

The ABA-1 allergen of the nematode *Ascaris suum*: epitope stability, mass spectrometry, and N-terminal sequence comparison with its homologue in *Toxocara canis*

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SUMMARY

ABA-1 is a major allergen of nematode parasites of the genus *Ascaris* which includes the large roundworms of humans and pigs, *A. lumbricoides* and *A. suum*, respectively. The allergen was purified from *A. suum* by immunoaffinity chromatography for immunochemical examination. The IgE antibody repertoire is under MHC control in infected rodents and the IgE-binding epitopes were robust to treatment with heat or periodate, and electroblotting on nitrocellulose. This implies that the IgE epitopes comprise primary peptide sequence or an unusually stable secondary or tertiary structure. The molecular mass of ABA-1 is controversial, but mass spectrometry analysis indicated that there were five components of similar size, with the major species being $14\,643.2 \pm 1.4$ D. Finally, N-terminal sequence analysis of ABA-1 and TBA-1 (the homologue in the canine nematode infective to humans, *Toxocara canis*) revealed a high degree of similarity, and we have previous evidence that ABA-1 homologues are widespread amongst ascaridid parasites of humans. ABA-1 and its homologues might, therefore, be important to the immunopathology of many infections with nematode parasites, upon which the genetic constitution of the hosts will also have a bearing.

Keywords *Ascaris* allergen ABA-1 nematodes MHC restriction IgE

INTRODUCTION

Parasitic nematodes are multicellular organisms which can cause chronic and highly debilitating diseases on a large scale. One of the unifying characteristics of nematodiasis is the induction of high IgE antibody responses, and much of the pathology they cause is associated with Type I hypersensitivities [1,2]. Possibly the most common and widespread of these parasites is *Ascaris lumbricoides*, which is a large gastrointestinal parasite thought to infect more than 1.2 billion people worldwide [3]. *Ascaris* is renowned for its content of potent allergens which presumably contribute to the lethal pulmonary hypersensitivity responses set up by the juvenile stages of the parasite during their migration through the lungs [1,2].

The potent allergenicity of nematode infections has prompted the idea that some characteristic of their antigens, or other feature of the infections, biases the immune system towards reaginic responses. Whether or not this is true, it is

important to understand the structure of their allergens and the factors which control responsiveness to them. This is particularly pertinent to the current effort to produce recombinant vaccines against nematodiasis of humans and domestic animals, in which it would be vital to minimize deleterious hypersensitivity responses initiated or recalled by vaccination.

The nematode allergen about which most information is available, and the first for which any amino acid sequence is available, is the ABA-1 molecule of *Ascaris* [4,5]. This allergen is probably synonymous with Allergen A [6,7] and is the major constituent of the Asc-1 reference allergen preparation provided by the National Institutes of Health (Bethesda, MD) [8]. ABA-1 has been variously sized at between 9 kD and 14 kD by SDS-PAGE [5,9,10], or a 25-kD complex with a 10-kD monomeric unit under high performance liquid chromatography (HPLC) [5]. Its N-terminal amino acid sequence to 41 residues has been reported [4], and, in this study, we extend this information.

The allergen is the major water-soluble protein constituent of the adult parasite and is released by the tissue-penetrative larval stages during culture *in vitro* [9,11]. Despite this relative abundance, most infected people do not appear to produce antibody to ABA-1, and, in experimentally infected rodents, the immune repertoire to it is under the control of the MHC. Only

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H-2^s mice and RT1^u rats have so far been found to respond [4,12] and reactivity in mice is determined by the class II I-A^s heterodimer [13]. Information on the structure of ABA-1 has, therefore, the combined value of contributing to an understanding of its allergenicity and of the genetics of immune responsiveness to its IgE-binding epitopes.

MATERIALS AND METHODS

Ascaris allergen

The source of the purified 14-kD allergen (ABA-1) used here was the pseudocoelomic body fluid (ABF) of adult female *A. suum*. This was collected from parasites recovered from pigs as previously described [9,11,12], centrifuged at 15 000 *g* for 10 min to remove particulate matter, and the supernatant dialysed against PBS overnight at 4°C, then stored at -70°C until used. ABA-1 was immunoaffinity purified using antibody raised in rabbits as follows. Small quantities of ABA-1 were obtained by separation of ABF (unboiled), laced with ¹²⁵I-labelled ABF, on 1.5 mm thick 20% SDS-PAGE gels. Gels were vacuum dried at 60°C, and the 14-kD region excised using an autoradiograph of the gels as a template. The gel matrix was rehydrated in 25 mM Tris, 192 mM glycine, 0.1% SDS buffer, and ABA-1 recovered by electroelution in the same buffer using a Bio-Rad (Richmond, CA) electroelutor (product no. 165-2976), with membrane caps having an exclusion limit of 3500 D (product no. 165-2986). SDS was removed from the protein sample by dialysis against 10% methanol over 48 h at 4°C using 2000-D exclusion limit dialysis membrane (Sigma, D2272), followed by passage through an AffinityPak Extracti-Gel D column (Pierce Chemical Co., N20346) to remove any remaining detergent. The protein sample was concentrated by nitrogen pressure ultrafiltration using a 5000-D exclusion limit membrane (Sartorius, SM14529-025N) and dialysed extensively against PBS.

