JOURNAL OF VIROLOGY, Aug. 1989, p. 3525–3528 Vol. 63, No. 8
0022-538X/89/083525-04$02.00/0
Copyright © 1989, American Society for Microbiology

Epitope Mapping of Monoclonal Antibodies against Human Immunodeficiency Virus Type 1 Structural Proteins by Using Peptides

M. NIEDRIG,[1]* J. HINKULA,[2] W. WEIGELT,[1] J. L'AGE-STEHR,[3] G. PAULI,[1] J. ROSEN,[4] AND B. WAHREN[2]

Robert Koch-Institut des Bundesgesundheitsamtes, Berlin, Federal Republic of Germany[3]; Johnson & Johnson, Biotechnology Center, La Jolla, California 92037[4]; AIDS-Zentrum am Bundesgesundheitsamt, D-1000 Berlin 65, Federal Republic of Germany[1]; and National Bacteriological Laboratory, Stockholm, Sweden[2]

Received 19 December 1988/Accepted 15 March 1989

Murine monoclonal antibodies directed against the structural proteins p17 and p24 of human immunodeficiency virus type 1 were investigated in an epitope mapping system. Overlapping peptides consisting of 15 amino acids of the p17 and p24 protein, respectively, were used as competitors in an enzyme-linked immunosorbent assay. Three different immunogenic regions (A, B, and C) could be defined, one on p17 and two on p24. Twenty monoclonal antibodies reacted with the human immunodeficiency virus type 1 peptides of region B, although differences in the reactivity of these antibodies with human immunodeficiency virus type 2 and simian immunodeficiency virus strain mac were detectable. Recognized epitopes were characterized by computer analysis as described by T. P. Hopp and K. R. Woods (Proc. Natl. Acad. Sci. USA 78:3824–3828, 1981) and P. Y. Chou and G. D. Fasman (Biochemistry 13:222–245, 1974).

The internal viral structural proteins p17 and p24 are major components of the human immunodeficiency virus (HIV). Whereas p24 represents the core protein, p17 is located adjacent to the lipid membrane of the virion (7, 8). Recent investigations suggest that p17 serves as a matrix protein involved in the shaping of the virus particle (13, 16). Both proteins seem to play a significant role as prognostic factors for the development of acquired immunodeficiency syndrome (17). The concentration of p17 antibodies might serve as a marker for the clinical status, whereas p24 antigenemia is frequently detectable in individuals developing acquired immunodeficiency syndrome (1, 2, 6, 12). The detection of p24 in the serum by antigen capture assays is one method to detect antigenemia in infected patients. For that purpose broadly reacting anti-p24 antibodies might be helpful to detect prototype p24 and its variants. This holds especially true when people infected with HIV type 2 (HIV-2) are investigated. Recently, monoclonal antibodies raised against HIV type 1 (HIV-1) that cross-react with the core proteins of HIV-2 and simian immunodeficiency virus strain mac (SIV_{mac}) were described (15).

To identify respective epitopes on p17 and p24 for some of the antibodies (Table 1), overlapping peptides were synthesized in solid-phase medium as described by Houghten et al. (11). The sequence used was based on published data (18). Each peptide corresponded to a sequence of 15 amino acids (aa) of p17 or p24. Each subsequent peptide overlapped with the previous one by 10 amino acids. Amino acid numbering was made according to the database (14). Seventy-seven *gag* peptides (p17/5 through 32 and p24/31 through 81) were synthesized starting with aa −7 LAEAR.

These peptides were investigated in direct and inhibition assays. Three different antigenic regions on the *gag* proteins could be defined. As expected, the monoclonal antibody 3-H-7 reacting with p17 in immunoblotting was inhibited by peptides originating in the p17 amino acid sequence (Table 2; peptides p17/28 and p17/29). Since peptides p17/28 and p17/29 both gave direct reactivity and blocked the monoclonal antibody (MAb) reaction, it is likely that the epitope is located in the 10-aa overlapping site KKAQQAAADT (aa 113 through 122; region A). The amino acid sequence of $HIV\text{-}1_{BH10}$ is quite distinct from those of $HIV\text{-}2_{ROD}$ and SIV_{mac} in this region (Fig. 1a). This difference is reflected by the reactivity of the antibody in different test systems where no crossreaction with $HIV\text{-}2_{ROD}$ and SIV_{mac} antigens were detectable.

