GENETIC CORRELATION OF A MOUSE LIGHT CHAIN VARIABLE REGION MARKER WITH A THYMOCYTE SURFACE ANTIGEN*

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Recent advances in the knowledge of antibody structure have prompted much discussion of the number and arrangement of the genes which code for immunoglobulin light and heavy chains (6, 12). In no case, however, have immunoglobulin structural genes been located in a genetic linkage map of any organism. Knowledge of the linkage groups containing immunoglobulin structural loci would be useful in further analyzing by genetic and other methods the number and disposition of germ line variable (V) region genes responsible for the enormous amino acid sequence diversity observed in immunoglobulin chains.

Edelman and Gottlieb have described a genetic marker in the V region of mouse immunoglobulin light chains (4, 5). The marker, called the I_B-peptide marker, was detected as a unique zone of radioactivity in peptide maps of tryptic digests of fully reduced [^{14}C]iodoacetic acid-alkylated light chains isolated from unimmunized mice. Amino acid sequence analysis showed that these peptides contain the half-cystinyl residue at position 23 of the light chains, and breeding studies indicated that the expression of these unique peptides segregates in a fashion consistent with the behavior of a classical Mendelian marker. In a I_B-positive strain, the I_B-peptide marker is present in approximately 5% of the light chains and it is absent from lambda chains. In heterozygotes between I_B-positive and I_B-negative strains, the marker is expressed at approximately half the level of I_B-positive homozygotes. The simplest interpretation for the existence of this light-chain V-region genetic marker is that it represents a polymorphism in structural genes coding for light-chain V regions. Alternatively, the presence or absence of the I_B-peptide marker may reflect polymorphism in a gene which influences the expression of light-chain V regions (e.g. a regulatory gene, an endogenous virus, etc.).

In order to identify the locus or linkage group responsible for expression of this light chain V-region marker, the inbred and congenic strain distribution of the I_B-peptide marker has been compared with the strain distribution of other known genetic markers (7). The present communication reports an absolute positive correlation between the I_B-peptide marker and the Ly-3.1 thymocyte

* This work was supported in part by a U. S. Public Health Service Training grant (5 PI GM 00196) from the National Institutes of Health to Stanford University Department of Biochemistry, by a U. S. Public Health Service Research grant (1 R01 CA 15808-01) from the National Cancer Institute to Paul D. Gottlieb, and by a U. S. Public Health Service Research grant (CA 14051) from the National Cancer Institute to the Massachusetts Institute of Technology Center for Cancer Research. Additional support for this research was provided by funds granted by the James and Lynelle Holden Fund.

cell surface antigen, and indicates genetic linkage of the I_B-peptide marker to the Ly-2 and Ly-3 loci in linkage group XI on chromosome 6 of the mouse. (1, 2, 8).

Materials and Methods

Mice and Sera. Inbred strains and pooled frozen serum were obtained from the Jackson Laboratories, Bar Harbor, Maine. Serum and breeding stock of the C57BL/6-Ly-2^aLy-3^a strain (9) were the generous gift of Dr. E. A. Boyse, Sloan-Kettering Institute for Cancer Research, New York. AKR.B6/1 mice, derived by Boyse and co-workers (9), were the gift of Dr. H. O. McDevitt, Stanford University School of Medicine, Stanford, Calif. Serum from AKR.M mice was kindly supplied by Dr. G. D. Snell, Jackson Laboratories, Bar Harbor, Maine.

Preparation of Immunoglobulins, Light Chains, and Peptide Maps. Immunoglobulins from pooled sera were obtained by starch zone electrophoresis in sodium barbital buffer, pH 8.6 (5), followed by two successive gel filtrations on Sephadex G-200 (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden). Isolation of light chains, complete reduction and alkylation with [^{14}C]iodoacetic acid, digestion with trypsin, and preparation and autoradiography of peptide maps have been described (5).

Absorption of AKR/J Serum. 4 ml of freshly prepared AKR/J serum were absorbed for 30 min at 4°C with a 0.6-ml packed vol of washed AKR/J thymocytes (7:1, vol/vol). The cells were then removed by centrifugation, and the absorbed serum was applied to a starch block along with a separate sample of 4 ml of untreated AKR/J serum. IgG and light chains were obtained from each sample, and peptide maps were prepared.