Adult female *Toxocara canis* were obtained by dissection of infected dogs or following expulsive chemotherapy and perienteric fluid (TBF) was collected in the same way as for ABF from *Ascaris*.

Antisera

Antiserum to ABA-1 was raised by intramuscular injection of 100 µg protein in Freund's complete adjuvant (FCA) into alternate thigh muscles on two occasions, 7 days apart, followed 28 days later by a boost of 100 µg protein in Freund's incomplete adjuvant (FIA) in 5-6 subcutaneous sites on the back. Serum was taken 7 days later at the peak antibody response predicted from previous immunizations assayed by ELISA. Serum from further boosting resulted in antibody of excessive affinity for elution of antigen from immunoaffinity chromatography columns. Mouse, rat and rabbit antisera to ABF or infection with *A. suum* were raised as previously described [4,11,13]. In all cases the rabbits used were Sandy-Lop, obtained from the National Institute for Medical Research, London, UK.

Immunoaffinity chromatography

Rabbit serum was first depleted of albumin and plasminogen by passage over CM Affi-Gel Blue (Bio-Rad 153-7304), and the IgG fraction affinity-purified using Protein-A sepharose CL4B (Sigma, P3391). The Protein-A purified rabbit anti-14-kD antibody was bound to Affigel 10 (Bio-Rad, 153-6046) and used to prepare an affinity chromatography column. Each chroma-

tography step was carried out according to the manufacturer's instructions. ABF (diluted 1:10 in 0.1 M 3-[N-Morpholino]propane-sulphonic acid pH 7.5) (MOPS; Sigma M9381) was applied to the column, followed by washing with 0.1 M MOPS pH 7.5, and the ABA-1 protein eluted with 0.1 M sodium borate pH 10. After adjusting the pH to 7.2, the protein was concentrated and dialysed against PBS as described above.

Radioiodination and immunoprecipitation

ABF, TBF or the immunoaffinity-purified ABA-1 were labelled with ¹²⁵I (Amersham) catalysed by the IODO-GEN reagent (Pierce Chemical Co.; product no. 28600) as previously described [11,12]. Immunoprecipitation was performed using *Staphylococcus aureus* [9] in which the iodinated purified ABA-1 was incubated with serum overnight at 4°C, and IgG immune complexes captured on heat-killed, formalin-fixed bacteria (Pansorbin Standardised 507861; Calbiochem-Behring, La Jolla, CA). The radioactivity in the washed pellets was measured in a gamma counter and prepared for SDS-PAGE, which was carried out with 0.7 mm thick, 5-25% acrylamide gradient gels in a Pharmacia (Uppsala, Sweden) GE-2/4LS electrophoresis apparatus as previously described [9], and dried gels exposed to preflashed x-ray film.

IgE-specific immunoblotting

ABF (300 µg/track) was separated by SDS-PAGE without preheating of samples and electroblotted onto nitrocellulose (Hybond-C extra, 45 µm; Amersham International) in Tris/glycine buffer pH 7.0 containing 0.1% SDS as transfer buffer. Strips were cut and blocked with 10% dried skimmed milk powder in 0.05% Tween in Tris-buffered saline for 1 h. Test antibody was added at 1:10 dilution in the above buffer for 90 min at ambient temperature. After washing three times, the blots were probed with ¹²⁵I-labelled mouse IgG1 anti-rat ϵ -chain specific MoAb clone MARE 1 (Serotec, Oxford, UK; Cat. no. MCA 193) at 250 000 ct/min per track. The blot was further incubated for 60 min, washed five times, dried and exposed to preflashed x-ray film.