On p24 two different regions of reactivity were observed. Region B (Table 2) comprised reactivity for 20 recloned MAbs, but with slightly different epitopic sites. Nine MAbs reacted with p24/47 and were also totally blocked by this peptide. Since neither neighbor peptide had any influence the reactivity of the MAbs, the centrally located aa EAAEW may carry the epitope site. Five MAbs reacted with p24/47 and p24/48 and were blocked by p24/47 and partially by p24/48. This group of MAbs must be assumed to react with a broader epitope, to be found in the sequence EAAEW-DRVHP. Six further MAbs of region B had reactivity patterns leading to the possibility that they were reactive with discontinuous epitopes, located within the sequences of p24/212 and/or p24/216 and p24/217. Either they did not react with any single linear small peptide, or they were not blocked in their reactivity.

Antibody 12-B-4, which cross-reacted with HIV-2 and SIV_{mac}, recognized peptides 64 and 65. From these results and sequence alignment with HIV-2 and SIV_{mac}, the sequence from aa position 293 through 302 can be predicted as the epitope recognized by 12-B-4. MAb 12-B-4 belongs to the region we call C on p24. Three MAbs were reactive to peptides of this region (Table 2).

* Corresponding author.

TABLE 1. Peptide aa sequences[a]

Peptide no.	Peptide aa sequence	Starting position[b]
28	QNKSK**KKAQQAAADT**	108
29	**KKAQQAAADT**GHSSQ	113
47	ETINE**EAAEWDRVHP**	203
48	**EAAEWDRVHP**VHAGP	208
212	GHQAAMQMLKETINE**EAAEWDRVHP**VHAGP	193
64	GPKEP**FRDYVDRFYK**	288
65	**FRDYVDRFYK**TLRAE	293
68	**QASQEVKNWMTETLL**	308
216	IVRMYSPTSILDRIRQGPKEP**FRDYVDRFYK**	272
217	**FRDYVDRFYK**TLRAE**QASQEVKNWMTETLL**	293

[a] Overlapping sequences are underlined. The published sequence of Ratner et al. (18) was used.
[b] Starting positions were taken from the database (14).

Comparison of the sequence alignment with cross-reactivity studies imply that a cluster of epitopes is located in this *gag* region. The sequence 205 through 208 forming the epitope is conserved in HIV-2_{ROD} and SIV_{mac}, sequence 207 through 211 seems to be responsible for the cross-reaction with HIV-2, whereas sequence 203 through 207 represents the type-specific epitope. This hypothesis has to be confirmed by investigations with peptides in this region.

The comparison of the epitope findings with the computer predicted antigenic epitopes as described by Hopp and Woods (10) (Fig. 1b) and Chou and Fasman (4) offers the possibility of proving the reliability of their predictions. The defined epitope of 3-H-7 is located in a region with great changes in hydrophilic quality (Fig. 1b) and a β turn. This seems to lead to a high antigenicity. Our data corroborate previous findings of Sternberg et al. (19). The defined epitope of 12-B-4 also shows total homology with the predictions. The sequence 293 through 302 is represented by a change in hydrophilicity (Fig. 1b), and also the secondary structure prediction points to a high antigenic region. Region B (aa 193

TABLE 2. Reaction of selected MAbs with peptides[a]

MAb	Coated peptide(s) no.	Blocking peptide(s) no.	Region	Reaction in immunoblot[b]		
				HIV-1	HIV-2	SIV_{mac}
3-H-7	28, 29	28, 29	A	+	–	–
1-E-9	212, 47	47	B_1	+	–	–
2-E-4	212, 47	47	B_1	+	+	–
2-H-4	212, 47	47	B_1	+	+	–
8-D-2	212, 47	47, (48)[c]	B_1	+	–	–
8-H-7	212, 47	47	B_1	+	–	–
8-G-9	212, 47	47	B_1	+	–	–
10-E-7	212, 47	47	B_1	+	+	+
10-G-9	212, 47	47	B_1	+	–	–
11-C-5	212, 47	47	B_1	+	–	–
1-B-7	212, 47, (48)	47, (48)	B_2	+	+	+
3-B-7	212, 47, (48)	47	B_2	+	+	–
6-D-12	212, 47, (48)	47, (48)	B_2	+	+	–
6-E-7	212, 47, 48	47, (48)	B_2	+	+	+
8-D-5	212, 47, (48)	47	B_2	+	–	–
5-B-11	212, –[d]	47	B_3	+	+	–
6-F-2	212, 47	–, 47	B_3	+	–	–
7-G-11	212, –	47	B_3	+	–	–
8-D-8	212, (217), 47	47	B_3	+	+	–
8-D-11	212, (216, 217), 47	47	B_3	+	–	–
11-G-7	212, –	–, 47	B_3	+	+	+
7-A-8	217, –	–	C	+	–	–
11-G-1	217, 68	–	C	+	–	–
12-B-4	216, 217	64, 65	C	+	+	+

