CHARACTERIZATION OF A NEW INTRA-*H-2* RECOMBINANT

SEPARATION OF THE *Ir-RE* AND *Ir-GLT* GENES*

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Since the first reports of crossing-over within the *H-2* complex (references 1 and 2, and footnote 1) approximately three dozen additional *H-2* recombinants have been observed (3–5). Analysis of these recombinant strains permitted the arrangement of genetic characters into a multipoint linkage map consisting of four regions, *K*, *I*, *S*, and *D* (6). Division of the *H-2* complex into regions was based on the genetic separation of the *H-2D* and *H-2K* private serological specificities, the *Ss-Slp* serum proteins, and the immune response (*Ir*) genes. The genetic region controlling immune responsiveness can be divided into subregions, *Ir-1A* and *Ir-1B*, based on the immune response patterns of the recombinant B10.A(4R) mouse strain (7). Thus, at least two distinct *Ir* loci exist within the *I* region.

In this report we describe a second *H-2* recombinant strain, D2.GD, in which crossing-over occurred between distinct *Ir* genes. Previous analysis of this recombinant localized the recombination event between *H-2K* and *H-2S* (8). This report tentatively localizes the crossover position between *Ir-1A* and *Ir-1B*, however, we cannot determine if recombination occurred at the same position within the *I* region as noted with the other intra-*I*-region recombinant strain B10.A(4R).

Materials and Methods

Mice.—All inbred mice were produced in our animal facilities or were purchased from Jackson Laboratories, Bar Harbor, Maine. Animals were between 8 and 26 wk of age at the beginning of immunization. Each experimental group consisted of at least four mice which were bled 7 days after secondary immunization. The D2.GD strain was produced at Albert Einstein College of Medicine, New York, from a backcross of (C57BL/6 × DBA/2)F_1 × DBA/2. It underwent 12 further generations of backcrossing to DBA/2 with selection for *H-2.2*-positive mice in each generation. In the N13 generation, the *H-2.2*-positive animals were intercrossed and a homozygous line was established. At this time complete characterization of the *H-2K* and *H-2D* serological specificities indicated that an intra-*H-2* recombination had occurred (8). The D2.GD congenic line lost the *H-2.33* antigen controlled by the $H\text{-}2K^b$ locus and gained the *H-2.31* specificity coded for by the $H\text{-}2K^d$ locus of the DBA/2 parent.

Antigens.—The random terpolymer L-glutamic acid^{57}-L-lysine^{38}-L-tyrosine^5 (GLT) was a gift of Dr. Paul H. Maurer, Jefferson Medical College, Philadelphia, Pa. The random terpolymer L-glutamic acid^{57}-L-lysine^{38}-L-phenylalanine^5 (GLϕ) was obtained from Dr. Elkan

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[1] The nomenclature for the *H-2*-gene complex used in this report is in accordance with the recent proposals by Klein et al. (6).

Blout, Harvard Medical School, Boston, Mass. The terpolymers were emulsified in complete Freund's adjuvant (CFA) containing 0.5 mg/ml *Mycobacterium butyricum*, from Difco Laboratories, Detroit, Mich. Primary and secondary immunizations of 0.2-ml emulsion containing 100 μg of antigen in CFA were given intraperitoneally on days 0 and 21, respectively. Mice were bled on day 28.

Ragweed extract (RE) was purchased from Greer Laboratories, Inc., Lenoir, N. C. (lot no. 56-36-3X12). Primary and secondary immunizations were performed by injecting 10 μg RE in 4 mg of aluminum hydroxide gel in a total vol of 0.5 ml. Mice received the secondary challenge on day 30 and were bled on day 37.

Antibody Titration.—The levels of reagenic (IgE) anti-RE antibodies were determined in pooled sera from at least four mice by passive cutaneous anaphylaxis (PCA) reactions using Sprague-Dawley rats as previously described (9). Reactions were read and recorded as the reciprocal of the highest dilution of serum evoking threshold PCA reactivity (5-mm diameter). Repeated titrations gave identical results.

The anti-GLT and anti-GLϕ responses were measured in individual sera by a modified Farr antigen-binding assay using ^{125}I-labeled GLT as described in detail elsewhere (10). Less than 10% binding was regarded as not significant, since this was the range of variation seen when nonimmune control sera was used.