Passive cutaneous anaphylaxis

Rat serum to infection with *A. suum* was raised in animals given three infections of 6000 infective eggs 28 days apart, and blood sampled 14 days after the last infection. All strains, with the exception of WLEP, were obtained from OLAC (1976) Ltd (Bicester, UK) and used at 2-3 months of age. The WLEP strain is an inbred strain of Wistar rat originally obtained from Nottingham University, and has been inbred at these laboratories since 1979 to 16 generations. These have been typed to RT1-A^u by Dr G. Butcher, of the Institute of Animal Physiology and Genetics Research, Babraham. In addition, we have found no mixed lymphocyte reaction between WLEP cells and those of PVG-RT1^u rats, but appropriate responses to PVG-RT1^a, PVG-RT1^c, and PVG-RT1^l cells. For the passive cutaneous anaphylaxis (PCA), normal, inbred WLEP were shaved on the back, and 100-µl samples of test serum, or dilutions thereof, injected intradermally. Twenty-four hours later, the rats received an intravenous injection of 50 µg antigen with 5 mg Evans Blue (BDH, Poole, UK; product no. 24115 2L) in PBS, and the diameters of the reaction spots were measured 30 min later. Normal serum controls were used on every occasion in each rat, and every assay point was carried out in duplicate on

separate animals. Sera were pooled from at least six animals per strain for the PCA, and no significant individual variability was found between the IgG response of individual donors to the allergen.

ABA-1 protein to be heat-treated before testing for PCA activity was autoclaved at 103 kPa pressure, the protein concentration of this and an untreated sample measured using the Bradford assay (Pierce & Warriner, Chester, UK; 23236) and adjusted to equivalence before use.

Amino acid sequencing

Fragments of affinity-purified ABA-1 were generated for sequencing by chemical cleavage with cyanogen bromide as follows. Freeze-dried protein (1 nmole) was incubated in the presence of CNBr (100 µg) dissolved in 500 µl of 70% aqueous formic acid in an atmosphere of argon gas overnight at 37°C. After digestion, the sample was freeze-dried to remove formic acid and to terminate the reaction.

After treatment, samples were freeze-dried and reconstituted in 100 µl 0.05% trifluoroacetic acid (TFA) before separation by HPLC. Digested samples (0.33 nmole) were chromatographed on an Aquapore RP-300 C-8 cartridge (2.1 × 30 mm). Elution was carried out at 100 µl/min over 70 min on a zero to 70% B gradient using a solvent A, 0.1% aqueous TFA and solvent B, 70% acetonitrile in 0.085% TFA.

The 14 kD molecule from *T. canis* perienteric fluid was separated by SDS-PAGE, transferred to PVDF membrane in a Mighty Small II SE250 Slab Gel Electrophoresis Unit (Hoefler Scientific Instruments) according to the manufacturer's instructions. Gels (20% acrylamide, 0.75 mm thick) consisting of 65 mm separating gel and 15 mm stacking gel (5%) were prepared and pre-run as recommended [14]. Samples (75 pmole) were prepared, electrophoresed and electroblotted onto PVDF membranes (Millipore IPVH 000 10) according to the method of Matsudaira [15].

Sequencing was carried out using an Applied Biosystems (Foster City, CA) Model 477A gas phase sequencer with a Model 120A on-line PTH analyser.

Mass spectrometry

Immunoaffinity-purified ABA-1 was analysed on a VG B10-Q biomedical mass spectrometer (VG Biotech, Altrincham, UK) using electrospray ionization at atmospheric pressure. Protein (500 pmol) was dissolved in 20 µl of 2% acetic acid, then 20 µl methanol added and 10 µl (125 pmol) used to obtain a preliminary spectrum. A further 10 µl methanol was added to the remaining solution, giving a final concentration of approximately 9.4 pmol/µl. Myoglobin solution (mol. wt 16950.5) was used as a standard to confirm calibrations.

RESULTS

The ABA-1 molecule and I-A restriction of antibody responses to it

ABA-1 is the major water-soluble protein constituent of ABF of adult *Ascaris*, and is also released by the tissue-invasive larvae of the parasite during culture *in vitro* [9]. It is likely, therefore, to be of significance to the immunobiology of the infection, particularly in view of its potent allergenicity in rodents and probably also humans [4,12]. The IgG response to the protein has previously been shown to be under MHC control in mice [12]

and in rats [4,16] in which the haplotypes involved are H-2^s and RT1^u, respectively. The purified ABA-1 protein, which will be dealt with below, was immunoaffinity purified from ABF, and, as shown in Fig. 1a, the response to the mol. wt 14 kD ABA-1 molecule from this source is under the control of the I-A^s heterodimer in mice. We have proposed elsewhere that the 14-kD component released by the infective larval stages of *Ascaris* (from both human and porcine infections) is identical to the ABA-1 from ABF [9,11], and Fig. 1b shows that the H-2^s restriction of the antibody response to the putative ABA-1 protein from parasite-secreted products is also I-A^s-restricted (see Table 1), as we have noted previously [13]. This further emphasizes the similarity, and probable identity, between the 14-kD component of ABF and that released by the parasite *in vitro*.

