Brief Definitive Report

DETECTION OF NON-H-2 ANTIGEN(S) WHICH, LIKE Ia ANTIGENS, ARE ASSOCIATED WITH THE Fc RECEPTOR OF B LYMPHOCYTES

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The B lymphocytes of several species, including mouse and man, have been shown to bear a receptor (termed the Fc receptor) which specifically binds heat-aggregated or antigen-complexed immunoglobulin (complexed Ig) (1, 2). The system of murine lymphocyte surface alloantigens determined by the *I* region of the *H-2* complex, and termed Ia (*Ir* associated) antigens (3), are also primarily expressed on B lymphocytes (4). Recently, in this laboratory, it has been shown that Fc receptors and Ia antigens are either identical or closely associated on the murine B-lymphocyte surface (5). Pretreatment of lymphocytes with anti-Ia antisera specifically inhibited the binding of complexed Ig while antibodies against alloantigens determined by the *K* and *D* regions of the *H-2* complex did not produce such inhibition. Inhibition studies on cells from F_1 mice further indicated that individual Ia molecules might be grouped in clusters on the B-lymphocyte surface.

We have recently suggested that the Ia/Fc receptor patch on B lymphocytes may play a role in regulation of the humoral immune response (6). Since a gene(s) which controls the immune response to at least one antigen has been shown to be neither allotype nor *H-2* linked (7), the prediction was made that this gene might likewise determine cell surface alloantigens located in the B-cell Ia/Fc receptor patch (6). Such molecules, like Ia antigens, could either be associated with, or a part of, Fc receptors. The present studies support this prediction and show that antibodies against alloantigen(s) determined by at least one non-*H-2* locus specifically inhibit the binding of Ig complexes to the Fc receptors of B lymphocytes.

Materials and Methods

Adult male mice of strains C57BL/10 Sn(B10), B10.A/SgSn, B10.D2/nSn, and $B6AF_1$/J, and female A/J mice were purchased from Jackson Laboratories, Bar Harbor, Maine. $B6AF_1$/J × A/J backcross mice were produced in our own animal colony.

Alloantibodies were prepared by immunizations as previously described (4), and were obtained either as serum or as ascites induced by Erlich's ascites tumor cells which had been prepassaged three or more times in the recipient strain. Ascites and serum from the same hyperimmunized animals showed identical specificities in extensive comparative testing. All antisera were decomplemented, ultracentrifuged to remove complexes, and used at dilutions which were on the plateau of cytotoxic killing for the strain of cells being tested as previously described (5). $F(ab')_2$ fragments were prepared by a modification[1] of the method of Nisonoff (8). This preparation was free of intact antibody and Fc fragments by the criteria of size, loss of complement-mediated cytotoxic activity,

[1] Sachs, D. H., J. L. Cone, and J. E. Ricks. Manuscript in preparation.

and failure to react with specific anti-γG1 Fc and anti-γG2 Fc reagents (kindly provided by Dr. R. Asofsky, NIH).

Preparation of spleen cells, fluorescent detection of surface Ig and bound antibody by fluorescein isothiocynate (FITC)-conjugated rabbit antimouse Ig (RAMIG), fluorescent detection of Fc receptors by FITC-conjugated heat-aggregated human Ig complexes, and inhibition of the latter by alloantisera were all performed as previously described (5). Spleen cells were serologically typed using a two-stage complement-mediated trypan blue exclusion microcytotoxicity assay as previously described (4).

Results

The congenic-resistant strain B10.A derived its *H-2* complex from the A strain (A/WySn) and the remainder of its genome from B10 (9). When tested on B10.A cells, the antiserum A/J anti-B10 should therefore detect only alloantigens determined by non-*H-2* genes. Spleen lymphocytes from B10.A were incubated with this antiserum, washed, and then assayed for the binding of aggregated Ig (Table I). The lymphocytes pretreated with this antiserum were markedly

TABLE I

Inhibition of Binding of Ig Complexes to Mouse B Lymphocytes by Antibodies against Non-H-2 Alloantigen(s) of B10.A Mice

Exp.	Antiserum	Specificity when tested on B10.A lymphocytes	Surface Ig	Aggregated Ig binding
			(% positive)	
A	Normal A/J	—	52.0	50.5
	B10.A anti-B10	—	53.0	51.0
	B10 anti-B10.A	H-2	98.5	6.5
	A/J anti-B10	Non-H-2	>99.5	6.0
B	Normal A/J	—	50.5	52.0
	B10 anti-B10.A	H-2	>99.5	6.5
	A/J anti-B10 IgG	Non-H-2	96.5	10.0
	A/J anti-B10 IgG $F(ab')_2$	Non-H-2	86.0	10.5
C	Normal A/J	—	40.0	38.5
	B10 anti-B10.A	H-2	>99.5	4.0
	A/J anti-B10	Non-H-2	89.0	7.0
	A/J anti-B10 absorbed with B10.D2 lymphocytes	—	54.0*	34.5

