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Homology and heterology between the secreted antigens of the parasitic larval stages of *Ascaris lumbricoides* and *Ascaris suum*

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SUMMARY

The materials released *in vitro* by the tissue-parasitic larval stages of the large roundworm of man, *Ascaris lumbricoides*, were analysed by radio-iodination, immunoprecipitation, and SDS-PAGE. The antigens were found to be heterogeneous, ranging in molecular weight from 14 to 410 kD, and were found to alter radically during the parasites' migration to the lungs. The antigens secreted by the infective and lung-stage larvae of the pig homologue, *Ascaris suum*, were compared with those of the human worms. This revealed a remarkable degree of homology between the products of the two, at both the molecular and immunological levels. The two species could be discriminated, however, on the basis of the SDS-PAGE profiles of the antigens secreted by both developmental stages of the parasites examined. Finally, antiserum to the canine ascarid infective to man, *Toxocara canis*, was found to precipitate a significant proportion of *Ascaris*-secreted molecules. These studies, therefore, confirm the potent antigenicity of excretory/secretory materials, and their potential for use in immunodiagnosis, but predict serious difficulties for seroepidemiology and the *specific* detection of ascariasis in man.

Keywords *Ascaris lumbricoides* *Ascaris suum* *Toxocara canis* excretory/secretory antigens cross-reactivity

INTRODUCTION

Over one billion people serve as hosts for the common roundworm, *Ascaris lumbricoides*, making it the most prevalent helminth infection of man (World Health Organisation, 1981). Ascariasis is, therefore, a major health problem on a global scale, and there is growing evidence that nutritional disturbances, morbidity and mortality associated with infection are far greater than formerly considered (Crompton, 1985; Crompton, Nesheim & Pawlowski, 1985).

A major constraint to serodiagnosis and seroepidemiology of nematode infections, such as ascariasis, is extensive immunological cross-reactivity between nematodes infective to man, which is notable even between distantly related forms (Ogilvie & Savigny, 1982). This problem is particularly evident among the ascarid nematodes, since, in many regions of the world, man is exposed to the infective stages of several species. The commonest of these are *Ascaris suum* and *Toxocara canis*, which are intestinal parasites of the pig and the dog respectively. These do not complete their life cycle in the human host, but their tissue-invasive larval stages can be highly pathogenic, especially in

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the latter case, which causes an insidious and chronic disease. To what degree exposure to these zoonotic parasites influences the pathology, immunology and epidemiology of human ascariasis remains unknown, but an understanding of their antigenic interrelationships is pertinent.

Here we have chosen to study the antigenic relationships between *A. suum* and *A. lumbricoides*. Such is their similarity in terms of life-cycle and structure that there has been a long-standing debate as to their status as strains, subspecies, or reproductively isolated true species. Discrimination between the two has been attempted on morphological, biochemical, and epidemiological grounds (Campbell, 1937; Sprent, 1952; Taffs & Voller, 1963; Kurimoto, 1974; WHO, 1981; Gibson, 1983), with some apparent success. However, the subject is controversial, and it remains to be determined whether host-specificity is consistent on a global scale.

In this report we have concentrated on the materials released by the tissue-invasive larvae during culture *in vitro*. We chose these so-called excretory/secretory (ES) materials, for several reasons. First, they are the targets of significant antibody responses in ascariasis (Kennedy & Qureshi, 1986) and other nematodiases (Ogilvie & de Savigny, 1982). Moreover, this response is greater than that elicited against worm somatic components, and, as we argue elsewhere (Kennedy & Qureshi, 1986), ES materials are the superior antigen source for the detection of humoral responses to ascariasis. Secondly, the ES products are thought to contain protective antigens (Stromberg, 1979a; Urban & Romanowski, 1985). Thirdly, they have been reported to contain allergens and IgE-potentiating factors which might contribute to the severe pulmonary hypersensitivity reponses which occur during heavy seasonal infection (Stromberg, 1979b; 1980; Ogilvie & de Savigny, 1982). Fourthly, as they are the released products of living parasites, ES would seem to be more appropriate for the detection of the occult tissue-invasive larvae, by screening for antigen in body fluids, than are the liberated components of dead worms. Finally, ES products are held to be of enhanced species specificity (Voller & de Savigny, 1981; Ogilvie & de Savigny, 1982), and, therefore, would seem to be a choice source of antigens for the immunological discrimination of antigenically and phylogenetically related parasites.

Here we present the first immunochemical comparison of the antigens released *in vitro* of the infective and lung-stage larvae of *A. suum* and *A. lumbricoides*. We find that there is extensive homology between their antigens at both the molecular and immunological levels, but the two parasites can, nevertheless, be discriminated. While this incomplete homology warrants a further search for species-specific markers, it also justifies the continued use of the more readily available pig parasite for immunological work on the human disease.