Stability of MHC-restricted ABA-1 epitope(s)

A central reason for investigating the structure of ABA-1 is in order to define IgE-binding regions. Such epitopes could depend either directly on linear amino acid sequences or on the folded protein's conformation. It is difficult to decide the relative importance of each class of epitope without very extensive analysis, but some indication can be provided by examining epitope stability in Western blotting or in heat treatment.

The immunoprecipitation experiment shown in Fig. 1 provides no information on whether or not the MHC-restricted antibody response is directed against a primary sequence or a conformational epitope, because radioiodinated, but otherwise native, antigen was used. In the next experiment, we addressed the question of whether the MHC-restriction of the antibody response in immunoblots on nitrocellulose was as would be expected from previous immunoprecipitation results. For this we examined sera from a range of rat strains, in which only those of RT1^u haplotype have previously been found to respond to ABA-1 in the context of infection [4,16]. Western blotting onto nitrocellulose membrane is a procedure that can result in loss of epitope integrity of some antigens [17]. Western blots of ABF were probed with rat serum and probed using a ¹²⁵I-labelled monoclonal anti-rat IgE (Fig. 2). This showed that indeed only RT1^u rats were found to respond, so the epitope(s) concerned is/are determined either by protein primary structure, or by stable conformational features.

Heat treatment of proteins is commonly regarded as causing irreversible conformational changes, and this is likely to be so for autoclaved proteins from organisms such as *Ascaris*, whose natural habitat never exceeds body temperature. The effect of autoclaving ABA-1, and the crude antigen from which it was prepared (ABF), was assayed by PCA (Table 2), using sera from rats which had been immunized by infection. The results showed that heat treatment of unfractionated ABF can diminish or eliminate its reactivity in the assay, but that this applied less to the strains bearing RT1^u. When the assay was carried out using the purified protein, it was clear that heat-treated material retained potent, although slightly diminished, activity. This could be taken to argue that whilst a proportion of the IgE-directed epitopes may be conformational, a substantial reactivity resided in heat-resistant determinants, or those which were reversible upon cooling.

While no data are presented, we also found that treatment of ABA-1 with sodium periodate did not alter its activity in the

