

MOLBIO 01291

Identification of the major *Ascaris* allergen and its purification to homogeneity by high-performance liquid chromatography

Angela M. McGibbon¹, Jacqueline F. Christie², Malcolm W. Kennedy² and Timothy D.G. Lee¹

¹Molecular Biology of Infectious Diseases Group, Faculty of Medicine, University of Calgary, Calgary, Canada; ²Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Glasgow, U.K.

(Received 4 August 1989; accepted 17 October 1989)

The major allergen from the body fluid of adult *Ascaris suum* (ABF) has been identified and purified to homogeneity using gel permeation high-performance liquid chromatography (HPLC). The purity of the *Ascaris* body fluid allergen (ABA-1) was confirmed by HPLC and SDS-PAGE. ABA-1 appears to be a 25-kDa dimer in its native form, which runs as a 10-kDa monomer on SDS-PAGE under reducing conditions. The allergenicity of the HPLC-purified protein was confirmed using isolated in vivo-sensitised mast cells from infected rats in an in vitro histamine release assay. ABA-1 is responsible for >80% of the allergenicity of ABF in this sensitive and specific system. Amino acid composition analysis and N-terminal amino acid sequencing were performed on ABA-1. Comparisons are made between ABA-1 and some of the heterogeneous *Ascaris* allergen preparations previously published. It is suggested that Asc-1 and allergen A both contain ABA-1 in large quantities and that discrepancies in the literature result from contaminating proteins in these preparations and technical differences in characterization of the predominant molecules present in the preparations. Compositional data suggests that the ABA-1 monomer is a molecule of 94 amino acids (based on a molecular weight estimate of 10 kDa) with a composition resembling that previously published for allergen A. The first 10 amino acids are identical to those of a protein affinity purified from the body fluid of *Ascaris suum* at the Wellcome Laboratories for Experimental Parasitology (WLEP-14K). The similarity between ABA-1 and WLEP-14k is also apparent on SDS-PAGE. ABA-1 appears also to be the major protein component in the body fluid of a viable *Ascaris lumbricoides* isolated from a hospital patient.

Key words: *Ascaris*; Nematode; Allergen; IgE

Introduction

There has been considerable interest in the identification of the major allergen or allergens present in extracts of *Ascaris*. This interest stems from the use of *Ascaris* extracts in a relevant model system of human allergy and asthma [1,2], from the interest in nematode allergens as activators of IgE responses (reviewed in ref. 3), and

from the possibility of the use of an *Ascaris* allergen as a vaccine candidate (McGibbon, A.M. and Lee, T.D.G., 1989. Isolation and characterization of *Ascaris* allergen. Proc. Can. Soc. Immunol., Abstr. No. 135). A primary concern with *Ascaris* crude extracts is the contamination of the soluble protein with non-specific immunomodulatory molecules such as vasoactive amines [4] and arachidonic acid metabolites [5], with non-specific degranulating agents [6] and with irrelevant proteins. Numerous laboratories have attempted to identify and characterise a molecule which is responsible for the majority of the allergenic potential of the crude extract derived from *Ascaris suum* (reviewed in ref. 7) but to date, all have had limited success. The two most cited potential allergens are allergen A, a 14-kDa molecule re-

Correspondence address: T.D.G. Lee, Department of Microbiology and Infectious Diseases, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada, T2N 4N1.

Abbreviations: ABA-1, *Ascaris* body fluid allergen; ABF, *Ascaris suum* body fluid; ALBF, *Ascaris lumbricoides* body fluid; FBS, foetal bovine serum; PBS, phosphate-buffered saline.

ported by Ambler and co-workers [8], and Asc-1, a 17–19 kDa molecule (dimer of 8.2 kDa) reported by Hussain and co-workers [9]. Asc-1 has been accepted as a reference allergen by many workers and is provided for *Ascaris* research through the National Institutes of Allergy and Infectious Diseases (NIAID), NIH, Bethesda, MD.

Unfortunately, none of the allergen preparations, including the two mentioned above, have been purified to homogeneity. Greenspon and co-workers [10] have probably come the closest to this with their enrichment of whole worm crude extract for a 8–10-kDa molecule which appears to be similar to the major protein present in the Asc-1 preparations. But even this preparation, by their own evidence, is contaminated with a 14-kDa protein on Coomassie Blue staining of gels and, as such, would probably reveal numerous contaminants on silver staining.

In this paper, we have taken advantage of the fact that *A. suum* body fluid (ABF) has high allergenic activity [11]. We used high performance liquid chromatography (HPLC) to isolate and purify the major protein component of ABF and confirmed its allergenicity in a sensitised mast cell degranulation assay *in vitro*.