MATERIALS AND METHODS

Parasites and hosts. Adult *Ascaris suum* worms were obtained from the intestines of infected pigs at an abattoir in Glasgow. For the collection of *Ascaris lumbricoides*, mass chemotherapy using pyrantel pamoate (Mexin Pharmaceuticals, Bombay) at 10 mg per kg body weight was administered to an infected fishing village community in Tamil Nadu, India. Adult female *Ascaris* worms were removed from faeces passed within the first 24 h after treatment. The worms were rinsed with water, placed in 4% formalin on ice and dispatched by airfreight to Glasgow within 24 h. Transit time was approximately 7 days.

Eggs were removed from the uteri of female worms and embryonated in a solution of 2% formalin and 125 units/ml of nystatin (Nystan, Paediatric Oral Suspension; Squibb, London) at 25°C for 40 days. The eggs were stored in the same solution at 4°C. Embryonated eggs were decoated in 25% NaOCl solution (BDH, Poole, Dorset), until microscopic examination showed removal of most of the shell. Following thorough washing in distilled water, the infective second-stage larvae (L2) were hatched by disruption of the remaining shell layers in a glass tissue homogenizer, and the larvae separated from shell fragments by allowing them to migrate through a cotton wool plug in culture medium (see below). The lung-stage larvae were recovered from the lungs of rabbits, 7 days after oral infection with 50,000 eggs. The larvae were separated from chopped lung tissue by migration through a 250 μm sieve (Endecotts, London) in PBS containing the same antibiotics used for their culture medium (see below). Further purification of the larvae was then carried out by an additional migration through a cotton wool plug in the complete culture

medium. The larvae so recovered were a mixture of third- and fourth-stage larvae, and were designated L3/4.

Ascaris body fluid (ABF) was collected on ice from freshly-collected adult *A. suum* worms, and stored at −70°C until used. Adult worms of the canine ascarid, *Toxocara canis*, were obtained from dogs, and their eggs embryonated using methods similar to those described above for *Ascaris*.

In vitro-*released parasite products*. The L2 and L3/4 stages were cultured in a defined serum-free medium modified from Stromberg, Khoury & Soulsby (1977) and Urban & Douvres (1984). It contained Medium 199, Dutch Modification (Flow Laboratories, Irvine) with 2 mM L-glutamine, 1 mg/ml glucose, 400 ng/ml glycyl-L-histidy-L-lysine (Sigma G 1887), 16 μM glutathione, 100 i.u./ml penicillin, 100 μg/ml streptomycin, and 1 mM sodium pyruvate. Larvae were cultured at concentrations of approximately 20,000/ml and 1,000/ml for L2 and L3/4 respectively at 37°C. Both stages survived in culture for about 3 weeks, during which time the medium was harvested and replenished weekly. These products are usually termed excretory/secretory materials and are abbreviated 'ES'.

Antisera. Antisera to both isolates of *Ascaris* were obtained from rabbits infected with 3000 viable eggs of the parasite at 28-day intervals, and bled at the peak of the antibody response, 14 days after the final infection.

Antiserum to *Toxocara canis* was collected from a rabbit infected with 20,000 viable eggs. Such infection gives rise to a persistent parasitism, inducing an antibody response which rises to a plateau at about 28 days, and decaying only slightly thereafter (unpublished observations).

Antiserum to the 14 kD protein in ABF was raised in rabbits with purified material, as follows: ABF was separated by SDS-PAGE on a 1·5 mm thick, 20% homogeneous gel which was stained with Coomassie Blue (Sigma, B 0630), destained, washed in three changes of distilled water over 30 min, and the appropriate band excised. The gel matrix containing the 14 kD component was ground in liquid nitrogen with a mortar and pestle. This was then homogenized with Freunds Complete Adjuvant (GIBCO Laboratories, Cat. No. 660-5721) and the emulsion was injected into multiple sites on the back of the rabbits. The procedure was followed 40 days later with a similar preparation made up in Freunds Incomplete Adjuvant (GIBCO, 660-5720) and the rabbits were bled 12, 14, 17 and 19 days later.

Radio-iodination. Iodine labelling of soluble ES materials was carried out by the IODOGEN method (Markwell & Fox, 1978). Two hundred microlitres of PBS-dialysed culture medium were incubated in a tube pre-coated wih 200 μg of dried 1,3,4,6-tetrachloro-3a,6a-diphenyl glycoluril (Pierce Chemical Co., Rockford, Illinois) and 100 μCi $Na^{125}I$ (West of Scotland Radionucleotide Dispensary, Glasgow) for 10 min on ice with repeated agitation. Excess iodine was consumed by the addition of 10% by volume of saturated tyrosine solution, and labelled macromolecules were isolated by gel filtration on Sephadex G-25.

Radio immunoprecipitation. Iodinated preparations were tested in an assay using formalin-fixed *Staphylococcus aureus* (SA) bacteria as a solid-phase absorbent (Kessler, 1975). To 50 μl PBS/0·5% Triton X-100 were added $70–200 \times 10^3$ ct/min of test antigen and 2·5 μl of serum. Following overnight incubation at 4°C, 50 μl of *S. aureus* suspension (Pansorbin Standardized: Calbiochem 507861) were added. The mixture was incubated for 1 h at room temperature, with occasional shaking, then 2 h at 4°C. The bacteria were washed three times in the cold, the radioactivity measured by an automatic gamma counter, and the pellet prepared for SDS-PAGE.

