H-2K MUTATION CONTROLS IMMUNE RESPONSE PHENOTYPE OF AUTOIMMUNE THYROIDITIS

Critical Expression of Mutant Gene Product in Both Thymus and Thyroid Glands*

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Experimental autoimmune thyroiditis (EAT) can be induced by injecting susceptible animals with thyroglobulin together with a suitable adjuvant (1). The disease is characterized by infiltration of the thyroid gland by mononuclear cells. Studies by Tomazic et al. (1) and Vladutiu and Rose (2) identified genes linked to the major histocompatibility complex (H-2) that regulated relative susceptibility or resistance to induction of EAT. The H-2 genotype was found to influence the incidence and degree of severity of disease in a population and did not code for an absolute yes-or-no disease phenotype. Mice genetically resistant to induction of EAT were found to produce antibodies to mouse thyroglobulin to a degree similar to that produced by mice genetically susceptible to EAT (2). Thus, the capacity to recognize mouse thyroglobulin as an immunogen was characteristic of most mice, irrespective of their H-2 genotype and EAT phenotype. Therefore, H-2 genes regulate the expression of EAT, not merely by defining high or low responders to the self-antigen, but by influencing immune mechanisms capable of damaging the thyroid gland after immunization. The fine structure or character of the response to thyroglobulin would appear to be critical for the disease phenotype.

We recently found that an apparent point mutation at the H-2K locus rendered mice susceptible to induction of EAT (3). C57BL/6 (B6) mice (H-2^b) are resistant to induction of EAT (2), whereas the B6.H-2^{ba} mutant strain was observed to be susceptible. In this paper, we report that expression of the mutant H-2K gene in two organs is critical for the EAT phenotype. These organs are the target thyroid gland and the thymus.

Materials and Methods

Mice. Inbred strains of mice were supplied by the Animal Breeding Center of the Weizmann Institute of Science, Rehovot, Israel. They included B6 (H-2^b), B6.H-2^k (H-2^k), B6.H-2^{ba} (H-2^{ba}), C3H/eB (H-2^k), BALB/c (H-2^d), B10.D2 (H-2^d), and (B6.H-2^{ba} × B6)F_1 hybrid strains. Nude mice that bore the nu/nu gene on three genotypes were used: B6, and the hybrids (C3H/eB × B6)F_1 and (BALB/c × B6)F_1 hybrids.

Implantation of Thyroid Glands. Parental-strain thyroid glands were implanted under the kidney capsules of (H-2^{ba} × H-2^b)F_1 hybrids at the age of 8 wk, as described elsewhere (4). EAT was induced 4–5 wk later.

Implantation of Thymus Glands. 4-wk-old nude mice were each implanted subcutaneously with

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six lobes of thymus glands obtained from 4-wk-old mice. Before implantation, the thymus glands were irradiated with a dose of 1,000 rad from a gamma-beam ^{60}Co source (Atomic Energy of Canada Limited Commercial Products, Ottawa, Canada). The mice were injected 4-6 wk later with thyroglobulin extract in adjuvant to induce EAT. Functional reconstitution of the nude mice was assayed by their ability to produce antibodies to thyroglobulin, a thymus-dependent antigen (5). About 80% of the nude mice implanted with thymuses produced these antibodies. Mice that did not do so were considered not to possess functional thymuses and were not included in the results.

Induction of EAT. EAT was induced as described (6) by injecting each mouse subcutaneously with thyroglobulin extract obtained from one thyroid gland. The extract was emulsified in incomplete Freund's adjuvant (Difco Laboratories, Detroit, Mich.), to which had been added 7 mg/ml *Mycobacterium tuberculosis*, H37Ra strain (Difco Laboratories). This injection was repeated 1 wk later. Donors of thyroglobulin extract were mice of the C3H/eB strain. 4-5 wk later, EAT was assayed by removing thyroid glands of recipient mice, fixing them in 10% formalin solution and then in 70% alcohol, and examining microscopic sections stained with hematoxylin and eosin. Microscopic slides were coded and examined without knowledge of their identity. A diagnosis of EAT was made by observing at least one uniquivocal focus of infiltration by mononuclear cells.

