The Journal of Immunology

RESEARCH ARTICLE | OCTOBER 01 1967

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J Immunol (1967) 99 (4): 675–678.
https://doi.org/10.4049/jimmunol.99.4.675

THE JOURNAL OF IMMUNOLOGY
Vol. 99, No. 4, 1967

Printed in U.S.A.

IMMUNOGLOBULIN ISOANTIGENS (ALLOTYPES) IN THE MOUSE

IV. Allotypic Specificities Common to Two Distinct Immunoglobulin Classes[1]

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Received for publication April 24, 1967

Immunoglobulin molecules have been shown to carry many types of antigenic determinants. These determinants have been in part responsible for the classification of the immunoglobulins and have also been of great use in studies on the structure, synthesis and genetic control of this group of proteins (1–3).

Antigenic specificities that have been found include those which are located in either all immunoglobulin classes of the species (e.g. L-chain specificities); those present in only one class of the species (e.g. H-chain class defining specificities); allotypic specificities which reflect genetic differences in either the L- or the H-chains among different individuals of a species (4, 5); and antigenic specificities which are probably associated with the variable regions of the molecule (e.g. idiotypic (6) antigens and myeloma specific antigens (7)). The subgroups or subclasses of human (8, 9) and mouse (10) immunoglobulins may be considered as separate immunoglobulin classes, but having considerable homology one to another. Because of this homology in structure, these classes (e.g. mouse γG_{2a} and γG_{2b}) have H-chain antigenic specificities in common as well as unique ones which define these two classes.

In both human and mouse immunoglobulin systems, heterologous antisera have been shown to be capable of detecting all five of these types of antigenic specificities. Alloantisera (isoantisera), however, have been found to detect allotypic antigens that are present on only one H-chain class in the human and mouse. Rabbit alloantisera detect allotypic antigens on the H-chain (Fd fragment) of all three immunoglobulin classes, i.e. the Aa locus (11–13).

This article reports the detection of several allotypic specificities common to two of the five (10, 14) mouse immunoglobulin classes.

MATERIALS AND METHODS

Several mouse alloantisera which detect allotypic specificities were used to determine the immunoglobulin class(es) carrying these allotypic specificities. Antisera were prepared in several strains of mice against the immunoglobulins of another inbred strain. The immunoglobulin used for immunization was either an anti-H-2 antibody (15) or a complex of *Hemophilis pertussis* with mouse anti-*H. pertussis* (16). The presence of anti-allotype antibodies in the resulting antiserum was detected by the ability of the serum to precipitate an I^{125}-labeled preparation of a purified (95% purity) mouse myeloma protein. The method of inhibition of precipitation with either mouse myeloma proteins or various inbred mouse sera was used to determine whether several antibody populations were present. A full description of all the methods used has previously been reported (15, 17). The antisera used were C57BL anti-CBA, LP/J anti-129/J, and NZB anti-NZC. These antisera were found to precipitate labeled myelomas of only two of the five mouse immunoglobulin classes, namely, γG_{2a} and γG_{2b}. RPC-5 was used as the labeled antigen representing the γG_{2a} class, and MPC-31, the γG_{2b} class. In each inhibition assay, two γG_{2a} myelomas (RPC-5 and 5563) and two γG_{2b} myelomas (MPC-11 and MPC-37) were used as inhibitors. All of these myelomas are derived from plasma cell tumors in BALB/c mice, except 5563 which is a C3H tumor. These myelomas are all of immunoglobulin allelic type "a." The methods used for isolation of the myeloma proteins have also been previously described (17, 18). Each of the three antisera were tested against the two

[1] This investigation was supported by National Institutes of Health, Public Health Service Grants CA-04681 and GM-12075, and Helen Hay Whitney Foundation F109.

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labeled myelomas, and all four inhibitor proteins were used with each inhibition assay. The results are presented as the percentage of inhibition for each myeloma protein inhibitor plotted against the concentration of protein used. The basic premise used in this work is that inhibition of precipitation occurs only with a protein which has at least one specificity in common with those specificities carried by the labeled protein and recognized by the antisera, and that complete inhibition is obtained only with a protein that carries all the antigenic specificities detected in the assay.

