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MHC class II (I-A) region control of the IgE antibody repertoire to the ABA-1 allergen of the nematode *Ascaris*

M. W. KENNEDY, E. M. FRASER & J. F. CHRISTIE
Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Glasgow

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SUMMARY

ABA-1 is an approximately 14,000 molecular weight (MW) allergen which is among the most abundant proteins synthesized by the nematode parasite *Ascaris*. IgG and IgE responses to it are major histocompatibility complex (MHC)-restricted in rodents and have only been found to occur in rats of the RT1^u haplotype and mice of the H-2^s haplotype. Humans infected with the parasite vary substantially in their immune response to the allergen, but the genetic basis for this is unknown. H-2 recombinant mice were used to identify the region within the MHC controlling antibody responses to the allergen. IgG antibody to immunoaffinity purified ABA-1 was assayed by radio-immunoassay and IgE by passive cutaneous anaphylaxis. This showed that the restriction element is the I-A molecule and that there was some evidence for I-E modulation of the level of response.

It has previously been established that the antibody repertoire to the antigens of the parasitic nematode *Ascaris* is under the control of the major histocompatibility complex (MHC) in infected rodents.[1,2] This effect was particularly clear for responses to a major allergen of the parasite which only elicits a response in H-2^s mice and RT1^u rats, of the haplotypes tested so far.[2,3] This allergen (ABA-1) has been variously sized at between 10,000 and 14,000 MW, is probably equivalent to Allergen A of the parasite[4] and can be purified to homogeneity either by immunoaffinity chromatography or by HPLC.[3,5]

Humans infected with *Ascaris lumbricoides* vary substantially from individual to individual in their IgG antibody responsiveness to this protein[6,7] and our unpublished results have shown that this also extends to the specificity of their IgE antibody responses. We predict that the MHC is in control of this specificity in humans and are currently attempting to confirm this using sera from HLA-typed individuals living in an endemic area for the infection.

In order to identify the region of the MHC involved in recognition of the ABA-1 allergen in mice, the antigen recognition profiles of H-2 recombinant strains (see Table 1 for the haplotypes used) were characterized. The mice were infected with the parasite on three occasions and serum was sampled 14 days after a tertiary exposure, as previously described.[1] Their antigen recognition profiles were characterized by protein A-based immunoprecipitation assay of radio-iodinated parasite antigen followed by gradient SDS–PAGE analysis of the immunoprecipitates. The target antigens used were either excretory–secretory materials (ES) from the lung stage of the parasite, which elicits pulmonary hypersensitivity in humans,[11] or the pseudocoelomic fluid of adult worms (ABF). Figure 1 shows a typical result using the first of these, and illustrates that only those strains which carried I-A^s responded to the 14,000 (MW) molecule. This component is the ABA-1 allergen and is known to be common to ABF and *in vitro*-released ES materials.[12–14]

In order to confirm this apparent class II region control of reactivity to ABA-1, the immunoprecipitations were repeated using the immunoaffinity-purified protein and the radioactivity precipitated by test sera was measured (Table 2). The strains which were recorded as responders by this assay were the same as those whose sera precipitated the M_r 14,000 MW from the heterogeneous mixture illustrated in Fig. 1. This immunoprecipitation assay would have detected antibody of only those subclasses of IgG which bind protein A. The profiles did not differ, however, when the assay was adapted to account for other isotypes.[2,7]

To establish whether this pattern of reactivity also applied to the IgE antibody response, the sera were further tested in a passive cutaneous anaphylaxis assay (PCA) in rats.[2] The results of this are also presented in Table 2, and show that the strains which produced IgE antibody were concordant with those which produced an IgG response as detected by immunoprecipitation. Although not detailed in Table 2, SJL (H-2^s) mice produced an IgE titre similar to the B10.S strain. SJL mice are

Abbreviations: ABA-1, *Ascaris* body fluid allergen-1; ABF, *Ascaris* body fluid (pseudocoelomic); ES, *in vitro*-released 'excretory–secretory' antigens of *Ascaris* lung-stage larvae; HPLC, high-performance liquid chromatography; MHC, major histocompatibility complex; MW, relative molecular weight; SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; PCA, passive cutaneous anaphylaxis.

Correspondence: Dr M. Kennedy, Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Bearsden, Glasgow G61 1QH, U.K.

Table 1. MHC haplotypes of the inbred and H-2 recombinant strains used

Strain	Haplotype	K	A_β	A_α	E_β	E_α	S	D	L
B10.BR	k	k	k	k	k	k	k	k	k
B10.D2	d	d	d	d	d	d	d	d	d
SJL	s	s	s	s	(s	s)	s	s	s
B10.S	s	s	s	s	(s	s)	s	s	s
A.TH	t2	s	s	s	(s	s)	s	d	d
B10.S(9R)	t4	s	s	s	s/k	k	d	d	d
B10.HTT	t3	s	s	s	s/k	k	k	d	d
A.TL	t1	s	k	k	k	k	k	d	d

Brackets represent inability to express the H-2E heterodimer because of the defective E_α^s chain gene. The B10.S(9R) strain will express the I-E molecule because the E_α^k gene product is expressed normally and assembles with the $E_\beta^{s/k}$ chain on the cell surface;[8] the same will presumably also apply to the B10.HTT strain. Taken from the listings in refs 9 and 10.