Polyacrylamide gel electrophoresis (SDS-PAGE). This was carried out in a Pharmacia GE-2/4LS slab gel apparatus according to the manufacturer's instructions. Gradient gels (5–25%) consisted of 120 mm separating and 10 mm stack gel, and were 0·7 mm thick. Samples were prepared according to Maizels, Meghji & Ogilvie (1983) with 5% mercaptoethanol or 1 mg/ml iodoacetamide for reducing or non-reducing conditions, respectively. Following electrophoresis, gels were fixed for 30 min in 25% methanol, 10% acetic acid, then dried. Dried gels were exposed to flashed Fuji film using intensifying screens, and the autoradiographs exposed at −70°C. Gels to be stained for protein were treated with Coomassie Brilliant Blue R (Sigma B 0630) overnight, destained, and dried. Apparent molecular weights were estimated by mobility relative to standard marker proteins (Pharmacia 17-0446-01 and Boehringer Mannheim 750 115), and are expressed in kilodaltons (kD).

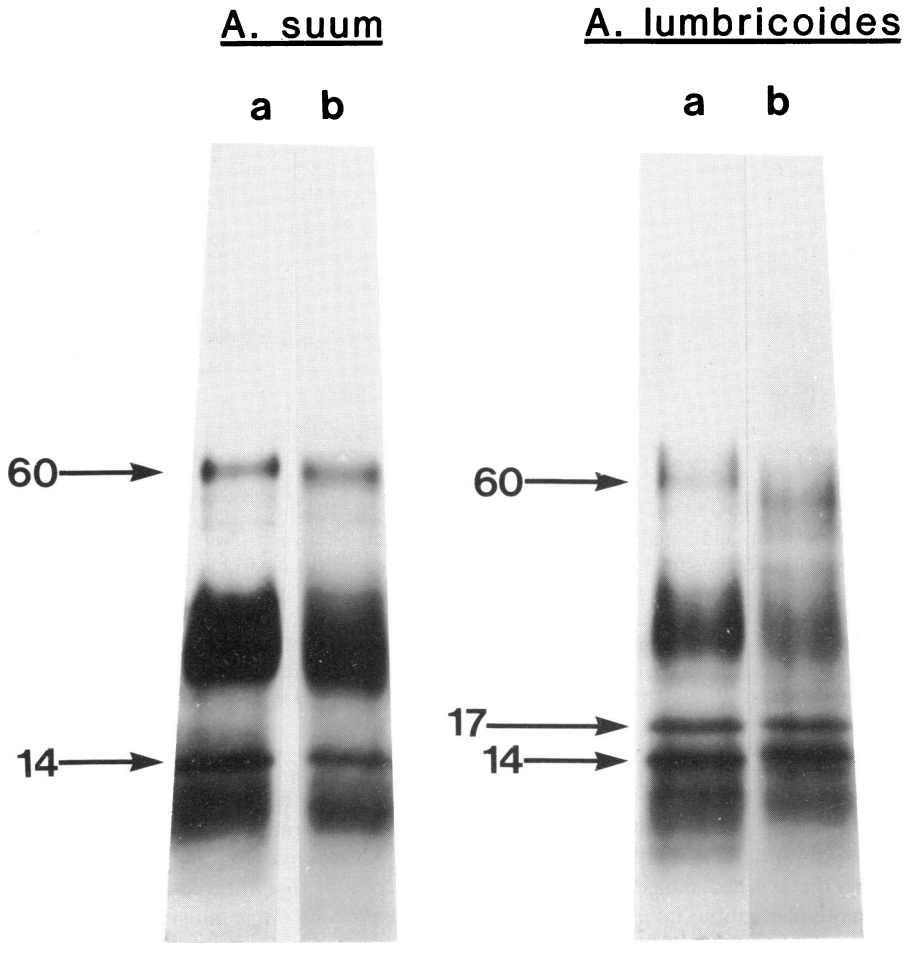

Fig. 1. SDS-PAGE and autoradiographic analysis of ^{125}I-labelled ES products of the infective larvae (L2) of *Ascaris suum* and *Ascaris lumbricoides* released *in vitro*. The samples were run under reducing (a) or non-reducing conditions (b). Apparent molecular weights are expressed in kilodaltons (kD).

RESULTS

Profile of the products of infective larvae (L2) released in vitro. The materials released by the infective larval stages of both *Ascaris suum* and *Ascaris lumbricoides* were radio-iodinated and analysed by SDS-PAGE and autoradiography (Fig. 1). The labelled molecules ranged in apparent molecular weight from 14 kD to 225 kD, with most of them falling in the range 14–60 kD, and the higher molecular weight components were only clear on gels exposed for longer than that illustrated. In the case of both the pig and human parasites, reducing conditions produced no significant change in the SDS-PAGE profile.

