GENETIC CONTROL OF THE IMMUNE RESPONSE TO NUCLEASE

V. Genetic Linkage and Strain Distribution of Anti-Nuclease Idiotypes

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Staphylococcal nuclease (nuclease) is a naturally occurring protein antigen, the sequence and crystallographic structure of which have been well characterized (1). Previous studies from this laboratory have shown that the ability of mice to produce antibodies directed against nuclease is dependent on the presence of an immune response (*Ir*) gene which has been mapped to the *I* region of the *H-2* complex (2). More recently, it has been possible to prepare antisera in rats that block the interaction of nuclease with mouse anti-nuclease antibodies (3). Such antisera have been shown to define idiotypic specificities related to the combining site of the anti-nuclease antibodies (3). The availability of these anti-idiotypic reagents allows the investigation of the possible relationships between the *Ir* gene for nuclease and genes coding for certain immunoglobulin variable regions. This communication presents a limited strain distribution of two anti-nuclease idiotypic markers and a genetic linkage study of one of these markers.

Materials and Methods

Mice. Adult mice, 8- to 12-wk old, of strains A/J, AKR/J, (B6 × A)F_1, SJL/J, C57BL/10Sn (B10), and B10.A/SgSn (B10.A) were purchased from The Jackson Laboratory, Bar Harbor, Maine. BALB/c and C3H/HeN mice were obtained from the Animal Production Unit, NIH. CB.20 mice were a gift from Dr. Michael Potter, NCI, NIH, Bethesda, Md , BAB.14 mice, a gift from Dr. L. A. Herzenberg, Stanford University, Stanford, Calif., and A.BY mice a gift from Dr. Ronald Schwartz, NIAID, NIH, Bethesda, Md. Backcross progeny of (B10.A × A/J) to B10 A were produced in our own breeding colonies.

Rats Adult male Lewis rats were purchased from Microbiological Associates, Bethesda, Md.

Preparation of Anti-Nuclease Antibodies. Nuclease was isolated from the extracellular broth of the Foggi strain of *Staphylococcus aureus* according to published methods (4) and was further purified as previously described (5) Mice were immunized with 100 μg of purified nuclease in complete Freund's adjuvant (CFA)[1] (Difco Laboratories, Detroit, Mich.) and bled from the tail 3 wk later. Hyperimmune sera or ascites were obtained after multiple weekly boosts of 25 μg of nuclease in saline as previously described (3). Assays for anti-nuclease antibodies were performed either on whole sera or ascites or after separation of antibodies by affinity chromatography on Sepharose columns bearing covalently bound nuclease.

Preparation of Anti-Idiotypic Antisera. Lewis rats were immunized every 2 wk with 500 μg of affinity column purified anti-nuclease antibodies in CFA. Anti-idiotypic activity was detected generally after the third to fourth immunization; subsequent immunization led to only small increments in activity

Assay for Nuclease. Nuclease was assayed according to the method of Cuatrecases et al. (6), in

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[1] *Abbreviation used in this paper.* CFA, complete Freund's adjuvant.

which the enzymatic cleavage of denatured DNA was measured by change in OD_{260} of the assay solution.

Assay for Anti-Nuclease Antibodies. The assay for anti-nuclease antibodies has been previously described in detail (2). Briefly, the activity of a known amount of nuclease was assessed after incubation with or without immune sera or affinity column purified antibodies. The antibody activity was expressed as the number of inactivating units per milliliter of antiserum and was calculated from the amount of inactivation obtained and from the dilution of antiserum used as previously described (2).

Assay for Anti-Idiotypic Antibodies. This assay has also been previously described (3) and involves the measurement of the inhibition of antibody-mediated inactivation of nuclease. Briefly, an anti-nuclease antibody preparation was preincubated with an anti-idiotypic antiserum for 5 min and then incubated with nuclease. The residual inactivating units were then determined and compared to that obtained by antibody not exposed to anti-idiotypic antisera. The inhibitory activity of an anti-idiotypic antiserum was calculated using the formula:

$$\% \text{ Inhibition} = \frac{^{A}[\text{Nase} + \alpha\text{-Nase} + \alpha\text{-ID}] - {}^{A}[\text{Nase} + \alpha\text{-Nase}]}{^{A}[\text{Nase}] - {}^{A}[\text{Nase} + \alpha\text{-Nase}]},$$

in which ^{A}[Nase + α-Nase + α-ID] indicates activity measured in the presence of nuclease (Nase), anti-nuclease (α-Nase), and anti-idiotype (α-ID), etc., as previously described (3). An antibody preparation was considered to contain a given idiotype if prior incubation with anti-idiotypic antiserum caused a statistically significant ($P < 0.05$) inhibition of activity.

