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AUTOIMMUNE MURINE THYROIDITIS

V. GENETIC INFLUENCE ON THE DISEASE IN BSVS AND BRVR MICE

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SUMMARY

Two inbred strains of mice, BSVS and BRVR, are similar in their antibody response to thyroglobulin but differ significantly with respect to thyroid gland damage. F_1 hybrids, like BSVS mice, respond well to thyroglobulin, exhibiting extensive thyroid lesions, whereas BRVR mice show minimal thyroid damage. *H*-2 typing of the two strains showed that BSVS mice have a type similar to *H*-2^{th}, with the *H*-2D-end of *H*-2^d and the *H*-2K-end of *H*-2^s, whereas BRVR mice are *H*-2^k. The cellular response to thyroglobulin appears to be influenced by a specific immune response gene located near the K-end of the *H*-2 complex.

INTRODUCTION

Recently it was found that the quantitative ability of different animals to make antibody to a particular antigen is a genetic trait, controlled by autosomal dominant genes, often at loci situated within the major histocompatibility region. Histocompatibility-linked specific immune response genes have been described in guinea-pigs, mice and rats (Benacerraf & McDevitt, 1972). While in the majority of experiments synthetic antigens were used, we have shown in mice a relationship between the immune response to an autoantigen, thyroglobulin, and the major histocompatibility type (Vladutiu & Rose, 1971).

In studying the cellular and humoral response to thyroid antigen in a large number of inbred strains of mice, we found that some strains of particular *H*-2 types were good responders, i.e. they developed high titres of thyroid autoantibody and severe thyroid lesions, while other strains were poor responders, having low titres of antibody and almost normal thyroids. A good correlation was found between the thyroid antibody titre and the severity of the thyroid lesions. An exception to this general finding was observed in two inbred strains, BSVS and BRVR. These were originally differentiated and bred for their immune response to *Salmonella enteritidis* and St. Louis encephalitis virus (Webster, 1937).

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They were later used as experimental animals for the induction of several autoimmune diseases (Boehme, 1966; Werdelin & Boehme, 1969). Surprisingly, the *H*-2 specificities of these two strains were not known. This report deals with the study of autoimmune thyroiditis in the BSVS and BRVR strains and the determination of their *H*-2 types.

MATERIALS AND METHODS

Animals

Female mice, 7–9 weeks old, of BSVS and BRVR strains were raised in our own animal unit, the breeding stock having been originally supplied by the Rockefeller University. They were kept in groups of ten in metal cages and fed Rockland food pellets and tap water *ad libitum*. F_1 hybrids were obtained using either BSVS or BRVR mice as mothers; progeny were weaned at 4 weeks of age.

H-2 typing

Twenty *H*-2 specificities were assayed in strains BSVS and BRVR by ^{51}Cr-cytotoxic method (Snell, Demant & Cherry, 1971). Some selected specificities were also checked by quantitative absorption with blood cells (David & Shreffler, 1972a).

TABLE 1. Results of direct cytotoxic tests of BSVS and BRVR

Reagent No.	Antiserum: Recipient	Antiserum: Donor	Cytotoxic titre against: BSVS	Cytotoxic titre against: BRVR	*H*-2 specificity	Other antibodies present
155	(C3H.Q × B10.D2)F_1	C3H.R3	160	1280	1	18?
143	(B10.D2 × A)F_1	HTH	0	0	2	18?
142	(B10.A.CA)F_1	B10.D2	320	20 VW	3	4, 13?, 31?
147	(B10 × AKR.M)F_1	B10.A	1280	0	4	
150	(B10.D2 × HTG)F_1	B10	160	160	5	33
137–1	C3H	C3H.B10	1280	—	6	2,...
149	A	A.SW	320	0	7	19
135	(B10 × A.SW)	A.CA	0	40 VW	8	9
136	(B10.A × A.SW)	A.CA	0	0	9	
145	(C3H.OH × A.SW)	C3H	0	320	11	23
141	(C3H × B10)	C3H.Q	20	0	13	30
S-13*	(B10.A. × A.SW)	B10.P	0	0	16	—
S-28*	(B10 × AKR.M)	B10.G	0	0	17	—
S-24+AS-	(B10 × A)	B10.S	320	0	19	7
C-23†	(B10 × LP.RIII)	B10.BR	0	1280	23	32
84–1	AKR	AKR.M	1280	0	28	6, 13, 27, 29, 30
146	(B10.A × A.KR)	AKR.M	0	0	30	
148	A	D2	160	160 W	31	34?
151	(A × B10.D2)	HTI	0	0	33	39
152	(A × B10.A	B10.K	0	40	32	

* Antisera supplied by Dr J. Klein.

† Antisera supplied by NIH Transplantation Immunology Branch.