[a] The MAbs originated from one fusion of spleen cells of a mouse immunized four times with 0.5 mg of purified human T-lymphotropic virus IIIB within 1 year (15). For the inhibition assays the hybridoma culture supernatants were concentrated 10-fold by ammonium sulfate precipitation. The titers of concentrated MAbs were determined on HIV-1 enzyme-linked immunosorbent assay plates (organon or recombinant p24-15; 20, 21). The dilution of the antibody corresponding to an optical density at 490 nm of 0.6 to 1.2 in the HIV-1 enzyme-linked immunosorbent assay was used for the incubation with different peptides in the blocking assay. The MAbs were incubated with a final concentration of 5 μg of each of the synthetic peptides per well, and 100 μl of the mixture was transferred to an HIV-1 enzyme-linked immunosorbent assay plate and incubated for an additional hour. The reactions were visualized by using anti-mouse immunoglobulin G conjugated peroxidase and the appropriate substrate H_2O_2-OPD.
[b] Western blot analysis was performed with purified virus. HTLV IIIB was grown in KE37 cells, LAV-2 was grown in CEM cells, and SIV_{mac} was grown in H9 cells. +, Reaction with *gag* protein; –, no reaction type detectable.
[c] Numbers within parentheses indicate partial reactivity with the peptide.
[d] –, No reactivity with an independent antibody preparation.

