H-2-LINKED IMMUNE RESPONSE (Ir) GENES

INDEPENDENT LOCI FOR *Ir-IgG* AND *Ir-IgA* GENES

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Studies of the genetic control of immune responsiveness of mice to BALB/c IgA and IgG (γ2a) myeloma proteins indicate that there are autosomal dominant immune response (Ir) genes associated with the capacity to respond to each of these classes of myeloma proteins and that these Ir genes are linked to different H-2 specificities (1, 2). It has previously been shown that the *Ir-IgA* gene, which controls the immune response to IgA myeloma proteins, maps essentially into the same chromosome region as the *Ir-1* gene of McDevitt et al. (3, 4) and is associated with the *H-2 K* region of the H-2 complex (1). The linkage of both *Ir-IgA* and *Ir-IgG* (the latter gene controls the immune response to γG myeloma proteins) to the H-2 locus raises the question of whether these are separate genes or alleles at a single locus.

As *Ir-IgA* is associated with the $H\text{-}2^a$ chromosome and *Ir-IgG* is associated with the $H\text{-}2^b$ chromosome, the existence of a series of recombinant mouse strains in which the H-2 region is derived from crossovers between the $H\text{-}2^a$ and $H\text{-}2^b$ chromosome types permits us to map the *Ir-IgG* gene in relation to the *Ir-IgA* gene. This study forms the basis of the current paper.

Recombinant strains derived from $H\text{-}2^a/H\text{-}2^b$ crossovers and congenic to the C57BL/10 were separately immunized with IgG and IgA myeloma proteins derived from BALB/c mice. Of the five recombinant strains tested, one was responsive to both IgA and IgG myeloma proteins. This indicates that *Ir-IgA* and *Ir-IgG* are separate genes. Moreover, the demonstration of the separation of these two genes in the small number of recombinant strains tested suggests that considerable genetic material exists between these genes and raises the possibility that many H-2-linked Ir genes may exist.

Materials and Methods

Preparation and Source of BALB/c Myeloma Proteins.—The BALB/c IgG (γ2a) myeloma proteins MOPC 173 (M173) and LPC1 and the IgA myeloma proteins MOPC 467 (M467) and MOPC 406 (M406) were obtained from Dr. Michael Potter, NIH, Bethesda, Md. The preparation of the myeloma proteins has been described previously (5).

The IgG myeloma proteins carry allotypic determinants $G^{1,6,7,8}$ on the constant regions

of their heavy chains in addition to myeloma-specific idiotypic (Fab) determinants (6). The IgA myeloma proteins carry heavy-chain constant region determinants $A^{12, 13, 14}$ in addition to the myeloma-specific idiotypic (Fab) determinants (6).

Description of Recombinant Strains of Mice.—The recombinant strains used in these studies possess H-2 chromosomes derived from exchanges between $H\text{-}2^a$ and $H\text{-}2^b$. They include the chromosomes $H\text{-}2^{h\text{-}Sg}$, $H\text{-}2^{h\text{-}2Sg}$, and $H\text{-}2^{h\text{-}3Sg}$ present in strains B10.A(1R), B10.A(2R), and B10.A(4R), respectively. The three H-2 combinations have the *H-2D* region of $H\text{-}2^b$ and the *H-2K* region of $H\text{-}2^a$. The H-2 chromosomes of strains 1R and 2R were derived from a