Antibodies to Thyroglobulin. Two methods were used to assay antibodies to purified thyroglobulin (7): Passive hemagglutination was done by coating mouse erythrocytes with thyroglobulin as described (8). Antibodies were also measured using a modification of a solid-phase radioimmunoassay, as described elsewhere (9).

Results

B6.H-2^{ba} Mutation Maps a Critical Gene to the H-2K Locus. We confirmed our earlier finding (3) that the B6.H-2^{ba} mutant was susceptible to induction of EAT in contrast to the wild-type B6, which was relatively resistant (Table I). The incidence and severity of EAT in B6.H-2^{ba} mice was similar to that of B6.H-2^{k} mice that have susceptible H-2^{k} alleles throughout the entire H-2 complex, or to that of C3H/eB mice that have the H-2^{k} genotype on a background of other genes independent of the B6 genetic background. Thus, the H-$2K^{ba}$ mutation was functionally equivalent to multiple H-2^{k} alleles present in the other EAT-susceptible mice.

Table I also demonstrates our finding that BALB/c and B10.D2 mice were susceptible to induction of EAT. These results were at variance with those published by Tomazic et al. (1). The reasons for this discrepancy are unknown, but have been confirmed using other strains with H-2^{d} alleles (DBA/2 and NZB) obtained from our animal facilities and elsewhere (R. Maron and I. R. Cohen. Manuscript in preparation.).

TABLE I

*H-2^{ba} Mutation of Resistant Strain B6 Leads to Susceptibility to EAT Equal to that of Susceptible H-2^{k} or H-2^{d} Genotypes**

Mouse strain	Non-H-2	H-2	Titer of antibodies to thyroglobulin	Incidence of EAT
			log₂	%
B6	B6	b	5	20 (10/49)
B6.H-2^{ba}	B6	ba	7	79 (27/34)
B6.H-2^{k}	B6	k	8	70 (7/10)
C3H/eB	C3H	k	5	82 (46/56)
BALB/c	BALB	d	6	94 (29/31)
B10.D2	B10	d	7	78 (29/37)

* EAT was induced by injection of thyroglobulin extract emulsified in adjuvant. Antibodies to purified thyroglobulin were measured by a passive hemagglutination assay.

TABLE II

*Susceptibility to EAT Controlled by the B6.H-2^{ba} Mutation Is Expressed at the Level of the Target Thyroid Gland**

Incidence of EAT in thyroids		
Endogenous (B6.H-2^{ba} × B6)F_1	Implanted parental	
	B6.H-2^{ba}	B6
%	%	
60 (31/51)	54 (26/48)	23 (7/30)‡

* (B6.H-2^{ba} × B6)F_1 hybrid mice were implanted under the kidney capsule with thyroid glands originating from either parental strain. 30 of the mice received glands of both types. EAT was induced 4–5 wk later and the incidence of lesions in each type of thyroid was determined.

TABLE III

*Susceptibility to EAT Controlled by B6.H-2^{ba} Mutation Is Expressed at the Level of the Thymus Gland Implanted into Thymusless Nude Mice**

Group	Genotype of nude recipient mice	Genotype of implanted thymus gland	Titer to antibodies to thyroglobulin‡	Incidence of EAT
				%
A	(C3H/eB × B6)F_1 H-2^k × H-2^b	None	<1/10	0 (0/4)
		C3H/eB (H-2^k)	1/100–1/250	73 (11/15)
		B6 (H-2^b)	1/50-1/250	8 (1/11)
		B6.H-2^{ba}	1/100-1/250	80 (8/10)
B	(BALB/c × B6)F_1 H-2^d × H-2^b	BALB/c (H-2^d)	1/100–1/1,000	70 (12/17)
		B6 (H-2^b)	1/100–1/1,000	17 (2/12)
		B6.H-2^{ba}	1/100–1/1,000	17 (1/6)
C	B6 H-2^b	B6.H-2^{ba}	1/100–1/250	13 (1/8)

* 4-wk-old nude mice were implanted with six lobes of irradiated (1,000 rad) thymus glands and EAT was induced 4–6 wk later.