Our results show that the major allergen of *Ascaris* is a 25-kDa molecule in its native form, which runs as a 10-kDa molecule on SDS-PAGE. We suggest that the native 25-kDa molecule results from the dimerisation of 10-kDa monomers. This 10-kDa protein bears great similarities to the major component of both the allergen A [8] and Asc-1 [9] allergen preparations. Moreover, the 10-kDa protein appears similar to the 9-kDa protein which is the major protein of Greenspon's [10] preparation, and is identical to the 14-kDa (WLEP-14k) protein referred to by Kennedy and co-workers [12].

Materials and Methods

Ascaris. Adult *A. suum* were obtained from a local pork slaughterhouse, washed well in warm (37°C) phosphate-buffered saline (PBS) and transported to the laboratory in warm (37°C) RPMI-1640 (Gibco) supplemented with 10% foetal bovine serum (FBS). We have found this medium to be able to support viable adult *Ascaris* in

vitro for at least 7 days (medium changed every day). ABF was obtained by removing the posterior tip of the worm and collecting the pseudocoelomic fluid. ABF was immediately spun at $100\,000 \times g$ for 1 h at 4°C to remove particulates. Low-molecular-weight non-specific mast cell degranulating factors were removed by Centricon (Amicon) centrifugal concentration (8-kDa cut-off) and the concentrate was subsequently diluted to the original volume with PBS. ABF was further centrifuged at $15\,000 \times g$ for 10 min before application to the HPLC.

Adult *A. lumbricoides* body fluid was obtained, in a similar manner to the description above, from a live female worm spontaneously passed by a patient at the Foothills Hospital, Calgary. The patient was not being treated with antihelmintic. Although there is a small possibility that the patient was bearing an *A. suum* infection, the patient history and appearance of the body fluids from this worm on SDS-PAGE reassures us that this worm was a human *A. lumbricoides*. All *Ascaris* body fluids were aliquoted and stored at –70°C if not used immediately. Asc-1 was obtained from Biotech Research Laboratories through the National Institutes of Health of the United States Government.

High-performance liquid chromatography. HPLC was performed on a Beckman System Gold HPLC apparatus. Size fractionation was accomplished on a TSK-3000SW silica-based column (7.5 mm $\times$ 30 cm, Beckman) utilising a 0.1 M Tris/0.1 M NaCl (pH 7.2) mobile phase. Ion exchange HPLC was carried out on a DEAE-5PW column (7.5 mm $\times$ 7.5 cm, Beckman) with a linear gradient from 20 mM Tris (pH 8.5) to 20 mM Tris/0.5 M NaCl (pH 8.5). BioRad HPLC molecular weight standards were used for molecular weight estimations.

SDS-PAGE. Gradient (5–20% acrylamide) 20-cm gels (1.5 mm) were run utilising a BioRad Protean II electrophoresis apparatus. Gels were run, at constant current (40 mA), to resolution for maximum separation of low-molecular-weight proteins. This required running the dye front off the bottom of the gel and subsequently running the gel for an additional 10 min. Proteins were made visible by both Coomassie Blue R250

(BioRad) staining and silver (BioRad) staining. Molecular weight was estimated, using both Sigma and BioRad molecular weight reference standards, by scanning wet gels on a BioRad scan densitometer (model 620).

Amino acid composition and sequencing. Composition analysis and sequencing of the HPLC-purified protein was performed at the University of Victoria, British Columbia, sequencing services. SDS-PAGE gels of the purified protein were first electro-blotted onto Immobilon P Transfer Membranes (Millipore) and the membrane cut-outs analysed directly [13]. An Applied Biosystems sequencer (model 470A) was used for sequence analysis, and compositional analysis was performed using an Applied Biosystems amino acid analyser (model 420).

Allergen assessment. Sensitised peritoneal mast cells were isolated from the peritoneal cavity of rats infected 20 days previously with 5000 *A. suum*

infective eggs (per os in a volume of 0.2 ml PBS). Peritoneal lavage and mast cell isolation was carried out as described elsewhere [14]. Cells were incubated for 5 min at 37°C in 1 ml Hepes-Tyroses (pH 7.5) solution with or without release agents. Supernatants and pellets were separated, after centrifugation ($200 \times g$, 5 min), and were separately assessed for histamine content by a fluorimetric assay as described previously [15]. Histamine release is expressed as mean % release ($\pm$ standard deviation from the mean, $N = 3$) in response to stimulation with *Ascaris* antigens as a percentage of the release seen with a positive release control (the calcium ionophore A23187, $10^{-6}M$), after subtraction of spontaneous release.