* A few T lymphocytes showed bound antibody by indirect immunofluorescence probably due to the fact that minimal residual antibody (measured by cytotoxic activity) against B10.A and B10.D2 spleen cells was present despite absorption with 5.5×10^9 B10.D2 spleen and thymus cells per ml.

inhibited in their ability to bind Ig complexes (exp. A). Controls indicated that: (*a*) neither normal serum nor immune serum not reactive with the B10.A cells caused inhibition, (*b*) antibody was indeed bound to the target lymphocytes as indicated by fluorescence of T cells with FITC-RAMIG and increased intensity of fluorescence of B cells resulting from the detection of both surface Ig and bound antibody, and (*c*) the inhibition was equal to that produced by anti-Ia antibodies in an anti-H-2 antiserum (5). Further, $F(ab')_2$ fragments of these non-H-2 antibodies produced equivalent inhibition of binding of Ig complexes (exp. B), thus showing that this inhibition was not due to the Fc portion of the bound antibody.

It seemed possible that this inhibition might be due to genetic drift within the *H-2* complex leading to an Ia antigen shared by B10 and B10.A, but not shared

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by A/J. Therefore, the A/J anti-B10 antiserum was absorbed with B10.D2 lymphocytes, which should share most if not all non-H-2 antigens with B10. The absorbed serum lost its ability to inhibit the binding of Ig complexes to B10.A B cells (exp. C), supporting the possibility that antibodies against non-H-2 antigens were responsible for the observed inhibition. However, the possibility of a mutation in B10.A producing an Ia antigen shared with B10 and B10.D2 but not A/J, while unlikely, could not be excluded by this experiment.

In order to exclude this latter possibility and also to obtain preliminary information about the number of non-*H-2* loci involved, a (B6A)F_1 × A/J backcross was performed. 10 backcross mice were analyzed for the presence of H-2^b, Ia^b (Ia.8), and non-H-2^b alloantigens serologically, and for inhibition of binding of Ig complexes to the Fc receptor by A/J anti-B10 antiserum (Table II). Four mice (1, 4, 7, and 10) inherited the $H\text{-}2^{a/b}$ genotype and inhibition of Fc receptors on their B lymphocytes was observed with the A/J anti-B10 antiserum (presumably due to anti-Ia^b antibodies). This left six mice with $H\text{-}2^{a/a}$ genotype for analysis. It was expected that if the inhibition seen with the B10.A mice was due to an Ia mutation, then no inhibition of the Fc receptor of B cells from the $H\text{-}2^{a/a}$ backcross mice would be seen. If antigens determined by a single independently segregating non-*H-2* locus were involved, approximately 50% of the animals would be expected to show inhibition. Numbers of loci greater than one would show increasing percentages of offspring in which inhibition would be seen.[2] Two animals (2 and 8) showed bound antibody by both cytotoxicity and indirect fluorescence with RAMIG, and inhibition of binding of Ig complexes (Table II). Four mice (3, 5, 6, and 9) showed neither bound antibody nor inhibition. The data thus suggest that antibodies against antigens determined by one non-*H-2* locus were associated with, or part of, the Fc receptor.

Discussion

The specificity of inhibition of the binding of heat-aggregated Ig complexes to the Fc receptors of B lymphocytes by alloantisera has previously been established by experiments which showed that: (*a*) inhibition was produced by F(ab') fragments of appropriate antibodies, and (*b*) inhibition was produced by antibodies to certain lymphocyte surface molecules (Ia antigens) but not others (*H-2K* and *D*-region antigens and surface Ig) (5). Similar results have been obtained using soluble antigen-antibody complexes (reference 10; Arbeit, R. D., and H. B. Dickler, unpublished observation; and B. Pernis, personal communication). In contrast, one laboratory employing a different Fc receptor assay (antibody-coated chicken erythrocytes) found inhibition of binding to the Fc receptor to be nonspecific (11). This discrepancy may reflect different binding affinities measured by the different assays. However, the studies reported here employed the same specific assay we have used previously. Thus, the inhibition of binding of Ig complexes to B lymphocytes from B10.A mice and two of six (B6A)F_1 × A/J backcross animals with $H\text{-}2^{a/a}$ genotype by anti-non-H-2 antibodies [and $F(ab')_2$ fragments thereof] indicates that antigens determined by at least one non-*H-2* locus are also associated with or a part of the Fc receptor.