RESULTS AND DISCUSSION

We have previously described three separate loci coding for mouse immunoglobulin H-chains, Ig-1 (15), Ig-2 (19) and Ig-3 (18), determining respectively the H-chains of γG_{2a}, γA and γG_{2b}. By an analysis of cross-reactions with normal mouse sera, the Ig-1 locus was shown to be composed of at least ten antigenic determinants, and the Ig-3 locus of at least three determinants. Different arrangements of these were found in the different allelic types, eight alleles having been recognized for Ig-1 and four alleles for Ig-3. Since the submission of our last article on this work (18), we have identified a total of 11 Ig-1 specificities, and 8 Ig-3 specificities (20). Some of these specificities had previously been demonstrated to be carried on only one class of immunoglobulin (i.e. Ig-1.1 on γG_{2a} (15)). This, however, has not been found to be true for all specificities. Since γG_{2a} and γG_{2b} have not been separated from normal mouse serum (owing to very similar physical and chemical characteristics (10)), analysis of the class location of the specificities has to be restricted to those specificities present in an allelic type for which a myeloma protein exists. With the exception of some recent tumors in (NZB × BALB/c) F_1 mice (18, 21), this limits analysis to those specificities present in the BALB/c strain, namely Ig-1.1, 2, 6, 7, 8, 9, 10 and Ig-3.1, 2, 4, 5, 6, 7, 8.

Specificities Ig-1.1, 2, 6, 7, 8 are found only on γG_{2a} immunoglobulins. Specificities Ig-3.1, 2, 7, 8 are found only on γG_{2b} immunoglobulins. The results obtained with assays which define the other specificities are presented in the figure. It should first be stated that no γM, γA or γG_1 myelomas have been found to inhibit any of the assays described in Figure 1, *A* to *F*. These classes therefore, do not carry any of the specificities detected in these assays. In Figure 1*A*, C57BL anti-CBA is reacted with I^{125}-labeled MPC-31 (a γG_{2b} protein). Both γG_{2a} and γG_{2b} myelomas completely inhibit this precipitation at similar protein concentrations. Therefore at least one allotypic specificity is common to these two distinct immunoglobulin classes and is detected by this antiserum. However, as described in the next paragraph, two specificities are actually detected in this assay and are found on both γG_{2a} and γG_{2b}.

When sera from the eight allelic type strains were tested in this assay, all were found to completely inhibit the precipitation of a labeled γG_{2b} myeloma, MPC-31, except DBA/2 which gave only partial inhibition and C57BL which failed

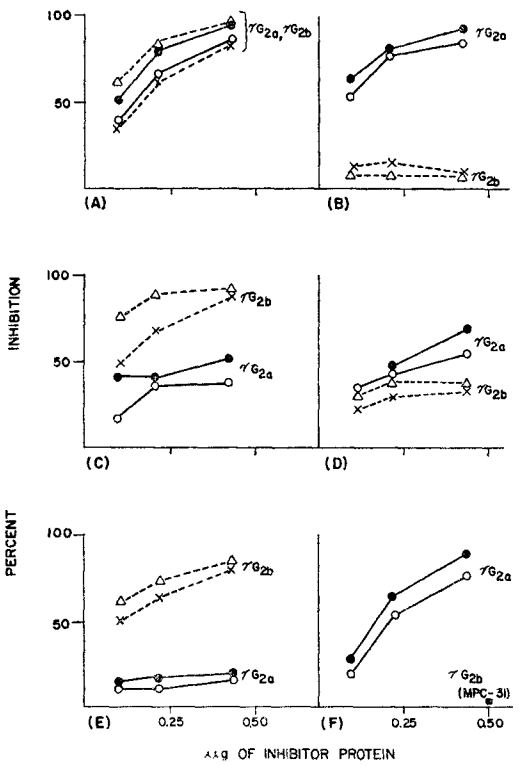

Figure 1. Inhibition of precipitation of I^{125}-labeled mouse myeloma proteins with purified γG_{2a} and γG_{2b} myelomas. The two inhibitor γG_{2a} proteins are RPC-5 (●——●) and 5563 (○——○). The two inhibitor γG_{2b} proteins are MPC-11 (X- - - -X) and MPC-37 (△- - - -△). The labeled antigen in *A*, *C* and *E* is MPC-31, and in *B*, *D* and *F* is RPC-5. The antiserum in *A* and *B* is C57BL anti-CBA, in *C* and *D* is NZB anti-NZC, and in *E* and *F* LP/J anti-129/J.

TABLE I

Allotypic specificities of mouse γG_{2a} and γG_{2b} immunoglobulins

Type Strain	Allele	Ig-1 Locus	Ig-3 Locus	γG_2 Common Allotypic Specificities		
BALB/c	a	1 2 – – – 6 7 8 10 –	1 2 – 4 7 8	1 (1.9)[a]	2 (3.5)[a]	3 (3.6)[a]
C57BL/10	b	– – – 4 – – 7 – – –	– – – 4 7 8	1	–	–
DBA/2	c	– 2 3 – – – 7 – – –	1 2 – 4 7 8	1	2	–
AKR	d	1 2 – – 5 – 7 – – –	1 – 3 – 7 or 8	–	2	3
A/J	e	1 2 – – 5 6 7 8 – –	1 – 3 – 7 –	–	2	3
CE	f	1 2 – – – – – 8 – 11	1 2 – 4 – –	1	2	3
RIII	g	– 2 3 – – – – – – –	1 2 – 4 ? –	1	2	3
SEA	h	1 2 – – – 6 7 – 10 –	1 2 – 4 7 8	1	2	3

