Immunology 1988 **63** 737–739

Genetic control of oral tolerance to ovalbumin in mice

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Accepted for publication 7 December 1987

SUMMARY

We have investigated the genetic basis of oral tolerance to OVA in a number of inbred mouse strains. Our results emphasise the efficiency of the oral route for inducing tolerance and provide evidence for both MHC and non-MHC linked control of oral tolerance.

It has been suggested that a breakdown in tolerance for delayed-type hypersensitivity (DTH) responses after oral administration of protein antigens allows the development of clinical food sensitive enteropathies, such as coeliac disease (Mowat, 1984; 1987). The association of the HLA-DQw2 locus in 80–90% of patients with coeliac disease (Howell *et al.*, 1986) suggests that a gene(s) within the human major histocompatability complex (MHC) influences the development of intestinal hypersensitivity to food proteins, such as gluten. Although there have been previous studies of genetic differences in oral tolerance in mice, these looked primarily at antibody responses or *in vitro* proliferative responses to antigen in a limited number of strains. Furthermore, there has been no attempt to establish MHC-linked control (Stokes, Swarbrick & Soothill, 1983; Tomasi *et al.*, 1983). Accordingly, we have examined the genetic control of oral tolerance by feeding the protein antigen ovalbumin (OVA) to a large panel of inbred mouse strains bearing several different MHC haplotypes. In addition, we have compared feeding and i.v. administration of antigen as methods of inducing tolerance, and have examined whether these routes share the same genetic basis.

Inbred female mice were purchased from Harlan Olac Ltd (Bicester, Oxon) and were maintained in the departmental animal facility on a standard laboratory rodent diet containing no OVA. All mice were first used at 8–10 weeks of age.

OVA (Sigma fraction V; Poole, Dorset) was dissolved in saline before use. Oral tolerance was induced and its effects on systemic immunity assessed as described elsewhere (Lamont, Gordon & Ferguson, 1987). Briefly, experimental groups received 25 mg OVA in 0·2 ml saline intragastrically (i.g.) via a rigid feeding tube, while control mice received 0·2 ml saline i.g. A further experimental group were given 25 mg native OVA by i.v. injection. Fourteen days later, all groups were immunized with 100 μg OVA in 50 μl complete Freund's adjuvant into one rear footpad. Three weeks post-immunization, the serum antibody and DTH responses of all groups were assessed. Animals were bled from the retro-orbital plexus and serum IgG anti-OVA antibodies were measured by an ELISA (Lamont *et al.*, 1987).

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DTH responses were assessed by the increment in footpad thickness 24 hr after challenge of the non-immunized footpad with 100 μg heat-aggregated OVA. DTH responses were expressed as means + 1 SD and were compared using the Student's *t*-test. Antibody responses are expressed as the geometric mean and were compared using Wilcoxon's rank sum test. Percentage suppression was calculated as follows:

$$\% \text{ suppression} = \frac{(\text{response in control group} - \text{response in experimental group})}{\text{response in control group}}.$$

The effects of feeding a standard dose of OVA on systemic immune responses were examined in a total of 13 inbred mouse strains. The results are shown in detail in Fig. 1 and are summarized in Table 1.

There was considerable strain variation in the degree of tolerance induced, confirming previous studies using limited numbers of mouse strains (Stokes *et al.*, 1983; Tomasi *et al.*, 1983). Although there was no clear overall correlation between the extent of tolerance and H-2 haplotype, several interesting points emerged from our studies. First, all strains examined in this study exhibited at least 44% suppression of systemic DTH responses after feeding (Table 1), an observation consistent with our previous suggestion that prevention of food antigen-specific DTH responses may be a major homeostatic role for oral tolerance (Mowat, 1987). Second, results in the three strains on the BALB background provide some evidence for MHC-linked control of oral tolerance, with BALB/c mice having approximately 90% suppression of both DTH and antibody responses, while the tolerance in BALB/B and BALB/K mice was considerably less marked. In subsequent experiments, we have confirmed these differences, and in particular, we have found that BALB/B mice often show no systemic tolerance after feeding OVA (Mowat, Lamont & Bruce, 1987). Further support for some degree of MHC-linked control of oral tolerance is suggested by the fact that mice bearing the H-2^d haplotype developed good tolerance of systemic DTH, irrespective of their background genes. However, it should be noted that the other H-2^b strain examined (C57Bl/10) had excellent tolerance of systemic DTH, in contrast to the findings in BALB/B mice.