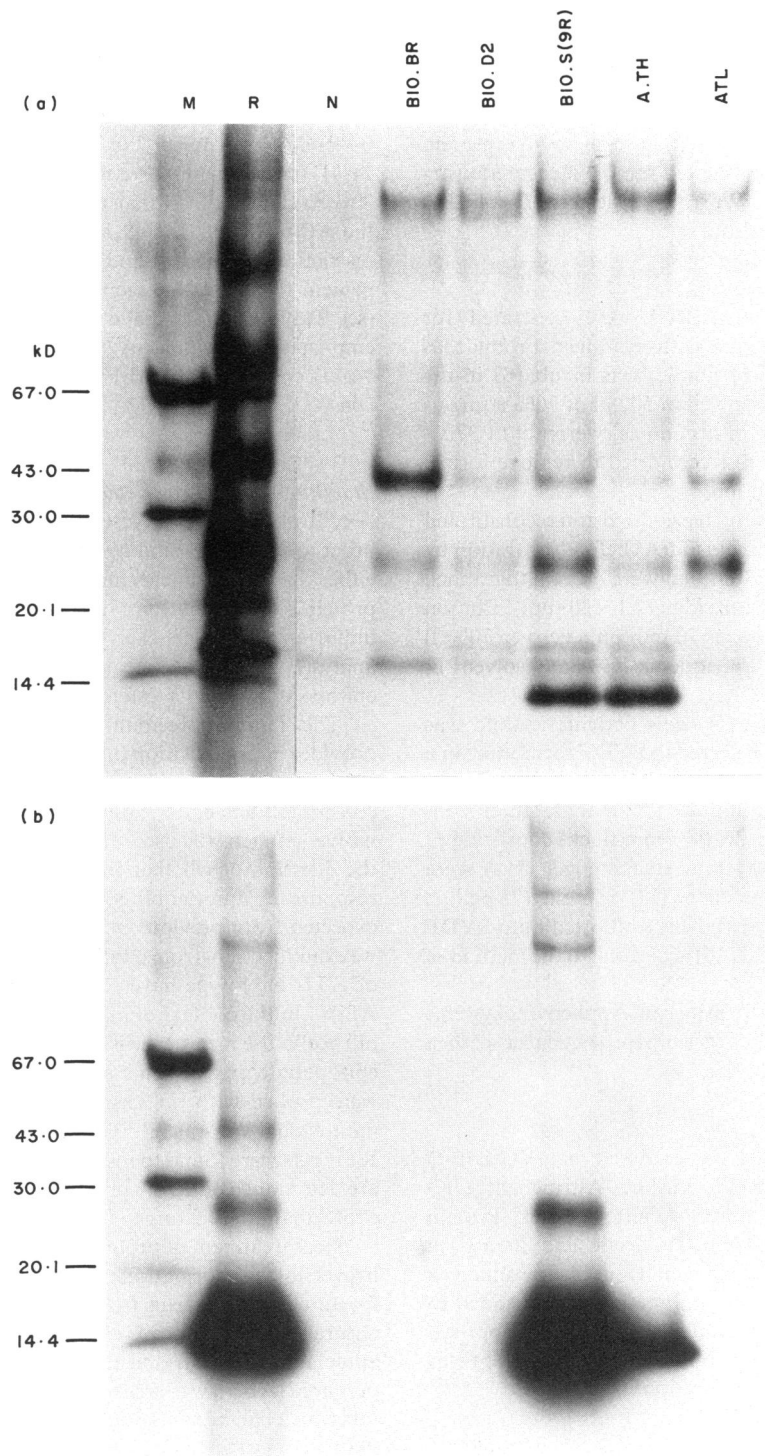


Fig. 1. MHC class II (I-A) control of immune recognition of ABA-1 in mice. Mice of the indicated strains were infected with *Ascaris suum* and pooled sera were assayed by immunoprecipitation of ^{125}I -labelled excreted-secreted (ES) material from lung stage larvae (a) of the parasite or perienteric fluid (ABF) of adult worms (b). See Table 1 for the MHC haplotypes of the mice used and the deduction that the I-A subregion of the class II region is involved. IgG immune complexes were analysed on SDS-PAGE and autoradiographed. The ABA-1 molecule has a mol. wt identical to that of the 14400 marker protein under the SDS-PAGE conditions used. The bands at approximately 28 kD and 43 kD in tracks M and B10.S(9R) in (b) represent dimeric and trimeric associations of the ABA-1 molecule which commonly appear under SDS-PAGE. M, Marker proteins whose relative molecular masses (mol. wt) are as indicated, expressed in kD; R, antigen preparation used in the assay, ^{125}I -ES for (a) and ^{125}I -ABF for (b); N, immunoprecipitation profile of serum from normal, uninfected mice.

Table 1. H-2 alleles of the mouse strains used

Strain	H-2 alleles carried						D
	K	A _β	A _z	E _β	E _z	S	
B10.BR	k	k	k	k	k	k	k
B10.D2	d	d	d	d	d	d	d
B10.S(9R)	<u>s</u>	<u>s</u>	<u>s</u>	s/k	k	d	d
A.TH	<u>s</u>	<u>s</u>	<u>s</u>	(s)	(s)	<u>s</u>	d
A.TL	<u>s</u>	k	k	k	k	k	d

Parentheses represent inability to express the H-2E heterodimer because of the defective E_z chain gene. The B10.S(9R) strain will express the I-E molecule because the E_β^k gene product is expressed normally and assembles with the E_β^k chain on the cell surface. Only those mice carrying H-2^s alleles at the I-A region were found to respond to the ABA-1 allergen (see Fig. 1).

Table 2. Allergenicity of ABA-1 is heat-stable

Strain	RT1 haplotype	ABF		ABA-1	
		Native	Autoclaved	Native	Autoclaved
WLEP	u	16; 32	32; 32	32; 64	16; 16
AGUS	l	16; 32	0; 0	0; 0	—
AO	u	32; 32	32; 32	64; 64	16; 32
PVG	c	16; 32	0; 0	0; 0	—
PVG-RT1 ^u	u	32; 64	32; 32	64; 64	16; 16
PVG-RT1 ^l	l	32; 64	8; 8	0; 0	—

IgE levels were measured by passive cutaneous anaphylaxis (PCA) as described in Materials and Methods. Each rat used in the assay received 50 µg purified ABA-1 allergen or 500 µg *Ascaris* body fluid (ABF) from which the ABA-1 was purified. The results are given as end point dilutions in a doubling dilution series of sera from the given strains, and the results of duplicate assays are presented.

PCA assay, implying that the epitopes concerned are not carbohydrate in nature.

