specific reactions, cross-reaction only to HIV-1, and a cross-reaction to HIV-1 and SIV_{mac}.

The 12 HIV-1-specific MAbs were detected in Western immunoblot analysis with purified HIV-1, p24 (CA-1), and its *gag* precursor, p55. The five MAbs cross-reacting in ELISA showed a strong reaction with CA-2 (p26) and a significant reaction with CA-SIV_{mac} (p28) when tested in immunoblotting. The MAbs generated against HIV-2 reacted with CA-2 as well as with CA-1 and CA-SIV_{mac}, varying in the binding intensity, which ranged from detectable to strong reactions (Table 1). Most of these cross-reactive MAbs reacted also with the *gag* precursor. Depending on the virus preparation and the antibody used, several minor bands of M_r between 24,000 and 55,000 were observed in addition to the main bands.

In immunofluorescence tests the MAbs were investigated on HIV-1-, HIV-2-, and SIV_{mac}-infected and uninfected cells (Table 1). The five MAbs against CA-1 with cross-reactions to CA-2 in ELISA reacted with cells infected with HIV-1, HIV-2, and SIV_{mac}, whereas the seven HIV-1-specific MAbs detected antigens only in HIV-1-infected cells. The CA-2-specific MAbs, except three antibodies (Table 1), reacted with HIV-1-, HIV-2-, and SIV-infected cells but not with uninfected cells.

The majority of the MAbs precipitated only the homologous CA. One HIV-1- as well as one HIV-2-specific MAb

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TABLE 1. MAb reactivities[a]

MAb	IgG[b]	Epitope[c]	ELISA[d]			Western blot[d]			Immunofluorescence[e]			Immunoprecipitation[f]			APAAP[g]	
			HIV-1	HIV-2	SIV_{mac}	HIV-1	HIV-2	SIV_{mac}	HIV-1	HIV-2	SIV_{mac}	HIV-1	HIV-2	SIV_{mac}	HIV-1	HIV-2
Anti-CA-1																
111/052	IgG1	B_1	++	−	−	++	(+)	−	−	NT	−	+	−	−	+	−
111/182	IgG1	A	+	−	−	++	(+)	−	−	NT	−	+	+	(+)	+	+
113/072	IgG1	B_3	++	−	−	++	(+)	−	−	NT	NT	−	−	−	+	−
112/027	IgG1	NT	++	−	−	++	(+)	−	+	(+)	+	+	+	+	+	−
111/056	IgG1	NT	++	−	−	++	−	−	−	−	−	+	−	NT	+	(+)
112/047	IgG1	A	++	−	−	++	−	−	+	−	−	+	NT	NT	+	−
112/021	IgG1	A	+	−	−	++	(+)	−	(+)	(+)	(+)	+	+	+	+	−
113/038	IgG1	B_2	++	+	−	++	+	(+)	+	+	+	+	+	+	+	−
110/015	IgG1	C_3	+	+	+	++	+	(+)	+	(+)	+	+	NT	(+)	+	+
108/03	IgG1	C_3	++	++	++	++	+	(+)	+	+	+	+	−	−	+	+
106/01	IgG1	C_4	++	++	++	++	+	−	+	+	(+)	+	NT	NT	+	+
111/073	IgG1	B_3	++	+	+	+	+	+	+	+	+	+	+	+	+	−
Anti-CA-2																
178/354	IgG1	C_3	−	+	−	(+)	+	+	(+)	+	(+)	−	+	+	+	+
178/108	IgG1	C_1	−	+	−	++	++	+	+	+	(+)	−	−	−	−	+
178/508	IgG1	C_2	−	+	−	++	++	+	(+)	+	+	−	+	+	(+)	+
178/165	IgG1	C_3	−	+	−	(+)	+	+	+	+	+	−	+	−	−	+
178/054	IgG1	C_2	+	++	−	++	++	++	−	++	−	−	(+)	(+)	(+)	+
178/088	IgG2b	C_2	+	+	−	+	++	+	−	++	(+)	−	+	+	(+)	+
178/192	IgG1	C_2	+	+	−	+	++	+	(+)	+	(+)	−	(+)	(+)	(+)	+
178/513	IgG1	C_3	+	++	−	(+)	+	+	−	+	(+)	−	+	−	−	+
178/023	IgG1	C_2	+	++	−	+	++	++	+	++	+	+	+	+	+	+
178/085	IgG1	C_2	+	++	−	++	++	++	(+)	++	(+)	+	+	+	−	+
178/029	IgG1	C_3	−	+	++	(+)	+	+	(+)	+	+	−	+	+	−	+
178/303	IgG1	C_2	+	++	+	+	++	+	+	++	+	+	+	−	(+)	+
178/249	IgG1	C_2	+	++	+	+	++	+	+	++	+	+	+	−	+	+
178/200	IgG1	C_2	+	++	+	+	++	+	+	+	+	+	+	−	+	+
178/129	IgG1	C_1	+	++	+	+	++	++	(+)	+	(+)	−	+	−	+	+