The most pertinent point here is that the secretions of the two kinds of larvae were remarkably similar, and that the only major difference was the major band of 17 kD, which was exclusive to *A. lumbricoides*. This was found to be consistent from batch to batch of culture fluid, although a faint band at this position could occasionally be discerned in the products of the pig parasite. It is possible, therefore, that the production of the 17 kD component is a quantitative rather than a qualitative difference between the two species.

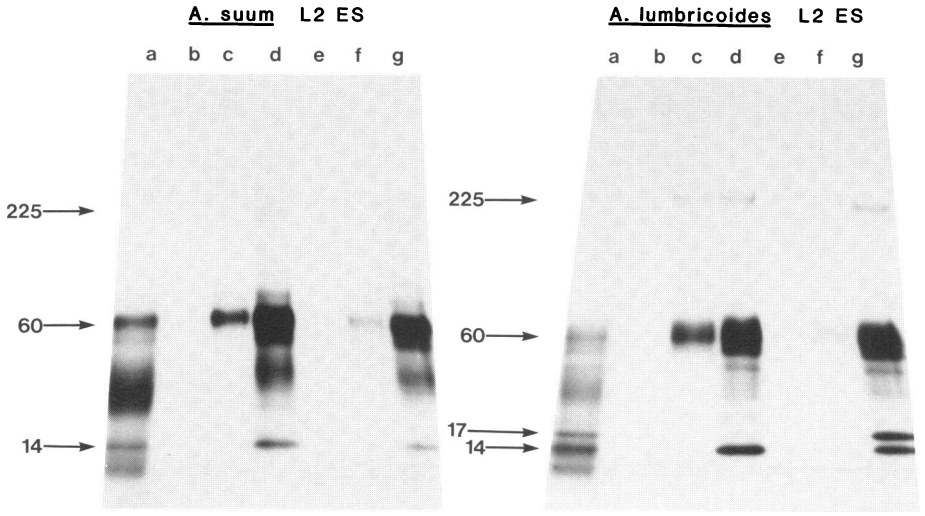

Fig. 2. Antigenicity of, and homology between, the ES products of *Ascaris suum* and *Ascaris lumbricoides* infective larvae (L2). Radioiodinated ES antigens (a) were reacted with serum from rabbits bled (b & e) before infection, or (c & d) after infection with *A. suum* or (f & g) *A. lumbricoides*. Serum from rabbits infected with the former parasite was taken (c) on day 21 of a primary infection or on (d) day 28 after a sixth infection. *Ascaris lumbricoides*-infected animals were bled (f) on day 21 of a primary, or (g) on day 28 of a tertiary infection. Immune complexes were absorbed onto *Staphylococcus aureus* (SA) bacteria and analysed by SDS-PAGE. The quantity of radioactivity precipitated under each of the above conditions is given in Table 1.

Antigenicity and reciprocal recognition of L2 ES components. The antigenicity of the L2 ES was examined qualitatively by immunoprecipitation with serum from rabbits infected with one or other of the ascarids, and the immunoprecipitates were analysed as above (Fig. 2). This showed, firstly, that all of the labelled molecules were antigenic. Secondly, that the recognition patterns of antiserum raised in rabbits infected with *A. suum* or *A. lumbricoides* were indistinguishable. That is, antibody to one species precipitated all the components secreted by both parasites. This figure also illustrates that the ES components were recognized sequentially; at 21 days after primary infection, the 60 kD entity was preferentially precipitated in both *A. suum* and *A. lumbricoides* infections. In this particular experiment, the rabbits infected with the former precipitated this component from both antigen preparations to an equal extent. The *A. lumbricoides*-infected rabbit did likewise, but less material was precipitated (see also Table 1). We have no reason to believe that this is a fundamental difference between the two infections, but is probably due to differences in the infectivity of the egg cultures used, or between the rabbits themselves. Whatever the cause, the pattern of recognition was identical.

Of possible significance is the fact that the *A. suum* rabbit did not recognize the 17 kD component of *A. lumbricoides* to the extent of the homologous antiserum. However, the 17 kD was nevertheless precipitated, and serum taken following reinfection of the same rabbit displayed an unambiguous recognition of this component (see below).