W, weak; VW, very weak.

TABLE 2. Alloantigenic profiles of the BSVS and BRVR strains and of *H*-2th and *H*-2^{k}*

	H-2 specificity																				
Strain	1	2	3	4	5	6	7	8	9	11	13	16	17	19	23	27, 28, 29	30	31	32	33	Ss	Slp
BSVS	1	–	3	4	5	6	?	–	–	–	13	–	–	19	–	?	–	–	–	–	h	a
H-2th	1	–	3	4	5	6	–	—	—	—	13	—	—	19	—	—	—	—	—	—	h	a
BRVR	1	—	3	—	5	—	—	8	—	11	—	—	—	—	23	—	—	—	32	—	1	o
H-2^{k}	1	—	3	—	5	—	—	8	—	11	—	—	—	—	23	—	—	—	32	—	1	o

* *H*-2th from David & Shreffler, 1972b; *H*-2^{k} from Snell, Demant & Cherry, 1971.

Skin grafting

The method of Billingham and Medawar was used (1951). Recipients of grafts (tail skin) were observed daily for the first 3 weeks and then weekly up to 60 days.

Thyroiditis assay

The immunization procedure consisted of two subcutaneous injections at 7-day intervals, of allogeneic thyroid extract from non-inbred CF-1 mice, emulsified in Freund's complete adjuvant, as previously described (Vladutiu & Rose, 1972). The animals were killed at different times after immunization and the haemagglutination antibody titre and thyroid pathology index were determined as reported (Vladutiu & Rose, 1971).

RESULTS

The results of direct cytotoxic tests with a panel of anti-*H*-2 sera are shown in Table 1. In most cases the antisera were monospecific. Quantitative absorptions with blood cells were performed to check *H*-2·4, *H*-2·19 and *H*-2·31. BRVR failed to absorb antibodies against all three specificities, while BSVS absorbed anti-*H*-2·4 and anti-*H*-2·19, but failed to absorb anti-*H*-2·31. The two strains were also typed for Ss and Slp. The *H*-2 alloantigenic specificities and Ss-Slp types of BSVS were found to be very similar to those of recombinant type *H*-2^{th} (David & Shreffler, 1972b) while BRVR had the *H*-2 specificities and Ss-Slp type of *H*-2^{k}. The alloantigenic profiles and Ss-Slp types of BRVR and BSVS along with those of H-2^{th} and *H*-2^{k} are shown in Table 2.

To test further whether the *H*-2 types of BRVR and BSVS are identical to the known *H*-2^{k} and *H*-2^{th} strains, several F_1 skin grafting experiments were carried out as shown in Table 3. (BRVR × B10)F_1 recipients accepted B10.K grafts, indicating that BRVR is not detectably different from *H*-2^{k}. (BRVR × C3H) F_1 recipients rejected C3H.OL grafts, eliminating a possibility that BRVR might have been *H*-2^{o1}. (BSVS × A)F_1 recipients accepted both A.TL and A.TH grafts, indicating that BSVS is very similar to *H*-2^{th} and H-2^{t1}; however, (BSVS × A.BY)F_1 recipients rejected both A.TH and A.TL grafts, indicating that there are some *H*-2-associated differences. Since the (BSVS × A)F_1s did not reject, and since A.TH and A.TL have the same *H*-2D region as A, those differences are apparently at the D-end of the *H*-2 complex.

The two strains differed sharply in their cellular response to thyroid antigen, but responded similarly with respect to thyroid antibody titre. The F_1 hybrids of the two strains

Table 3. Skin graft tests of F_1

Recipient	No.	Donor	Donor's type	Result
(BRVR × B10)F_1	7	B10.K	H-2^{k}	Accepted
(BRVR × C3H)F_1	7	C3H.OL	H-2^{o1}	Rejected
(BSVS × A)F_1	7	A.TH	H-2^{th}	Accepted
(BSVS × A)F_1	7	A.TL	H-2^{t1}	Accepted
(BSVS × A.BY)F_1	7	A.TH	H-2^{th}	Rejected
(BSVS × A.BY)F_1	7	A.TL	H-2^{t1}	Rejected

TABLE 4. Haemagglutination titre and pathology index in BSVS and BRVR strains and F_1 hybrids*

Strain	Antibody titre (log_2)	Pathology index
BSVS	4·9±0·9	3·5±0·3
BRVR	4·8±0·3	0·8±0·3
(BSVS × BRVR)F_1	4·4±0·9	2·5±0·4

* Ten animals in each group, injected twice at 7-day intervals with thyroid extract and killed 16 days after the first injection.

were relatively good responders with respect to thyroid pathology, although their response was intermediate between the two parental strains (Table 4).