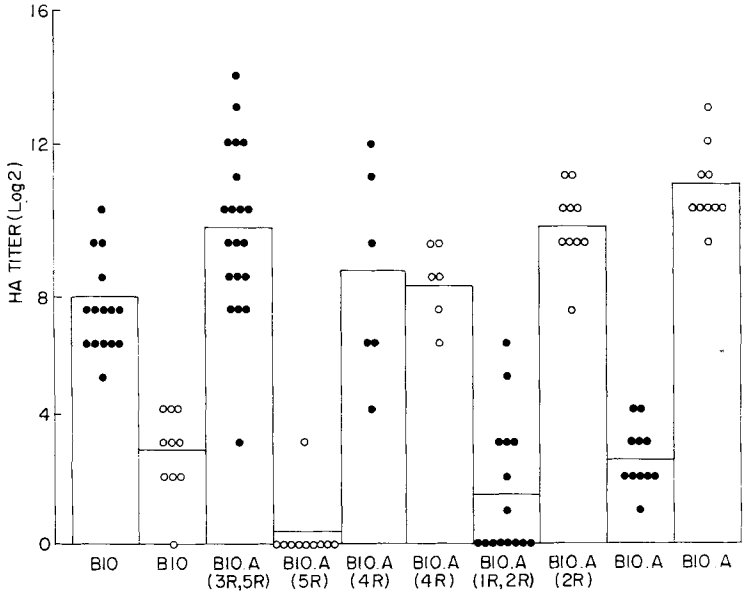

FIG. 1. The immune response of B10 ($H\text{-}2^b$), B10.A ($H\text{-}2^a$), and $H\text{-}2^a/H\text{-}2^b$ crossover mice B10.A(1R), B10.A(2R), B10.A(3R), B10.A(4R), and B10.A(5R) to IgG (γ2a) and IgA myeloma proteins of BALB/c origin is shown. The 1R and 2R are combined as the H-2 products have not yet been distinguished. This is also true of 3R and 5R. Solid circles indicate HA titer to IgG myeloma proteins; open circles to IgA myeloma proteins. Geometric mean titers are shown for each strain.

crossover between the *H-2D* and *SsSlp* subdivision of the *H-2* complex, while the H-2 chromosome of strain 4R resulted from a crossover between *SsSlp* and *H-2K*. Two other recombinant strains, B10.A(3R) and B10.A(5R), bear the H-2 chromosomes $H\text{-}2^{i\text{-}Sg}$ and $H\text{-}2^{i\text{-}2\ Sg}$ derived from two independent crossovers between the *SsSlp* and *H-2K* regions of the *H-2* complex. In this case, both combinations possess the *H-2D* subdivision of $H\text{-}2^a$ and the *H-2K* subdivision of $H\text{-}2^b$. All the recombinant strains used are congenic to C57BL/10 (B10).

All of the mice used in this study share none of the recognized allotypic markers with the IgA or IgG myeloma proteins of the BALB/c strain.

Immunization.—Procedures used to immunize mice with BALB/c IgG and IgA myeloma proteins M173 and M467, respectively, were previously described (1, 2).

Antibody Assay.—Each immune serum was tested to the respective myeloma proteins M173 or M467 used for immunization in addition to a different myeloma protein of the same immunoglobulin class (LPC1 or M406) that was not used for immunization. The antibody to the immunizing myeloma protein was determined by titration with chromic chloride-treated

erythrocytes coated with the specific myeloma protein (7). These antibodies may identify allotypic determinants present on the constant region of the heavy chain in addition to idiotypic determinants that are myeloma specific and are on the Fab segment. Anti-allotypic antibody alone was determined by titration of chromic chloride-treated erythrocytes coated with a different BALB/c myeloma protein (LPC1 or M406, respectively) that was not used for immunization.

RESULTS

Immune Response of H-2^a and H-2^b Mice and of H-2^a/H-2^b Recombinant Mice to BALB/c IgG (γ2a) and IgA Myeloma Proteins.—The antibody response of B10 (H-2^b) and B10.A (H-2^a) and of a series of B10 strains possessing H-2