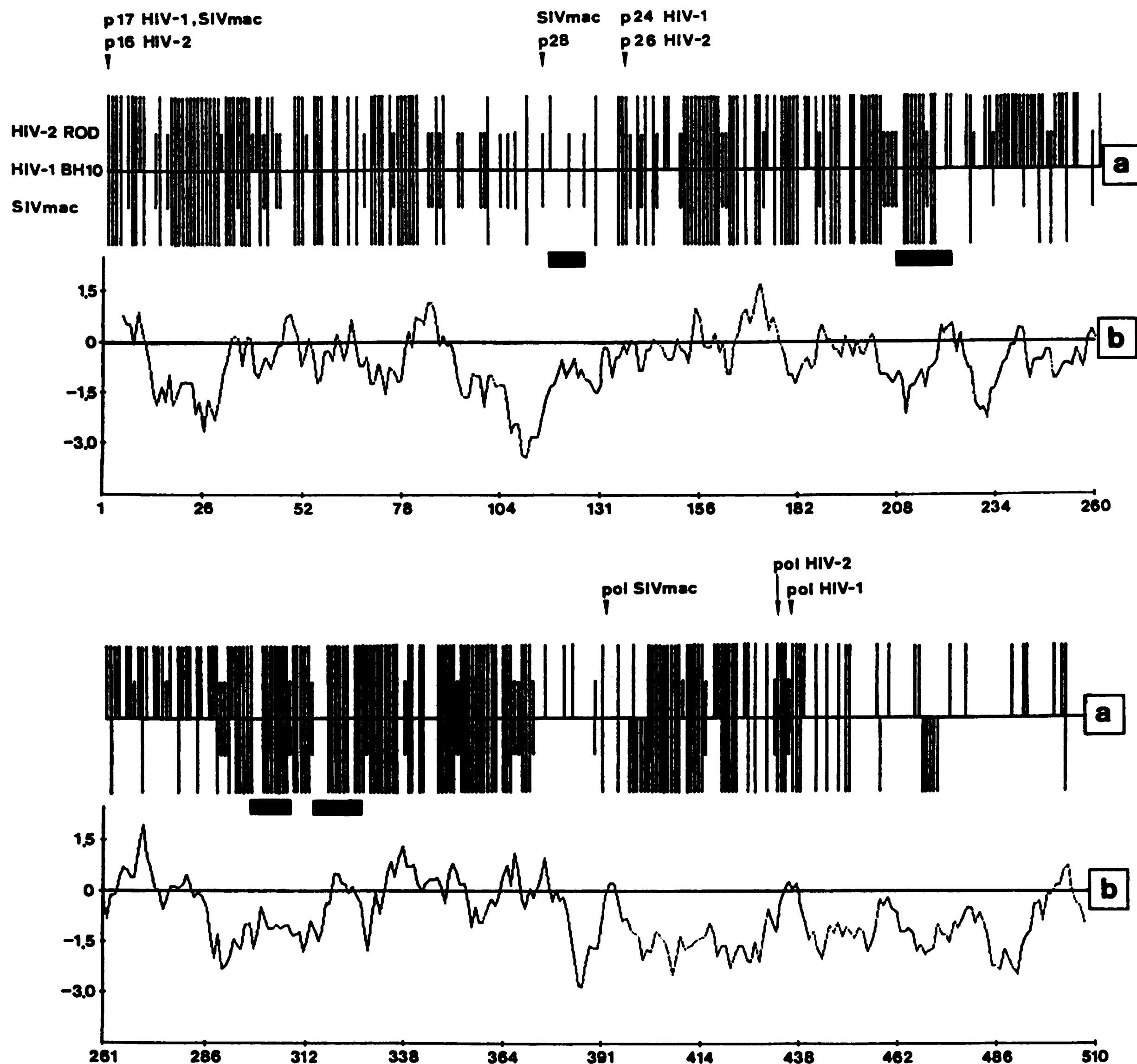

FIG. 1. (a) The amino acid sequences of the three different immunodeficiency virus isolates, HIV-1_{BH10} (18), HIV-2_{ROD} (9), and SIV_{mac} (5, 3), were compared. The sequence of HIV-1 is used as a standard. Identity in amino acids of a given position to the sequence of prototype HIV-1_{BH10} is depicted by a full-length vertical bar, whereas homologous exchange is marked by a half-length bar. Horizontal bars label the sequences inhibiting the reaction of the MAbs (Tables 1 and 2). (b) The hydrophobicity profile of the *gag* protein was calculated by the method of Hopp and Woods (10). Positive values identify hydrophobic regions and negative values identify hydrophilic regions within the protein.

through 222) contains β turns on each side of a hydrophilic region that is also predicted to be of α-helical configuration.

We thank Y. Kassner and K. Kretschmer for assistance in raising the monoclonal antibodies and B. Thiele for providing the secondary structure prediction.

LITERATURE CITED

1. **Bolton, V., N. C. Pedersen, J. Higgins, M. Jennings, and J. Carlson.** 1987. Unique p24 epitope marker to identify multiple human immunodeficiency virus variants in blood from the same individuals. J. Clin. Microbiol. **25**:1411–1415.
2. **Caruso, A., L. Terlenghi, R. Ceccarelli, R. Verardi, I. Foresti, G. Scura, N. Manca, C. Bonfanti, and A. Turano.** 1987. Liquid competition radioimmunoassay for the detection and quantitation of the HIV p24. J. Virol. Methods **17**:199–210.
3. **Chakrabarti, L., M. Guyader, M. Alizon, M. D. Daniel, R. C. Desrosiers, P. Tiolais, and P. Sonigio.** 1987. Sequence of simian immunodeficiency virus from macaque and its relationship to other human and simian retroviruses. Nature (London) **328**: 543–547.
4. **Chou, P. Y., and G. D. Fasman.** 1974. Prediction of protein conformation. Biochemistry **13**:222–245.
5. **Daniel, M. D., N. L. Letvin, N. W. King, M. Kannagi, P. K. Sehgal, and R. D. Hunt.** 1985. Isolation of T-cell tropic HTLV-III like retrovirus from macaques. Science **228**:1201–1204.
6. **Forster, S. M., L. M. Osborne, R. Cheingsong-Popov, C. Kenny, R. Burnell, J. D. Jeffries, A. J. Pinching, J. R. Harris, and J. N. Weber.** 1987. Decline of anti-p24 antibody precedes antigenaemia as correlate of prognosis in HIV-1 infection. AIDS **1**:235–240.
7. **Gelderblom, H. R., E. H. S. Hausmann, M. Özel, G. Pauli, and M. A. Koch.** 1987. Fine structure of human immunodeficiency virus (HIV) and immunolocalization of structures proteins. Virology **156**:171–176.
8. **Gelderblom, H., H. Reupke, T. Winkel, R. Kunze, and G. Pauli.**

1987. MHC-Antigens: constituents of the envelopes of human and simian immunodeficiency virus. Z. Naturforsch. Sect. C Biosci. **42**:1328–1334.