‡ Antibody titers were assayed by the solid-phase binding assay (9). The titer was measured as the last fivefold dilution giving a counts per minute value threefold greater than the counts per minute value of the control sera. The cpm of control sera ranged from 552 to 846. Maximal cpm of test sera ranged from 6,131 to 8,209.

Critical Expression of the B6.H-2^{ba} Mutation at the Level of the Target Thyroid Gland. The comparison (Table I) between the mutant B6.H-2^{ba} and the wild-type B6 emphasizes the decisive importance of the H-2K gene and, hence, of the H-2K glycoprotein in regulating the pathophysiology of EAT. Because the H-2K glycoprotein is expressed on all the cells of the body (10), we decided to see if any particular cells whose expression was signficant for the EAT phenotype could be detected.

We used the following experimental preparation (4) to investigate the role in EAT of the H-2K glycoprotein expressed in the target thyroid gland. F_1 hybrids were bred between B6.H-2^{ba} mutant mice and B6 wild type mice, because F_1 hybrids between susceptible and resistant strains of mice were shown to be susceptible to induction of EAT (1). Under the capsules of the kidneys of these F_1 mice, we implanted thyroid glands originating from either parental type. In this way, the F_1 mice were outfitted with three different thyroid glands: their endogenous glands expressing both H-$2K^b$ and H-$2K^{ba}$, one implanted gland expressing the H-$2K^b$ product and one implanted gland expressing the H-$2K^{ba}$ product. 4–5 wk later, we induced EAT in these mice. The results are shown in Table II. The glands orginating from the B6.H-2^{ba} parent

had an incidence of EAT (54%) similar to that of the endogenous F_1 thyroid glands (60%). In contrast, the glands originating from the wild-type B6 parent had a significantly lower incidence of EAT (23%).

Expression of H-2K in the Thymus Gland Regulates Inducibility of EAT. Zinkernagel et al. (11) and Bevan (12) found that the genotype of the thymus was important in defining the H-2 products seen by cytotoxic T lymphocytes in associative recognition with antigens on target cells. We therefore investigated the possible role of the H-2K locus expressed on radioresistant thymic epithelial cells in regulating the EAT phenotype to determine what would be the effect on EAT of a thymus gland of H-$2K^{ba}$ compared with that of H-$2K^{b}$ origin.

Nude mice of three genotypes were available: B6, (C3H/eb × B6)F_1, and (BALB/c × B6)F_1. Thymus glands were obtained from mice of various donor strains, irradiated to kill donor thymus lymphocytes, and implanted into the nude mice. These thymus-restored nude mice were then tested for their EAT phenotypes by injecting them 1 mo later with mouse thyroglobulin extract in adjuvant.

Table III shows the results of these groups of experiments. The mice in group A, (C3H/eB × B6)F_1, had the susceptible Ir genotype of hybrids between susceptible H-2^k and resistant H-2^b parental mice. In the absence of an implanted thymus, antibodies to thyroglobulin could not be detected and EAT was not observed. The presence of thymus glands of H-2^k origin led to a 73% incidence (11/15) of EAT. Mutant B6.H-2^{ba} glands achieved a similar high incidence of EAT (80%; 8/10). In contrast, thymus glands of B6 origin produced a low incidence of EAT (8%; 1/11), although all the mice demonstrated reconstitution of thymus-dependent antibody production to thyroglobulin. Hence, we could conclude that the incidence of EAT was a function of the type of H-2K glycoprotein expressed in thymus grafts. In contrast, the ability to recognize thyroglobulin as an immunogen for helper T lymphocytes and antibody production was insensitive to the difference between H-2^{ba} and H-2^b.

To extend these findings, we implanted thymus glands into (BALB/c × B6)F_1 nude mice (Table III, group B). Implantation of thymus glands of BALB/c (H-2^d) origin led to a susceptible EAT phenotype (70%; 12/17). However, thymus glands of either B6 (H-2^b) or B6.H-2^{ba} origin failed to generate a susceptible EAT phenotype (16%; 2/12 and 1/6). Moreover, B6.H-2^{ba} thymus glands were not effective in permitting EAT in B6 nude mice (group C). Hence, B6.H-2^{ba} thymus epithelial cells restored the EAT phenotype of nude mice of the susceptible (H-2^k × H-2^b)F_1 genotype but not of the susceptible (H-2^d × H-2^b)F_1 or resistant H-2^b genotype.