DISCUSSION

Years ago, it was shown that the BRVR and the (BRVR × BSVS)F_1 mice are completely resistant to experimental allergic encephalomyelitis. The resistance was considered to be due to two equipotent genetic factors, either of which, in single dosage, by simple dominance, was sufficient to confer resistance (Lee *et al.*, 1954). In sharp contrast to BSVS, the BRVR mice were also resistant to induction of allergic orchitis and allergic myositis (Boehme, 1965a, b). BSVS mice were much more sensitive to anaphylaxis after sensitization with crystalline bovine serum albumin and had an increased sensitivity to histamine and serotonin following the injection of pertussis vaccine (Lee, 1964). However, basis of the differences in the response of the two strains to the induction of autoimmune diseases was not found. The response of the reticuloendothelial system to antigens was rather similar in BSVS and BRVR mice (Boehme, 1965a, b). It was suggested that the resistance of BRVR mice to Arbor B virus infection was expressed at the cellular level in a type of macrophage (Goodman & Koprowski, 1962).

The response of different strains of mice to mouse thyroid antigen is a trait related to their *H*-2 types (Vladutiu & Rose, 1971). The F_1 transmission of the trait and the association with *H*-2 suggested, but did not definitely establish, a single gene autosomal dominant mode of inheritance. The immune responses of mice to purified thyroglobulin or to a crude preparation of thyroid extract as used in this experiment are quite similar, regardless of the antigen preparation (Vladutiu & Rose, 1972). It is known that immune response genes controlling responsiveness to different polypeptides are distinct. Since thyroglobulin is a large molecule, with multiple antigenic determinants, it is not possible at this time to determine which sequence has a major role in the genetically controlled immune response.

Of particular interest is the mapping of the trait, within or adjacent to the *H*-2 complex. As emphasized by McDevitt *et al.* (1972) the mapping of *H*-2-linked specific response genes for different antigen has a value in explaining the mechanism of action of the specific immune response genes. Our previous results (Vladutiu & Rose, 1971), gave some suggestion that the trait maps at the K-end of the *H*-2 complex. *H*-2^a strains have the K-end of *H*-2^k and the D-end of *H*-2^d and show a pattern of response like *H*-2^k. *H*-2^i strains have the K-end of *H*-2^d and the D-end of *H*-2^a and respond poorly, as do *H*-2^b mice. The present results with strain BSVS are also consistent with a K-end location, since the strain apparently

has the K-end of H-2^s and the D-end of H-2^d and responds like H-2^s. The findings with the BRVR strain, however, present some difficulties in interpretation. Serologically and by skin graft tests, BRVR is H-2^k; yet it gives a low pathology index, while all other H-2^k strains gave a high index. Three possible explanations may be offered for this inconsistency: (1) there may have been a mutation in strain BRVR in an immune response gene from high to low responsiveness; (2) through one or more recombination events, a new poorly responding immune response allele may become associated with the H-$2K^k$ and H-$2D^k$ alleles of BRVR (this is quite possible if the trait maps outside the H-2 complex; it is much less likely if the trait is between H-2K and H-2D as is Ir-1); (3) there may be, in the BRVR background, a non-H-2 gene which regulates the typical H-2^k associated response to thyroid antigen. If such a suppression took place only at the level of thyroid tissue damage, it could explain the lack of correlation between antibody titre and pathology index with this strain. Crosses are in progress to test for such a non-H-2 effect.* The possibility exists that BRVR and BSVS mice may also differ at other loci.

Ir-1 was shown to be within the H-2 complex, between the H-2K locus and the Ss-Slp loci (McDevitt *et al.*, 1972). Susceptibility to Gross murine virus leukaemia is mediated by Rgv-1, a gene located at the K-end of the H-2 complex (Benacerraf & McDevitt, 1972). It seems that susceptibility to autoimmune thyroiditis may also be dependent on a factor either within the H-2 complex or closely linked to H-2. This could be an Ir-1 or a new gene.

The cellular immune response to an autoantigen is a most important feature since the characteristic of an organ-specific autoimmune disease is the typical mononuclear infiltration of the target organ. A clear cut quantitative difference between responder and non-responder animals is seen particularly well with respect to cellular immunity and carrier function (Benacerraf & McDevitt, 1972). It was assumed that Ir genes are expressed in thymus-derived lymphocytes (Green, Paul & Benacerraf, 1972). These genes influence the specificity of antigen recognition by thymus-derived and possibly by bone marrow-derived cells (Benacerraf & McDevitt, 1972). The possible importance of the thymus in experimental and human autoimmune diseases has been pointed out many times (Allison, Denman & Barnes, 1971). A subject of future investigation will be the elucidation of the role of the hypothetical gene in controlling the thymus-derived cells in self-recognition.

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* The results so far obtained suggest both an H-2 and a non-H-2 effect.

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