Mass spectrometry of ABA-1

No consensus for the true molecular mass of ABA-1 is available in the literature. A reliable mass determination is necessary to estimate how much of the protein remains to be sequenced. For a more definitive measurement than heretofore available, therefore, we carried out mass spectrometric analysis using immunoaffinity-purified material. This identified three major and two subsidiary peaks with molecular weights as follows:

Peak	Mol. wt. ± s.d.
A	14 643.2 ± 1.4
B	14 801.2 ± 1.6
C	14 759.8 ± 1.0
D	14 573.4 ± 2.1
E	14 710.7 ± 1.2

The peaks are listed in order of abundance and the principal peak had a molecular mass of 14 643.2, which approximates to

that estimated by SDS-PAGE (Fig. 1). The subsidiary peaks are presumably slightly modified forms of the protein, derived either from differential post-translational modification or suggesting that ABA-1 might be encoded by a polymorphic gene. Contamination with another species of protein is probably an unlikely source of the apparent diversity, considering the fact that the N-terminal sequencing did not produce ambiguities of a scale commensurate with a significantly contaminated sample.

Sequence analysis of ABA-1 and its homologue in *Toxocara canis*

We previously published the sequence of residues 1–41 of ABA-1 [4] and here provide further sequence information. N-terminal sequencing was carried out as described in Materials and Methods and the data obtained are presented in Fig. 3. Sufficient ABA-1 was available in affinity-purified form for direct sequencing to be performed and a complete sequence of residues 1–68 was obtained directly, and part of it was confirmed from an internal sequence from a CNBr fragment. Attempts to obtain further sequence from trypsin and clostripain digestion fragments have proved fruitless.

The canine parasite *T. canis*, which is a causative agent of visceral and ocular larva migrans in humans [1,18], synthesizes a protein of similar mol. wt to the ABA-1 which is precipitated by antisera to *Ascaris* infection [11]. When radio-iodinated TBF from *T. canis* is immunoprecipitated with specific polyclonal antibody to ABA-1, only the suspected homologue of ABA-1 was precipitated (Fig. 4). We have also found indications that another ascaridid nematode which infects humans, *Anisakis simplex*, synthesizes a 14-kD protein which is immunologically cross-reactive with ABA-1 [19]. ABA-1 and its homologues might, therefore, be of importance in the immunobiology of several infections with this group of nematodes. In order to examine the putative homologues more closely, the N-terminal amino acid sequence of the ABA-1-like component of the pseudocoelomic fluid of *T. canis* (here designated TBA-1) was analysed (Fig. 3). This showed that the two protein sequences have regions of identity, and that where this is not so, the properties of the amino acids are often conserved. This clearly argues for the TBA-1 protein as a homologue of ABA-1.

These sequences bear no significant resemblance to any other protein in recent issues of the GenEMBL or SwissProt databases, although there is similarity with that encoded by the cloned gene for a 15-kD antigen of certain filarial nematodes (see below).

DISCUSSION

More is known of the ABA-1 molecule of *Ascaris* than of any other nematode allergen, although preliminary amino acid sequence information has only recently become available [4,5]. The sequences we have now obtained for ABA-1 and its homologue from *T. canis* (TBA-1) revealed no apparent similarity with any other protein whose sequence is in the data banks, apart from the homologues in filarial nematodes (see below). This also applies to allergens from sources such as pollen, house dust mite, etc., and it is unlikely, therefore, that the molecule's allergenicity can be explained by a common structural motif, unless conformational features not immediately apparent from primary sequence are important. It might be, therefore, that allergens such as ABA-1 have no intrinsic

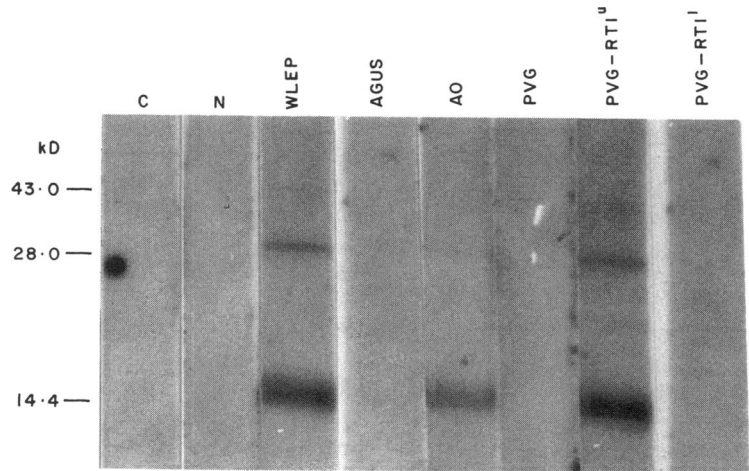


Fig. 2. MHC restriction of IgE antibody response to electroblotted ABA-1. *Ascaris* perienteric fluid (ABF) was electroblotted to nitrocellulose, strips were incubated with sera from a range of inbred strains of rat, and probed with radio-iodinated MoAb to rat IgE. C, No primary serum; N, uninfected rat serum; and sera from the strains of rat indicated. See Table 2 for the RTI haplotypes of these strains. The mol. wt values are for those of the presumed monomeric, dimeric and trimeric aggregates of the ABA-1 molecule appearing on the immunoblot.