RESULTS

Preliminary investigations in our laboratory established the conditions of immunization required for the three *H-2*-linked *Ir*-gene systems described in this report to distinguish between the I^b and I^d alleles. The *H-2*-linked *Ir*-gene controlling responsiveness to ragweed pollen extract (*Ir-RE*) has been described in detail elsewhere (9). Briefly, the strain distribution of IgE and IgG antibody responses to RE and its dinitrophenylated derivative (DNP-RE) characterize a new *Ir* gene, *Ir-RE*. Responder strains show higher primary and secondary levels of both antibody classes than nonresponders after immunization with these antigens in aluminum hydroxide gel, the differences being most marked at low doses, but present over a wide dose range (10–500 μg/mouse). High responses are associated with $H\text{-}2^{a,\,d,\,h,\,k,\,m,\,n,\,p}$ types; low responses with $H\text{-}2^{b,\,f,\,i,\,j,\,u}$. The *Ir-RE* gene has been mapped to the *H-2* region by the use of congenic resistant strains. The high response allele is dominant and appears, by its regulation of antihapten responses in the DNP-RE hapten-carrier system, to act at the cellular level of thymus-derived lymphocytes. T cells possessing this gene control responses of IgG and IgE B lymphocytes in a parallel fashion (9).

After secondary immunization with 10 μg of RE, mice carrying the $H\text{-}2^a$, $H\text{-}2^d$, and $H\text{-}2^k$ haplotypes make a high level of PCA antibody to RE, in contrast mice homozygous for the $H\text{-}2^b$ haplotype make low levels of PCA antibody (Table I). The B10.A(4R) and D2.GD recombinant strains which are used to map the *Ir-RE* locus, also produced high levels of PCA antibody after immunization with RE.

The other *Ir* systems utilized in this study employed the synthetic linear random terpolymers GLT and GLϕ. The immune responses to both polypeptides have been previously characterized by Merryman et al. (11, 12). The

TABLE I

Immune Responses To RE and the Terpolymers GLT and GLϕ

Strain	*H-2* type	Immune responses		
		RE (PCA titer)*	GLT	GLϕ
			% *binding*‡	% *binding*‡
B10.A	a	1,280	0	8
C57BL/10	b	40	3	−1
B10.D2	d	1,280	74	72
DBA/2	d	640	79	40
B10.BR	k	640	2	2
B10.A (4R)	h4	1,280	NT§	NT
B10.HTG	g	NT	50	65
D2.GD	g2	640	4	−3

* Values represent reciprocals of the highest serum dilution evoking threshold PCA activity in pooled sera from at least four mice per group. Repeated titrations gave identical results.

‡ Values represent the percent binding of a 1:5 serum dilution with radiolabeled ligand in the Farr antigen-binding assay. The mean percent binding from four to seven individual sera in each group are presented.

§ NT, not tested.

GLT and GLϕ polypeptides induced similar response patterns in all strains tested (Table I). The uniformly high titered antibody responses in mice bearing the $H\text{-}2^d$ haplotype can be clearly distinguished from the responses of congenic $H\text{-}2^a$, $H\text{-}2^b$, and $H\text{-}2^k$ homozygous mice which made no detectable antibody response. Recombinant mice of the B10.HTG strain were responders to these synthetic polypeptide antigens, while the B10.A and D2.GD animals failed to make an antibody response after immunization with GLT or GLϕ (Table I).

DISCUSSION

Previous studies have established that the genes controlling immune responsiveness to the RE, GLT, and GLϕ antigens were *H-2*-linked *Ir* genes (9, 11, 12). The use of C57BL/10 and DBA/2 congenic mice in the current study confirm the *H-2* linkage of each of these *Ir* genes. After immunization with RE high serum levels of IgE antibody were produced by the B10.A(4R) congenic recombinant strain, which derived its *K* and *Ir-1A* subregions from the high responder $H\text{-}2^a$ haplotype and the remainder of its *H-2* complex from the low responder $H\text{-}2^b$ chromosome. This localizes the *Ir-RE* gene in the $K^k\text{-}Ir\text{-}1A^k$ subregion of the *H-2* complex (Table II). The $H\text{-}2^d/H\text{-}2^b$ recombinant strain, D2.GD, is also a high responder to RE indicating this strain bears the *Ir-RE* gene derived from the $H\text{-}2^d$ allele. Assuming the locus for a given *Ir* gene is always situated at the same linear position in the *H-2* haplotype on different *H-2* chromosomes, we can tentatively designate the *Ir-1A* subregion of the D2.GD strain as derived from the responder $H\text{-}2^d$ haplotype (Table II).

TABLE II

Genetic Fine Structure of the H-2-Gene Complex

Strain	*H-2* haplotype	Regions of the *H-2* complex				
		K	Ir-1A (Ir-RE)	IR-1B (Ir-GLT)	S	D
C57BL/10	b	b	b	b	b	b
B10.D2 or DBA/2	d	d	d	d	d	d
B10.BR	k	k	k	k	k	k
B10.A	a	k	k	k | *	d	d
B10.HTG	g	d	d	d	d |	b
B10.A(4R)	h4	k	k |	b	b	b
D2.GD	g2	d	d ?⟷	b	b	b

* Points of recombination are indicated by a vertical bar. The precise location of the crossover in the D2.GD strain relative to that in the B10.A(4R) strain has not been determined.

