Specificity of *anti*-MHC Class II Antibody Binding to Synthetic Peptides

Alberto Chersi, Teresa F. Romano, and Emilio Ruocco

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

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Synthetic Peptides, *anti*-HLA Antibodies

This study indicates that antibodies raised against a DR4,w6; DQw1,3 positive cell line may bind to synthetic peptides selected from the polymorphic amino acid sequences 51–59 and 63–79 on the DQw2 beta chain. This cross-reaction may be explained by the relatively high sequence homology of these sequences in the beta chains of class II histocompatibility antigens, and suggests that antibody binding to small peptides may be scarsely selective.

Based on the observations of the reactivity of the antibodies with several cell lines, and comparison of the amino acid sequences of beta chains of DR and DQ molecules, an attempt to identify the cross-reacting epitope is presented.

Introduction

A novel approach for identifying epitopes of a given protein is to systematically synthesize all peptide units in its sequence, and measure their reactivities with antibodies against the native molecule [1, 2]. This approach has been recently employed for the isolation of *anti*-Class II antibodies from the serum of a rabbit immunized with human lymphoblastoid B cells, using immunoadsorbents prepared from Sepharose and synthetic peptides selected from HLA-DQ polymorphic regions [3]. Those affinity-purified antibodies, however, resulted scarsely specific for the DQ alloantigens of cells expressing different phenotypes: this could be ascribed to a low specificity of antibody binding to synthetic peptides, with consequent adsorption, onto the Sepharose-peptide immunoadsorbents, of several antibody subpopulations directed to different Class II antigens.

In order to confirm this hypothesis, we immunized two rabbits (113 and 566) with M14 cells (HLA-DR4,w6; DQw1,3) and assayed whether, alike peptides selected from DQw1 or DQw3 glycoprotein sequence, also synthetic fragments from HLA-DQw2, a specificity not expressed by the cells used for immunization, might be able to adsorb *anti*-Class II antibodies. The isolation of such molecules would confirm the limitations of this approach for isolating truly specific reagents.

Reprint requests to Dr. Alberto Chersi.

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Methods

The rabbits were injected a first time with 40×10^6 cells, and then with 20×10^6 cells, at 10-day interval. Details are similar to these reported in a previous study [3]. The first blood sample was collected after the third immunization, then regularly every second injection. The *anti*-Class II activity of the immune sera was controlled by direct binding of highly purified, iodine-labeled Class II glycoproteins (data not shown). Since rabbit 113 was a weak responder, only rabbit 566 was studied in detail.

The immune serum was loaded in 5-ml aliquots on adsorbents prepared by linking Sepharose-AH to synthetic peptides selected from HLA-DQ glycoproteins, as well as from unrelated proteins (Table I). Peptide C (DQw2β, 63–79) and T (DQw2β, 51–59) corresponded to fragments of DQw2 antigens, a specificity not expressed by M14 cells used for immunization, peptide A (DQw3β, 63–79) to an exposed region in HLA-DQw3; peptide G was selected from a monomorphic buried region of DQ beta chains (residues 96–110), peptides 17 and 21 from two unrelated proteins. Details of the coupling procedure of peptides to Sepharose-AH have been reported elsewhere [4].

The columns were washed first with PBS, then with 0.5 M NaCl in PBS, and finally with 0.2 glycine-HCl buffer, pH 2.6, to elute the antibodies. The protein recovery from the immunoadsorbents was 0.08 mg (Seph-17), 0.10 mg (Seph-21), 0.11 mg (Seph-G), 0.21 mg (Seph-C), 0.26 mg (Seph-T) and 0.85 mg (Seph-A).

Results and Discussion

The six samples obtained by affinity chromatography of the immune serum on the Sepharose-peptide adsorbents were first tested with an ELISA on peptides A, B, C and T, as well as on cells with different DR and DQ specificities, using preimmune rabbit *IgG* as negative control.

The binding data (Fig. 1) indicate that samples 566-A, 566-T and 566-C reacted with cell lines expressing Class II antigens, but not with ALL1 cells. The affinities of 566-T and 566-C for peptides T and C, however, were lower than that of sample 566-A

