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N-terminal amino acid sequence identity between a major allergen of *Ascaris lumbricoides* and *Ascaris suum*, and MHC-restricted IgE responses to it

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SUMMARY

A protein allergen of the parasitic nematode *Ascaris* has been purified to homogeneity by immunoaffinity chromatography. It is the most abundant protein species in the parasite's body fluid and has been named ABA-1. The allergen's molecular weight (MW) has been previously estimated at 14,000, but this sizing is currently under re-evaluation. The immunological activity of the protein was intact after purification, as attested by immunoprecipitation and passive cutaneous anaphylaxis. The IgE response to ABA-1 was under major histocompatibility complex (MHC) restriction in the rat, in which only $RT1^u$ strains were found to respond following infection with the parasite. The tissue-invasive and intestinal stages of both *Ascaris lumbricoides* (of humans) and *Ascaris suum* (of pigs) have an antigen of similar MW to ABA-1 in their secretions or among their somatic antigens. These are antigenically indistinguishable; they were found to have similar amino acid compositions, and their N-terminal amino acid sequences were identical to 41 residues. Finally, the apparent MW, amino acid composition and isoelectric point of ABA-1 all argue for close similarity to the previously described Allergen A of the parasite.

INTRODUCTION

The nematode parasite *Ascaris lumbricoides* is among the most widespread and prevalent of all the helminthiases of humans (World Health Organization, 1981), and it can present more foreign biological material to an infected individual than any other nematode. As is typical in nematode parasitisms, ascariasis induces hypersensitivity responses which contribute significantly to disease manifestations. The most important of these occurs in the lung during the pulmonary stage of the infection, and has the characteristics of an immediate-type hypersensitivity response (Ogilvie & de Savigny, 1982; Coles, 1985). The degree to which the intestinal stage of the infection contributes to pathology by immunological mechanisms is largely unknown, but there is one report of a lethal anaphylactic intestinal response following chemotherapy for the infection (Odunjo, 1970), and changes in the architecture of the intestine during infection are typical of immune-mediated events (Coles, 1985; Ferguson & Jarrett, 1975; Ogilvie & Parrott, 1977).

Abbreviations: ABA-1, *Ascaris* body fluid allergen-1; ABF, *Ascaris* worm body fluid; MHC, major histocompatibility complex; PBS, phosphate-buffered saline; PCA, passive cutaneous anaphylaxis; PTC, phenylthiocarbanyl; PVDF, polyvinylidene diflouride.

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More allergens have been described from *Ascaris* than from any other nematode (Jarrett & Miller, 1982). This study concentrates on an allergen which has previously been described as the most abundant protein in the parasite's body fluid (Tomlinson *et al.*, 1989). The molecular weight (MW) of this is approximately 14,000 by gradient SDS–PAGE (Kennedy & Qureshi, 1986), although it can run at 10,000 MW under different SDS–PAGE conditions and on HPLC (McGibbon *et al.*, 1990), and has now been named ABA-1 (McGibbon *et al.*, 1990). It is produced by all developmental stages of the worm, and is released during culture of the tissue-parasitic larvae *in vitro* (Kennedy & Qureshi, 1986; Kennedy *et al.*, 1987b, 1989). It is, therefore, potentially significant in immunopathology, both pulmonary and gastrointestinal. Of further interest is the observation that the antibody response to the protein, both IgG and IgE, is under precise control of the major histocompatibility complex (MHC) in mice (Kennedy *et al.*, 1987a; Tomlinson *et al.*, 1989). This genetic restriction operates in the context of infection but can be overruled in adjuvant-assisted immunization (Tomlinson *et al.*, 1989; Kennedy *et al.*, 1990). The antibody response to the antigen varies considerably amongst infected humans (Haswell-Elkins *et al.*, 1989; Kennedy, 1989), presumably on a genetic basis. If so, then detailed study of the immune response to ABA-1 might aid the understanding of heterogeneity of humans to immunopathology in ascariasis. It might also have implications for hypersensitivity responses to transmissible disease agents in general.

