Do Monoclonal Antibodies Recognize Linear Sequential Determinants?

Matilde Camera, Eleonora Muratti, Maria Luisa Trinca, and Alberto Chersi

Regina Elena Institute for Cancer Research, Viale Regina Elena 291, I-00161 Rome, Italy

Z. Naturforsch. **43c**, 967–970 (1988); received March 28/September 9, 1988

Monoclonal Antibodies, Linear Sequential Determinants

A group of 19 anti-class II monoclonal antibodies produced in different laboratories were tested in ELISA for their ability to bind to a panel of synthetic peptides selected from HLA-DQ alpha and beta chains. No one of the antibodies tested was found to react with the synthetic fragments, thus confirming the common finding that MoAbs generally fail to recognize fragments of the native antigen. The possibility that this result might be partly due to the procedure used for screening hybridoma supernatants is discussed.

The antigenic sites of proteins are architecturally of two types: they may comprise residues that are on a continuous segment of the protein chain [1], or are distant in sequence but, due to the folding of the polypeptide chain, come into close spatial proximity (discontinuous sites) [2]. Immune serum antibodies to an intact protein may be directed against both alternative site architectures, and their relative proportions may vary considerably from protein to protein [3, 4]; monoclonal antibodies (MoAbs), on the contrary, appear to react preferentially with native conformations or discontinuous determinants [5].

Since antibodies that bind to a peptide do exist in conventional antisera raised against a protein or a cell [3, 6, 7], some of the MoAbs prepared by the hybridoma technique might occasionally recognize a linear amino acid stretch of the immunogen. Hence, by testing the ability of several MoAbs to bind to synthetic peptides selected from the amino acid sequence of the immunogen, it might be possible to select these that bind to a known epitope of the protein. Such MoAbs would be of considerable help in determining the biological and functional properties of different regions of the antigen.

In this investigation, we tested a group of 19 anti-class II MoAbs from different sources against a panel of synthetic peptides corresponding to known amino acid sequences of HLA-DQ membrane glycoproteins. The antibodies, kindly provided by different laboratories, or prepared in our institute, were named as follows:

1 JJ 623
2 D4-22
3 MCS 7D
4 DI-12
5 2-2 7/10
6 M6 3/4
7 IIA-B5
8 YE 2/36
9 13A-B6
10 PTF 29-12
11 XIV-466
12 XII-358
13 SDR 4-1
14 KUL-01
15 GPB
16 GPA
17 GPC
18 H-21-03
19 H-21-17

The ten synthetic peptides used for the binding assays were selected from published amino acid sequences of DQ membrane glycoproteins [8, 9] and were synthesized by the aid of a Beckman Synthesizer, model 990 B, or by manual synthesis. Some of the fragments were available from previous investigations [10, 11]. Highly polymorphic regions were represented by two peptides with alternative sequences. Peptides T, A(B), P(Q), G, W and V, covered approximately 30% of the DQ beta chain. Only two peptides (E and N), for a total of 23 residues, were selected from the alpha chain; the low representation of this chain was in accordance with the recent finding that the large majority of MoAbs investigated until today have been reactive with the

Table I. Synthetic peptides selected from HLA-DQ histocompatibility antigens, and used for binding assays.

α-Chain	Position	No. of residues
E	44– 58	15
N	77– 84	8
β-Chain		
T	51– 58	9
A/B	63– 79	17
P/Q	82– 93	12
G	96–110	15
W	118–130	13
V	131–143	13

Abbreviations: MoAb, monoclonal antibody; PBS, phosphate-buffered saline; RIA, radioimmunoassay; CLL, chronic lymphocytic leukemia; GP, glycoproteins; MBS, *m*-maleimidobenzoyl-N-hydroxysuccinimide ester.

Reprint requests to Dr. A. Chersi.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/88/1100–0967 $ 01.30/0

beta chains [12], suggesting therefore that most of the potentially immunogenic epitopes of the alpha chain are probably inaccessible due to the three-dimensional folding of the alpha-beta dimer [13].

For the binding assay, microtiter plates were coated with 1 μg of peptide. The MoAbs were diluted 1:50 (supernatants) or 1:1000 (ascites); the assay was performed in duplicates using 50 μl of antibody dilutions. Goat anti-mouse horseradish peroxidase (GAM-HRP) Ig was used as the secondary antibody, and *o*-phenylendiamine as the substrate. The reaction was allowed to proceed for 15 min, then the plates were read at 492 nm in a Titertek Multiskan. As a positive control for antibody reactivity, the samples were simultaneously assayed on membrane glycoproteins extracted by non-ionic detergents from chronic lymphocytic leukemia cells (CLL 54) with known phenotypes (HLA-DR 1,5; HLA-DQw1w3), and purified by affinity chromatography on Lens Culinaris Lectin-Sepharose [14].