Anti-Allotype Antisera and Allotype Determination The anti-allotype antisera used for these studies were produced by injecting anti-pertussis/pertussis complexes into allotypically dissimilar inbred mice as described by Herzenberg and Herzenberg (7). Allotypes of individual animals were determined in an Ouchterlony double-diffusion system.

Results

Rat Anti-Idiotypic Antibodies. Rat anti-A/J anti-nuclease antisera have been previously shown to contain anti-idiotypic specificity by the following criteria: (*a*) Extensive affinity column absorption with normal A/J globulins did not diminish the ability of these antisera to cause inhibition of enzyme inactivation; (*b*) while antisera with anti-allotypic or anti-isotypic specificities could interact with anti-nuclease antibodies as demonstrated by their ability to form complexes that could be sedimented by high-speed centrifugation, they could not mediate inhibition of nuclease inactivation. Thus, only antisera reactive with the antigen-combining site appeared to cause such inhibition. Using similar methods, we have prepared rat antisera which inhibit SJL antibody-mediated inactivation of nuclease. The results obtained with this serum are presented in Table I, where they are compared with results obtained with the previously described rat anti-A/J anti-nuclease. As can be seen, each antiserum was capable of causing inhibition of inactivation only with the antibodies against which it had been prepared. Within the limits of the assay, there was no cross-reaction between the anti-nuclease antibodies from A/J and SJL animals with respect to either anti-idiotypic serum. These antisera thus defined two sets of idiotypic markers.

Lack of Relationship Between the H-2 Complex and the Expression of Anti-Nuclease Idiotypes. The presence of the A/J idiotype was assessed in reciprocal pairs of congenic-resistant mice, A/J vs. A.BY and B10.A vs. B10. A/J and A.BY differ at the *H-2* locus (*H-2*^{a} vs. *H-2*^{b} haplotypes) but share other genes in common, including the heavy chain allotype locus (*Ig-1*^{e}). Similarly, B10 and B10.A differ at the *H-2* locus (*H-2*^{b} vs. *H-2*^{a}) but share other genes in

TABLE I
Reactivity of Anti-Idiotypic Antisera to A/J and SJL Anti-Nuclease Antibodies

Strain tested	Anti-idiotype tested	Inhibition
		%
Pooled A/J anti-nuclease antibody	Rat α-A/J Id^n	49.6 ± 2.6
Pooled SJL anti-nuclease antibody	Rat α-A/J Id^n	−0 3 ± 1.6
Pooled A/J anti-nuclease antibody	Rat α-SJL Id^n	36.3 ± 4.2
Pooled SJL anti-nuclease antibody	Rat α-SJL Id^n	−2.4 ± 1.8

Pooled A/J and SJL anti-nuclease antibodies were assessed for activity in the presence and in the absence of anti-idiotypic antisera directed against either A/J or SJL antibodies. The percent inhibition of inactivation was calculated by the formula described in the Materials and Methods. In this and subsequent tables α-AJ Id^n and α-SJL Id^n refer to anti-idiotypic antisera to A/J and SJL-anti-nuclease antibodies, respectively

TABLE II
Relationship of H-2 Haplotype to Expression of A/J Idiotype

Strain	*H-2* haplotype	*Ig-1* allotype	Inhibition of Inactivation	Significance of inhibition ($P <$ 0.05)
			%	
A/J	a	Ig-1^e	66.9 ± 4.5	+
B10.A	a	Ig-1^b	5 1 ± 7.1	−
B10	b	Ig-1^b	5.4 ± 9.1	−
A.BY	b	Ig-1^e	42.0 ± 4.6	+