The structure and biochemistry of the allergen are under investigation in order to understand MHC control of antibody and cellular responses to it, with particular reference to hypersensitivity reactions. The intention is also to place the molecule in context with previously described allergens of the parasite. Most work on antigens of *Ascaris* has centred around those of *Ascaris suum*, the porcine homologue, and little has been done using the human parasite, *A. lumbricoides*. It is shown here that the ABA-1 molecule from the two parasites is similar, if not identical, and resembles the previously described allergen of the parasite, Allergen A (Ambler *et al.*, 1972, 1973a, 1973b; Ambler, Miller & Orr, 1974).

MATERIALS AND METHODS

Ascaris *allergen*

The source of the purified 14,000 MW allergen (ABA-1) used here was the perienteric body fluid (ABF) of adult female *Ascaris suum*. This was collected from parasites recovered from pigs as described previously (Kennedy *et al.*, 1987b, 1989), centrifuged at 15,000 *g* for 10 min to remove particulate matter, and the supernatant dialysed against phosphate-buffered saline (PBS) overnight at 4°, then stored at −70° until used. ABF from *Ascaris lumbricoides* was obtained from infected people in Nigeria, flown to Glasgow on ice, and treated upon arrival as above. 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 ^{125}I-labelled ABF, on 1·5 mm thick 20% SDS–PAGE gels. Gels were vacuum dried at 60°, and the 14,000 MW 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 number 165-2976), with membrane caps having an exclusion limit of 3500 (product number 165-2986). SDS was removed from the protein sample by dialysis against 10% methanol over 48 hr at 4° using a 2000 MW exclusion limit dialysis membrane (Sigma D 2272; St Louis, MO), followed by application onto an AffinityPak Extracti-Gel D column (Pierce Chemical Co., Rockford, IL; N20346) to remove any remaining detergent. The protein sample was concentrated by nitrogen pressure ultrafiltration using a 5000 MW exclusion limit membrane (Sartorius, Göttingen, FRG; SM 14529–025 N) and dialysed extensively against PBS.

Antisera

Antiserum to ABA-1 was raised by intramuscular injection of 100 μg protein in Freund's complete adjuvant 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 in five to six subcutaneous sites on the back. Serum was taken 7 days later at the time of peak antibody response predicted from previous immunizations assayed by ELISA. Serum from further boosting resulted in antibody of excessive affinity. Rabbit antisera to infection with *A. lumbricoides* and *A. suum* were raised as described previously (Kennedy *et al.*, 1987a, b, 1989). In all cases the rabbits used were Sandy-Lop, obtained from the National Institute for Medical Research, London.

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 CL 4B (Sigma P3391). The protein A-purified rabbit anti-14,000 MW antibody was bound to Affigel 10 (Bio-Rad 153-6046) and used to prepare an affinity chromatography column. Each chromatography step was carried out according to manufacturers instructions. ABF (diluted 1:10 in 0·1 M 3-[N-Morpholino] propane-sulphonic acid, pH 7·5; MOPS; Sigma M 9381) 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.

Protein detection—Coomassie and silver staining

Sample preparation and SDS–PAGE was carried out as described previously (Kennedy & Qureshi, 1986) with 5% β-2-mercaptoethanol or 1 mg/ml iodoacetamide for reducing and non-reducing conditions, respectively. Following electrophoresis, gels were fixed in 25% methanol, 10% acetic acid, stained in 0·1% Coomassie Brilliant Blue R-250 (Sigma B0630) in fixative solution, then destained in fixative. In addition, gels were silver stained using Amersham's Quick-Silver Stain Kit (Amersham RPN. 17; Amersham, Bucks), then dried.

Radioiodination and immunoprecipitation of ABA-1

The immunoaffinity-purified ABA-1 was labelled with ^{125}I (Amersham) catalysed by the IODO-GEN reagent (Pierce Chemical Co.; product number 28600) as described previously (Tomlinson *et al.*, 1989). Immunoprecipitation was performed using a *Staphylococcus aureus*-based assay (Kennedy & Qureshi, 1986) in which the iodinated purified ABA-1 was incubated with serum overnight at 4°, 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.