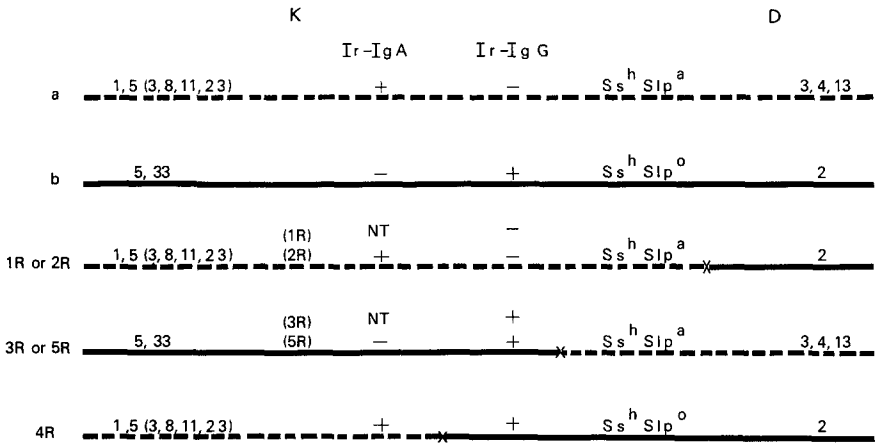

FIG. 2. Schematic representations are shown of the chromosomes of B10.A (H-2^a), B10 (H-2^b), and of the H-2^a/H-2^b crossover chromosomes of B10.A (1R, 2R), B10.A (3R, 5R), and B10.A (4R). The region of crossover in the generation of each recombinant chromosome is indicated by an *X*. It has previously been established that the B10.A(4R) chromosome resulted from a crossover event between the *K* region of *H-2* and the *SsSlp* genes. The data presented in Table I indicate that the crossover separated the *Ir-IgA* and *Ir-IgG* genes which occur in this region. This indicated that *Ir-IgA* and *Ir-IgG* are at separate loci. The 1R and 2R are combined as the H-2 products have not yet been distinguished. This is also true of 3R and 5R.

regions derived from H-2^a/H-2^b recombinations to the BALB/c IgG (γ2a) myeloma protein MOPC 173 and to the IgA myeloma protein MOPC 467 is illustrated in Fig. 1. As was previously reported, the B10 mice have a high response to IgG myeloma proteins and a low response to IgA myeloma protein (2). The B10.A, on the other hand, manifests a low response to IgG myeloma proteins and a high response to IgA (2). The B10.A recombinant strains 3R and 5R demonstrate a high response to an IgG protein and the 5R demonstrates a low response to an IgA protein. The data from 3R and 5R were combined since the H-2 products of these strains have not yet been distinguished. On the other hand, the B10.A (1R) and (2R) strains manifest a poor response to IgG and the 2R has a high response to IgA. Data of 1R and 2R have also been

combined since their H-2 products have not yet been distinguished. Inspection of chromosome maps of Fig. 2 reveal a linkage between the *H-2K* region and the *Ir-IgA* and *Ir-IgG* in these recombinant strains. Thus, B10.A (1R) and B10.A (2R) possess the H-2K region gene of the $H\text{-}2^a$ and have the same responsiveness that the B10.A ($H\text{-}2^a$) displays. Similarly, the B10.A (3R) and 5R share the same H-2K region gene of the $H\text{-}2^b$ and have the same immune responses to IgG and IgA myeloma proteins that the B10 ($H\text{-}2^b$) exhibits.

The immune response of the B10.A (4R) strain is exceptional. As it shares the *H-2K* region gene of the $H\text{-}2^a$ type, it would have been anticipated that it

TABLE I

Immune Response of $H\text{-}2^a/H\text{-}2^b$ Recombinant Strains to BALB/c IgG ($\gamma 2a$) (M173) and IgA (M467) Myeloma Proteins: Specificity of Response

