Antipeptide Antibodies: Do They Distinguish HLA-Alloantigens?

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Three immune sera, raised in rabbits against synthetic peptides corresponding in sequence to predetermined regions of the HLA-DQ histocompatibility antigens, were tested for their ability to recognize phenotypically distinct human lymphoblastoid cell lines.

The immune sera readily recognize the immunogen. When tested on the cells, they react poorly but seem to exhibit a certain degree of specificity.

The possibilities in developing true selective reagents for HLA-alloantigens are considered and discussed.

The extensive use of synthetic peptides as immunogens has revealed their ability to induce antibodies that bind to almost any exposed region of a protein [1, 2].

Those antipeptide antibodies, because of their predetermined sequence specificity, might also offer a good tool for the selective recognition of closely related structures, such as the products of allelic genes. It was recently reported that a synthetic peptide, whose sequence corresponds to the region 61–83 of HLA-B7 heavy chain, is able to induce rabbit antibodies that specifically recognize the HLA-B7 antigen, and do not react with membrane glycoproteins from cell lines bearing different HLA-B specificities [3]. This selective recognition might be a quite rare event: crossreactions seem to occur quite often between antipeptide antibodies and related or even not related antigens: it has been reported that antisera raised in a rabbit against a 14-residue fragment from a variable region of HLA-DR2 glycoproteins (β-chain, pos. 61–73) are not able to discriminate LG-2 cells (HLA-DR1) from GM 3107 cells (HLA-DR2) [4].

Abbreviations: MBS, *m*-maleimidobenzoyl N-hydroxysuccinimide ester; PBS, phosphate-buffered saline.

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In the present investigation, three antisera to three synthetic peptides from the variable region of HLA-DQ1, HLA-DQ2 and HLA-DQ3 glycoproteins, were tested on a panel of cell lines with distinct phenotypes.

The three peptides, sharing from 11 to 12 of the 17 amino acid residues (Table I), were conjugated to a carrier protein by the aid of MBS [5], and injected into NZW rabbits. After a first immunization with 200 μg of complex in complete Freund's adjuvant, rabbits received bimonthly intracutaneous injections of 100 μg of complex in incomplete adjuvant. Blood samples were collected after two months, and the immune sera recovered by centrifugation.

Table I. Amino acid sequence of the peptides selected from HLA-DQ glycoproteins (pos. 63–79) and used as immunogens.

		Rabbit
A) HLA-DQ3	S Q K E V L E R T R A E L D T V C	202
B) HLA-DQ1	S Q K E V L E G A R A S V D R V C	203
C) HLA-DQ2	S Q K D I L E R K R A A V D R V C	207

The antipeptide antibodies were first tested in ELISA in their ability to recognize the immunogen (Fig. 1), then against the two other "parent" peptides. Antiserum 203 (anti-B) reacted extensively also with peptide C, and antiserum 207 (anti-C) with peptide B. In contrast, only slight crossreaction (≈ 10%) was observed between antiserum 202 (anti-A) and peptides B and C, and peptide A was very scarsely recognized by antisera 203 and 207.

The antipeptide antibodies were then assayed against different lymphoblastoid cell lines with known phenotypes. For this test, in order to minimize nonspecific binding of IgG to cells and heavy background, the $F(ab')_2$ fragments of each affinity-purified antibody were used instead of the immune sera.

The test was carried out in duplicates as previously described [6]. The cells (5×10^4 in 50 μl PBS) were placed in flatbottom 96-wells plates, and dried at 37 °C for 10 hours under gentle air stream. The $F(ab')_2$ fragments were pretreated with protein A-Sepharose, and used at a concentration of 0.03 mg/ml. The $F(ab')_2$ fragment of preimmune rabbit IgG was simultaneously tested as a control.