allergenicity, but that it and other nematode allergens become the subject of IgE responses in infected hosts by virtue of some other characteristic of these infections, such as their ability to induce immune responses in which Th2-type activities predominate, resulting in high IgE, mast cell and eosinophilic responses [20,21]. In our unpublished results, we have found that ABA-1 is poorly allergenic when presented in the context of Freund's adjuvant immunization in rodents (in contrast to its potent allergenicity in the context of infection), again arguing against an intrinsic allergenicity.

The molecular mass of ABA-1 has been estimated by several groups, providing estimates ranging from 9 to 14 kD using a variety of techniques, but no firm consensus has emerged [5,9,10]. We have repeatedly found the protein to co-run with a 14.4 kD marker protein (α lactalbumin) in SDS-PAGE, but in a recent collaborative study it was found that it could be sized at 25 kD by HPLC, with subunits which run at 10 kD in the presence of SDS and that, under certain conditions, SDS-PAGE could provide values of between mol. wt 9 and 10 kD [5]. Our present mass spectral analysis gave a major peak at

$14\,643.2 \pm 1.4$, which corresponds closely to our original estimate and to the value of approximately 14 000 obtained for Allergen A of *Ascaris* by sedimentation equilibrium analysis almost 20 years ago [6,7]. Allergen A has a similar pI and amino acid composition to ABA-1 [4] and the latter is probably the major constituent of both the Allergen A and Asc-1 preparations [5-8]. Our own recent unpublished work has indicated that ABA-1 runs at approximately 28 kD on fast protein liquid chromatography (FPLC) gel permeation chromatography and differential scanning calorimetry analysis confirmed that it associates as a homodimer.

As mentioned above, we have found no protein, whose sequence is in recent editions of the protein or gene sequence data bases, to bear any similarity to ABA-1. The genes encoding 15-kD antigens of the filarial nematodes *Dirofilaria immitis* and *Brugia malayi* have, however, recently been cloned [22-24] and the predicted amino acid sequences show clear similarities with that of ABA-1. These have not been confirmed by direct N-terminal sequencing, but the predicted amino acid sequences have runs of identical residues as in the shared sequences of

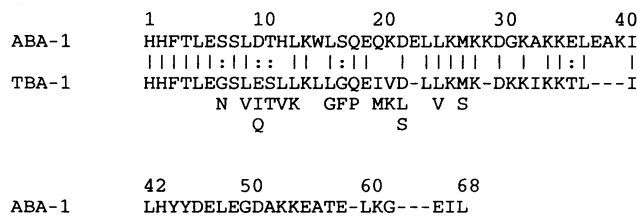


Fig. 3. Partial sequence of ABA-1 of *Ascaris suum* and comparison with its homologue TBA-1 of *Toxocara canis*. Direct N-terminal amino acid sequencing of affinity-purified ABA-1 identified residues 1-68. Cyanogen bromide cleavage provided an internal overlapping fragment of residues 28-55. TBA-1 was sequenced directly from material transferred to PVDF membranes from SDS-PAGE-separated *T. canis* body fluid. The single letter code for amino acids is used, with '-' representing an unidentified residue. For TBA-1, letters below the line represent an alternative amino acid identified at a given position, although the most abundant residue is placed on the line. Amino acid identities are indicated by a vertical bar (|), and similar amino acids by a colon (:).