Pooled sera from immune animals were tested for the presence of A/J idiotype by comparing inactivating activity in the presence and in the absence of anti-idiotypic antibody

common, including the *Ig-1^b* heavy chain allotype locus. Both A/J and B10.A have been shown to be high responders to nuclease while A.BY and B10 are low responders. Significant quantities of anti-nuclease antibodies can be obtained, however, by hyper-immunizing the low responder strains (8). Table II shows the results of the inhibition of inactivation of nuclease on equivalent amounts of pooled antibodies from these strains. Significant inhibition by the anti-A/J idiotypic antiserum was obtained only with antibodies from A/J and A.BY mice, indicating that these strains both express the A/J idiotype although they differ at the *H-2* locus. This result, suggesting independence of expression of the idiotype from *H-2*-linked *Ir* genes, was substantiated by the failure to identify significant amounts of the A/J idiotype in the B10.A strain which shares the *H-2^a* haplotype with the A/J strain. Conclusions derived from these results, however, are limited by the fact that anti-idiotypic antisera available do not inhibit A/J antibodies 100% (in these experiments, a maximum of 66.9% was obtained). Thus, there may be idiotypes not recognized in this assay that do demonstrate dependence on *H-2*-linked *Ir*-gene function.

Allotype Linkage. While the above results failed to support linkage of the A/J idiotype with the *H-2* locus, they did not necessarily indicate linkage to the heavy chain allotype locus since other loci shared by strains demonstrating

TABLE III

Expression of A/J Idiotype in (B10.A × A/J) × B10.A Backcross

Animal No.	Allotype	Inhibition of inactivation	Significant inhibition
		%	
2	Ig-1^b/Ig-1^e	27.0	+
6	"	42.2	+
8	"	55.8	+
10	"	24.7	+
11	"	33 3	+
13	"	54.3	+
16	"	55.3	+
1	Ig-1^b/Ig-1^b	4.0	−
3	"	0 0	−
4	"	0.8	−
5	"	−2 4	−
7	"	1.9	−
9	"	2.4	−
12	"	0.3	−
14	"	27.1	+
15	"	42 3	+
17	"	−4.7	−
18	"	−0.9	−
19	"	−5.6	−

Progeny of backcross (B10.A × A/J) × B10.A were immunized with nuclease and bled at 3 wk. Allotypes of individual animals were determined by Ouchterlony double-diffusion analysis and the presence of A/J idiotype was determined by the inhibition of inactivation assay using rat anti-A/J anti-nuclease

the idiotype could be responsible for its expression. To examine further linkage relationships, the presence of the A/J idiotype in the offspring from a backcross of (A/J × B10.A) × B10.A was determined (Table III). All animals from this mating would be expected to be informative as (*a*) all animals would possess the *H-2*^{a} haplotype and should be responders to nuclease; and (*b*) the expression of the idiotype is dominant (codominant) as shown in Table IV where the presence of the A/J idiotype is demonstrated in all members of a sample of F_1 animals from the cross of A/J × B6. Sera from backcross animals were tested for allotype using Ouchterlony double-diffusion analysis. Of 19 backcross animals tested, 7 were heterozygotes (*Ig-1*^{b}/*Ig-1*^{e}). All animals heterozygous at the allotype locus showed the presence of the A/J idiotype while two of the homozygous *Ig-1*^{b} animals produced significant amounts of idiotype, verified by repeated analysis of these sera and multiple bleedings. These data indicate linkage between genes coding for the A/J idiotype and those for the heavy chain allotype ($P < 0.005$ by chi-square analysis). The explanation of the apparently high recombination frequency (2/19) is unclear. While it remains likely that the two idiotype-positive *Ig-1*^{b} homozygotes represent heavy chain recombinants, other interpretations are also possible. Further backcross analyses and progeny testing of these animals are in progress and hopefully will clarify the origins of the observed phenotypes.

Strain Distribution. Results presented here and previously (3) have sug-

TABLE IV
Expression of A/J Idiotype in (B6 × A)F_1

Strain	No.	Inhibition with α-A/J Id^n
		%
(B6A)F_1	1	24.2
	2	54.2
	3	44.3
	4	43 8
	5	36.1

Immune sera from individual (B6 × A)F_1 animals were assayed for the presence of A/J idiotype by methods described.

gested that A/J and SJL idiotypes showed strain specificity in contrast to individual or species specificity. To determine the distribution of the available idiotypic markers more fully, animals from various strains were immunized with nuclease and their sera tested individually for the presence of the A/J and SJL idiotypes. Table V presents the results of a limited survey.