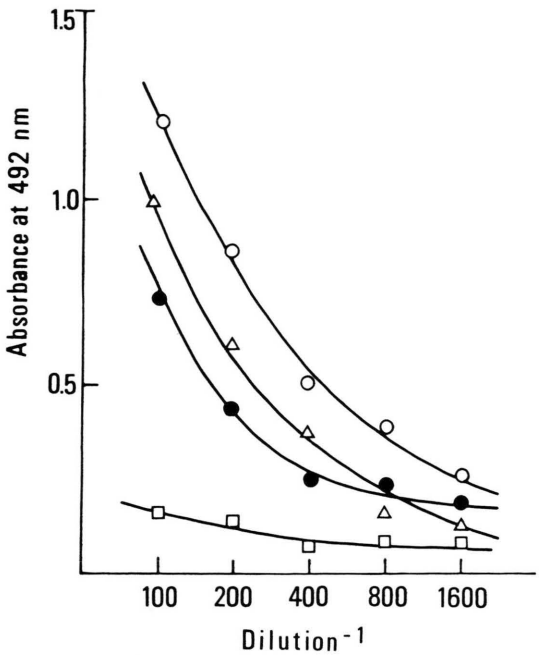

Fig. 1. Binding of immune sera 202 (○), 203 (●) and 207 (△) to the corresponding antigen peptides, evaluated by ELISA. Into wells of microtiter plates were placed 1 μg of peptide. The immune sera, diluted as indicated, were used in a volume of 25 μl. Preimmune rabbit serum (□) was diluted and tested simultaneously.

Table II reports the degree of binding of the fragments to the panel of cells with different phenotypes, as evaluated in ELISA: only very few cell lines are recognized by a single antibody. All antibodies react, to different extent, with several cell lines. The reactivity pattern of antibodies 203 and 207 are surprisingly similar, but this could be predicted from the binding assays of the two immune sera on peptides B and C.

More difficult to explain is the binding of $F(ab')_2$ fragment of serum 202 to cell lines such as MDV(DQw1) or BRU(DQw1), or that of the $F(ab')_2$ of sera 203 and 207 to cell lines such as JUM(DQw3). This might be an indication that the antipeptide antibodies recognize on the cell surface other binding sites that do not correspond to sequences of peptides A, B, or C, either on DQ molecules or in other membrane proteins. Finally, some cell lines are not recognized by any of the three antibodies; the reason is unclear.

In any case, the binding of the antipeptide antibodies to the cells is poor, and seldom exceeds 50% of the unspecific binding shown by preimmune sera, when evaluated by ELISA. The use of the $F(ab')_2$ fragments lowers substantially the background activity, but this technique requires additional antibody purification, enzymatic digestion, and isolation of the fragments.

The specificities could be improved by absorption and immunodepletion of the antipeptide antibodies by Sepharose-bound peptides mimicking the same region of other HLA-DQ alloantigens. Another possibility is the selection, as immunogens, of shorter,

Table II. Relative reactivity of antipeptide antibody $F(ab')_2$ fragments with phenotypically distinct cell lines.

Cell line	Phenotype		% Binding 202 (anti-DQw3)	203 (anti-DQw1)	207 (anti-DQw2)
D147	HLA-DR1,7	DQw1,2	0	70	100
PGF	HLA-DR2	DQw1	0	0	0
WT49	HLA-DR3	DQw2	0	0	0
BM14	HLA-DR4	DQw3	100	N.T.	N.T.
JUM	HLA-DR5	DQw3	38	23	33
MDV	HLA-DRw6	DQw1	44	100	59
BRU	HLA-DR6,w9	DQw1	10	33	43
MAN	HLA-DR7	DQw2	5	0	63
LUY	HLA-DRw8	–	0	0	0
BM9	HLA-DRw8	–	43	0	0

The test was carried on using 50 μl of $F(ab')_2$ preparations at a concentration of 0.03 mg/ml. The last step of the ELISA assay (peroxidase-substrate reaction) was allowed to proceed for 5 minutes only, in order to have absorbance at 492 nm not higher than 1.9.
The absorbance given by preimmune rabbit IgG $F(ab')_2$ fragment on each cell line was subtracted. For each antibody, the cell line presenting the highest binding was given the value 100.

more representative peptides having unique amino acid sequences, and low homology with related antigens. Finally, the production of monoclonal antibodies, directed against a single suitable epitope, might allow the selective recognition of HLA alloantigens.

All those techniques are presently under investigation and development in our laboratory.

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