The *H-2*^{d} haplotype also codes for immune responsiveness to the GLT and GLϕ terpolymers. The D2.GD recombinant mice which inherited the *Ir-RE* gene from the *H-2*^{d} chromosome did not inherit the *Ir-GLT* or *Ir-GLϕ* genes from the *H-2*^{d} parent. The intra-*H-2* crossover event which occurred during the establishment of the D2.GD line separated the *Ir-RE* and *Ir-GLT* (Ir-GLϕ) loci, thereby establishing that these are distinct loci and map independently of each other.

The tentative mapping of the *Ir-GLT* and *Ir-GLϕ* genes within the *I* region is indicated in Table II. This tentative localization places these genes in the *Ir-1B* subregion, based on the failure of D2.GD and B10.A mice to make a detectable immune response after hyperimmunization with the GLT or GLϕ polypeptides. We assume that responsiveness to either of these terpolymers is controlled by a single locus. The antibody responses elicited in the B10.HTG strain after immunization with either terpolymer support the provisional fine structure mapping. In data not shown, we observed strong immune responses in C3H.OL mice after immunization with the GLϕ polypeptide; the latter strain has the *K*^{d} and *I*^{d} regions, but its *S* and *D* regions are derived from the nonresponder *H-2*^{k} haplotype.

A serological analysis of the *H-2*^{g2} haplotype carried by the D2.GD strain has been reported by Lilly and Klein (8). These investigators serologically identified the *K*^{d} and *D*^{b} regions and presumed that the crossover occurred to the left of the *S* region since the strain was *S1p*^{o}, like the *H-2*^{b} (8). Shreffler and David (5) tested D2.GD lymphocytes with a battery of anti-Ia antisera. The data demonstrated that the *H-2*^{g2} haplotype lacked certain *Ia*^{d} and *Ia*^{b} specificities, Shreffler and David tentatively placed the crossover between *Ir-1A*^{d} and *Ir-1B*^{b} (5). Their localization of the crossover event based on serology is similar to that reported here.

We cannot determine whether the *Ir-GLT* and *Ir-GLϕ* genes represent

genetically separable loci or if responsiveness to both polypeptides is controlled by a single *Ir* locus. The GLT and GLϕ terpolymers are chemically similar, each is a random linear polypeptide consisting of 57 mole percent L-glutamic acid and 38 mole percent L-lysine. The tyrosine and phenylalanine residues, which constitute the remaining 5% of each polypeptide differ only by an oxygen atom. The present data do not distinguish between the *H-2* distribution of GLT and GLϕ responsiveness. However, when animals bearing different *H-2* haplotypes were tested differences in GLT and GLϕ responsiveness were apparent.[2]

An important issue to consider is the number of specific *Ir* loci in the genome. The data from the mouse (7, 9, 10, 13) and the Rhesus monkey[3] have established that at least two genetic regions separable by recombination can control immune responsiveness. If there is a large cluster of specific *Ir* genes we would not expect the crossover in the $H\text{-}2^{g2}$ and $H\text{-}2^{h4}$ chromosomes to have occurred at identical positions along the parental $H\text{-}2^{b}$ chromosome, as indicated in Table II. On the other hand, if only two or three loci are coded for the the *I* region crossing-over could functionally have occurred at the same position in the formation of the $H\text{-}2^{g2}$ and $H\text{-}2^{h4}$ chromosomes. If a very small number of *Ir* loci exist in the *H-2* complex, each *Ir-1* allele must have pleiotropic effects (i.e., a single allele controls responsiveness or nonresponsiveness to a large number of different antigens) to account for the unique patterns of immune responsiveness to a variety of antigens characteristic of each haplotype. Continued analysis of the immune response patterns of the D2.GD and B10.A(4R) strains with additional antigens will provide further data on the fine structure of the *H-2* complex and an indication of the complexity of the *I* region. This report represents the first step in this new approach for the fine structure mapping of the *I* region.

SUMMARY

This report characterizes the intra-*H-2* crossover in the D2.GD mouse strain. Recombination occurred within the *I* region between the immune response (*Ir*) gene controlling immune responsiveness to ragweed extract (RE), *Ir-RE*, and the *Ir* gene governing responsiveness to the random linear terpolymers of glutamic acid and lysine with either tyrosine or pheylalanine (*Ir-GLT* and *Ir*-GLϕ). The *Ir-RE* gene was tentatively located in the *Ir-1A* subregion and the *Ir-GLT* and *Ir-GLϕ* gene(s) tentatively placed in the *Ir-1B* subregion. The importance of this recombinant strain to further studies on the fine structure of the *I* region is discussed.

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