Two results are of note: (*a*) the SJL idiotype was absent in sera from B10 animals, although both strains belong to the same heavy chain allotype group (*Ig-1^b*), and (*b*) both the A/J and SJL idiotypes were present in sera from BALB/c animals. To investigate the relationship of the two idiotypic markers in the BALB/c strain, their presence was assesed in animals of the CB.20 and BAB.14 strains (also shown in Table V). These strains, derived by backcrosses designed to transfer the *Ig-1^b* allotype to the BALB/c background, have been shown to differ in their expression of various variable region gene markers, suggesting a recombination event between variable region genes and constant region allotype marker genes of the BAB.14 strain (9). As shown in Table V, CB.20 animals failed to express either the A/J or SJL idiotype, whereas the BAB.14 animals expressed the A/J idiotype. This result is consistent with the hypothesis that the genes for the SJL and A/J idiotypic markers were separated by a crossover between variable region genes during the development of the BAB.14 strain. By this hypothesis, a tentative map for these markers would place the genes for the SJL idiotype marker closer to the heavy chain allotype gene locus.

Discussion

A failure to find a linkage relationship between *H-2* and the A/J anti-nuclease idiotypic marker was perhaps to be expected on the basis of previous linkage studies on idiotypic markers (9). Such studies, however, have involved antigens not demonstrably under the control of *H-2*-linked *Ir* genes. The influence of *Ir* genes on the expression of variable region genes could thus be formally tested in this system. This seemed particularly relevant in light of recent studies indicating sharing of idiotypes between T-cell and B-cell receptors (10–12) and suggesting possible interactions between idiotype-bearing molecules and products of *H-2*-linked genes. Our results, however, do not entirely rule out an influence of *H-2*-linked *Ir* genes on expression of heavy chain variable region idiotypes. It is possible that there are antibodies that cannot be detected by our antisera and that it is these antibodies that show

TABLE V
Strain Distribution of A/J and SJL Anti-Nuclease Idiotypes

Strain	Animal No.	Inhibition with α-A/J Id^n	Inhibition with α-SJL Id^n
		%	%
A/J	1	35.4	−2.3
Ig-1^e	2	22 7	−0 4
H-2^a	3	38.9	−1 2
	4	39.8	1.0
	5	46.1	−3 2
SJL	1	0.2	23 7
Ig-1^b	2	−2.8	24.7
H-2^s	3	−2.2	39.1
	4	1 2	39.0
	5	−4.6	22.4
BALB/c	1	34 0	30.0
Ig-1^a	2	32.8	46 8
H-2^d	3	25.4	21.8
	4	43.4	44.6
	5	50 8	19 3
CB.20	1	2.1	−2.0
Ig-1^b	2	3 2	0.0
H-2^d	3	2.0	−6 0
	4	0 0	−2.4
	5	1 8	0.0
BAB.14	1	29.5	−2.0
Ig-1^b	2	33.0	1.6
H-2^d	3	36 3	0 8
	4	22 6	−2.1
	5	47.5	5.0
B10	1	−2 7	−3 4
Ig-1^b	2	−0.8	1 1
H-2^b	3	−1.2	−0 8
	4	1 5	−1.2
	5	2.6	−5 4
AKR/J	1	−2.9	−4.0
Ig-1^d	2	6.0	−4.0
H-2^k	3	−1.3	5 5
	4	5.7	−1 5
C3H/HeN	1	0.0	3.2
Ig-1^a	2	0.0	0 4
H-2^k			

Immune sera from individual animals of strains listed were tested for the presence of A/J and SJL idiotypes by use of the inhibition of inactivation assay

dependence on *Ir*-gene function. Further experiments involving fractionation of the anti-nuclease antibodies and anti-idiotypic antisera are in progress to identify more precisely the specificity of the predominant antibodies detected in our assay.