Strain	H-2* type	IgG			IgA		
		No. of mice	HA titer (log 2)		No. of mice	HA titer (log 2)	
			M173‡	LPC1§		M467‡	M406§
B10.A	a	11	2.88 ± 0.08	2.70 ± 0.19	10	10.8 ± 0.37	2.00 ± 0.39
B10.A (1R)	ha	9	1.64 ± 0.66	0.03 ± 0.21	NT	NT	NT
B10.A (2R)	ha	6	2.70 ± 0.31	0.04 ± 0.30	10	9.50 ± 0.37	2.00 ± 0.22
B10.A (4R)	ho	6	8.33 ± 1.19	1.58 ± 0.71	6	7.83 ± 0.47	3.16 ± 0.79
B10	b	15	7.28 ± 0.35	6.83 ± 0.25	10	2.38 ± 0.32	0.1 ± 0.00
B10.A (3R)	ia	10	9.77 ± 1.11	5.25 ± 0.89	NT	NT	NT
B10.A (5R)	ia	10	9.25 ± 0.59	2.12 ± 0.33	10	0.30 ± 0.30	0.00 ± 0.00

* Stimpfling, J. H., and A. Richardson. 1965. Recombination within the histocompatibility-2 locus of the mouse. *Genetics.* **51**:831.

‡ Antisera reacted to the specific myeloma protein immunogens (M173 or M467) may identify the myeloma specific Fab idiotypic determinants and also the allotypic determinants on the constant region heavy chain.

§ Antisera reacted to different myeloma proteins of the same class (LPC1 or M406) as the specific immunogens identify only the allotypic determinants. All the myeloma proteins were of BALB/c origin. B10 = C57BL/10. NT = not tested.

should be a high responder to IgA and a low responder to IgG proteins, just as the B10.A and the B10.A (2R) are. However, B10.A (4R) mice consistently demonstrate high responses to both IgA and IgG proteins. On the basis of this finding, the crossover event resulting in the H-2 complex of the B10.A (4R) strain appears to have occurred between the *Ir-IgA* and the *Ir-IgG* genes. The ordering of *Ir-IgA* and *Ir-IgG* in Fig. 2 and the assignment of both these genes to the region between H-2K and SsSlp was chosen because it involves only a single crossover, as illustrated. Other orders would require more complex genetic events but they cannot yet be formally excluded.

Specificity of Antibody Produced by $H\text{-}2^a/H\text{-}2^b$ Recombinants in Response to BALB/c IgG ($\gamma 2a$) and IgA Myeloma Proteins.—The sera of mice immunized

with IgG (M173) or IgA (M467) myeloma proteins were examined for antibodies capable of agglutinating red cells coated with the immunizing myeloma protein and for antibodies which agglutinate red cells coated with a different myeloma protein of the same class (IgG; LPC1 and IgA; M406) (Table I). The latter antibodies are directed at allotypic determinants on the constant region of the heavy chain and are referred to as anti-allotype antibodies.

The antibodies which agglutinate red cells coated with the immunogen may identify either anti-allotype antibodies or antibodies to myeloma-specific determinants on the Fab portion of the immunoglobulin molecule. These latter antibodies are specific for idiotypic determinants. Thus, agglutination of erythrocytes coated with the immunogen may reflect anti-idiotype and/or anti-allotype antibody.

B10 and B10.A (3R), 4R, and 5R are high responders to γG myeloma proteins. The antibodies of the B10.A (4R) and 5R are largely directed at idiotypic determinants as the agglutination titers for these sera are quite low to an IgG myeloma protein (LPC1) different from the immunogen (M173). On the other hand, the B10 and the B10.A (3R) produce conisderable amounts of anti-allotype antibody. This pattern of responsiveness is particularly interesting in that it reveals a difference between the B10.A (3R) and the B10.A (5R) mice which in other respects are indistinguishable. The response to IgA (M406) in strains B10.A, B10.A (2R), and B10.A (4R) was very low for anti-allotype antibody although the hemagglutination titer of their sera to erythrocytes coated with the immunizing IgA protein (M467) was quite high. This implies the response to IgA in each of these strains is largely directed at idiotypic determinants and is in accord with previously reported findings (1, 2).