Results

Allergen purification. ABF has been shown by others to contain significant allergenic activity [11,16] and, as such, was used as the source of our

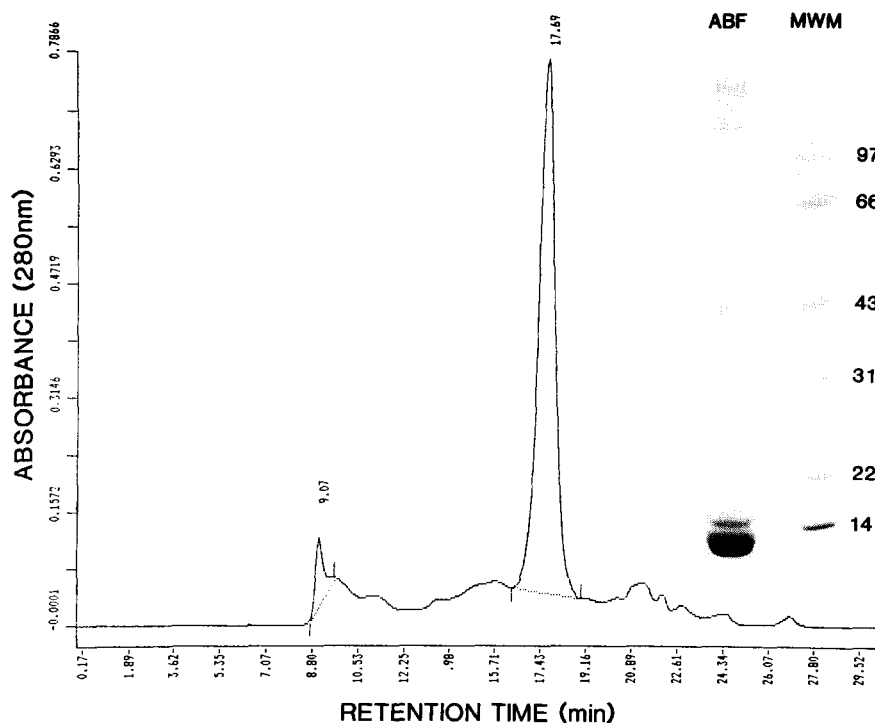


Fig. 1. Profile of *Ascaris* body fluid proteins using gel permeation HPLC. The major protein component runs at a relative molecular weight of 25 kDa in comparison to HPLC molecular weight standards. Inset, 5–20% gradient SDS-PAGE of ABF under reducing conditions stained with Coomassie Blue. The major protein is resolved at approximately 10 kDa, suggesting that the 25-kDa protein is a dimer of 10-kDa monomers. MWM, molecular weight markers (in kDa).

purified allergen. ABF is a heterogeneous mixture of proteins with the major protein in the mixture representing more than 60% of the total protein (by computer calculation of area under the HPLC peaks). The protein concentration of the crude ABF we obtained was 40–50 mg ml⁻¹. Using gradient SDS-PAGE (reducing conditions) on long (20 cm) gels the calculated molecular weight of the major protein, by scan densitometric comparison to known standards, is 10 kDa (Fig. 1, inset). Gel permeation HPLC, however (Fig. 1), showed that the major protein in ABF runs at 25 kDa. Dimerisation of the 10-kDa protein into a non-globular form is a possible reason for this apparent discrepancy.

HPLC purification of the major ABF protein yielded a homogeneous 25-kDa protein (Fig. 2) with no detectable contamination even when run on ion exchange (DEAE) HPLC (data not shown). Using a 250- μ l sample loop, and a 30 cm gel permeation HPLC column, up to 1 mg of purified protein could be obtained from ABF per run. When the purified peak was run on gradient SDS-PAGE under reducing conditions its relative mobility was 10 kDa (Fig. 3a; similar results were obtained under non-reducing conditions – data not

shown). Fig. 3a demonstrates the relationship between this purified protein and the major protein component of Asc-1 which, using our gradient SDS-PAGE conditions resolves as a 10-kDa molecule. Fig. 3b shows the ion exchange (DEAE) HPLC profile of Asc-1 indicating the presence of a range of different molecular species in this commercially available Asc-1 which has been prepared in a similar manner to the original description of Hussain et al. [9,17].

Confirmation of allergen activity. The purified 25-kDa molecule from HPLC-fractionated ABF caused specific release of histamine from sensitised mast cells at 250 μ g ml⁻¹ (Fig. 4). The level of release varied from experiment to experiment (range 12%) due to differences in sensitivity of the mast cells obtained from different rats, but release from the 25-kDa peak was never less than 80% of the release from ABF (protein concentrations adjusted for equivalence). No non-specific release was observed (Fig. 4) and very limited release was seen with any other HPLC fraction (data not shown). Although ABF obtained directly from fresh worms caused release of histamine from non-sensitised mast cells, this