While our results indicate linkage to the heavy chain allotype at a locus statistically significant level, they also suggest an apparently high level of recombination (10.5%). This recombination frequency should be viewed as tentative, as only 19 animals have been examined so far and the 2 *Ig-1*^{b}/*Ig-1*^{b} homozygotes bearing the A/J idiotype have not yet been formally proven to be recombinants by progeny testing. The observed phenotype may have resulted, for example, from suppression of the *Ig-1*^{e} allotype in a *Ig-1*^{e}/*Ig-1*^{b} heterozygote to levels that could not be detected in our assay systems; tests are in progress to consider this possibility. In addition, since the precise mechanisms by which variable region expression is controlled have not been established, it remains possible that mechanisms other than genetic crossover events could lead to the expression of the A/J idiotype in *Ig-1*^{b}/*Ig-1*^{b} homozygotes. In this context, the possible operation of regulator genes controlling the level of expression of structural genes for idiotypes must be considered. Similar mechanisms have recently been suggested for the expression of human, mouse, and rabbit allotypes (13–16). Progeny testing of our putative recombinants should clarify the nature of these apparent recombinant animals, and the analysis of more backcross animals will allow calculation of a more accurate recombination frequency.

The strain distribution of the A/J and SJL idiotypes indicates some interesting relationships. B10 and B10.A mice did not express the SJL idiotype despite the fact that all three strains are *Ig-1*^{b}. In contrast, BALB/c mice, belonging to the *Ig-1*^{a} allotype group, expressed both the A/J and SJL idiotypes.

These observations may indicate the operation of different selective pressures on the evolution of variable region and constant region genes. The SJL-B10 relationship could result from either convergence during constant region evolution or divergence during variable region evolution. While it might be possible to propose a common origin for the A/J idiotype in the A/J and BALB/c strains on the basis of the ancestral background (17), the BALB/c strain is not known to be ancestrally related to SJL. The expression of the SJL idiotype by the BALB/c may thus indicate convergent evolution of variable region genes.

The expression of the A/J idiotype by the BAB.14 strain is consistent with the presumed recombination between the *Ig-1*^{b} constant region and the BALB/c variable region which apparently occurred during the development of this congenic strain (9). However, the absence of the SJL idiotype in immune sera from the BAB.14 strain suggests that the crossover event may have occurred within the V_H region rather than between the V_H and V_C regions, and thus separated the genes coding for the antibodies bearing the A/J and SJL idiotypes.

It should be noted that this map order presumes that the genes coding for the SJL anti-nuclease idiotypes are linked to the heavy chain allotype in the BALB/c strain, a hypothesis that has not yet been formally tested by backcrossing. While the CB.20 data makes this seem likely, the data must be

considered preliminary until such linkage is established. Furthermore, this order may pertain only to the BALB/c heavy chain locus, as it has not been shown that idiotype markers occupy identical positions in the map of variable region genes in different strains. Finally, studies are in progress to characterize BALB/c antibodies bearing the SJL and A/J idiotypes, and to determine whether the idiotypes are on the same or different molecules.

Summary

Rat antisera raised against anti-nuclease antibodies from mouse strains A/J and SJL detect strain-specific idiotypic determinants related to the antigen-combining site. These antisera have been used to investigate the genetic linkage and strain distribution of the anti-nuclease idiotypes. Despite the existence of an *H-2*-linked immune response gene controlling the humoral response to nuclease, expression of the A/J anti-nuclease idiotype has been shown to be independent of genes in the *H-2* region: the A/J idiotype was present in immune sera from strains A/J ($H\text{-}2^a$) and A.BY ($H\text{-}2^b$) but absent in sera from strains B10 ($H\text{-}2^b$) and B10.A ($H\text{-}2^a$). An analysis of the segregation of the A/J idiotype in offspring of the backcross (A/J × B10.A) × B10.A demonstrated linkage to the $Ig\text{-}1^e$ heavy chain allotype markers. In a small sample of backcross animals a very high apparent recombination frequency was observed, but further backcross analyses and progeny testing of putative recombinant animals will be required to substantiate this observation. Analysis of the A/J and SJL anti-nuclease idiotype markers in the BALB/c, CB.20, and BAB.14 strains indicate that these idiotypic markers may permit mapping of distinct variable region genes.

The authors thank Dr John Ricks for the preparation of anti-allotype reagents, and Laura Kunz and Judy Steckel for excellent secretarial assistance.

Received for publication 25 October 1976.

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