Results

Genetic Correlation of I_B and Ly-3.1. The distribution of the I_B-peptide marker among 27 inbred and congenic strains of mice is shown in Table I. The four inbred strains, AKR/J, C58/J, RF/J, and PL/J, that express the I_B-peptide marker in their immunoglobulin light chains also express the Ly-3.1 antigenic specificity on the surface of their thymocytes. None of the I_B-negative strains expresses the Ly-3.1 antigenic specificity. The C57BL/6-Ly-2^aLy-3^a strain (Ly-2.1, Ly-3.1 positive) was derived by Boyse and co workers to be congenic with the I_B-negative C57BL/6 strain (Ly-2.2, Ly-3.2 positive) at all but the region of the Ly-2 and Ly-3 loci (9). The Ly-2.1 and Ly-3.1 alleles were supplied by strain RF and the mice were inbred after 16 backcrosses to C57BL/6 (E. A. Boyse, personal communication). C57BL/6-Ly-2^aLy-3^a immunoglobulin light chains were found

TABLE I
Expression of I_B-Peptide Marker in Inbred Strains of Mice

Express I_B-peptide marker*	Lack I_B-peptide marker‡		
AKR/J	DBA/2J	CE/J	C57Br/cdJ
C58J	DBA/1J	SJL/J	C57L/J
RF/J	A/J	SWR/J	CBA/J
PL/J	A/HeJ	NZB	BALB/cJ
AKR.B6/1	C3H/HeJ	ST/bJ	MA/J
C57BL/6-Ly-2^aLy-3^a	C57BL/6J	BDP/J	SEA/GnJ
	C57BL/KsJ	129/J	AKR.M

* Thymocytes from strains in this column have been determined to be positive for the Ly-3.1 antigenic specificity (1).

‡ Thymocytes from strains in this column have been determined to be positive for the Ly-3.2 antigenic specificity (1, 13).

to be I_B positive, indicating that expression of the I_B-peptide marker is closely linked to the Ly-2 and Ly-3 loci selected in the derivation of this strain.

Since three of the four inbred strains expressing the I_B-peptide marker also possess the *H-2*^{k} genotype (Table II), it was important to test for any possible linkage to the *H-2* locus, the major histocompatibility locus of the mouse. The inbred strain AKR.B6/1 is congenic to AKR at all but the *H-2* locus where it is *H-2*^{b} instead of *H-2*^{k} (9). This strain was I_B positive, indicating that expression of the I_B-peptide marker is not linked to the *H-2*^{k} genotype. In addition, the non-*H-2*^{k} strains, PL/J (*H-2*^{u}) and C57BL/6-Ly-2^{a}Ly-3^a (*H-2*^{b}) are I_B positive. Since the *Ir-1* locus governing the immune response to a number of antigens is closely linked to the *H-2* locus (10), the I_B-peptide marker would appear to be unlinked to it as well.

TABLE II

Characteristics of Several Inbred Strains of Mice at Selected Genetic Loci

STRAIN	I_B*	Ly-1‡	Ly-2‡	Ly-3‡	H-2‡	θ‡
AKR/J	+	2	1	1	k	AKR
C58/J	+	2	1	1	k	C3H
RF/J	+	2	1	1	k	AKR
PL/J	+	2	1	1	u	AKR
C57BL/6-Ly-2^{a}Ly-3^a	+	2	1	1	b	C3H
AKR.B6/1	+	2	1	1	b	AKR
C57BL/6J	−	2	2	2	b	C3H
CE/J	−	2	1	2	k	C3H

* I_B, expression of I_B-peptide marker in light chains; (+) present and (−) absent.

‡ (Data from references 1 and 13). Ly-1, Ly-2, and Ly-3, number indicates allele present; 1, allele 1 and 2, allele 2. *H-2*, haplotype at *H-2* locus. θ, θ-antigen allele.

Absorption with Thymocytes. The possibility was considered that immunoglobulin containing I_B-positive light chains might be autoantibody directed against the Ly-3.1 antigenic specificity. Extensive absorption of serum from the I_B-positive strain AKR/J with washed AKR/J thymocytes (Ly-3.1 positive) failed to remove the I_B-containing immunoglobulin components as revealed by subsequent peptide mapping of light chains.