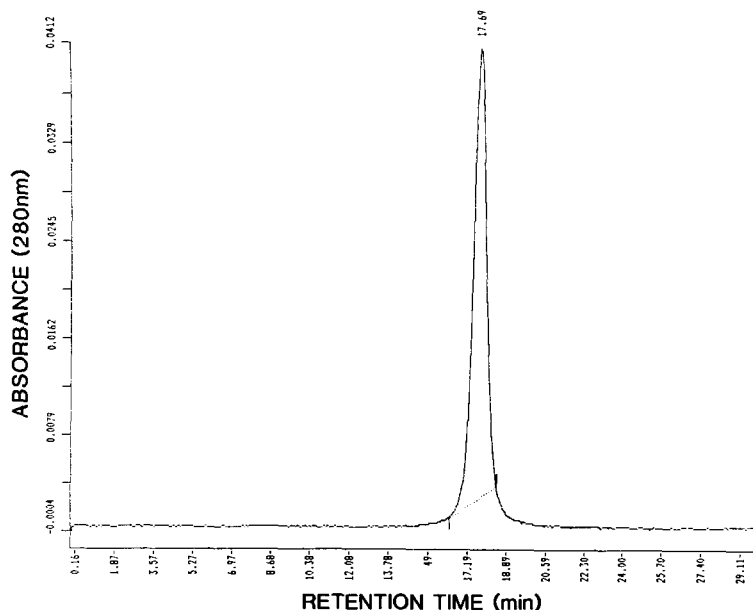


Fig. 2. 25-kDa peak from *Ascaris* body fluid (Fig. 1) purified by gel permeation HPLC to homogeneity, shown re-run on gel permeation HPLC (0.1 M Tris/NaCl buffer, pH 7.2) to demonstrate purity.

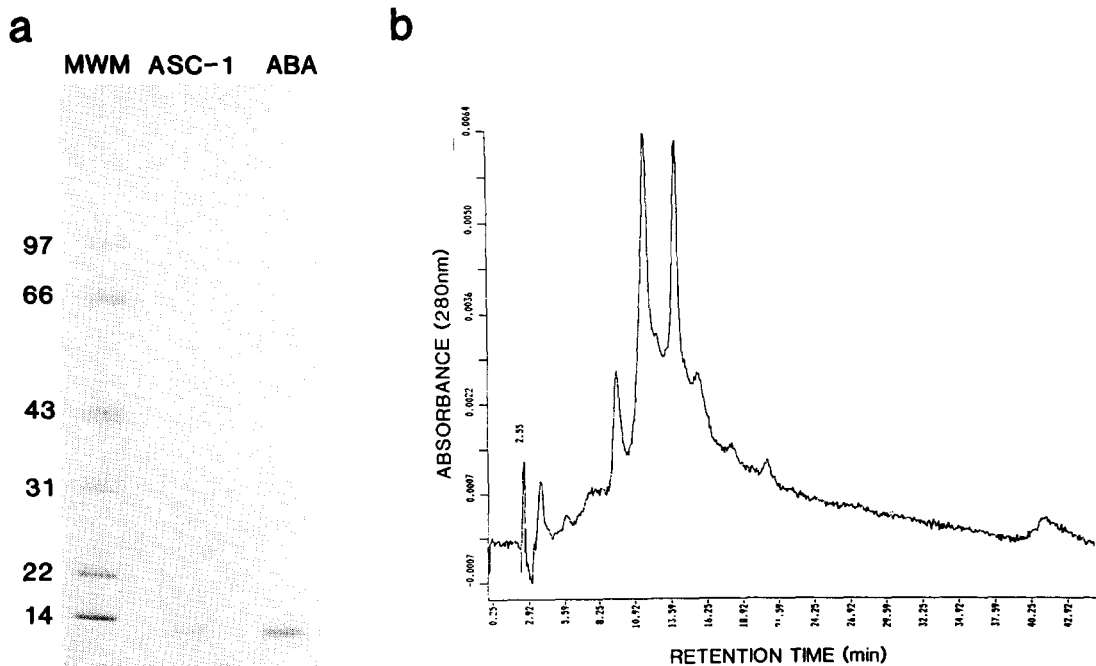


Fig. 3. (a) Asc-1 (NIH) subjected to SDS-PAGE (5–20% gradient under reducing conditions). At the level of protein added, only the major protein is evident. This protein runs at the same molecular weight as ABA-1. MWM, molecular weight markers (in kDa); (b) Ion exchange HPLC of Asc-1, demonstrating the heterogeneity of this preparation. ABA = ABA-1

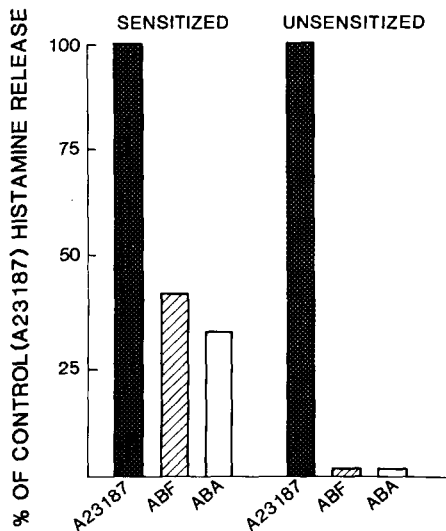


Fig. 4. Histamine release from peritoneal mast cell populations isolated from *Ascaris* infected or non-infected control rats. Release is expressed as a % of release mediated by the calcium ionophore A23187 (positive control) after subtraction of the spontaneous release. ABA = ABA-1

activity could be removed by concentrating the ABF 25-fold in a Centricon concentrator (molecular weight cut-off approximately 8 kDa) and re-constituting it to the original volume with PBS. The 25-kDa HPLC purified allergen (and 10-kDa putative monomer) are subsequently referred to as the *Ascaris* body fluid allergen (ABA-1).