9. **Guyader, M., M. Emerman, P. Songio, F. Clavel, L. Montagnier, and M. Alizon.** 1987. Genome organization and transactivation of the human immunodeficiency virus type 2. Nature (London) **326**:662–669.
10. **Hopp, T. P., and K. R. Woods.** 1981. Prediction of protein antigenic determinants from amino acid sequences. Proc. Natl. Acad. Sci. USA **78**:3824–3828.
11. **Houghten, R. A.** 1985. General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. Proc. Natl. Acad. Sci. USA **82**:5131–5135.
12. **Lange, J. M. A., D. A. Paul, H. G. Huisman, F. Wolf, H. Van der Berg, R. A. Coutinho, S. A. Danner, J. van der Noordaa, and J. Goudsmit.** 1986. Persistent HIV antigenaemia and decline of HIV core antibodies associated with transition to AIDS. Br. Med. J. **293**:1459–1462.
13. **Marx, P. A., R. J. Munn, and K. J. Joy.** 1988. Computer emulation of thin-section electron microscopy predicts an icosadeltahedral nucleocapsid for human immunodeficiency virus. Lab. Invest. **68**:112–118.
14. **Myers, G., S. Josehs, A. Rabson, T. Smith, and F. Wong-Staal (ed.).** 1988. Database human retroviruses and AIDS. National Laboratory, Los Alamos.
15. **Niedrig, M., J. P. Rabanus, J. L'age Stehr, H. Gelderblom, and G. Pauli.** 1988. Monoclonal antibodies directed against human immunodeficiency virus (HIV) gag proteins with specificity for conserved epitopes in HIV-1, HIV-2 and simian immunodeficiency virus. J. Gen. Virol. **69**:2109–2114.
16. **Özel, M., G. Pauli, and H. Gelderblom.** 1988. The organization of the envelope projections on the surface of HIV. Arch. Virol. **100**:255–266.
17. **Ranki, A., S. H. Weiss, S.-L. Valle, J. Antonen, and K. J. E. Krohn.** 1987. Neutralization antibodies in HIV (HTLV-III) infection: correlation with clinical outcome and antibody response against different viral proteins. Clin. Exp. Immunol. **69**:335–344.
18. **Ratner, L., W. Haseltine, R. Patarca, K. J. Livak, B. Starcich, S. F. Josephs, E. R. Dorane, J. A. Rafalski, E. A. Whitehorn, K. Baumeister, I. Ivanoff, S. R. Petteway, M. L. Pearson, J. A. Lautenberger, T. S. Papas, J. Ghrayeb, N. T. Chang, R. C. Gallo, and F. Wong-Staal.** 1985. Complete nucleotide sequence of the AIDS virus, HTLV III. Nature (London) **313**:277–284.
19. **Sternberg, M. J. E., G. J. Barton, M. J. Zvelebil, J. Cookson, and A. R. M. Coates.** 1987. Prediction of antigenic determinants and secondary structures of the major AIDS virus proteins. FEBS Lett. **218**:231–237.
20. **Wahren, B., F. Chiodi, K. Ljunggren, S. Putney, R. Kurth, R. C. Gallo, and E. M. Fenyö.** 1988. B and T cell reactivities after immunization of macaques with HIV subcomponents. AIDS Res. Hum. Retroviruses **4**:199–210.
21. **Wahren, B., L. Morsfeldt-Mansson, G. Biberfeld, L. Moberg, P. Ljungman, S. Nordlund, U. Bredberg-Raden, A. Werner, A. Löwer, and R. Kurth.** 1986. Impaired specific cellular response to HTLV-III before other immune defects in patients with HTLV-III infection. N. Engl. J. Med. **315**:393–394.