Comparison of Ir-IgA and Ir-IgG to Ir-1 in H-2 Complexes Derived from $H\text{-}2^a/H\text{-}2^b$ Crossovers.—The data presented in Fig. 1 in regard to Ir genotype of the B10.A series of recombinant strains were compared with the published data on the immune responses of these mice to the branched-chain polymers (tyrosine, glutamic acid)-D-L-alanine--lysine [(T,G)-A-L][1] and (histidine, glutamic acid)-D-L-alanine--lysine [(H,G)-A-L] (Table II). These latter responses are controlled at the *Ir-1* locus (4).

Ir-IgA and the Ir gene controlling (H,G)-A-L were associated to each other and to the *H-2K* gene characteristic of the $H\text{-}2^k$ type in each of the recombinants tested, including B10.A (4R). *Ir-IgG* and the Ir gene controlling (T,G)-A-L were both found in the B10 strain but the B10.A (4R) possessed *Ir-IgG* but did not respond to (T,G)-A-L. This suggests that *Ir-IgG* and the Ir gene controlling (T,G)-A-L are at different loci. Whether *Ir-IgA* and the Ir genes controlling the response to (T,G)-A-L and (H,G)-A-L are individual genes or alleles at a single locus is still unresolved from this analysis.

[1] *Abbreviations used in this paper:* DNP, 2,4-dinitrophenyl; GL, copolymer of L-glutamic acid and L-lysine; (H, G)-A-L, (histidine, glutamic acid)-D-L-alanine--lysine; PLL, poly-L-lysine; T, thymus-derived; (T, G)-A-L, (tyrosine, glutamic acid)-D-L-alanine-lysine.

H-2 Chromosome Types and Ir-IgA, Ir-IgG, and Ir-1.—A further clarification of the relation between *Ir-IgA*, *Ir-IgG*, and *Ir-1* was sought by comparing the responses of a series of strains of different H-2 types to IgA and IgG myeloma proteins and to (T,G)-A-L and (H,G)-A-L (Table III). The response to the branched-chain polymers was obtained from published reports (4).

This analysis revealed three chromosome types in which a discordance between the response to IgG myeloma proteins and that to (T,G)-A-L was noted and one clear instance in which the response to IgA myeloma proteins and the response to (H,G)-A-L was discordant. The H-2 types associated with these

TABLE II

Immune Response Genes Associated with Histocompatibility Regions Derived from H-2^a/H-2^b Crossovers

Strain	H-2* type	Derivation of chromosome regions			Ir genes			
		K	D	SS-Slp	IgG	IgA	(T,G)-A-L‡	(H, G)-A-L‡
B10.A	a	k	d	h-a	−	+	−	+
B10.A (1R)	ha	k	b	h-a	−	NT	NT	NT
B10.A (2R)	ha	k	b	h-a	−	+	NT	+
B10.A (4R)	ho	k	b	h-o	+	+	−	+
B10	b	b	b	h-o	+	−	+	−
B10.A (3R)	ia	b	d	h-a	+	NT	NT	−
B10.A (5R)	ia	b	d	h-a	+	−	NT	−

NT, = not tested.

* Stimpfling, J. H., and A. Richardson. 1965. Recombination within the histocompatibility-2 locus of mouse. *Genetics.* **51**:831.

‡ McDevitt, H. O., and B. Benacerraf. 1969. Genetic control of specific immune responses. *Adv. Immunol.* **11**:31.

discordances were *H-2^p*, *H-2^r*, and *H-2^s*. These data reinforces the notion that more than one distinct Ir loci exists within the H-2 complex and suggests, indeed, that there may be more than two such loci.

DISCUSSION

Histocompatibility-linked immune response (Ir) genes play an important role in the induction of immune responses to a wide variety of antigens in several species (8). To explain the differing pattern of association of various Ir genes with histocompatibility loci, a large number of alleles would be required if only a single Ir locus existed. A plausible alternative would be a series of linked Ir genes.