Allergen characterisation. Primary characterisation of ABA-1 involved running the HPLC-purified 25-kDa molecule on a gradient SDS-PAGE reducing gel, blotting onto Immobilon P Transfer Membranes, staining for protein, and performing composition analysis and amino acid sequencing directly from 10 kDa bands excised from the Immobilon P membranes. Compositional data, assuming an approximate size of 10 kDa, indicates a molecule composed of 94 amino acids with an estimated formula weight of 10321 Da. As both Ambler et al. [18] and Hussain et al. [9] speculated, with considerably less pure preparations, the protein is rich in glutamic acid and lysine but has low detectable levels of methionine and cys-

teine. This, however, may be because the acid hydrolysis technique of amino acid detection does not resolve methionine and cysteine residues. The amino acid composition of ABA-1 bears a strong resemblance to the allergen A preparation of Ambler et al. [18] (r value of calculated best fit regression line is 0.782, Fig. 5) giving credence to the idea that ABA-1 is the major component of allergen A. The discrepancy in the amino acid composition probably arises from additional protein species in the allergen A preparation.

Amino acid sequencing was carried out to confirm that the affinity-purified WLEP-14k protein being studied in Glasgow by Christie and Kennedy (unpublished results and refs. 19 and 20), which has a strong resemblance to that of Ambler and co-workers [18], was the same as the HPLC-purified allergen. It is clear from the SDS-PAGE profile (Fig. 6a), from the mobilities on HPLC (data not shown) and from the homogeneity of the first 10 N-terminal amino acids (Fig. 6b), that both laboratories are working with the same protein species. Preliminary evidence from further sequencing has revealed identity up to amino acid 32 (Christie et al., unpublished results).

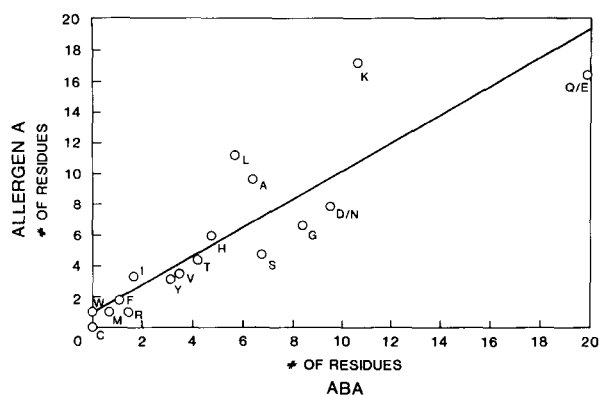


Fig. 5. Comparison of amino acid composition of *Ascaris* body fluid allergen and allergen A using the one letter code for each amino acid (see text). Using the acid hydrolysis technique of amino acid compositional analysis, it is impossible to distinguish aspartic acid (D) from asparagine (N), or glutamic acid (E) from glutamine (Q). Calculated best fit similarity line has regression coefficient (r value) of 0.782, indicating a significant degree of similarity between the two preparations. ABA = ABA-1

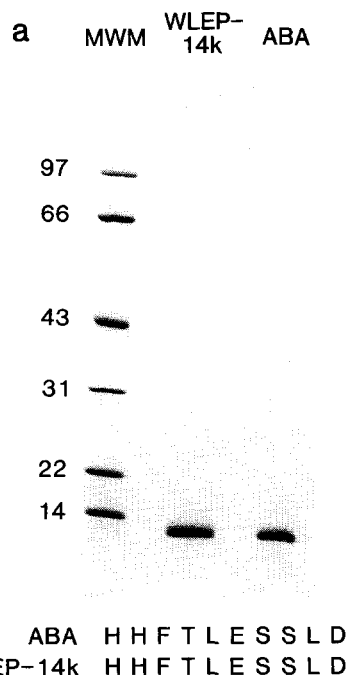


Fig. 6. (a) Comparison by 5–20% gradient SDS-PAGE of *Ascaris* body fluid allergen (lane 3) and WLEP-14k (lane 2). Both proteins migrate identically and have an M_r of approximately 10 kDa compared to the molecular weight markers (MWM) (lane 1). (b) Further evidence that these two proteins are identical is found in comparing the first 10 N-terminus acids of ABA-1 and WLEP-14k (using one-letter code for amino acids). ABA = ABA-1

A. lumbricoides. *A. lumbricoides* body fluid (ALBF) was run on HPLC and SDS-PAGE to confirm that the allergen from *A. suum* is also present in *A. lumbricoides*. Fig. 7a demonstrates by SDS-PAGE that although there are some differences between ALBF and ABF, there are also major similarities including the abundance of a 10-kDa protein. HPLC of the ALBF (Fig. 7b) showed that the major protein had an M_r of 24 kDa, suggesting that the 10-kDa protein in ALBF may be dimerised in a similar way to the major (25-kDa) protein in ABF. We have also demonstrated (manuscript in preparation) that monospecific polyclonal rabbit anti-ABA-1, raised against ABA-1 excised from nitrocellulose blots of SDS-PAGE gels, reacts strongly with the 10-kDa protein in ALBF. This is further evidence that ABA-1 is present in an at least similar, if not identical form in human *Ascaris*.