Passive cutaneous anaphylaxis (PCA)

Rat serum to infection with *A. suum* was raised in different strains of rats which were 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, Oxon, 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, Cambridge. In addition, it has been found that there is no mixed lymphocyte reaction between WLEP cells and those of PVG-RT1^{u} rats, but there are appropriate responses to PVG-RT1^{a}, PVG-RT1^{c}, and PVG-RT1^{l} cells. For the 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 were given an intravenous injection of 50 μg antigen with 5 mg Evans Blue (BDH, Poole, Dorset; product number 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.

Electrophoresis and electroblotting of proteins onto polyvinylidene difluoride (PVDF) membranes

This was carried out in a Mighty Small II SE250 Slab Gel Electrophoresis Unit (Hoeffer Scientific Instruments, San Francisco, CA) 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 prerun according to Yeun *et al.* (1986). Samples (75 pmol) were prepared, electrophoresed and electroblotted onto PVDF membranes (Millipore, Bedford, MA; IPVH 000 10) according to the method of Matsudaira (1987).

Amino acid composition and sequence analysis

Amino acid analysis of the immunoaffinity-purified ABA-1 and the ABA-1 molecule excised from PVDF membranes were determined using an Applied Biosystems 420A amino acid analyser with automated hydrolysis and derivatization. The phenylthiocarbanyl (PTC) amino acids generated were identified on-line with 130A separation system employing a C18 reverse-phase narrow bore cartridge. Sequence analysis was carried out on an Applied Biosystems (Foster City, CA) Model 470A gas phase sequencer with a Model 120A on-line PTC analyser.

RESULTS

Purification of the ABA-1 allergen

The perienteric fluid (ABF) of *Ascaris* comprises a mixture of molecular species detectable by Coomassie blue staining of gradient SDS–PAGE-separated material. These range in MW up to about 400,000, the highest being susceptible to reducing conditions (Fig. 1). The protein profiles of the ABF of *A. suum* and *A. lumbricoides* are similar, and vary slightly between individual parasites of both species (not shown). The most abundant component of these is an entity whose MW is approximately 14,000 according to the gradient SDS-PAGE gel system used in this study, although it has been found to have an apparent MW of less under certain SDS–PAGE conditions and by HPLC (Greenspon *et al.*, 1986, McGibbon *et al.*, 1990). The protein was isolated to homogeneity by the immunoaffinity technique described in the Materials and Methods, and Fig. 1 shows the result of a typical purification. No other parasite proteins were detectable by silver staining of the gel illustrated or in gels loaded with larger quantities of purified protein. The proteinaceous nature of the molecule was confirmed by its complete susceptibility to digestion with pronase and proteinase K as indicated by loss of the band in enzyme-treated samples (not shown).

Antigenic integrity of the purified allergen

To establish the credentials of the affinity-purified protein, ABA-1 was radioiodinated and immunoprecipitated with rabbit antiserum to ABA-1 or to infection with *A. lumbricoides* and *A. suum*. The protein A-mediated immunoprecipitate was analysed by SDS–PAGE (Fig. 2), in which the protein displayed its expected reactivity to antiserum raised in the context of infection or adjuvant-assisted immunization.

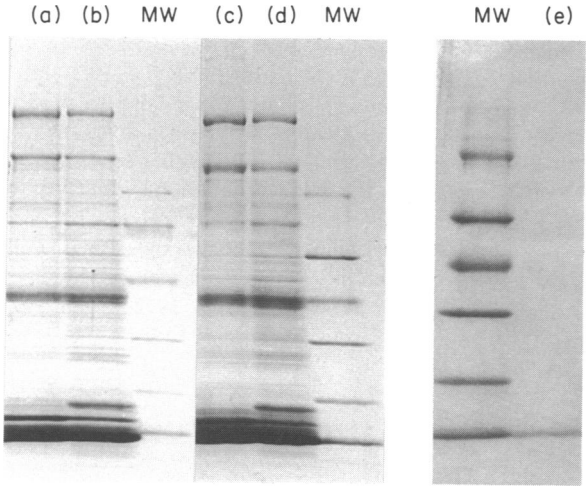

Figure 1. SDS–PAGE analysis of the pseudocoelomic fluid (ABF) of adult *Ascaris suum*, to show the abundance of the 14,000 MW component and its purification to homogeneity. ABF was run on a 5–25% gradient gel under reducing (a) and (b) and non-reducing conditions (c) and (d), and stained with Comassie blue. The profiles of female parasites are given in (a) and (c) and of males in (b) and (d). Males have at least one major additional band in the low MW region, so only the products of females were used in the purification. (e) Was loaded with immunoaffinity-purified ABA-1. Standard MW marker proteins (Pharmacia 17-0446-01) were run under reducing and non-reducing conditions as appropriate. The relative MW for the marker proteins, as given by the manufacturer, are 14,400, 20,100, 30,000, 43,000, 67,000 and 94,000, reading from the bottom of the gel.