Discussion

The results indicate that a locus governing the expression of the I_B-peptide marker is genetically linked to the locus governing the expression of the Ly-3.1 thymocyte surface antigen. Moreover, preliminary peptide mapping studies of light chains from AKXL recombinant inbred strains derived by Taylor et al. (14) indicate that the expression of the I_B-peptide marker is genetically linked to the Ly-2 and Ly-3 loci in these strains as well (P. D. Gottlieb and B. A. Taylor, unpublished observations). Itakura and co-workers have shown (8) that the Ly-3 locus is extremely closely linked to the Ly-2 locus in linkage group XI of the mouse such that no recombinants have been observed in over 370 backcross

progeny. Since the Ly-3.1 allele appears always to be associated with the Ly-2.1 allele, all I_B-positive mice are also Ly-2.1 positive. The I_B-peptide marker is therefore linked to the Ly-2.1, Ly-3.1 genotype. That the I_B-peptide marker is correlated with Ly-3.1 rather than Ly-2.1 is demonstrated by such strains as CE/J (Table II), which is Ly-2.1 positive, Ly-3.2 positive, and I_B negative.

A primary goal of these studies is to discern the nature of the locus governing expression of the I_B-peptide marker. The simplest hypothesis is that it is a structural locus coding for light-chain V regions. The expression in I_B heterozygotes of approximately half as much I_B-peptide marker as in I_B homozygotes could then be simply explained as a gene dosage effect. Alternatively, a genetic locus linked to or identical to Ly-3.1 may regulate the expression of light-chain V-region structural genes encoded elsewhere in the genome (7).

The possibility was considered that I_B-positive immunoglobulin might represent autoantibody to the Ly-3.1 thymocyte antigenic specificity since autoantibodies to thymocytes have been detected in several strains of mice (11). However, attempts to adsorb I_B-positive immunoglobulin with Ly-3.1-positive thymocytes were unsuccessful, making autoantibody to Ly-3.1 an unlikely explanation.

The extremely close genetic linkage observed between the Ly-2 and Ly-3 loci led Itakura et al. to suggest (8) that they comprise a complex genetic locus (Ly-2, 3) characterized by multiple specificities. Moreover, studies by Boyse and coworkers (1, 3) using the antibody blocking method suggested a close topological relationship between Ly-2 and Ly-3 antigenic specificities on the thymocyte surface, and led to the suggestion that the Ly-2 and Ly-3 antigens may reside on the product of a single gene (8). These characteristics of the Ly-2,3 chromosomal region and antigenic specificities are remarkably similar to what one might expect for a complex chromosomal region coding for immunoglobulin V and C regions (6). The intriguing possibility must be considered that the I_B-peptide marker in immunoglobulin light chains may be coded for by the Ly-3.1 locus, and that the Ly-3 and perhaps Ly-2 antigenic specificities might reside on molecules structurally related to immunoglobulins.

Finally, it may be significant that in all the characterized inbred strains, the Ly-2.2, Ly-3.1 haploid chromosome has not been observed, whereas the other three combinations have been identified (Table II). The absence of the Ly-2.2, Ly-3.1 chromosome may reflect some inherent lethality of this chromosome, or may be due to chance in the derivation of laboratory mice. In this regard, a careful survey of wild mice for the missing chromosome would be informative. However, the absence of the Ly-2.2, Ly-3.1 chromosome may reflect the evolutionary pathway that gave rise to the chromosomes bearing linkage group XI of the mouse. For example, the pathways shown in Fig. 1 would preclude the existence of the missing chromosome.

The close genetic linkage of the I_B-peptide marker in mouse immunoglobulin light chains to the Ly-2 and Ly-3 loci (or Ly-2,3 complex locus) specifying thymocyte surface antigens strongly suggests that this chromosomal region in linkage group XI may be intimately involved in the function of the immune system. Further dissection of this genetic region and of the molecules specified by it should therefore bear upon the mechanisms which underlie the immune response.

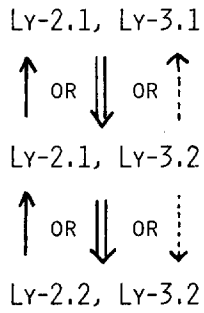

FIG. 1. Evolutionary model to account for absence of Ly-2.2, Ly-3.1 chromosome. Single, double, and dotted arrows denote three possible evolutionary pathways which would preclude the existence of the Ly-2.2, Ly-3.1 chromosome.

Summary

The inbred and congenic strain distribution of the I_B-peptide marker in the variable region of mouse immunoglobulin light chains has been compared with other known genetic markers. A positive correlation was noted between the I_B-peptide marker and expression of the Ly-3.1 thymocyte cell surface antigen. This suggests that the locus responsible for I_B-peptide expression is genetically linked to the Ly-2 and Ly-3 loci in linkage group XI on chromsome 6 of the mouse.

Received for publication 13 August 1974.

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