The 14 kD and 17 kD molecules are not related. Most of the major components of *Ascaris* ES tend to give diffuse bands on SDS-PAGE, with the notable exception of the 14 and 17 kD entities. Their similar appearance on autoradiographs prompted us to ask whether the two exhibit some homology. The findings illustrated in Fig. 2 would seem to argue for heterology, but a repeat of this experiment (Fig. 3), using serum from the same rabbit given further infections, compelled us to re-examine this point using reagents with greater powers of discrimination. For this we used a serum, again raised in rabbits (see Materials and Methods), to the dominant 14 kD component of the body fluid of adult *A. suum* worms, which is known to be homologous to that of the larval ES (Kennedy & Qureshi, 1986). When this serum was reacted with *A. lumbricoides* L2 ES, the 14 kD component was

Table 1. Antigenicity of *Ascaris* ES materials to homologous and heterologous antisera

	^{125}I-antigen precipitated			
		A. lumbricoides L2 ES		
Rabbit serum	*A. suum* L2 ES (Fig. 2)	(Fig. 2)	(Fig. 3)	*A. suum* L3/4 ES (Fig. 5)
Normal	2·6±0·3	2·2±0·3	1·7±0·2	4·3±0·4
Single *A. suum* infection	7·6±0·8	6·5±0·5	—	—
Multiple *A. suum* infections	25·2±1·4	17·1±2·6	20·0±1·6	61·7±2·6
Single *A. lumbricoides* infection	5·5±0	3·2±0·2	—	—
Multiple *A. lumbricoides* infections	24·1±7·0	16·6±1·3	29·4±2·5	35·4±1·3
Single *T. canis* infection	—	—	12·6±0·2	—
Anti-14 kD molecule	—	—	4·3±0	—

Results presented are those from the experiments used for the Figures indicated, using the *S. aureus*-based radioimmunoassay described in Materials and Methods. Results are expressed as the percentage (mean±s.d.) of TCA-precipitable input ct/min per assay tube. The total activity added varied from 70×10^3 to 200×10^3 ct/min per duplicate tube.

precipitated to the exclusion of all others, including the 17 kD. This disparity was confirmed by using an antibody with the complementary specificity from a rabbit infected with a related ascarid nematode, *Toxocara canis*. This recognized a subset of *Ascaris* ES molecules, including the 17 kD, but *excluding* the 14 kD.

The experiment also clearly illustrated the homology between the 14 kD somatic component of *A. suum* and that of the corresponding molecule in the products of the infective larvae released *in vitro* of both *A. suum* and *A. lumbricoides*. Although not shown in this paper, this also applies to the 14 kD found in the ES of the lung-stage larvae, whose secretory products we now go on to describe.

Profiles of the products of the lung-stage larvae (L3/4) released in vitro. Culture fluid from *Ascaris* larvae obtained from rabbits was radioiodinated and analysed as before. These larvae are in a later developmental stage than the infective larvae, having moulted once or twice en route to, or in, the lungs, and are a mixture of third and fourth stage larvae (L3/4). The products of these were quite different from those of the earlier larval stage, and ranged from 14 to 410 kD (Fig. 4), the highest molecular weight component only being clear in over-exposed autoradiographs. Furthermore, these larger molecules were susceptible to reducing conditions, giving rise to several components of lesser molecular weights.

This stage-specificity was apparent in both *A. suum* and *A. lumbricoides* L3/4 ES, but the most significant finding was the clear similarity in the profiles of the two preparations, yet with distinct differences. Several molecules were shared, but at least two major components, of 25·5 kD and 118 kD, were unique to *A. lumbricoides*, and one of 28 kD was apparently unique to *A. suum*. It is possible that there are some homologies between these molecules, in spite of differences in both abundance and molecular weight, but the resolution of this possibility will require methods of greater specificity and discrimination.

One notable point about Fig. 4 is the difference in abundance of the 14 kD present in the two cultures, this molecule being more apparent in *A. lumbricoides* ES than in that of *A. suum*. However, we find that the abundance of this component varies considerably from culture to culture of both parasites, and we do not consider this variability to be of significance. Such is the abundance of this molecule among the somatic components of these nematodes that we have not been able to eliminate the possibility that its source in the culture medium is the small number of dead or moribund larvae which are inevitably found in these cultures, in spite of every effort to maintain and select for their viability.