Its activity as an allergen was assayed by homologous passive cutaneous anaphylaxis (PCA) in rats using serum from a panel of inbred strains infected with *A. suum* (Table 1). This showed that the protein retained its potent allergenicity following purification. Moreover, the response to it was MHC-restricted in the rat, as it is in the mouse (Tomlinson *et al.*, 1989),

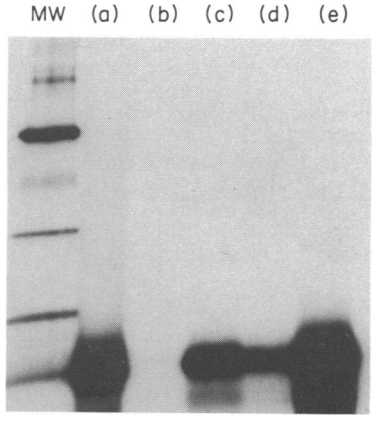

Figure 2. Immunoprecipitation of affinity-purified ABA-1. Radioiodinated immunoaffinity-purified ABA-1 (a) was immunoprecipitated with normal rabbit serum (b) or serum from rabbits infected three times with *A. suum* (c), *A. lumbricoides* (d) or immunized with purified ABA-1 from *A. suum* in adjuvant (e). Antigen–antibody complexes were captured on protein A-coated *Staphylococcus aureus* bacteria, analysed by SDS–PAGE on 5–25% gradient gels and visualized by autoradiography. Protein standards were run and were as for Fig. 1.

Table 1. Integrity of the purified ABA-1 allergen, and MHC restriction of response to it in the rat

Strain of rat	RT1 haplotype	PCA titre	^{125}I counts precipitated
Uninfected WLEP	u	0	2·5
Infected			
PVG	c	0	2·6
AGUS	l	0	1·6
PVG-RT1^{l}	l	0	2·4
WLEP	u	32,64	31·0
AO	u	64	21·9
PVG-RT1^{u}	u	64	20·5

PCA was carried out using inbred WLEP rats for the assay as detailed in the Materials and Methods. Rats were each given 50 μg of ABA-1 as challenge antigen. The sera used were pooled from at least six animals per group, 14 days after the last of three oral infections with 6000 infective eggs of *A. suum*. The assays were performed in duplicate, and if the end-point titres were not identical, both are given. All strains gave a positive reaction to the unpurified ABF antigen. ^{125}I-labelled ABA-1 antigen was immunoprecipitated in the protein A-based assay described in the Materials and Methods, and is expressed as the mean of duplicates for each serum pool.

only RT1^{u} strains responding on any genetic background. The efficacy of immunization was controlled for by using whole ABF as challenge antigen in PCA, and all ABA-1 non-responder strains reacted to this unpurified material (not shown). This implies that there are more allergens in *Ascaris* body fluid than ABA-1; allergens of different biochemical characteristics have been identified by other workers (reviewed by Ogilvie & de Savigny, 1982; Jarrett & Miller, 1982). Details of the MHC restriction to this and other *Ascaris* antigens in the rat will be presented elsewhere, but of immediate concern is that the pattern of responsiveness to the purified protein did not differ between purified and unpurified preparations.

N-terminal amino acid sequence identity between ABA-1 from human and porcine *Ascaris*

The major protein in *A. lumbricoides* body fluid cross-reacts immunologically with that of *A. suum*, and is of the same MW (Kennedy *et al.*, 1987b, 1989). Given the extensive work performed on the antigens of *A. suum* as a more readily available substitute for human-derived parasite material, it was important to establish the relationships between the two proteins. The N-terminal amino acid sequences were, therefore, compared and found to be identical (Fig. 3). This was despite one molecule being sequenced from affinity-purified protein, and the other from material separated by SDS–PAGE and transferred to PVDF membrane.

