Brief Definitive Report

A RECURRENT IDIOTYPE ON MONOCLONAL ANTI-HUMAN Ia ANTIBODIES*

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Anti-idiotypic antibodies have been used in various systems to study both the spectrum of the immune response (1) and the nature of cellular receptors to antigens (2). Because of the evidence that idiotypic determinants can be associated with the antigen-combining site (3), idiotypy of antibody may be used as a mirror image for defining antigenic determinants of molecules otherwise difficult to elucidate. Moreover, the demonstration of idiotypic markers on lymphocyte receptors (2) suggests that anti-idiotypic (anti-receptor) antibodies can be used for functional studies of immune recognition.

Ia molecules, HLA-DR in man, are an extensively polymorphic family of cell-surface antigens of key importance in regulating antigen responsiveness and immune cell cooperation (4). From studies in the mouse system (5), it appears that single Ia antigenic determinants can affect recognition events and immune performance. In man, the definition of single Ia determinants cannot be achieved through genetic recombination or isolation of mutant strains, as in the mouse, thus posing a major problem for the functional study of Ia antigens.

We describe here an initial investigation of the idiotypes of murine monoclonal antibodies (mAb) to human Ia antigens, in an effort to define Ia determinants and their cellular interaction pathways.

Materials and Methods

Monoclonal Antibodies. Monoclonal antibodies to human Ia (HLA-DR) antigens are listed in Table I. Monoclonal antibodies to HLA-A,B antigens Q1/28 (6), Q6/64 (7), and W6/32 (8) have been previously described. W6/32 was a kind gift of Dr. Peter Parham. The mouse myeloma protein MOPC21 was obtained from ascites of BALB/c mice carrying intraperitoneally the mouse myeloma line P3X63Ag8.

Cells. Ia-positive human B lymphoblastoid cell lines LG-2 (HLA-DR1), Daudi (HLA-DRW6), and WI-L2 (HLA-DR4,7) were cultured in RPMI 1640 media supplemented with 10% fetal calf serum, 2 mM glutamine, and 50 μg/ml gentamicin.

Anti-Idiotypic Sera. A New Zealand white rabbit was immunized with purified mAb Q5/13 (IgG2a,k) (9), as described (10). The immune serum was sequentially absorbed over a pooled normal mouse gamma globulin immunoadsorbent and an mAb Q1/28 (IgG2a,k) immunoadsorbent. The absorption procedure was repeated three times, at a ratio of 5 ml of packed immunoadsorbent beads per 10 ml of serum.

Binding Assay. Polyvinyl microtiter wells (Dynatech Corp., Alexandria, VA) coated with either purified mAb or immunoglobulins were incubated at 4°C overnight with 50 μl of serum

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TABLE I

Monoclonal antibody	Ia specificity	Donor mouse strain	Source	Reference	Competitive inhibition of binding of HP-Q5/13 to anti-idiotype 3496	Inhibition of binding to Ia$^+$ cells by anti-idiotype 3496*
					%	%
Q2/47	Monomorphic	NZB/W	V. Quaranta	9	—	—‡
Q2/70	Monomorphic	NZB/W	V. Quaranta	9	—	—
Q2/80	Monomorphic	NZB/W	V. Quaranta	9	95	—
Q5/13	Monomorphic	BALB/c	V. Quaranta	9	95	98
Q6/22	Monomorphic	BALB/c	V. Quaranta	9	—	—
417.1	Monomorphic	BALB/c	G. A. Molinaro	—	95	98
S1.5/1	Monomorphic	DBA2/J	M. Trucco	20	—	—
S1.19/9	Monomorphic	DBA2/J	M. Trucco	20	—	—
T2143	Monomorphic	BALB/c	R. F. Fox	21	—	—
T2171	Monomorphic	BALB/c	R. F. Fox	21	—	—
T2172	Monomorphic	BALB/c	R. F. Fox	21	—	—
SCX7	Monomorphic	BALB/c	R. F. Fox	21	—	—
L203	Monomorphic	BALB/c	R. Levy	22	—	—
L243	Monomorphic	BALB/c	R. Levy	22	—	—
OKIa2	Monomorphic	—	Ortho	—	—	—
CA2.206	Monomorphic	BALB/c	H. O. McDevitt	23	62§	54
DR.2	Monomorphic	BALB/c	BRL	24	—	—
NE1-011	Monomorphic	B6 × BALB/c	NEN	25	—	—
DA.2	Monomorphic	BALB/c	F. M. Brodsky	8	—	—
Genox 3.53	DR1,2,W6	C3H	F. M. Brodsky	8	—	ND‖
MRC.OX3	DR1,2,W6	—	F. M. Brodsky	26	—	ND
MCLB.8	Neg to DR3,5,7	—	F. M. Brodsky	27	—	—
Q5/6	Neg to DR7	BALB/c	V. Quaranta	9	—	—
E3.15/4	DR3,5,W6	BALB/c	M. Trucco	20	—	—