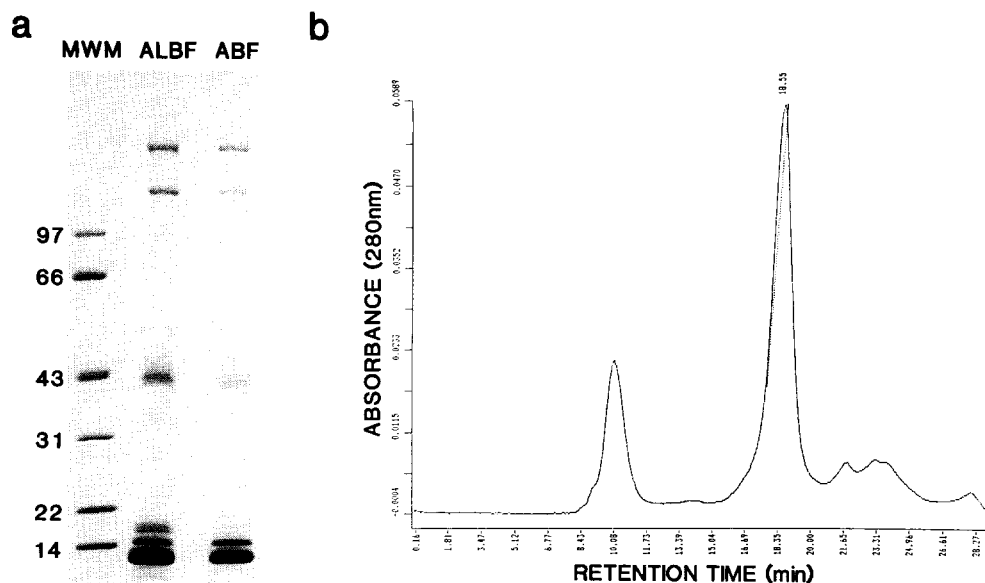


Fig. 7. (a) Comparison of the proteins in body fluids of *A. lumbricoides* (ALBF, lane 2) and *A. suum* (ABF, lane 3) on 5–20% gradient reducing gels indicates the major component of both runs at 10 kDa compared to standards (MWM, lane 1). (b) Gel permeation HPLC of ALBF indicates that the major protein, in its native form, is 24 kDa. This suggests that the 10-kDa protein in ALBF dimerises in a similar way to ABA-1 in ABF.

Discussion

Since IgE-mediated responses have been implicated in protection against helminth infection [3], we are investigating (i) the potential of the major *Ascaris* allergen as a vaccine candidate against pig and human *Ascaris* (Calgary); and (ii) the immunogenetics of the responsiveness to allergens of the parasite (Glasgow). Both of these areas of study require allergen preparations purified to homogeneity.

In this study, we have shown that the most abundant protein present in *Ascaris* body fluids, which co-migrates and is probably identical to the major protein present in crude soluble extracts [10], is responsible for >80% of the allergenic activity of ABF. This protein exists as a 25-kDa molecule in its native state and appears as a 10-kDa molecule when subjected to SDS-PAGE with or without 2-mercaptoethanol present as a reducing agent. This suggests that the 25-kDa molecule may be a dimer composed of 10-kDa monomers which are not disulphide-bonded. Additional support for this hypothesis comes from gel permeation HPLC which reveals a shift in molecular

weight of the 25-kDa native molecule to about 10 kDa in the presence of SDS (unpublished results). The 25-kDa dimer form is stable to heating at 100°C for 10 min, in that the protein retains its migratory characteristics on HPLC (data not shown). Whether it also retains its allergenicity after boiling has yet to be determined. A non-globular form for the dimer is the most likely reason for its slightly higher than expected molecular weight with respect to the monomer.

Amino acid compositional analysis predicts a molecular weight for the putative monomer of 10321 Da. The level of glycosylation of ABA-1 is yet to be determined but since interruption of sequencing by glycosylation has apparently not occurred up to residue 55 (Christie et al., unpublished results), heavy glycosylation is unlikely. There are however, a number of potential glycosylation sites on the molecule.

ABA-1 appears to be identical to the WLEP-14k protein which was used by Kennedy and co-workers to study genetic control of differential responsiveness in mice and shown to be an allergen [21,22]. The discrepancy in the molecular weight determinations may be explained by dif-

ferences in SDS-PAGE running conditions. Affinity-purified WLEP-14k has the same N-terminal amino acid sequence, and in this collaborative study runs identically on HPLC and SDS-PAGE to the HPLC-purified ABA-1.