The above comparison of ABA-1 from *A. lumbricoides* and *A. suum* only extended to 41 residues of the protein. While the sequence identity was impressive, the argument for homology was strengthened by amino acid compositional analysis of the molecule as a whole. Figure 4a shows a comparison of the two, in which the correlation coefficient was 0·98. This negligible

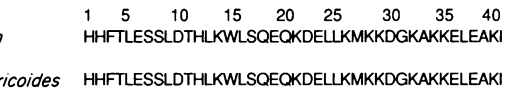

Figure 3. N-terminal sequences of the ABA-1 molecules of *A. suum* and *A. lumbricoides*. Affinity-purified *A. suum* protein was sequenced directly, and that of *A. lumbricoides* was taken from PVDF membranes as described in the Material and Methods.

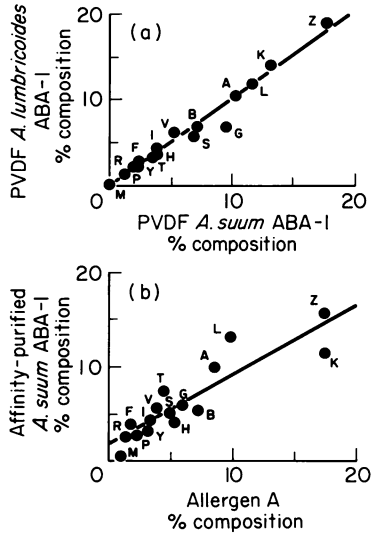

Figure 4. Comparison of the amino acid compositions of the affinity-purified ABA-1 of *A. suum*, *A. lumbricoides* and Allergen A. (a) Comparison of the ABA-1 protein of *A. suum* with that of *A. lumbricoides*. ABF from each parasite was run on mini-gels and electroblotted onto PVDF membranes for analysis of the 14,000 MW region. (b) Comparison of the immunoaffinity-purified molecule with that published for Allergen A (Ambler *et al.*, 1973b). In both panels the percentage composition for each residue in one protein is plotted against that for the residues occurring in the other protein, and each point is labelled with the single letter code for amino acids (Table 2). Acid hydrolysis renders aspartic acid (D) indistinguishable from asparagine (N), and glutamic acid (E) from glutamine (Q); these pairs are given the code letters B and Z, respectively. Acid hydrolysis of PVDF-bound protein destroys methionine residues. Regression analysis produced best-fit lines; equations and regression coefficients (R) were as follows: (a) $y = 0{\cdot}162 + 1{\cdot}0251x$; $R = 0{\cdot}98$; (b) $y = 1{\cdot}7714 + 0{\cdot}7322x$; $R = 0{\cdot}90$.

difference was within the range of results for duplicate analytical runs on the same protein. Acid hydrolysis of PVDF membrane-bound proteins eliminates methionine residues, and hence there was none found in those assays.

ABA-1 is similar to Allergen A

In the early 1970s Ambler and co-workers carried out extensive work on the purification and biochemical characterization of a major allergen of *A. suum* which they named Allergen A (Ambler *et al.*, 1972, 1973a, b, 1974). The MW of Allergen A was estimated to be 14,000 by gel filtration and sedimentation equilibrium centrifugation, and its isoelectric point 5·2. The molecular mass clearly draws comparison with the ABA-1 protein, as does its isoelectric point, which was found to be identical, as estimated using a Pharmacia PhastSystem (Upp-

Table 2. Amino acid analysis of affinity purified ABA-1 of *A. suum* and its comparison to Allergen A