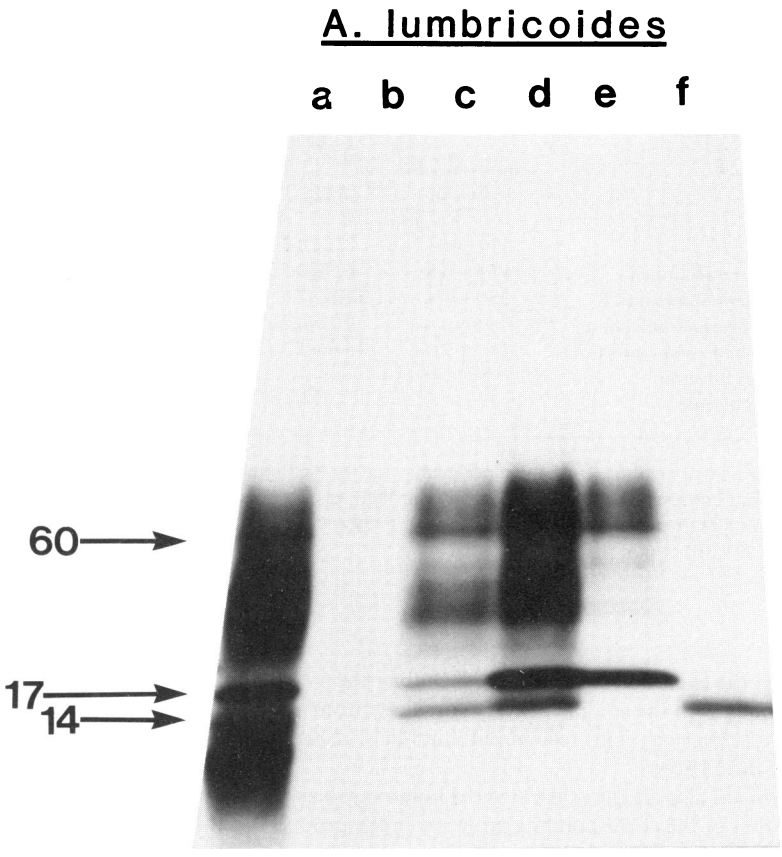

Fig. 3. The 14 kD and 17 kD components of *A. lumbricoides* L2 ES are not related. (a) Radio-iodinated products of infective larvae were reacted with serum from (b) a normal rabbit, or (c) rabbits infected seven times with *A. suum*, (d) six times with *A. lumbricoides*, (e) once with *Toxocara canis*, or (f) immunized with the 14 kD component of the body fluid of adult *Ascaris* worms. SA-mediated immunosorbates were processed for SDS-PAGE analysis as described in Materials and Methods. The quantity of radioactivity precipitated under each of the above conditions is given in Table 1.

Reciprocal recognition of L3/4 ES components. As for L2 ES, all of the L3/4 ES components of both *A. suum* and *A. lumbricoides* lung larvae were recognized by both homologous and heterologous antisera (Fig. 5 and Table 1). We only illustrate this here using serum raised against the pig parasite, but the results are indistinguishable when anti-*A. lumbricoides* serum is used.

The important point here is that the difference between the L3/4 ES of the two parasites appears to be more qualitative than for L2 ES, but this does not affect the heterologous recognition. In the experiment illustrated in Fig. 5, the molecules of the lowest molecular weight appear as a doublet, one of 14 kD, the other migrating slightly more slowly. When this immunoprecipitation was repeated in the presence of excess ABF, the 14 kD component disappeared from the precipitate, but not its partner (not shown). The two are, therefore, not homologous, and the upper might be related to the 17 kD antigen of *A. lumbricoides* L2 ES, although their positions on the gels do not quite correspond. This molecule is only seldom found in *A. suum* L3/4 culture supernatant, unlike the 17 kD of *A. lumbricoides* L2 ES, and we have, therefore, not investigated it further.

DISCUSSION

We report here the first characterization of the materials released by the living, tissue-invasive larvae

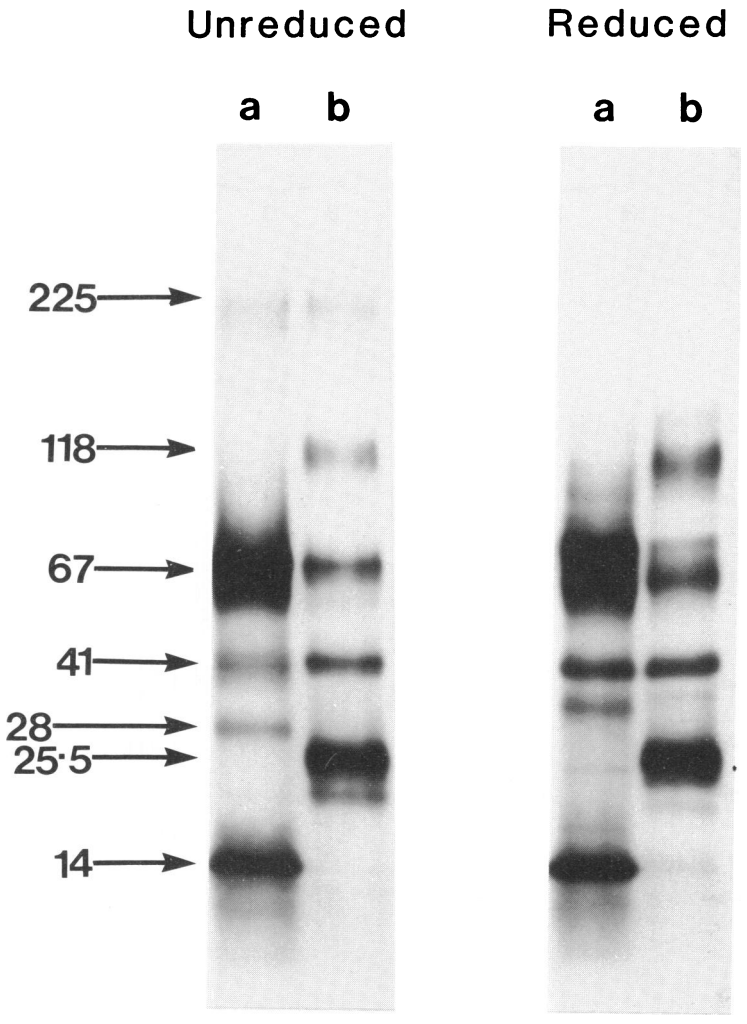

Fig. 4. Isolate-specific profile of ES products of the lung-stage larvae (L3/4) of *Ascaris* released *in vitro*. Radioiodinated ES from (a) *A. lumbricoides* and (b) *A. suum* were analysed by SDS-PAGE under non-reducing or reducing conditions.

