(Reprinted from Nature, Vol. 279, No. 5715, pp. 715-716, June 21 1979)

Mutation at H-2K locus influences susceptibility to autoimmune thyroiditis

GENES in the major histocompatibility complex (MHC) have been found to be associated with the development of autoimmune diseases, including spontaneous diseases in man[1,2] and spontaneous[3] or experimentally induced autoimmunity in animals[4]. Experimental autoimmune thyroiditis (EAT) can be induced in susceptible strains of mice, such as those with the MHC H-2^k haplotype, by injecting them with mouse thyroglobulin together with complete Freund's adjuvant[5]. In contrast, injection of mice homozygous for the H-2^b haplotype fails to induce the mononuclear cell infiltration of the thyroid gland characteristic of EAT. We report here that susceptibility to induction of EAT results from an apparent point mutation which evidently occurred at the H-2K locus of the resistant H-2^b haplotype. This indicates that the H-2K glycoprotein can serve to regulate the autoimmune response to thyroglobulin.

Table 1 shows the results of injecting mouse thyroid extract in adjuvant into mice of strains C3H/eb (H-2^k), C57BL/6 (H-2^b) or its HZ1 mutant (B6.C-H-2^{ba})[6,7]. C3H/eb mice demonstrated the response of high-responder H-2^k strains with about 80% incidence of development of EAT. Only about 20% of C57BL/6 mice developed lesions of EAT, as expected for low responder mice of H-2^b strains[5,8]. However, the HZ1 (H-2^{ba}) mutant showed an incidence of EAT (79%) comparable with that of high-responder H-2^k mice. Hence, the HZ1 mutation led to susceptibility to EAT in mice whose original genome coded for resistance.

The immunogen used in these experiments was thyroid extract obtained from C3H/eb mice. Mouse strains differ in the immunogenicity of their thyroglobulin[9] and we have found that thyroid extract from C3H/eb mice is strongly immunogenic whereas that of both C57BL/6 and HZ1 mice is poorly immunogenic for either high- or low-responder strains of mice (in preparation). These findings suggest that the increased incidence of EAT in the HZ1 mutant mice was not due to a change in the immunogenicity of their thyroglobulin.

Note that all groups developed antibodies to purified thyroglobulin after injection with thyroid extract in adjuvant. This confirms the observation that mice develop specific antibodies to determinants of thryoglobulin after injection with extract, whether or not they are genetically susceptible to histological EAT[10].

Table 1 Susceptibility to EAT of the HZ1 mutant (H-2^{ba}), and H-2^b low- and H-2^k high-responder strains of mice

Mouse strain	H-2 haplotype	Injection of thyroid extract plus adjuvant	Incidence of EAT	Antibody titre (log_2)
C3H/eb	k	Yes	82% (46/56)	5.0
C57BL/6	b	Yes	20% (10/49)	5.0
HZ1	ba	Yes	79% (11/14)	7.0
		Adjuvant alone	0 (0/5)	<1.0

Mice were injected twice subcutaneously at 7-d intervals with thyroid extract emulsified in complete Freund's adjuvant, as described by Twarog and Rose[21]. The extract was prepared from thyroid glands of C3H/eb mice. Each mouse received the equivalent of one thyroid gland. The adjuvant[21] was prepared by adding 7 mg ml^{-1} of *Mycobacterium tuberculosis* H37Ra (Difco) to incomplete Freund's adjuvant (Difco). The animals were killed 5 weeks after the second injection and histological sections of the thyroid glands were examined in a blind fashion by two independent observers. A thyroid gland was considered to be positive for EAT if it showed at least one unequivocal focal infiltrate of mononuclear cells[9]. Antibodies to purified thyroglobulin, prepared as described by Tomazic and Rose[9], were measured in the pooled serum of mice in each group, using a haemagglutination of formalinised tanned sheep red blood cells[22] coated with thyroglobulin. The results represent three separate experiments. Difference in incidence of EAT between C57BL/6 and HZ1 was highly significant by the χ^2 test ($P<0.0003$).

The HZ1 mutant has been studied in several laboratories[7,11-14] and it is generally concluded that a point mutation occurred at the H-2K locus of the C57BL/6 genome. This conclusion is supported by the finding of differences in the peptide map of the H-2K glycoprotein of the HZ1 mutant, compared with the wild-type C57BL/6 strain[15]. This molecular difference would seem to account for the mutual T-cell reactivity of C57BL/6 and HZ1 (refs 13, 16). However, the HZ1 mutant seems to preserve other determinants on the H-2K molecule which are identical to those of the H-$2K^b$ wild type[7,12].

There is no evidence of any differences between C57BL/6 and HZ1 in the I region to the right of the H-2K locus, as the immune response (Ir) genes which functionally define the I region are the same in the mutant and wild-type mice[17]. Thus, it seems very likely that the only genetic differences between the wild-type C57BL/6 and the HZ1 mutant mouse are at the H-2K locus of the MHC[7]. Hence, our results indicate that the H-2K glycoprotein can regulate the pathological expression of EAT following an autoimmune response to thyroglobulin. This implies a major role for cytotoxic T cells in EAT. Furthermore, the limited portion of the H-$2K^b$ gene in which the mutation occurred, the Z1 locus[6], seems to be critical in determining susceptibility to EAT.

Previously, H-2K or H-2D gene products have been observed to restrict the cytotoxic effects of T lymphocytes against target cells infected with certain viruses[18]. T lymphocytes obtained from mice immunised against viruses will often kill virus-infected target cells only when the target cells and the lymphocytes have H-2K or H-2D genes in common. Note that HZ1 and C57BL/6 differ in the specificity of virus-H-2K-associated cytotoxicity[13]. It is thought that associative recognition by T lymphocytes of viral antigens together with MHC gene products is based on the molecular association of viruses with MHC gene products at the surface of the infected cell[19,20]. It remains to be determined whether susceptibility to EAT also involves physical association between H-2K glycoprotein and thyroglobulin on the surface of thyroid epithelial cells. Such an association could occur if MHC gene products served to control movement of macromolecules across cell membranes.

The different susceptibilities to induction of EAT of the C57BL/6 strain and its HZ1 mutant suggest that these mice may be helpful in determining how MHC gene products regulate immune responses. Any differences between the mice in the handling or presentation of thyroglobulin by macrophages, lymphocytes or other cells could be related to the specific modification of the H-2K glycoprotein. Furthermore, isolation of purified H-2K glycoproteins may allow study of the interaction of these molecules with specific determinants of thyroglobulin.

We thank Professor M. Feldman for his support. This work was supported by grants from the Stiftung Volkswagenwerk and the US-Israel Binational Science Foundation, Jerusalem.

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Received 2 March; accepted 15 May 1979.

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Printed in Great Britain by Macmillan Production Ltd., Basingstoke, Hampshire