The interpretation of the binding data had to take into consideration the fact that some peptides (notably P) were apparently recognized by all MoAbs: this binding was interpreted as a non-specific reaction between peptide P and the GAM-HRP Ig used as the secondary antibody (Morganti, personal communication). For most MoAbs, the profiles of the binding to the ten synthetic fragments were similar, but few of them apparently displayed a low affinity for one of the peptides. Those were MoAb 5 (for Q), MoAb 7 (for E), MoAb 9 (for N), MoAb 10 (for A), MoAb 14 (vor V). Examples are shown in Fig. 1. Those five antibodies were then processed further. 50 μl of ascites or 1000 μl of supernatants, diluted to 2 ml with PBS, were loaded on 0.8 × 4 cm columns packed with immunoadsorbents prepared by linking Sepharose-AH to the synthetic peptides with MBS or glutaraldehyde [7]. Those conjugates had previously been successfully employed for the isolation of anti-HLA-class II antibodies from the serum of a rabbit immunized with B lymphoblastoid cells [7]. The columns were washed first with PBS, then with 0.2 M glycine-HCl buffer, pH 2.6. Most of the protein amount loaded (90–95%) was eluted with PBS; a minor peak (2–4%) with the acidic buffer. Both fractions, adjusted to the same protein concentration, were then tested with an ELISA on soluble membrane glycoproteins from CLL 54 cells. All antibody activities were detected in the first fractions. Additional investigations were carried on using ^{14}C-labeled peptide Q and V (Chersi *et al.*, submitted for publication) testing MoAbs 5 and 14 in immunoprecipitation, as well as by RIA using ^{125}I-labeled MoAbs 7 and 14 and peptides P and V (data not shown). Both experiments indicated that the antibodies tested failed to bind to the fragments. These experiments, therefore, confirmed that although the probability of detecting MoAbs reactive with one peptide might have been relatively high, no one of the 19 reagents tested apparently bound any of the 10 synthetic fragments assayed. This confirmed the common view that MoAbs raised against a native protein are conformation-specific and thus generally do not bind peptide fragments of the molecule [5].

We suppose that the extreme rarity of peptide-reactive anti-class II MoAbs might be explained by the fact that, in most protocols, hybridoma supernatants are screened by binding assays with intact cells or solubilized glycoproteins as targets and not with peptides. In other words, this procedure selects for those clones that secrete antibodies with high affinity for complex conformational determinants as opposed to MoAbs with low affinity for those sites. The latter probably include most, if not all, hybridomas that secrete antibodies directed to linear amino acid sequences. These MoAbs, like antipeptide antibodies [11], probably exhibit low affinity for complex discontinuous determinants. The paucity of peptide-reactive MoAbs described hitherto might, therefore, be a consequence of the screening procedure used. It is predictable that, in analogy to rabbits, sera of mice immunized with cells or intact proteins contain a low but measurable proportion of antibodies to linear sequential sites, and that clones-producing antibodies to those continuous epitopes might be isolated by suitable technique. The main problem in detecting those peptide-reactive antibodies in hybridoma supernatants probably concerns the number of screening tests to be performed: since the statistical probability of finding a peptide-reactive antibody increases with the number of peptides assayed, the synthetic fragments to be tested should ideally cover the whole protein length, and overlap each other by a couple of residues. For practical reasons, the synthesis might be limited to ⅓ or ¼ of the theoretical fragments, using as criteria for the selection of the fragments the hydrophilicity index [15], or the occurrence of multiple allotypic variations in the amino acid sequence, since those fragments are more likely exposed on the surface of the