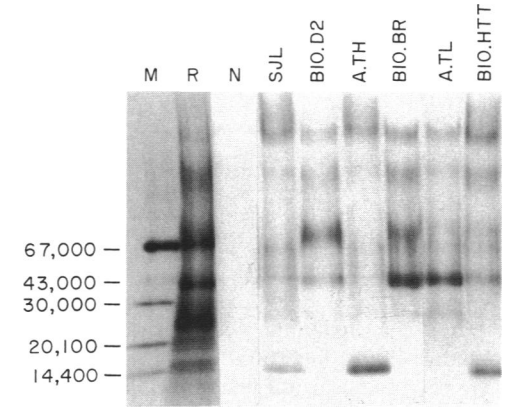

Figure 1. Strain differences in immune recognition of the ABA-1 molecule. Mice of the indicated strains were infected with 2000 eggs of *Ascaris suum* on three occasions 28 days apart and blood was sampled 14 days after the final infection. Pools of serum, to which all members of groups of at least three animals contributed equally, were used in protein A-based immunoprecipitation of ^{125}I-labelled ES material from lung stage larvae of *Ascaris suum*. Protein A-bound (IgG) immune complexes were analysed on 5–25% gradient SDS–PAGE, the gels were fixed, dried and autoradiographed. The ABA-1 molecule has a relative MW of 14,000 under the SDS–PAGE conditions used. MW marker proteins (Pharmacia 17-0446-01) whose relative molecular masses are as indicated (M); R, antigen preparation used in the assay; N, immunoprecipitation profile of serum from normal uninfected mice.

known to have unusual IgE responses and we have previously found it difficult to detect IgE antibody to *Ascaris* infection in this strain.[2]

The above findings argue that responses to the allergen are under I-A control, for the following reasons. First, the D and L regions can be eliminated from involvement because the A.TH strain responded yet does not bear s alleles at these loci. The same argument applies for the E and S regions because the

Table 2. I-A control of IgG and IgE responses to the ABA-1 allergen of *Ascaris*

Strain/serum	H-2 haplotype	% ^{125}I-ABA-1 precipitated	PCA titre
Normal mouse serum		0·9	0
B10.BR	k	1·2	0
B10.D2	d	1·0	0
B10.S	s	10·8	256
A.TH	t2	24·8	256
B10.S(9R)	t4	4·3	128
B10.HTT	t3	3·9	64
A.TL	t1	0·7	0

The ABA-1 allergen was purified by immunoaffinity chromatography as described previously[2,3] and used in both the radio-immunoprecipitation and passive cutaneous anaphylaxis (PCA) assays. For the former, 2 μg protein were labelled with 3·7 mBq ^{125}I and used as target antigen for IgG antibody in a protein A based immunoprecipitation assay.[12] The results are expressed as the radioactivity precipitated as a percentage of the trichloro-acetic acid precipitable activity, and are the means from duplicate assay tubes. For IgE antibody, a PCA assay was carried out in inbred WLEP (Wistar) rats which were given 50 μg ABA-1 protein as challenge antigen and the results are expressed as the last doubling serum dilution to show a reaction. Each test serum was assayed in duplicate rats and the results were identical between duplicates.

B10.S(9R) and B10.HTT strains responded while bearing k or d alleles in these regions. The first of these two strains is known to express the I-E molecule because the E_α^k gene product will substitute for that of the defective E_α^s gene in the assembly and cell-surface expression of the heterodimer.[8] This is also likely to apply to the B10.HTT strain. The response to ABA-1 cannot, however, be I-E-restricted because mice of the H-2^s haplotype always respond to the allergen despite being defective in the expression of the H-2E heterodimer.[8,15] Finally, when an s allele is only present at the K locus, no response occurs to ABA-1. By this process of elimination, therefore, the I-A region is implicated in the control of the response.

There appeared, however, to be some evidence of a role for the I-E molecule in that the B10.S(9R) and B10.HTT strains (both H-2E$^+$) were noticeably poorer responders than were the B10.S or A.TH (both H-2E$^-$). It could be argued, therefore, that the lower responsiveness of these strains was due to I-E-mediated suppressor activity. The I-E molecule has been associated in the past with suppression of I-A-restricted T-cell responses to purified antigens,[16,17] with deletion of substantial parts of the T-cell repertoire,[18,19] presumably through cross-tolerance to the I-E molecule,[20,21] and with enhanced susceptibility to infection with the nematode *Trichinella spiralis*[22] and to the protozoan *Leishmania donovani*.[23] In these cases, the I-E^k molecule was implicated, as it could be in the present study, but no mechanism has yet been defined for the putative I-E-mediated effects.

There are, however, other possibilities, including the modulatory roles played by cytokines, some of which are encoded by genes in the vicinity of the S and D regions. Alleles of these cytokines, or regulatory regions controlling their synthesis and/or secretion, could influence the level of the I-A-restricted anti-ABA-1 response. Moreover, the genes for complement components encoded within the S region are polymorphic[24] and might

vary from haplotype to haplotype in their function as accessories to the immune response.

The response to ABA-1 is, therefore, under conventional class II-associated Ir gene control in the context of infection and of all the haplotypes tested so far (H-2 haplotypes a, b, d, f, g, k, m, q, r, s, and z; ref. 2 and unpublished results) only the $I\text{-}A^s$ molecule permits responsiveness to the allergen in mice. Responsiveness in infected humans is similarly rare despite the relatively large quantities of the protein to which the parasite is likely to expose its host. This would perhaps not be surprising were the molecule to be very small, comprising repeated epitopes or having a homologue in mammalian tissue such that any non-self epitopes will be limited in number and/or subject to tolerance along with self-like epitopes on the molecule. It appears that none of these apply—our knowledge of the N-terminal amino acid sequence, which is currently about 75% complete (ref. 3 and unpublished data), has revealed a sequence with neither repeated motifs nor similarity with any protein whose sequence is known. The rarity of immune recognition is, therefore, particularly surprising but it is probably a general phenomenon in immune responses to the antigens of other parasitic nematodes.[25] These findings have implications, therefore, to the immune responses to complex antigens and allergens of pathogens in general.

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