Allergen activity of ABA-1 was confirmed using an in vitro sensitised mast cell assay. This assay has greater analytical potential than the in vivo passive cutaneous anaphylaxis (PCA) assay, and can be argued to have greater relevance because of the IgE sensitisation of mast cells in situ in infected animals.

The spectrum of potential allergens derived from *Ascaris* has been reviewed elsewhere [7,23]. The majority of the suspected allergens have been in the low molecular weight range (<50 kDa) but some workers [23,24] have described high-molecular-weight (>100 kDa) allergens. Previous studies, however, have used relatively crude column chromatography and slab gel purification techniques for the isolation and purification of potential allergens, and have usually tested these molecules using PCA. The isolation techniques have resulted in preparations contaminated with irrelevant proteins at best, and non-specific immunomodulatory factors at worst. Asc-1 for example, as issued as a standard *Ascaris* allergen preparation by NIH is described as a protein mixture containing 4 bands on SDS-PAGE, the most abundant of which co-migrates with our 10-kDa monomer on gradient SDS-PAGE. In our hands, Asc-1 consists of at least 20 bands on immunoblotting against rabbit anti-Asc-1 antiserum (unpublished observations). The extent of this contamination is highly relevant, since it is known that the allergenic molecule of *Nippostrongylus* is only a small fraction of the allergen-rich excretory/secretory proteins (ref. 25, and Lee, T.D.G. and Adams, E.J., Identification of the allergen of *Nippostrongylus brasiliensis* and its cloning in *E. coli*. AHFMR Research. 1988. Abstracts, p. 20). The contamination of previous allergen preparations along with the inherent inaccuracy of the less sophisticated column chromatography systems

available to earlier workers and the differences in SDS-PAGE running conditions among different groups has yielded a confusing picture in the literature. Given the data presented here it may well be that a number of groups have been working independently on the same protein and minor variations in techniques and the presence of contaminant proteins may be responsible for the discrepancies in results. A good example of this is the comparison of allergen A and Asc-1. Using shorter gels and shorter running times with a gradient system, it is possible to reproduce, approximately, the 14-kDa banding pattern seen for allergen A with ABA-1. Moreover, the methods used for molecular weight determination of Asc-1 by Hussain et al. [8] (Sephadex G-100 and SDS-PAGE on 10% slab gels) are inaccurate in the molecular weight range under examination. It is most likely that Asc-1 and allergen A contain, as well as irrelevant contaminating proteins, the same major protein, and that this protein is similar to the allergen we have purified to homogeneity.

We are currently characterising the allergen more fully and have initiated experiments to clone it into an expression vector for further research.

Acknowledgements

We wish to thank Ms. Sandy Kielland at the University of Victoria, British Columbia, sequencing services laboratory, Mr. Bryan Dunbar and Mr. Ian Davidson of the Department of Biochemistry, Marischal College, University of Aberdeen, Dr. J.F. Urban, United States Department of Agriculture, Maryland, and Dr. G. Evans, Foothills Hospital, Calgary, for their contributions to this study. We thank Ms. Ivy Lee for the preparation of the manuscript. This work was funded by the Alberta Heritage Foundation for Medical Research and the Wellcome Trust, through grants to TDGL and MWK, respectively.

References

- 1 Eady, R.P., Greenwood, B., Jackson, D.M., Orr, T.S.C. and Wells, E. (1985) The effect of nedocromil sodium and sodium cromoglycate on antigen-induced bronchoconstriction in the *Ascaris*-sensitive monkey. *Br. J. Pharmacol.* 85, 323-325.