[a] Reaction of MAbs with the CAs of HIV-1 (CA-1), HIV-2 (CA-2), and SIV_{mac} (CA-SIV_{mac}). ++, strong reaction (in ELISA, optical density at 497 nm of >0.4); +, positive reaction (in ELISA, optical density at 497 nm of >0.2); (+), weak positive reaction; −, negative reaction; NT, not tested.

[b] IgG, mouse immunoglobulin subclass determined by the Ouchterlony technique.

[c] Binding region on HIV-1 *gag* peptides.

[d] HIV-1 (strain BH10) grown in KE37 cells, HIV-2 (strain ROD) grown in CEM cells, and SIV_{mac} (strain SIV_{mac} 251) grown in Molt4/8 cells were purified by centrifugation through a 10% sucrose cushion. Purified virus (5 to 10 μg/100-μl well) was coated on 96-well microtiter plates (Nunc, Roskilde, Denmark). The reactions were visualized by rabbit anti-mouse conjugated peroxidase and the appropriate substrate H_2O_2-TMB.

[e] Immunofluorescence studies were performed on KE37 cells infected with HIV-1 or HIV-2 or Molt4/8 cells infected with SIV_{mac}. Cells were spotted on slides, air-dried, and fixed overnight in acetone-methanol, 1:1 (vol/vol). Hybridoma culture supernatant affinity purified or 10-fold concentrated by ammonium sulfate precipitation was used. The detecting antibody was fluorescein-conjugated goat anti-mouse Fc antibody (Dianova).

[f] Partially purified HIV-1, HIV-2, and SIV_{mac} were used as antigens in the immunoprecipitation tests by the method of Conraths et al. (3). Proteins of the immunoprecipitate were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Detection of antigen was performed by high-titer human sera reacting with HIV-1 and SIV and anti-human immunoglobulin G coupled to alkaline phosphatase (Dianova).

[g] HIV-1-infected cells were attached to slides by using a Shandon Cytospin. Cells were fixed with acetone-methanol, 1:1 (vol/vol). As a second antibody, rabbit anti-mouse immunoglobulin G–alkaline phosphatase (1:40; Dianova), followed by the preformed APAAP complex (1:50; Dianova), was used. Uninfected cells served as a control. The method of Cordell et al. (4) was used.

cross-reacted in precipitation tests. However, cross-reactivity with the CA of the other viruses was less pronounced in this test in comparison with the broad reaction in the other test systems (Table 1).

All of the anti-CA-1 MAbs were reactive in the alkaline phosphatase anti-alkaline phosphatase assay (APAAP) (4) with HIV-1-infected H9 cells. Five of these MAbs showed a cross-reaction with HIV-2-infected (H9) cells. All MAbs raised against HIV-2 reacted with HIV-2-infected cells, and 10 of these MAbs showed a more or less strong reaction with HIV-1-infected cells. Reactions with uninfected cells could not be observed.