of an important parasite of man, *Ascaris lumbricoides*. We have analysed the products of both the infective and lung-stage larvae, and found them to be heterogeneous and specific to the developmental stage of the parasite. Moreover, all of the labelled molecules were recognized by antibody from infected hosts.

We also examined the (ES) antigens of the pig roundworm, *Ascaris suum*, released *in vitro* and found that these exhibited extensive homology to those of the human-derived worms. However, there was also clear evidence of molecules unique to each species of parasite.

The two forms of *Ascaris* have been previously discriminated on several grounds (see Introduction), and our present findings would further support the argument that the two represent distinct species. However, a caveat must be added to our study and all others: the parasite material used has been restricted in both the numbers of worms examined, and/or the area from which samples have been obtained. In our case, the isolates were obtained from widely separated regions (*A. lumbricoides* from India, and *A. suum* from Scotland), each of which presumably comprises genetically heterogeneous parasite populations. We cannot, therefore, eliminate the possibility that

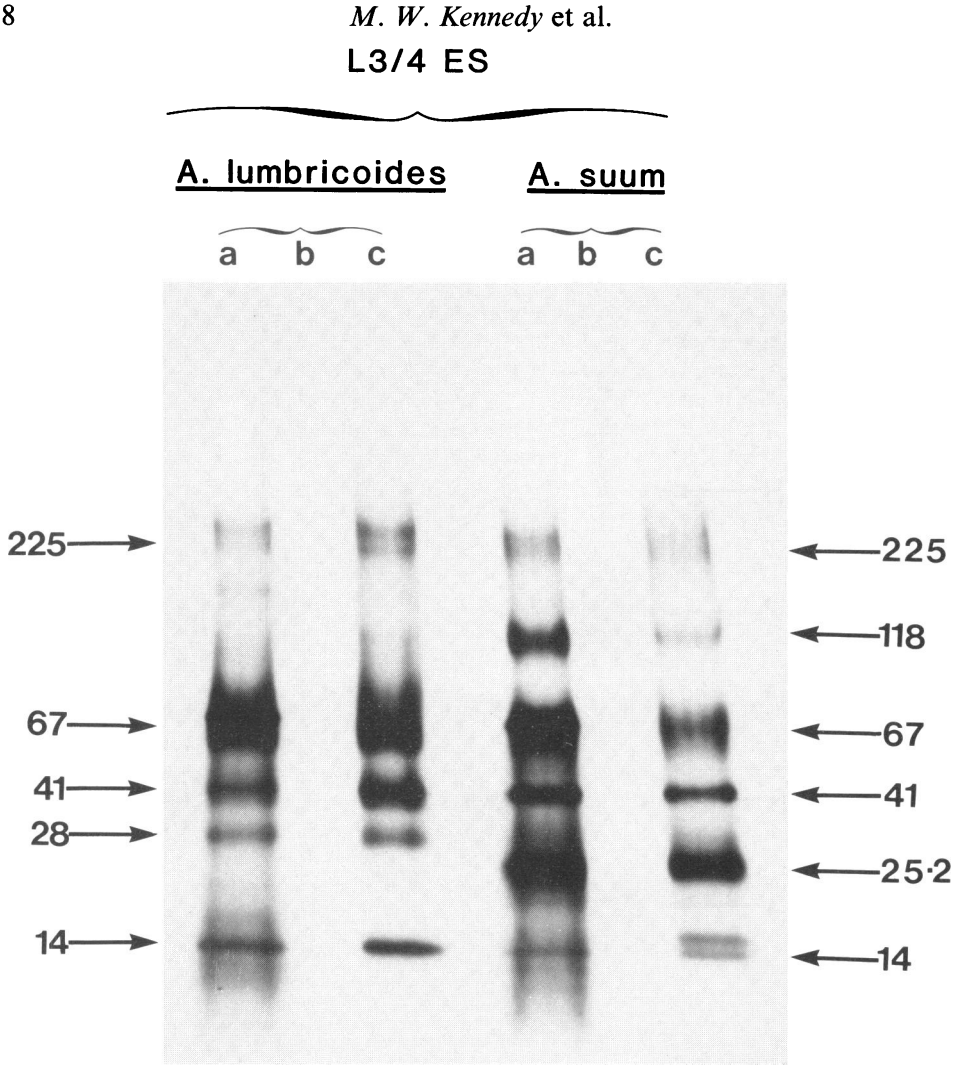

Fig. 5. Antigenicity of, and cross-reactivity between, the ES products of the lung-stage larvae (L3/4) of *Ascaris*. (a) Radiolabelled ES of *A. lumbricoides* or *A. suum* were reacted with serum from (b) normal or (c) *A. suum*-infected rabbits, and the SA-mediated absorbates analysed by SDS-PAGE. The percentage of labelled ES (TCA precipitable) bound by these sera is given in Table 1.