* These tests were performed as described in Materials and Methods with three human B lymphoblastoid cell lines, LG-2, Daudi, and WI-L2.
‡ No detectable inhibition.
§ Inhibition caused by 100 ng of purified antibody.
‖ Not done.

3496, appropriately diluted. After washing, the wells were incubated at 4°C for 3 h with ^{125}I-labeled sheep IgG anti-rabbit Fc, then washed extensively, and radioactivity bound was determined with a gamma counter. The binding of preimmune serum to Q5/13 was used as a background control.

Inhibition of Binding to Target Cells. A previously determined limiting dilution of mAb (25 μl) was incubated overnight with either serial dilutions of antiserum 3496 (25 μl) or normal serum (25 μl) and then added to microtiter wells coated with human cells WI-L2 (5×10^4 cells/well) by drying overnight at 37°C. After 1 h at 4°C, the plate was washed and incubated with 50 μl of an optimal dilution of horse-radish peroxidase (HP)-conjugated rabbit anti-mouse Ig (Tago, Inc., Burlingame, CA), for 1 h at 4°C. Bound peroxidase activity was revealed by addition of *o*-phenylenediamine and H_2O_2. The reaction was stopped after 30 min in the dark by 4 M H_2SO_4, and optical absorbance was determined at 492 nm with a Multiskan ELISA reader (Flow Laboratories, Inc., Inglewood, CA). Incubation of Q5/13 with preimmune serum 3496 was used as a further control. Results are expressed as percent inhibition: 100 − ([average A_{492} of duplicate wells in which 3496 plus mAb were tested] × 100/average A_{492} of duplicate wells in which normal rabbit serum plus mAb were tested). In the latter wells, A_{492} ranged between 0.200 and 0.800, with a background of 0.020. Phosphate-buffered saline, pH 7.4, containing 0.5% Tween 20 and 2% bovine serum albumin was used throughout the assays.

Competitive Inhibition Assays. mAb Q5/13 conjugated to HP (HP-Q5/13) was prepared according to Harper and Orengo (11).

Inhibition of Binding of Q5/13 to Anti-Idiotypic Serum 3496 by Human Ia Molecules. A limiting dilution of HP-Q5/13 was mixed in a final volume of 50 μl with the indicated amounts of either immunologically active human Ia molecules purified by high performance liquid chromatography (L. E. Walker, unpublished data) or purified β_2-microglobulin (Immunochemical Engineering, Inc., Minneapolis, MN). After incubating overnight at 4°C, the mixture was allowed to react at 4°C for 3 h in wells coated with anti-idiotypic serum 3496 diluted 1/2,000.

After extensive washing, the bound HP activity was measured as described above. Tests were done in triplicate. Results are expressed as percent inhibition of the binding of HP-Q5/13 in the presence of Ia molecules, as compared with the maximum binding observed in wells to which no inhibitor was added. In the wells lacking inhibitor, A_{492} averaged 0.400, i.e., >10 times the reading of blank background wells.

Inhibition of Binding of Q5/13 to Anti-Idiotypic Serum 3496 by Other mAb. This assay was performed as described above, with the following modifications: 25 μl of a limiting dilution of HP-Q5/13 was mixed with an equal amount of mAb (either ascites fluid or 10 × concentrated hybridoma supernatant) rather than with purified Ia. The first overnight incubation was omitted. Most of the antibodies obtained from other laboratories were understandably received in limited quantities and in a nonpurified form, either as ascitic fluid or hybridoma supernatant; consequently, it was not possible to test them quantitatively. However, because stoichiometry of idiotype-anti-idiotype binding indicated that 1 ng of Q5/13 idiotype could induce 50% inhibition of binding (data not shown), we estimate that each mAb was tested at a concentration at least 100-fold greater than that of Q5/13 idiotype.