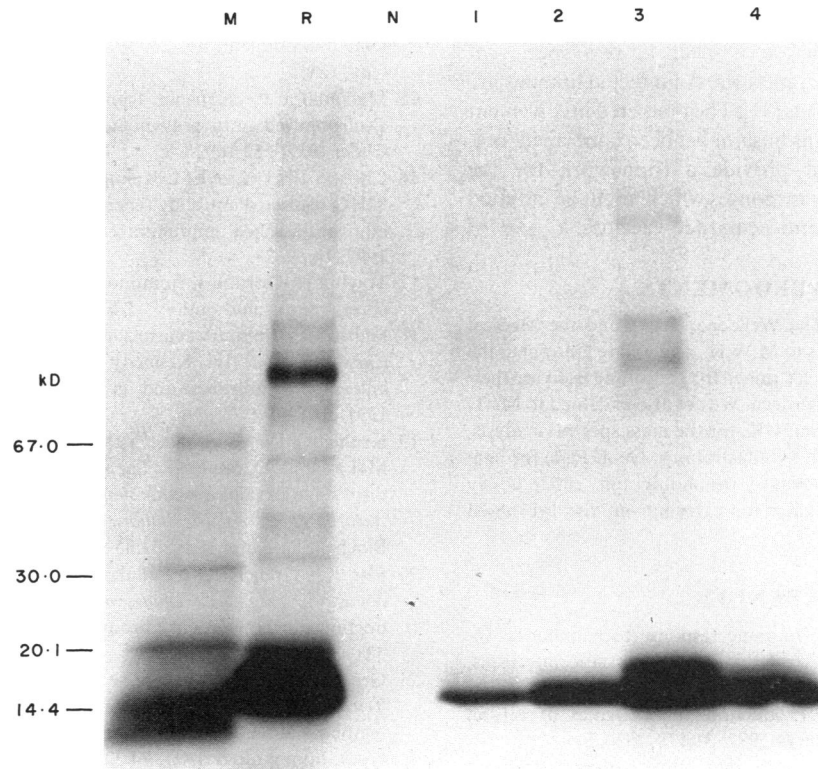


Fig. 4. *Toxocara canis* contains an immunological homologue of the ABA-1 of *Ascaris*. Pseudocoelomic fluid (TBF) from adult *T. canis* parasites was radio-iodinated (lane R) and immunoprecipitated with sera from an untreated rabbit (N), a rabbit infected on three occasions with *Ascaris* (1), and rabbits immunized with gel-excised ABA-1 (2 and 4) or *Ascaris* perienteric fluid (ABF) (3). Immunoprecipitates were analysed by gradient SDS-PAGE. Marker proteins were run in lane M, with relative masses as indicated.

ABA-1 and TBA-1. The homologues from the filarial nematodes appear to be synthesized by the parasite as a polypeptide of approximately 400 kD which is then cleaved into 15-kD units [22–24]. There is also evidence from the same laboratories that these polypeptides are encoded by tandemly repeated coding units, and we now have indications that this also applies to the ABA-1 of *Ascaris* [25]. Such amplification would provide a mechanism for the production of a protein in the relative abundance exhibited by ABA-1 and its respective homologues. It might also explain the multiple peaks found by mass spectrometry and the few ambiguities which arose in the amino acid sequencing of ABA-1; tandemly repeated coding units could presumably accumulate mutations independently within the copies, and, although divergences will probably have limits, some persistence of these may be inevitable. There may also be several alleles of the protein in any given repeat locus such that slightly different forms of the molecule will be detected in samples pooled from a number of individual organisms. The fact that we obtained good N-terminal sequence for ABA-1 would, however, argue that this region is invariant and/or is representative of the most abundant species synthesized by the parasite.

Another alternative would be that the protein undergoes heterogeneous post-translational modification. One obvious cause of such modification is glycosylation, but we have found that ABA-1 binds to none of a wide range of lectins screened, and its mol. wt by SDS-PAGE is unaffected by treatment with N-glycanase [26].

The biological function of ABA-1 is not known, but its relative abundance within the parasite presumably reflects an important role in its physiology. Alignments of the sequences of ABA-1 and its various homologues could reveal conserved regions which will provide an indication of the protein's structure and function. Other reasons for seeking the full sequence would be to provide a basis for epitope mapping of the protein in order to establish the nature of its IgE-binding regions. This has been carried out for other allergens using nested synthetic peptides [27,28], and for *Plasmodium* antigens and the *Derp1* allergen of house dust mites using random cleavage of cloned cDNA encoding the antigen/allergen and subcloning fragments into an expression vector to produce recombinant bacteria synthesizing parts of the complete polypeptide [29,30]. These can then be screened for colonies which still react with antibody, positive clones sequenced, and the epitopes thereby defined. Our present findings are apposite, therefore, in that some if not all of the IgE-binding epitopes of ABA-1 can survive harsh treatment (SDS treatment, binding to nitrocellulose and autoclaving) and that the conformation of recombinant polypeptides need not, therefore, be critical for epitope mapping. To this end, we have obtained a cloned cDNA encoding ABA-1 [25] which is currently being expressed for allergen testing and epitope mapping.

ABA-1 is the first nematode allergen in which MHC restriction of the IgG and IgE antibody repertoire has been described. This pronounced genetic restriction of IgE responses to ABA-1 makes it important to understand the crucial

fragments of the polypeptide which interact with the class II molecule. The MHC restriction is clear in two species of experimental animal, and the repertoires of infected humans are also known to be heterogeneous [31]. The predicted involvement of HLA in infection of humans has not yet been confirmed, but, if true, then ABA-1 might provide a framework for the prediction of hypersensitivity responses which might be initiated or recalled by recombinant anti-nematode vaccines.

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