The results of the peptide mapping are listed in Table 2. All MAbs reacted with one to three consecutive linear synthetic peptides representing HIV-1 p24. The antibodies could be grouped in three different groups, i.e., A, B, and C, depending on their reactivity patterns with the peptides. Anti-CA-1 MAbs to each of the epitopes were found (Table 2), whereas all the anti-CA-2 MAbs reacted only with the C epitope. Most of the MAbs reacted with one or two of the overlapping peptides in the respective region. Only one MAb (108/03) showed a broad reaction with three consecutive peptides.

Domain A, recognized by three CA-1 MAbs (111/182, 112/047, and 112/021), is located at the amino terminus of p24. From the reactivity with the overlapping oligopeptides it can be concluded that the binding site covers the sequence amino acid (aa) 134 to 153. The second immunogenic region (B) is defined by three overlapping peptides ranging from aa 173 to 242. Within this region three reaction patterns could be observed. MAb 111/052 reacted exclusively with peptide 211, MAbs 111/073 and 113/038 reacted with peptide 212, and

TABLE 2. Reactions of MAbs with 30-aa oligopeptides[a]

MAb	No. Coated peptide	Region	aa sequence[a]	Starting position
111/182	209	A	PIVQNIQGQMVHQAISPRTLNAWVKVVEEK	133
112/021	209	A		
112/047	209	A		
111/052	211	B1	SALSEGATPQDLNTMLNTVGGHQAAMQMLK	173
113/038	212	B2	GHQAAMQMLKETINEEAAEWDRVHPVHAGP	193
111/073	212	B2		
113/072	212 / 213	B3	GHQAAMQMLKETINEEAAEWDRVHPVHAGP	193
			DRVHPVHAGPIAPGQMREPRGSDIAGTTST	213
178/129	216	C1	IVRMYSPTSILDIRQGPKEPFRDYVDRFYK	273
178/108	216	C1		
178/023	216 / 217	C2a	IVRMYSPTSILDIRQGPKEPFRDYVDRFYK	273
178/054	216 / 217	C2a	FRDYVDRFYKTLRAEQASQEVKNWMTETLL	293
178/085	216 / 217	C2a		
178/088	216 / 217	C2a		
178/192	216 / 217	C2a		
178/508	216 / 217	C2a		
178/200	216 / 217	C2b	IVRMYSPTSILDIRQGPKEPFRDYVDRFYK (discont.)	
178/249	216 / 217	C2b		
178/303	216 / 217	C2b		
178/29	217 / 218	C3a	FRDYVDRFYKTLRAEQASQEVKNWMTETLL	293
178/165	217 / 218	C3a	VKNWMTETLLVQNANPDCKTILKALGPAAT	313
178/354	217 / 218	C3a		
178/513	217 / 218	C3a		
110/015	217 / 218	C3a		
108/03	217	C3b	FRDYVDRFYKTLRAEQASQEVKNWMTETLL	293
	218	C3b	VKNWMTETLLVQNANPDCKTILKALGPAAT (discont.)	313
	219	C3b	ILKALGPAATLEEMMTACQGVGGPGHKARV	333
106/01	219	C4	ILKALGPAATLEEMMTACQGVGGPGHKARV	333

[a] Reactions of MAbs with consecutive 30-aa peptides are according to the published sequence of Ratner et al. (23). Overlapping amino acid sequences are underlined. Starting positions were taken from the data base (20). Linear peptides were synthesized by the method of Houghten et al. (10) and donated by J. Rosen, Johnson Pharmaceutical Research, La Jolla, Calif. MAbs were titrated on HIV-1 ELISA plates coated with recombinant p24. The dilution of the antibody corresponding to an A_{490} of 2.0 was used for the incubation with different synthetic peptides coated on an ELISA plate (Nunc) at a final concentration of 5 μg per well. The reaction was visualized with peroxidase-conjugated anti-mouse immunoglobulin G and the appropriate substrate H_2O_2–*o*-phenylenediamine dihydrochloride.