Results and Discussion

Rabbit antiserum 3496 to the murine mAb Q5/13, specific for HLA-DR monomorphic determinants, was effectively rendered idiotype specific by appropriate absorptions as it markedly bound to Q5/13, but failed to react with either pooled normal mouse gamma globulin or antibodies sharing isotype, allotype, or MOPC21 determinants with Q5/13.

Idiotypic determinants recognized by this antiserum were shown to be associated with the antigen-combining site of Q5/13 by the results of two independent sets of experiments: (*a*) anti-idiotypic antibodies were able to completely block the binding of Q5/13 to Ia-positive target cells (Fig. 1 a); (*b*) conversely, purified human Ia molecules inhibited the binding of enzyme-linked Q5/13 to microtiter wells coated with anti-idiotype 3496 (Fig. 1 b). In both instances, the inhibitions were specific because antiserum 3496 did not affect the binding of HLA-A,B monoclonal antibodies to target cells, and the binding of Q5/13 to anti-idiotype 3496 was not diminished by either a control protein (Fig. 1 b) or cell lysates from Ia-negative human lymphoid cells (data not shown).

We examined the presence of Q5/13 idiotype on other anti-Ia mAb to ascertain the degree of idiotypic relatedness among antibody molecules obtained from different immunizations and fusion experiments. As indicated in Table I, three mAb (417.1, CA2.206, and Q2/80), when tested for competitive inhibition of binding of Q5/13 idiotype to anti-idiotype 3496, were found to be inhibitory and, therefore, shared idiotype with Q5/13. These mAb were all directed against monomorphic Ia determinants. In contrast, none of five mAb directed to Ia polymorphic specificities inhibited the binding reaction (Table I). Inhibition by mAb CA2.206 was not complete, even with 100-fold excess amounts, indicating only partial idiotype cross-reactivity with Q5/13. Interestingly, not only were the four mAb bearing similar idiotypes obtained in distinct fusion experiments, but in one instance, Q2/80, the donor mouse was of NZB/W strain, as opposed to the BALB/c strain donors for the other three antibodies.

To confirm and further define the idiotype cross-reactivities observed, we tested the ability of antiserum 3496 to inhibit the binding of mAb to target cells (Table I). Thus, the binding of both mAb 417.1 and CA2.206 was inhibited, the latter to a similar extent as in the competitive binding assay, demonstrating sharing of idiotype at the level of the antigen-combining site. In contrast, the binding of Q2/80 to target cells was not affected by antiserum 3496, indicating that this assay system is highly

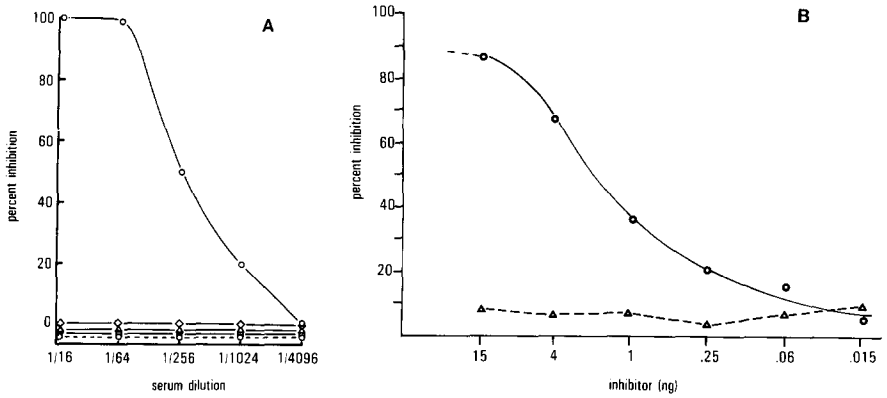

FIG. 1. (a) Inhibition of binding to Ia-positive human B lymphoblastoid cell lines by anti-idiotype serum 3496. Tested mAb are anti-Ia Q5/13 (O——O), anti-HLA-A,B Q1/28 (□——□), W6/32 (◇——◇), and Q6/64 (△——△). Background activity by preimmune serum on Q5/13 is also indicated (O-----O). (b) Competitive inhibition of the binding of Q5/13 to anti-idiotype serum 3496 by purified human Ia molecules (O——O) and control β_2-microglobulin (△-----△).

discriminating and that Q2/80 bears a Q5/13 idiotype, however, not in association with its antigen-combining site.