- 2 Patterson, R., Harris, K.E. and Krell, R.D. (1988) Effect of a leukotriene D₄ (LTD₄) antagonist on LTD₄ and *Ascaris* antigen-induced airway responses in Rhesus monkeys. *Int. Arch. Allergy Appl. Immunol.* 86, 440–445.
- 3 Ogilvie, B.M. and De Savigny, D. (1982) Immune responses to nematodes. In: *Immunology of Parasitic Infection* (Cohen, S. and Warren, K., eds.), pp. 715–757, Blackwell, Oxford.
- 4 Pawlowski, Z.S. (1982) Ascariasis: host-pathogen biology. *Rev. Infect. Dis.* 4, 806–814.
- 5 Dworski, R., Wendelborn, D.F., Sheller, J.R., Fitzgerald, G.A. and Roberts II, L.J. (1988) Prostaglandins in *Ascaris* antigen. *J. Allergy Clin. Immunol.* 82, 484.
- 6 Uvnas, B. and Wold, J.K. (1967) Isolation of a mast cell degranulating polypeptide from *Ascaris suum*. *Acta Physiol. Scand.* 70, 269–276.
- 7 Coles, G.C. (1985) Allergy and immunopathology of ascariasis. In: *Ascariasis and its Public Health Significance* (Crompton, D.W.T., Nesheim, M.C. and Pawlowski, Z.S., eds.), pp. 167–184, Taylor and Francis, London.
- 8 Ambler, J., Doe, J.E., Gemmell, D.K., Roberts, J.A. and Orr, T.S.C. (1972) Biological techniques for studying the allergenic components of nematodes, I. Detection of allergenic components in *Ascaris suum* extracts. *J. Immunol. Methods* 1, 317–328.
- 9 Hussain, R., Bradbury, S.M. and Strejan, G. (1973) Hypersensitivity to *Ascaris* antigens, VIII. Characterization of a highly purified allergen. *J. Immunol.* 111, 260–268.
- 10 Greenspon, L.W., White, J., Shields, R.L. and Fugner, A. (1986) Purification of *Ascaris suum* antigen: Its allergenic activity in vitro and in vivo. *J. Allergy Clin. Immunol.* 77, 443–451.
- 11 Hogarth-Scott, R.S. (1967) The molecular weight range of nematode allergens. *Immunology* 13, 535–537.
- 12 Kennedy, M.W. and Qureshi, F. (1986) Stage-specific secreted antigens of the parasitic larval stages of the nematode *Ascaris*. *Immunology* 58, 515–522.
- 13 LeGendre, N. and Matsudaira, P. (1988) Direct protein microsequencing from Immobilon-P transfer membrane. *Biotechniques* 6, 154–159.
- 14 Atkinson, G., Ennis, M. and Pearce, F.L. (1979) The effect of alkaline earth cations on the release of histamine from rat peritoneal mast cells treated with compound 48/80 and peptide 401. *Br. J. Pharmacol.* 65, 395–402.
- 15 Lee, T.D.G., Shanahan, F., Miller, H.R.P., Bienenstock, J. and Befus, A.D. (1985) Intestinal mucosal mast cells: isolation from rat lamina propria and purification using unit gravity velocity sedimentation. *Immunology* 55, 721–728.
- 16 Kuo, C.Y. and Yoo, T.J. (1977) A new allergen from the perienteric fluid of *Ascaris suum* with respect to charges. *Int. Arch. Allergy Appl. Immunol.* 54, 308–314.
- 17 Hussain, R., Strejan, G. and Campbell, D.H. (1972) Hypersensitivity to *Ascaris* antigens, VII. Isolation and partial characterization of an allergen. *J. Immunol.* 109, 638–647.
- 18 Ambler, J., Miller, J.N. and Orr, T.S.C. (1974) Some properties of *Ascaris suum* allergen A. *Int. Arch. Allergy* 46, 427–437.
- 19 Kennedy, M.W., Qureshi, F., Haswell-Elkins, M. and Elkins, D.B. (1987) Homology and heterology between the secreted antigens of the parasitic larval stages of *Ascaris lumbricoides* and *Ascaris suum*. *Clin. Exp. Immunol.* 67, 20–30.
- 20 Kennedy, M.W., Qureshi, F., Fraser, E.M., Haswell-Elkins, M.R., Elkins, D.B. and Smith, H.V. (1989) Antigenic relationships between the surface-exposed, secreted and somatic materials of the nematode parasites *Ascaris lumbricoides*, *Ascaris suum*, and *Toxocara canis*. *Clin. Exp. Immunol.* 75, 493–500.
- 21 Kennedy, M.W., Gordon, A.M.S., Tomilson, L.A. and Qureshi, F. (1986) Genetic (major histocompatibility complex?) control of the antibody repertoire to the secreted antigens of *Ascaris*. *Parasite Immunol.* 9, 269–273.
- 22 Tomlinson, L.A., Kennedy, M.W., Christie, J.F., Fraser, E.M., McLaughlin, D. and McIntosh, A.E. (1989) MHC restriction of the antibody repertoire to secretory antigens, and a major allergen, of the nematode parasite *Ascaris*. *J. Immunol.* 143, 2349–2356.
- 23 O'Donnell, I.J. and Mitchell, G.F. (1978) An investigation of the allergens of *Ascaris lumbricoides* using a radioallergosorbent test (RAST) and sera of naturally infected humans: comparison with an allergen for mice identified by passive cutaneous anaphylaxis test. *Aust. J. Biol. Sci.* 31, 459–487.
- 24 McWilliam, A.S., Stewart, G.A. and Turner, K.J. (1987) An immunochemical investigation of the allergens from *Ascaris suum* perienteric fluid. Cross-reactivity, molecular weight distribution and correlation with phosphorylcholine-containing components. *Int. Archs. Allergy Appl. Immunol.* 82, 125–132.
- 25 Petit, A., Pery, P. and Luffau, G. (1980) Purification of an allergen from culture fluids of *Nippostrongylus brasiliensis*. *Mol. Immunol.* 17, 1341–1349.