Discussion

Differences between the EAT phenotypes of wild-type B6 and mutant B6.H-2^{ba} mice must be related to the H-2^{ba} mutation. The present evidence, although not yet absolute, compels us to conclude that the H-2^{ba} mutation occurred exclusively in the H-2K gene (13, 14). Thus, it is the H-2K glycoprotein whose expression is decisive for the EAT phenotype of the mutant mice. H-2K is, therefore, an Ir gene and the H-2K glycoprotein is its molecular agent. Ir genes regulating the responses to trinitrophenyl-modified cells (15) and to the Thy-1 alloantigen (16) have been mapped to the H-2K locus using H-2 recombinant mice. Our findings using the B6.H-2^{ba} mutant mice strengthen the conclusion that an Ir regulatory function and a target cell H-2K

syngeneic signal are pleiotropic effects coded for by a single H-2K structural gene expressed in both the target thyroid and the thymus.

How does expression of the H-2K glycoprotein in both the thymus and thyroid regulate the EAT phenotype? It is conceivable that damage to cells in the thyroid gland in EAT depends on an attack by cytotoxic T lymphocytes against a critical antigenic determinant of thyroglobulin. We propose that such a determinant might be made more available to cytotoxic T lymphocytes when thyroglobulin is associated with H-$2K^{ba}$ than with H-$2K^{b}$ glycoprotein.

A prerequisite for recognition of thyroglobulin in association with a particular H-2K glycoprotein is the ability to see the H-2K glycoprotein as a legitimate associated signal for cytotoxic T lymphocytes. The experiments of Bevan (12) and of Zinkernagel et al. (11) would suggest that the role of the thymus implants in our experiments was to induce functional differentiation of such T lymphocytes. It is interesting that thymuses of B6.H-2^{ba} origin contributed to a susceptible EAT phenotype in (H-2^{k} × H-2^{b})F_1, but not in (H-2^{d} × H-2^{b})F_1 nude mice (Table III). The reason for this is unknown, but may be explained by similarity of structure between H-2^{k} and H-2^{ba} gene products. It was found that cytotoxic T lymphocytes of B6 origin immunized against B6.H-2^{ba} cells caused lysis of H-2^{k} target cells (17).

The association of thyroglobulin with H-2K in the thyroid suggests a more fundamental role for H-2 gene products than that of presenting viruses (11) or foreign antigens. Thyroglobulin is not an exogeneous antigen but an endogenous product exported from epithelial cells of the thyroid itself. Conceivably H-2 gene products might have a primary, nonimmune function in the transport of macromolecules across cell membranes. Substances leaving or even entering cells might do so associated with H-2 gene products. Hence, by focusing on H-2 products, T lymphocytes would be able to efficiently survey the transactions of the cells of the body. Associative recognition of antigens with H-2 products may be seen as a secondary exploitation by the immune system of a molecular mechanism first developed for another purpose.

The results presented in this paper shed light on a number of issues: they illustrate the pleiotropic effects of a single H-2K structural gene on the early antigen-independent differentiation of T lymphocytes in the thymus and on the terminal performance of fully mature cytotoxic T lymphocytes in the antigenic target; they show that a single point mutation in an Ir gene product can have far-reaching consequences for the pathophysiology of autoimmunity; they imply that cytotoxic T lymphocytes have a critical role in the lesions of EAT; they demonstrate associative recognition of antigen plus H-2K in vivo; and they suggest a fundamental association between H-2K and export of an endogenous cell product.

Summary

Autoimmune thyroiditis (EAT) can be induced by immunizing mice against mouse thyroglobulin. A gene critical to the phenotypical expression of EAT was mapped to the H-2K locus by studying B6 mice and its mutant strain B6.H-2^{ba}. To identify organs in which expression of the gene was decisive for the EAT phenotype, we transplanted thyroid or irradiated thymus glands into various strains of normal mice or thymusless nude mice. We found that the pathophysiology of EAT was controlled by the expression of specific H-2 genes in both the target thyroid gland and the thymus gland.

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