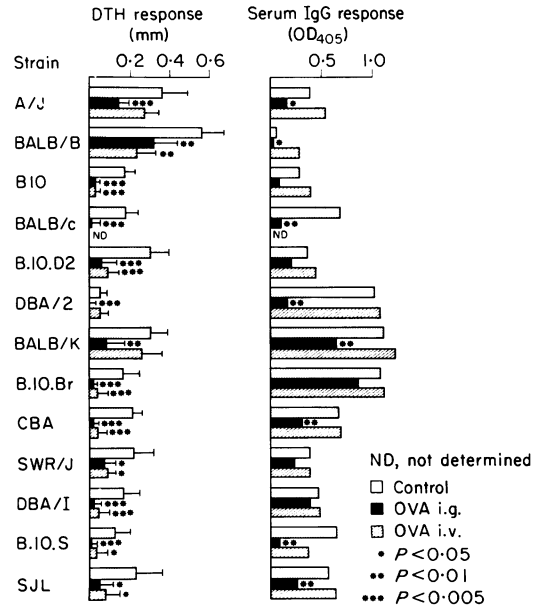

Figure 1. Degree of tolerance induced in different inbred mouse strains by a single feed or i.v. injection of 25 mg OVA 14 days before immunization. DTH responses were assessed by the increment in footpad thickness 24 hr after challenge with 100 μg heat-aggregated OVA, and the results are shown as the mean + 1 SD for groups of six to eight mice. Serum IgG anti-OVA antibody responses were assessed in an ELISA using, as a positive control sample, antiserum collected from control CBA mice immunized 20 days previously, and, as a negative control sample, normal mouse serum. Antibody results are represented as the geometric mean of OD_{405} readings for groups of six to eight mice.

Table 1. Summary of the results presented in Fig. 1.

		DTH		Antibody	
Strain	H-2	I.g.	I.v.	I.g.	I.v.
A/J	a	59%	23%	59%	<0%
BALB/B	b	44%	58%	54%	<0%
B.10	b	88%	86%	68%	<0%
BALB/c	d	94%	ND	87%	ND
B.10.D2	d	78%	68%	46%	<0%
DBA/2	d	110%	11%	82%	<0%
BALB/K	k	68%	11%	41%	<0%
B.10.Br	k	95%	85%	21%	4%
CBA	k	94%	79%	59%	<0%
SWR/J	k	61%	57%	34%	<0%
DBA/1	q	77%	66%	17%	<0%
B.10.s	s	95%	62%	87%	40%
SJL	s	69%	60%	51%	<0%

The figures represent percentage suppression of the responses of the experimental groups relative to the control group, calculated by the formula in the text. The groups which show significant suppression of the responses are underlined.

ND, not determined.

Furthermore, H-2^k mice of different backgrounds showed marked variation in the induction of tolerance, and, together, these results indicate an additional role for genetic factors outwith the MHC.

Another feature of our study is that at this dose of OVA feeding, there was little correlation between tolerance for systemic DTH and antibody responses. This is shown by the lack of suppression of serum antibody responses in several strains (B.10; B.10.D2; B.10.Br; SWR/J; DBA.1) which had varying degrees of DTH tolerance. These data support the hypothesis that different mechanisms are active in suppressing these limbs of the immune response after feeding (Mowat, 1987).

Finally, virtually no attention has been paid previously to the relative efficiencies to inducing tolerance by oral or i.v. administration of proteins of different strains of mice. The present study shows that feeding OVA is more efficient than i.v. injection at inducing systemic immune tolerance. This was particularly noticeable for antibody responses, where no suppression was observed in any strain given OVA i.v. while 9/12 strains showed significant DTH tolerance. Furthermore, in these strains there was a good correlation between the degree of tolerance induced when OVA was given either i.g. or i.v., suggesting that the final mechanism of suppression in both cases may be similar. However, the difference in tolerogenic potential between the two routes indicates that they may activate this mechanism by different means. This possibility requires to be examined further using doses of i.v. OVA in the range equivalent to that found in the circulation after feeding.

Tolerance induced by the systemic administration of deaggregated antigen has been shown to be controlled by both MHC and non-MHC genes (Azar *et al.*, 1978; Ranges & Azar, 1979). The present results suggest a similar form of control for oral tolerance, with the influence of non-MHC genes being underlined by the similar degree of DTH tolerance found in different MHC haplotypes on the B.10 background. These findings therefore argue against a simple model of Ir gene control of tolerance induction after OVA feeding. Nevertheless, some evidence to support the role of MHC genes in the control of DTH tolerance was obtained, and we suggest that further examination of the BALB/B and BALB/c strains may provide a useful model to study the influence of MHC genes in the development of food hypersensitivity.

ACKNOWLEDGMENTS

This work was supported by MRC Grant G8513090CA. The authors acknowledge the support of the Coeliac Society and the Wellcome Trust in the completion of this work.

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