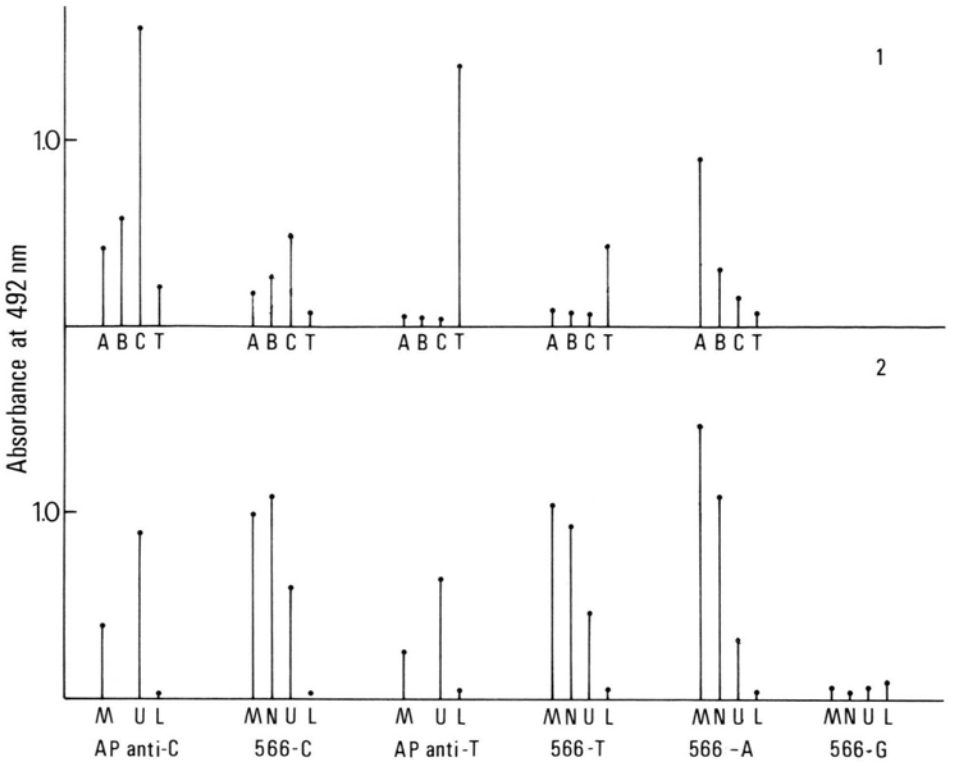

Fig. 1. Binding of antibodies to peptides A, B, C, and T, (1) and to cells with different phenotypes, (2) as evaluated in ELISA. Wells of microtiter plates were coated with 1 μg of peptide, or 2×10^4 cells in 50 μl PBS, and dried carefully at 30 °C. Antibodies were adjusted to the same concentration (10 μg/ml) and used in the amount of 50 μl. Goat *anti*-rabbit horseradish peroxidase was used as second antibody, and *o*-phenylendiamine as substrate. The reaction was stopped after 10 min by addition of 25 μl of 4 N sulfuric acid. The plates were then read at 492 nm in a Titertek Multiskan. Rabbit preimmune *IgG*, at the same dilutions, was used as negative control. The phenotype of the cells used as antigens, as defined by RIA assay [8], was: M14 (M): HLA-DR4,w6; DQw1,w3. U937 (U): HLA-DR3; DQw2,w2. Namalwa (N): HLA-DR2,4; DQw1,w3. ALL1 cells, (L), that do not express Class II antigens, were used as a control. The binding to the same antigens by antipeptide (AP) antibodies *anti*-C and *anti*-T, elicited by KLH-peptide conjugates, is also reported for comparison. The assay was performed in duplicates, and the values reported are the mean of two determinations. Values of preimmune rabbit *IgG* were subtracted.

for A. On the contrary, 566-G, 566-17 and 566-21 were unreactive with every antigen.

The results suggested that 566-T and 566-C were effectively directed against Class II antigens, and apparently bound to peptides T and C of the DQw2 sequence. This result might be explained in terms of relevant homologies among corresponding regions of Class II beta chains.

Comparison of the primary structures of DR and DQ antigens might provide an indication of the specificities of *anti*-Class II antibodies selected by Seph-C and Seph-T affinity adsorbents. This theoretical approach, however, is arduous, since the DR4 and DRw6 subtypes of the immunizing cells were not known: in fact, the amino acid sequences of DR beta chains of the various DR4 subtypes differ in the stretch 51–79, while the DRw6 specificity is associated with different DQw1 beta chains depending on the Dw subtype.

The phenotype of the M14 cells used for immunization was HLA-DR4,w6; DQw1,3: this suggests that the haplotypes were DR4,DQw3 and DRw6,DQw1. If we assume that the immune response of the rabbit might have produced antibodies to all four specificities, comparison of the sequences 51–79 of all DR and DQ chains possibly represented on the cell membrane, with fragments T and C might indicate the most probable cross-reacting epitope. In

Table I. Antigens, immunogens, and source of rabbit antibodies.