In the studies reported here, an *H-2^a/H-2^b* recombinant mouse strain was re-

sponsive to both IgG and IgA myeloma proteins although the "parental" strains responded to either IgG or IgA proteins but not to both. This result demonstrates that the crossover event which was responsible for the chromosome type found in the *H-2*$^{h\text{-}3Sg}$ recombinant strain B10.A (4R) occurred between the *Ir-IgA* and *Ir-IgG* loci, thus providing evidence for two separate loci.

TABLE III

H-2 Chromosome Types Associated with Various Immune Response Genes

Strain	H-2 type	Derivation of chromosome region*			Ir genes			
		K	D	SS-Slp	IgG	IgA	(T, G)-A-L‡	(H, G)-A-L‡
B10.BR	k	k	k	1-o	−	+	−	+
B10.A	a	k	d	h-a	−	+	−	+
B10	b	b	b	h-o	+	−	+	−
B10.D2	d	d	d	h-a	−	−	?(±)	?(±)
B10.F	n	n	b		+	?(±)	NT	NT
B10.P	p	p	p	h-a	+	?(±)	−	−
DBA/1	q	q	q	h-o	−	−	−	−
LP.RIII	r	r	r	h-o	+	+	−	NT
A.Sw; SJL	s	s	s	h-a	+	+	−	−
SM	v	v	v	h-a	+	−	NT	NT

?(±), moderate response with a wide range of variation.

NT, not tested.

* Stimpfling, J. H., and A. Richardson. 1965. Recombination within the histocompatibility-2 locus of the mouse. *Genetics.* **51**:831.

‡ McDevitt, H. O., and B. Benacerraf. 1969. Genetic control of specific immune responses. *Adv. Immunol.* **11**:31.

A comparison of the pattern of association of *Ir-IgA* and *Ir-IgG* with that of the *Ir-1* locus demonstrated certain clear differences. Although many similarities exist between responsiveness to IgA myeloma proteins and to (H,G)-A-L, mouse strains of the *H-2*^{s} type are responsive to IgA and unresponsive to (H,G)-A-L. Furthermore, the response to IgG proteins and (T,G)-A-L has a similar association pattern when mice possessing *H-2* chromosomes of the a, b, d, and k types are considered but the response of mice bearing chromosomes of the n, p and s types are different.

Although the number of H-2-linked Ir loci is still unknown, it is of interest that those genes which have been mapped thus far all appear to be located be-

tween the *H-2K* and *SsSlp* regions of the *H-2* complex. The failure to localize Ir genes to the region between the serum substance genes and the D region gene may simply reflect the relatively small number of systems studied thus far. Alternatively, the region between K and Ss may be a specialized Ir area and the *Ss-D* interval may subserve another function or the amount of genetic material in the two regions may be very different.

A recent provocative finding has suggested that differences in Ir genotype may have a role in mixed lymphocyte reactivity (9). The precise significance of this finding is uncertain, but it suggests that Ir gene products may be important lymphocyte surface structures.

The chemical nature and biologic function of the Ir gene product are currently unknown. Evidence from the study of Ir genes of guinea pigs and mice indicate that these genes function primarily in thymus-derived (T) lymphocytes (8, 10). Moreover, recent data obtained by Shevach et al.[2] in the study of guinea pig Ir genes suggest that the gene product functions at the cell surface and demonstrate that alloantisera block this function in vitro.

The demonstration that Ir genes function at the cell surface, that they are largely expressed on T cells, and that more than one and probably many loci exist raise the possibility that the Ir gene product is the receptor of the T cell. This suggestion merits particular consideration in view of the fact that the chemical nature of the antigen-binding receptor of the T cell is still controversial.

The possibility that the Ir genes codes for T cell receptors, although tempting, is probably not great. If immunoglobulin is the T cell receptor, then the Ir gene would have to code for variable regions of immunoglobulin light and/or heavy polypeptide chains. Although definitive genetic evidence excluding this possibility is not yet available, the association of genes for Fab and Fc in the rabbit (11), and the lack of association of Fc genes with *H-2* complex in the mouse (12, 13) make it very unlikely that variable region genes for mouse immunoglobulin are within the *H-2* complex.