Amino acid	Single letter code	Allergen A		ABA-1	
		% composition	Integer	% composition	Integer
Aspartic acid/asparagine	B	7·21	7	5·30	5
Glutamic acid/glutamine	Z	17·51	17–18	15·60	16
Serine	S	4·95	5	5·10	5
Glycine	G	5·90	6	5·90	6
Histidine	H	5·34	5	4·10	4
Arginine	R	1·31	1	2·50	2–3
Threonine	T	4·41	4–5	7·40	7–8
Alanine	A	8·59	8–9	9·80	10
Proline	P	2·21	2	2·70	3
Tyrosine	Y	3·15	3	3·10	3
Valine	V	3·80	4	5·50	5–6
Methionine	M	1·00	1	0·50	0–1
Isoleucine	I	3·29	3	4·30	4
Leucine	L	9·94	10	13·10	13
Phenylalanine	F	1·71	2	3·80	4
Lysine	K	17·54	17–18	11·30	11

The analysis of Allergen A is taken from Ambler *et al.* (1973b), and that for the affinity-purified ABA-1 protein is the mean from two separate analyses. Glutamic acid and glutamine are indistinguishable in the analyses used, as are aspartic acid and asparagine, and each pair is given the single letter code conventionally used to signify this.

sala, Sweden) and pI markers (cat. no. 17-0472-01). It is possible, therefore, that the major constituent of Allergen A is ABA-1. This similarity prompted a comparison of the amino acid composition of the ABA-1 preparation with that published for Allergen A (Table 2). The two assays did not give identical results, but when the number of each residue was compared graphically (Fig. 4b), the resemblance was striking, with a regression value of 0·9. This is greatly in excess of the correlation coefficient resulting from a similar comparison of ABA-1 with other *Ascaris* allergen preparations (Hussain, Bradbury & Strejan, 1973; O'Donnell & Mitchell, 1978) and the non-specific mast cell degranulating substance of *Ascaris* (Thompson, 1972), and it also differs from the published composition of whole ABF (Hussain *et al.*, 1973). It is not as close a correlation as for the analysis illustrated in Fig. 4a, but this could be due to differences in the analytical methods used. The SQΔ value for the comparison, calculated according to Marchalonis & Weltman (1971) is 78, which falls within the range for related proteins, although relatively high for a close relationship. Lastly, although the analytical method used does not resolve methionine, the parasite will incorporate ^{35}S-methionine into ABA-1 *in vitro* (J. F. Christie and M. W. Kennedy, unpublished data).

DISCUSSION

The relationship between the biochemical or structural characteristics of a given molecule and its allergenicity is unclear (Aas, 1978; King, 1976; Lind & Løwenstein, 1988). Some loose correlations have been made with regard to general physicochemical and immunochemical characteristics, but these might reflect a bias in widely used extraction methods rather than a fundamental relationship between structure and allergenicity (Lind & Løwenstein, 1988). It might be that no correlation is possible, but the problem is compounded by genetic differences between individuals in their susceptibility to allergic responses in general and reactivity to a given potential allergen in particular. For instance, the induction of a reaginic response to ABA-1 of *Ascaris* is dependent at the outset on the control exerted by the MHC on the immune repertoire (Tomlinson *et al.*, 1989 for mice; this paper for the rat). Unpublished findings (M. W. Kennedy *et al.*) suggest that the means of presentation of ABA-1 is also critical in that an IgG response, but no IgE response, to ABA-1 can be induced by Freund's adjuvant-assisted immunization in mice. A similar effect has been reported for allergens of *Ascaris* and other sources (O'Donnell & Mitchell, 1987; Jarrett *et al.*, 1972, 1980). Therefore, a proper understanding of responses to allergens of transmissible disease agents requires study in the context of infection, rather than exposure by other means.

The way in which nematode infections elicit their characteristic high IgE antibody levels remains unknown, but it could be argued that structural studies on a defined allergen will be instructive if combined with an immunogenetic approach. The ABA-1 molecule is ideal for this purpose because of its availability, ease of purification, and the genetic control of IgE antibody responses to it. The control of immune specificity by the MHC has already been mentioned, but there is also evidence for non-MHC gene control of the *level* of the IgE antibody response (Tomlinson *et al.*, 1989). This kind of approach could, therefore, fill the gap left by previous work on antigen structure and allergenic potential.