These data are consistent with the possibility that the mouse antibody response to human Ia antigens is based upon a discrete number of recurrent idiotypes. This interpretation needs to be confirmed by examining the idiotypes of those anti-Ia mAb that do not bear the Q5/13 idiotype. It is possible that the techniques that are used to generate hybridomas may introduce a bias towards certain idiotypes; however, this does not seem to be the case here because preliminary data indicate that the Q5/13 idiotype is found as a significant component in the serum of both BALB/c and NZB/W mice immunized against human Ia-positive cells.

Results similar to ours have been reported in the homologous murine Ia system. Thus, the idiotype of a mAb to Ia.7, a monomorphic specificity of murine I-E molecules, was readily detectable in anti-Ia.7 alloantisera (12). Similarly, idiotypic cross-reactivity among mAb directed to monomorphic but not polymorphic specificities of Ia^k has been recently reported (13). Taken together, these observations suggest that Ia monomorphic determinants stimulate antibody-forming cells displaying related idiotypes.

It is tempting to propose that mAb Q5/13, 417.1, and CA2.206 define a monomorphic Ia specificity because their shared idiotype is associated with the antigen-combining site. However, one has to critically evaluate this conclusion because a shared idiotype at the level of the antigen-combining site may (3, 14, 15) or may not (16, 17) relate directly to a particular epitope specificity. Conversely, idiotype sharing between Q5/13 and Q2/80, restricted to determinants outside the antigen-combining site, does not definitively rule out the possibility that Q5/13 and Q2/80 may react with a similar Ia antigenic site. Verification of such an association in the human Ia system is especially hampered by the lack of definition of private and public Ia specificities. Nevertheless, by using Western blotting techniques, we demonstrated additional similarities among the reactivity patterns of Q5/13, 417.1, and CA2.206, i.e., they all react with an epitope that is located on the Ia β subunit and is resistant to subunit dissociation (V. Quaranta, unpublished work). Because of their differential reactivity with antiserum 3496 (See Table I), Q5/13 and CA2.206 may be directed to

overlapping but not identical antigenic sites.

The frequency of the Q5/13 idiotype among anti-Ia mAb from separate immunizations may either be the effect of human Ia molecule domains immunodominant to the mouse antibody response or it may represent an integral part of a regulatory idiotype pathway (18) in the mouse immune system. Determining whether or not mAb Q5/13, 417.1, CA2.206, and Q2/80 react with the same or similar epitopes will help in distinguishing between these two alternatives. It is possible that both of them might turn out to be correct.

A potential application of anti-idiotypic serum 3496 is as a probe of recognition units for Ia antigens on T cells. Thus, the role of Ia monomorphic determinants in immune cell interplays could be elucidated. In this regard, it is relevant that Q5/13 has an inhibitory effect both on allogeneic and autologous mixed lymphocyte reactions (19). Consequently, alloreactive and autoreactive T cells may recognize the Q5/13 monomorphic determinant and possibly bear idiotypic structures similar to the Q5/13 idiotype.

Summary

We report a recurrent idiotype on a remarkably high fraction (4/19) of murine monoclonal antibodies specific for human Ia monomorphic determinants and elicited by separate immunizations. For three of them, the shared idiotype is associated with the antigen-combining site. These results indicate that the spectrum of mouse antibody responses to human Ia antigens may be based on recurrent idiotypes, suggesting a limited potential repertoire of murine monoclonal antibodies to human Ia antigens. Anti-idiotypic reagents might be helpful in dissecting this repertoire and to generate a mirror image of a human Ia antigenic map. Furthermore, antisera to the idiotype of antibodies specific for human Ia monomorphic determinants might help in elucidating the interactions between Ia molecules and receptors on immune cells.

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