Peptides				Antibodies
A	HLA-DQw3	β chain	63–79	–
B	HLA-DQw1	β chain	63–79	–
C	HLA-DQw2	β chain	63–79	AP *anti*-C
T	HLA-DQw2	β chain	51–59	AP *anti*-T
G	HLA-DQw1,2,3	β chain	96–110	–
17	Influenza virus matrix prot.		17–29	–
21	*Mycobacterium* tub.		180–190	–
Cells				
Namalwa	HLA-DR2,4; DQw1,3			–
U937	HLA-DR3; DQw2,2			–
ALL1	HLA-DR –; DQ –			–
M14	HLA-DR4,w6; DQw1,3			566–A 566–C 566–T 566–G

Peptides were synthesized by aid of a Backman synthesizer, Model 990 B (peptides A, B, C, G), by manual synthesis (T), or by aid of a Dupont-Vega coupler, Model 1000 (peptides 17 and 21).

the stretch 63–79, peptide C shares 58–70% homology with DQw1 sequences, 64% with DQw3, and from 70 to 76% with DR4 and DRw6 (Table II). Sepharose-C adsorbent, therefore, might have preferably selected subsets of *anti*-DR4 or *anti*-DRw6 antibodies rather than the others. Thus, the reaction of 566-C with M14 and Namalwa cells, that share the DQw1, DQw3 and DR4 specificities, might be due to the binding of *anti*-DR4 antibody subsets to DR4 glycoproteins of both cell lines. Moreover, as these antibodies bound peptide C in affinity chromatography, they recognize this fragment in DQw2 membrane proteins of U937 cells (DR3,3; DQw2,2). It should be added that antipeptide antibodies (AP) elicited by KLH-C complexes, display high affinity for peptide C and U937 cells, but bind poorly to M14 cells (Fig. 1).

On the other hand, because of the relevant homology between peptide T and DQw3 sequence in the segment 51–59 of the beta chain (77%), Sepharose-T might have selected subsets of *anti*-DQ3 anti-

Table II. Comparison of amino acid sequences of beta chains of HLA-DR and HLA-DQ histocompatibility antigens (pos. 51–79).

	60	70
DQ2	T L L G L P A A E	Y W N S Q K D I L E R K R A A V D R V C
DQw3.1	- P - - P - D - - - - - - - - -	E V - - - T - - E L - T - -
DQw3.2	- P - - P - - - - - - - - - -	E V - - - T - - E L - T - -
DQw1.9	- P Q - R - D - - - - - - - - -	E V - - G A - - S - - - - -
DQw1.18	- P Q - R - D - - - - - - - - -	E V - - G T - - E L - T - -
DQw1.19	- P Q - R - V - - - - - - - - -	E V - - - T - - E L - T - -

DQ2	T L L G L P A A E Y W N S Q K D I L E R K R A A V D R V C
DR4 Dw4	- E - - R - D - - - - - - - - - - L - - Q - - - - - - T Y -
DR4 Dw10	- E - - R - D - - - - - - - - - - D E - - - - - T Y -
DR4 Dw13	- E - - R - D - - - - - - - - - - L - - Q R - - - - - T Y -
DR4 Dw14	- E - - R - D - - - - - - - - - - L - - Q R - - - - - T Y -
DR4 Dw15	- E - - R - S - - - - - - - - - - L - - Q R - - - - - T Y -
DR4 Dw53	- E - - R - D - - - - - - - - - - L - - - R - - E - - T Y -
DRw6	- E - - R - D - - - - - - - - - - D E - - - - - T Y -
DRw6	- E - - R - - - D D - - - - - - - L - - - R - - E - - T Y -

Peptides T (51–59) and C (63–79), selected from a DQw2 sequence, are boxed. Three DQw1 beta chain sequences may be associated with the Dw-subtype of DRw6 of the cells used for immunization, and two DQw3 with the DR4 subtype. Sequences of DR4 and DRw6 exhibit slight differences in primary structures according to the subtype.

bodies, that, in turn, react with cells expressing the DQ3 specificity. The homology between T and DQw1 sequences is only 55%, that with DR4 and DRw6, 66%. These lower homologies might prevent *anti*-DQw1, *anti*-DR4 and *anti*-DRw6 antibodies from cross-reacting with peptide T.

The low level of specificity of antibody binding to small peptides arises the problem of the validity of this approach for the isolation of truly specific reagents, and are in agreement with recent findings obtained by reacting monoclonal antibodies with replacement sets of synthetic fragments: Geysen and collaborators [5–7] have reported significant antibody binding to related fragments when three amino acid residues within the sequence of six had both the correct identity and position. It is predictable, however, that only subsets of antibodies with scarse specificity, as those elicited by sequential determinants, might bind to peptides not corresponding exactly to sequences of the immunogen. Binding of antibodies directed to complex conformational sites, and with strict requirements of both sequence and conformation, might be prohibited.

The data presented indicate that antibodies isolated from immune sera by aid of peptides may comprise several populations reacting with different related molecules. When using cells as immunogens, a careful selection of the peptides used for affinity chromatography, or the utilization of cells with known phenotypes, and possibly homozygous at both DQ and DR loci, might reduce considerably the possibility of cross-reaction. This might result in the isolation of reasonably specific reagents.

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