The MHC restriction has implications for hypersensitivity responses to the antigens of pathogens in general, and might also provide a model for responses to environmental allergens. It is widely accepted that genetics play a substantial role in predisposition to allergies, and there is some evidence for

association between certain HLA alleles and such susceptibility (Marsh *et al.*, 1982; Tiwari & Terasaki, 1985; Frick, 1987; Thomson, 1986). Moreover, HLA-DR loci have been shown to be restriction elements for T-cell clones in proliferative responses to purified allergens (O'Hehir *et al.*, 1988). One difficulty in allergy research is that many experimental systems for the study of Type 1 hypersensitivity reactions involve the use of adjuvants to elicit reaginic responses to defined allergens in rodents. As noted above, adjuvant-assisted immunization can over-ride MHC control and generate responses that are quite unlike those elicited by natural routes of exposure. Infection with *Ascaris* is obviously quite different from exposure to environmental allergens, but it could nevertheless provide valuable insight into genetic control of hypersensitivities.

The first step in the dissection of the response to *Ascaris* allergens requires structural information. This study was primarily concerned with the relationship between the ABA-1 antigen of *A. suum* and *A. lumbricoides*. The case for homology between the proteins is convincing in terms of amino acid sequence and compositional analyses, and confirms a previous suggestion of similarity based on their immunological and physicochemical characteristics (Kennedy *et al.*, 1987b, 1989). It is difficult to obtain allergen in sufficient quantities and quality from human-derived *Ascaris*, and these findings, therefore, amply justify the use of *A. suum* allergen as a substitute.

The function of the protein in the biology of the parasite is unknown, and computer searches of the NBRF Protein, EMBL, and GenBank data bases have so far revealed no significant overall sequence similarity between ABA-1 and any other protein. Knowledge of its full sequence might provide information on its biological role, although this is unlikely to provide any generalizations as to allergenicity. The sequence will also be necessary for the identification of B- and T-cell epitopes for the IgE response and how these might vary with the genetic constitution of infected individuals. It would be important, for example, to look for variation in epitope recognition between syngeneic individuals, as is known to occur for influenza haemagglutinin in infected mice (Barnett *et al.*, 1989), and the bearing that this might have on allergic reactions.

The analyses presented here also place the protein in context with previously described allergens of the parasite. The data argue for close similarity between ABA-1 and the Allergen A preparation described by Ambler *et al.* (1972, 1973a,b, 1974) in terms of MW, pI and amino acid composition. These authors also reported that their preparation comprised only 1% hexose and 0·5% hexosamine. This low carbohydrate content is paralled by the inability to find a lectin to which the molecule will bind (J. F. Christie, E. M. Fraser and M. W. Kennedy), unpublished observations).

There are discrepancies between published estimates of the MW of ABA-1. In gradient SDS–PAGE system ABA-1 was found to consistently migrate at MW 14,000 relative to a 14,400 MW marker potein (Kennedy & Qureshi, 1986; Kennedy *et al.*, 1987a, b, 1989; Tomlinson *et al.*, 1989), but it will exhibit a smaller MW (11,300) when run on long 15% acrylamide homogeneous gels under reducing conditions (M. W. Kennedy, unpublished observations). In a separate study, it was found that the protein will migrate at 10,000 MW (McGibbon *et al.*, 1990) and that ABA-1 bears some similarity to the major protein present in Asc-1, which is an allergen preparation reported by Hussain *et al.* (1973). It is probable that this molecule is similar to the molecule partially purified by Greenspon *et al.* (1986) and sized at 9000 MW. The mass of ABA-1 is, therefore, operationally defined, and full sequence data for definitive sizing is awaited. In the meantime, there is some preliminary evidence that the protein might comprise a non-covalently linked dimer *in situ*, which dissociates in the presence of SDS (McGibbon *et al.*, 1990).

With regard to the heterogeneity of immune recognition of ABA-1 by *Ascaris*-infected individuals (Haswell-Elkins *et al.*, 1989; Kennedy, 1989, 1990), it would be anticipated from work on rodents that this is under genetic control. Whether this in turn has a bearing on heterogeneity in susceptibility to immunopathology and immunity to infection, or whether it is irrelevant, remains to be seen. What is remarkable, however, is that certain people do not respond to the substantial quantities of the ABA-1 allergen to which they are potentially exposed during years of continuous infection and reinfection with such a large parasite.

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