[a] Previous designated name of specificity.

to inhibit at all. Thus, this assay detects specificities Ig-3.5 and 6, Ig-3.6 being defined as the specificity absent from DBA/2 immunoglobulins. Since the γG_{2a} myelomas completely inhibit this precipitation, both Ig-3.5 and 6 are common to the γG_2 classes. No allotypic specificities unique to γG_{2b} were found in this assay. However, when this same antiserum is used with a labeled γG_{2a} myeloma (Figure 1*B*), γG_{2b} myelomas give only marginal inhibition, even though this antiserum contains antibodies directed to common allotypic antigens. This apparent nonreciprocality between the two assays is due to the preponderance of several antibodies directed to unique γG_{2a} antigenic specificities which mask the smaller amount of antibody to the common antigens.

Another antiserum used in Figure 1, *C* and *D* (NZB anti-NZC), defines specificities Ig-1.9 and 10. In each assay, at least two specificities are involved, as γG_{2a} and γG_{2b} respectively give only partial inhibition. The specificity common to γG_{2a} and γG_{2b} detected in Figure 1*D* is termed Ig-1.9, since C57BL/10 carries 1.9 and not 1.10 (18). Only a limited quantity of this antiserum was available and detailed testing of the reaction in Figure 1*C* with normal sera as inhibitors could not be done. It is, therefore, possible that the specificity designated Ig-3.4 is the same as Ig-1.9, and that this is the common specificity detected in Figure 1, *C* and *D*. This would imply that a unique γG_{2b} specificity that has not been designated is also detected in this assay. Figure 1, *E* and *F* show the typical pattern of most other alloantisera which detect only allotypic specificities unique to one immunoglobulin class. The slight inhibition given by the nonreciprocal antigen, i.e. γG_{2a} in Figure 1*E*, is that expected from a 5% contamination with normal immunoglobulins.

We conclude, therefore, that the specificities previously designated Ig-1.9, Ig-3.5 and Ig-3.6 are present on immunoglobulins of two distinct classes, γG_{2a} and γG_{2b}. The designation of a single locus number to these specificities is clearly unsuitable, and we therefore list these specificities as γG_2 common allotypic specificities 1, 2 and 3 (Table I). The previously named γG_{2a} and γG_{2b} unique specificities will remain unaltered and 1.9, 3.5 and 3.6 will no longer be used. Specificities which are carried only in strains other than BALB/c (Ig-1.3, 4, 5, 11) may or may not be present on both immunoglobulin classes, and this question will have to remain open until a method of separating γG_{2a} and γG_{2b} in strains with these specificities is available.

We have previously reported (18) that specificity Ig-1.5 is present on two different myeloma proteins arising in (NZB × BALB/c) F_1 hybrid mice, one of which typed unequivocally as a γG_{2a} myeloma, and the other carrying both γG_{2a} and γG_{2b} class specific antigens. Since this second myeloma protein was very abnormal, in also lacking several of the Ig-1 and Ig-3 allotypic specificities, we do not feel that Ig-1.5 is necessarily a common γG_2 allotypic specificity. This will also have to remain unanswered until a typical γG_{2b} myeloma of allelic type "e" can be found.

The basic finding presented in this article is the detection of allotypic antigens common to two distinct mouse immunoglobulin classes. Since these allotypic specificities were not present on γA, γM or γG_1 myelomas, it is unlikely that they are located on the L-chains of the γG_2 molecules, but rather on the H-chains. All previously tested mouse allotypic specificities (those unique to only one γG_2 class) were found to be on the Fc fragment (22 and unpublished observations) and ex-

amination of these new common specificities will have to be carried out. The submolecular localization of these common allotypic antigens is of importance since the only other H-chain allotypic specificities described to be present in more than one Ig class are those of the Aa locus in rabbits (12, 13) and these are present in the Fd portion of the H-chain.

The evolutionary origin of these common specificities is obscure. However, in view of the close linkage of the Ig-1 and Ig-3, and the many physicochemical and antigenic similarities of the polypeptide chains determined by these loci, it is very probable that a gene duplication was responsible for the development of these two loci. Whether the duplication was followed by or preceded by mutational changes giving rise to the common allotypic specificities or indeed whether an entirely different mechanism was responsible will have to remain unanswered at present.

SUMMARY

Three heavy chain allotypic specificities have been found on two of the mouse immunoglobulin classes, γG_{2a} and γG_{2b}. The other described immunoglobulin allotypic specificities in the mouse are restricted to one or another H-chain class. The possible evolutionary origins of shared or common specificities are discussed.

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