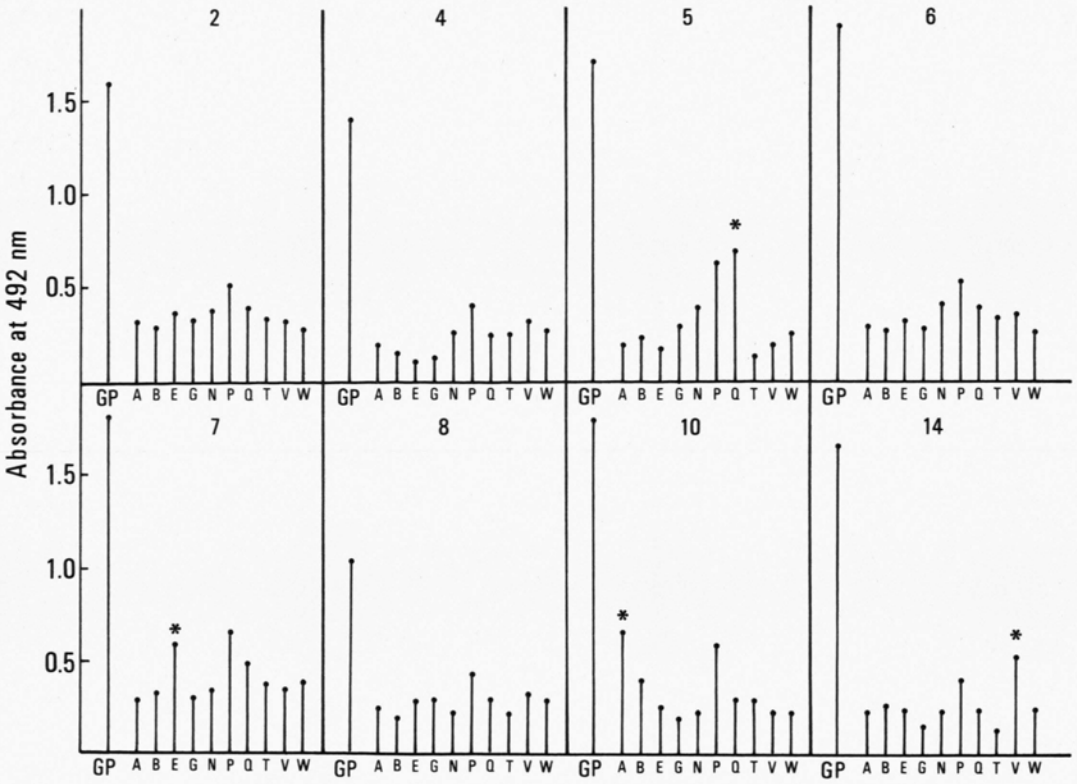

Fig. 1. Binding of 8 of 19 MoAbs to synthetic peptide fragments, as evaluated in ELISA. Wells of microtiter plates were coated with 1 μg of peptide, or 2 μg of soluble membrane glycoproteins (GP) extracted by non-ionic detergents from CLL 54 cells (HLA-DR 1,5; DQw1w3). MoAbs were diluted 1:50 (supernatants) or 1:1000 (ascites) and used in the amount of 50 μl. The test was performed in duplicate. Goat anti-mouse horseradish peroxidase (GAM-HRP) Ig was used as the second antibody, and *o*-phenylendiamine as the substrate. The reaction was stopped after 5 min (glycoproteins) or 15 min (peptides). The plates were then read at 492 nm in a Titertek Multiskan. Peptides apparently recognized by one of the MoAbs are indicated by an asterisk.

native molecules. Even so this would imply, for a protein of MW 30,000, the preparation of 12–15 fragments, each to be tested in duplicate or triplicate on each of the several hundreds hybridoma supernatants. This practical problem may be partly overcome by testing the hybridoma supernatants by ELISA on mixtures of 4–5 peptides, and then by retesting each positive well on each of the 4–5 fragments, in order to individuate the peptide that is effectively recognized by the respective MoAb. This procedure would reduce the time required for screening to an acceptable level.

Acknowledgements

The authors thank Roberto Tosi, Patrizio Giacomini, and all scientist whose MoAbs were used for this investigation.

[1] M. Z. Atassi, Immunochemistry **12,** 423 (1975).
[2] M. Z. Atassi, Immunochemistry **15,** 900 (1978).
[3] M. Z. Atassi (ed.), in: Immunochemistry of Proteins, **Vol. 2,** p. 77, Plenum Press, New York 1977.
[4] K. Denis, R. H. Kenneth, N. Kliman, C. Melinaro, and L. Sherman, in: Monoclonal Antibodies (R. H. Kenneth, T. J. McKearn, and K. B. Bechtol, eds.), p. 49, Plenum Press, New York 1980.
[5] J. A. Berzofsky, G. K. Buckenmeyer, G. Hicks, F. R. N. Gurd, R. J. Feldman, and J. Minna, J. Biol. Chem. **257,** 3189 (1982).
[6] R. G. Ulrich, H. Atassi, P. Lutz, P. Cresswell, and M. Z. Atassi, Eur. J. Immunol. **17,** 497 (1987).
[7] A. Chersi, M. C. Morganti, R. A. Houghten, and F. Chillemi, Scand. J. Immunol. **26,** 723 (1987).
[8] J. M. Boss and J. L. Strominger, Proc. Natl. Acad. Sci. **81,** 5199 (1984).
[9] K. Okada, J. Boss, H. Prentice, T. Spiest, R. Mengler, C. Auffray, J. Lillie, D. Grossberger, and J. L. Strominger, Proc. Natl. Acad. Sci. **82,** 3410 (1985).
[10] A. Chersi, R. A. Houghten, F. Chillemi, R. Zito, and D. Centis, Z. Naturforsch. **41c,** 613 (1986).
[11] A. Chersi, M. C. Morganti, F. Chillemi, R. A. Houghten, and C. Cenciarelli, Clin. Exp. Immunol. (in press).
[12] M. Igarashi, L. Imberti, M. Maio, M. Tsujisaki, F. Perora, K. Sagakuchi, P. G. Natali, and S. Ferrone, in: HLA Class II Antigens (B. G. Solheim, E. Moller, and S. Ferrone, eds.), p. 224, 1986.
[13] B. B. Cohen, D. N. Crichton, and C. M. Steel, Immunology **61,** 255 (1987).
[14] J. P. Allison, S. Ferrone, L. E. Walker, M. A. Pellegrino, J. Silver, and R. A. Reisfeld, Transplantation **26,** 451 (1978).
[15] T. P. Hopp and K. R. Woods, Proc. Natl. Acad. Sci. U.S.A **78,** 3824 (1981).

Verantwortlich für den Inhalt: A. KLEMM
Satz und Druck: Allgäuer Zeitungsverlag GmbH, Kempten