If the T cell receptor is not immunoglobulin, the above argument, of course, is no bar to the Ir gene product being the receptor. However, evidence from the analysis of apparent specificity of control of Ir genes and specificity of T-dependent immune responses weighs against the identity of the Ir gene product and the T cell receptor. Thus, in the guinea pig, the immune response to poly-L-lysine, poly-L-arginine, protamine, the copolymer of L-glutamic acid and L-lysine (GL), and their haptenic derivatives are controlled by a gene linked to the gene(s) controlling histocompatibility specificities of strain 2 guinea pigs (10). Nonetheless, animals immunized with 2,4-dinitrophenyl (DNP) poly-

[2] Shevach, E. M., W. E. Paul, and I. Green. 1972. Histocompatibility-linked immune response gene function in guinea pigs. Specific inhibition of antigen-induced lymphocyte proliferation by alloantisera. *J. Exp. Med.* **136**:1207.

L-lysine display very meager cellular immune responses to DNP-GL although their responses to DNP-PLL are marked (14). Although there is the possibility that this specificity difference may be explained by a large series of linked Ir genes, the great heterogeneity in specificity of T cell reactions would require this series to be very large indeed.

Although it seems unlikely that the Ir gene functions as the T cell's prime antigen receptor molecule, it may serve an auxilliary role lending a moderate additional energy of interaction between antigen and the cell surface so that the net energy of binding between the antigen and a cell bearing a specific antigen-binding receptor would be very high. This might help to explain how the T cell, although bearing apparently few receptors (15), could be the exquisitely antigen-sensitive cell which it is.

Finally, one further distinction of an unexplained nature is evident from the data presented in this study and the preceding paper. Thus, although the genetic control of responsiveness is very clear when antibody capable of binding to the immunizing myeloma protein is considered, additional differences are observed when the anti-allotype response is studied. In the previous paper, it was shown that different strains of mice of the same histocompatibility type, although having generally similar antibody titers when tested against the immunizing γG protein, showed marked differences when the antisera were tested against other γG myeloma proteins not used for immunization. One possible explanation for this distinction lay in the range of receptors possessed by the precursors of antibody-secreting cells in the various strains. The examination of the B10.A recombinant strains yields a similar finding. Thus, strains B10, B10.A (3R), B10.A (4R), and B10.A (5R) all produce comparable amounts of anti-idiotype antibody but B10 and B10.A (3R) make much more anti-allotype antibody than do strains B10.A (4R) and the B10.A (5R). As these mice are congenic, the reason for this distinction presumably lies within the *H-2* complex and suggests a new level of genetic complexity.

SUMMARY

Two H-2-linked autosomal dominant immune response (Ir) genes *Ir-IgG* and *Ir-IgA* were demonstrated to be at separate loci. *Ir-IgG* controls the immune response to IgG (γ2a) myeloma proteins and *Ir-IgA* the immune response to IgA meyloma proteins. Both genes are associated with the *H-2K* region specificities of the *H-2* chromosome, specifically *Ir-IgG* with $H\text{-}2^b$ and *Ir-IgA* with $H\text{-}2^a$. Different recombinants derived from $H\text{-}2^a/H\text{-}2^b$ crossovers were examined for their immune responsiveness to BALB/c IgG (γ2a) and IgA myeloma proteins. B10 ($H\text{-}2^b$) parental type responded only to IgG; B10.A ($H\text{-}2^a$) responded only to IgA. All the recombinants except for B10.A (4R) responded to either IgG or IgA. B10.A (4R), however, responded to both IgG and IgA. This indicated that the crossover event giving rise to B10.A (4R) occurred between the *Ir-IgG* and *Ir-IgA* loci.

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