MAb 113/072 reacted with peptides 212 and 213. From these results we could conclude that the binding site of MAb 111/052 is located between aa 183 and 192 (B1), that of MAbs 111/073 and 113/038 is located between aa 202 and 211 (B2), and that of MAb 113/072 is located between aa 213 and 222 (B3).

In the fine mapping with overlapping 15-aa peptides, MAb 178/085 reacted with two consecutive peptides (GPKEPFRD YVDRFYK and FRDYVDRFYKTLRAE). The three MAbs 178/200, 178/249, and 178/303 reacted with two different peptides, indicating a discontinuous epitope. The eight MAbs exhibiting cross-reactivity with HIV-1 and HIV-2 showed different homologies in the conservation of the amino acid sequence, ranging from 6 aa (110/015) to 17 aa (106/01) in identical positions in the respective epitope. Most of the common epitopes were localized in the C region.

There is only a limited amino acid sequence homology among CA-1, CA-2, and CA-SIV in the regions of the epitopes A, B1, and B3. The MAbs reacting with these epitopes also show very weak cross-reaction in the immunological tests.

The third domain (C) is located at the C terminus (aa 293 to 362). In this relatively broad region four binding sites can be distinguished: C1, aa 283 to 292; C2, aa 293 to 302; C3, aa 313 to 342; and C4, aa 243 to 362. The overlapping peptides 217 to 219 were recognized by three MAbs (110/015, 106/01, and 108/03) generated against CA-1 but cross-reacting with CA-2 and CA-SIV (Table 1). All anti-CA-2 MAbs selected in this study reacted with one or two of the three overlapping peptides 216 to 218.

In a previous investigation we found that there is only a limited correlation between the amino acid sequence homology and the cross-reaction observed in different test systems (21). This also became obvious with the MAbs reacting with peptides 216 and 217. Depending on the test used, a type-specific reaction or a pronounced cross-reaction could be observed. The mixed immunization scheme with HIV-1 and -2 antigen induced cross-reacting MAbs reacting within the sequence aa 273 to 343, corresponding to a conserved region found in all sequenced primate immunodeficiency viruses (21).

The murine CA epitopes described here are in agreement with those of other investigations (6, 9, 21, 25). In contrast to epitope mapping with recombinant polypeptides, our peptide mapping allowed a more precise location of the binding sites. However, it is worthwhile to note that with this method only

limited information on conformational epitopes can be achieved. Although the antibodies all reacted in the peptide-mapping system, differences in reactivity patterns would be observed in other test systems, suggesting conformation-dependent epitopes for the antibodies.

The large antigenic variations of HIV strains require specific MAbs for subtyping of different HIV isolates and broadly reacting MAbs for diagnostic purposes. The set of antibodies described above might be useful to develop broadly reactive test systems for the detection of CA of different primate immunodeficiency viruses in infected cells and tissues as well as in the sera of infected individuals or tissue culture supernatants of infected cells. In preliminary immunofluorescence studies with four HIV-1 field isolates we found complete correlation of these reactivities compared with those in the HTLV-IIIB laboratory strain. The applicability of the antibodies in the APAAP technique offers the possibility for their use in immunocytochemistry staining of histological specimens derived from various origins.

The MAbs directed against the antigenic structures defined here may be also helpful in investigations of cell surface expression of CA, which was reported by Ikuta et al. and Laurent et al. (11, 14). These regions of CA seem to play an important role in cell-mediated immunity (17, 28). All three epitopes, A, B and C, were reported to have cell proliferation inducing capacity (29). The murine B-cell epitopes described in this study correspond to human T-cell epitopes (16, 26, 31). The epitopes B3 and C2, for example, show T-cell proliferation in response to 20-aa peptides (24), and the epitope B1 is described as a restricted epitope for *gag*-specific cytotoxic T lymphocytes from SIV_{mac}-infected rhesus monkeys (30). In addition, the C epitope is recognized by T-cell clones obtained from p24-immunized cynomolgous macaques (18). The analysis of the immune response to the CA of HIV is one main goal for the characterization of a protective immune response for infection and/or disease.

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