[2] i.e., for n non-*H-2* loci the fraction of offspring expected to show inhibition would be given by: $1-(1/2)^n$

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TABLE II

Analysis of Lymphocytes from A/J × (B6A)F$_1$ Backcross Mice for H-2^b Ia^b, and Non-H-2^b Alloantigens and Inhibition of Binding of Ig Complexes to B Lymphocytes by A/J Anti-B10 Antiserum

Animal No.	Sex	Cytotoxicity		Fluorescence		
		Antiserum*	Maximum specific cell death*	Antiserum‡	Surface Ig	Aggregated Ig binding
			%		% positive	
1	F	B10.A × A/J anti-B10	>80	Normal A/J	47.0	44.0
		B10.A anti-B10.D2	24	A/J anti-B10	99.5	5.5
		A/J anti-B10	>80			
4	F	B10.A × A/J anti-B10	>80	Normal A/J	53.0	52.0
		B10.A anti-B10.D2	24	A/J anti-B10	>99.5	10.5
		A/J anti-B10	>80			
7	M	B10.A × A/J anti-B10	>80	Normal A/J	47.5	51.0
		B10.A anti-B10.D2	33	A/J anti-B10	>99.5	7.5
		A/J anti-B10	>80			
10	M	B10.A × A/J anti-B10	>80	Normal A/J	48.0	46.5
		B10.A anti-B10.D2	35	A/J anti-B10	>99.5	9.5
		A/J anti-B10	>80			
2	F	B10.A × A/J anti-B10	<10	Normal A/J	49.5	53.0
		B10.A anti-B10.D2	<10	A/J anti-B10	97.5	14.0
		A/J anti-B10	>80			
8	M	B10.A × A/J anti-B10	<10	Normal A/J	53.0	54.5
		B10.A anti-B10.D2	<10	A/J anti-B10	95.0	18.5
		A/J anti-B10	>80			
3	F	B10.A × A/J anti-B10	<10	Normal A/J	55.0	53.5
		B10.A anti-B10.D2	<10	A/J anti-B10	56.0	52.5
		A/J anti-B10	<10			
5	F	B10.A × A/J anti-B10	<10	Normal A/J	53.0	54.5
		B10.A anti-B10.D2	<10	A/J anti-B10	55.0	55.0
		A/J anti-B10	<10			
6	F	B10.A × A/J anti-B10	<10	Normal A/J	48.0	47.0
		B10.A anti-B10.D2	<10	A/J anti-B10	48.5	46.5
		A/J anti-B10	<10			
9	M	B10.A × A/J anti-B10	<10	Normal A/J	52.0	54.0
		B10.A anti-B10.D2	<10	A/J anti-B10	56.0	54.5
		A/J anti-B10	<10			

* Percentage obtained by subtraction of percentage of cell death in complement controls from percentage cell death in experimental wells.

‡ The specificity of the antisera when tested on (B6A)F$_1$ × A/J backcross animals was: B10.A × A/J anti-B10 detects H-2^b; B10.A anti B10.D2 detects Ia^b (Ia.8); and A/J anti-B10 detects both H-2^b and non-H-2^b.

The number of lymphocyte surface antigens determined by the non-*H-2* locus detected in these experiments is unclear. Since bound antibody was observed on both T and B cells, and since both Fc receptors and Ia antigens are expressed primarily on B lymphocytes, it seems possible that there may be two or more antigens determined by this locus, at least one of which is primarily expressed on B cells. The results of preliminary experiments in which the A/J anti-B10 antiserum was absorbed with EL4 (an H-2^b T-cell tumor) and tested on B10.A

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spleen cells were consistent with this possibility (Sachs, D. H., and J. L. Cone, unpublished observation).

A precise determination of the number of non-*H-2* loci which determine lymphocyte surface antigens associated with Fc receptors will be important for functional interpretations. While the present backcross data are consistent with the interpretation that the A/J anti-B10 antiserum is detecting one such locus, the number of animals tested was small. Further backcrosses and intercrosses are in progress to confirm this result. In addition, there may be other loci which determine similar antigens but which are not detected by the A/J anti-B10 antiserum, either because these loci are identical in the A/J and B10 strains, or because the antigens themselves are only weakly antigenic in this immunization combination. Studies with other antisera and strain combinations will be necessary to clarify this point.

A possible interpretation of an association between non-H-2 alloantigens and Fc receptors is that such antigens are similar in structure and function to Ia antigens, and play a role in the regulation of the immune response by non-*H-2*-linked genes. A hypothetical mechanism for regulation by such molecules has recently been described (6). Studies are in progress which will attempt to determine linkage between the non-*H-2* gene(s) which determine the antigens associated with Fc receptors and non-*H-2*-linked *Ir* genes and genes determining other known alloantigens.

The present results illustrate the necessity of using antisera raised between congenic resistant strains of mice which are identical at non-*H-2* loci when attempting to define *H-2*-linked Ia antigens by correlative criteria (12). Also, in attempts to define Ia-like antigens in outbred populations such as man, it will be necessary to show that the genes determining such antigens are linked to the major histocompatibility complex.

Summary

Antibodies contained in an A/J anti-B10 antiserum, when tested on lymphocytes from B10.A mice, were shown to bind to both B and T cells and to inhibit the binding of Ig complexes to the Fc receptors of B lymphocytes. These antibodies could be removed by absorption with B10.D2 lymphocytes. Similar results were obtained with lymphocytes from two of six $(B6A)F_1 \times$ A/J backcross mice which had $H\text{-}2^{a/a}$ genotype. These data indicate that alloantigens determined by at least one non-*H-2* locus are associated with or a part of Fc receptors. These antigens may be similar in structure and function to Ia antigens.

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