the limited species-specificity which we observed was due to geographical and/or intrinsic variability of *Ascaris* populations. However, the ease with which the infective stages of this parasite can be transported, and the availability of techniques which require small quantities of material, permits the rapid screening of isolates on a global scale. Such methods should eventually be superseded by molecular genetic techniques, and the identification of candidates for species-specific marker antigens might then provide powerful tools for a combined approach to the questions of speciation and immunobiology of human ascariasis. Of particular importance would, for example, be the comparison of sympatric populations of the human and porcine parasites.

One possible source of the observed differences between the two parasites is that, until arrival in this laboratory, they were subjected to different conditions. The porcine worms were dealt with within hours of removal from their hosts, whereas the human parasites differed in several respects. First, they were recovered following anthelmintic treatment of infected individuals, rather than by dissection; second, they were in transit for 1 week, under conditions which were beyond our control.

It is likely, for example, that their temperature was such that the eggs would have begun to develop while still inside the female worm. We find that this can, if allowed to proceed for too long, result in the death of the larvae, presumably through oxygen depletion. On the other hand, we found that the eggs transported from India embryonated successfully, although they did so more slowly, and a smaller percentage completed their development, than the locally obtained *A. suum*. Moreover, it could be argued that the antigens used here were quality-selected by two criteria: first, they were the products of living larvae, and, second, these were in their turn selected for viability by passage through a cotton wool plug, or by their ability to undergo their programmed migration to the lungs of infected rabbits, for the L2 and L3/4 larvae respectively.

The close homology between the two ascarids was further emphasized at the immunological level. Polyvalent antisera raised against the two infections displayed complete reciprocal cross-reactivity. This implies that those molecules which were apparently unique to one parasite, by the criterion of molecular weight, shared epitopes with other ES components. This is known for *Toxocara* ES molecules (Maizels *et al.*, 1983), and is presumably due to internal homologies, e.g. shared peptide sequences or carbohydrate residues.

The use of polyvalent infection serum is open to the criticism that the observed cross-reactivity between ES is due to antigenic similarity between ES and worm somatic components. The host will presumably be exposed to the latter during moulting, or following death, of the migrating larvae. However, the similarity in the gel profiles of the ES components in question would seem to argue in favour of true homology.

This immunological homology would seem to render serological discrimination of *A. suum* and *A. lumbricoides* futile. Indeed, such is its extent that we have been discouraged from attempting cross-absorption studies. However, it is conceivable that methods based on monoclonal antibodies or cloned peptides might provide the required specificity.

We have presented elsewhere a detailed analysis of the ES products of *A. suum* larvae (Kennedy & Qureshi, 1986), and two of our findings on these products are likely to apply to those of *A. lumbricoides*. Firstly, there are two entities of identical apparent molecular weight (67 kD) in L3/4 ES. One of these is of parasite origin, and the other is serum albumin derived from the rabbits from which the larvae were recovered. Secondly, while the overall SDS-PAGE profile of ES components is clearly stage-specific, there is evidence of molecules common to both preparations. The clearest example of this is the 14 kD component, which is also abundant in the body fluid of adult worms. This homology was originally inferred by a cold target inhibition experiment, and is confirmed by our present results using a monospecific antiserum raised against the 14 kD molecule.

This antiserum was also used to show homology between the 14 kD component in the ES of the two species of *Ascaris*, as well as to discriminate it from the 17 kD molecule which is apparently released only by the second-stage larvae of *A. lumbricoides*. This disparity between the two molecules was further underlined by the converse result using an antiserum raised against a closely related ascarid parasite, *Toxocara canis*. Antiserum to this recognized several *Ascaris* ES molecules, including the 17 kD, to the exclusion of the 14 kD. This cross-reaction was fortuitious for its analytical use, but it has wider implications for the seroepidemiology of both ascariasis and toxocariasis in man. We have pursued this observation further, as we will report elsewhere, but it is pertinent to point out here that this cross-reactivity is apparent in spite of the contrasting SDS-PAGE profiles of *Ascaris* and *Toxocara* ES (this paper; Maizels, de Savigny & Ogilvie, 1984).

The few serological studies which have examined the antibody response to *Ascaris* infection in man have all relied on the use of whole worm homogenates of adult *Ascaris*, or, more commonly, their body fluid (O'Donnell & Mitchell, 1978; 1980; Tsuji *et al.*, 1977; Welch, Dobson & Chopra, 1986). We have argued elsewhere (Kennedy & Qureshi, 1986) that ES products are superior for the detection of *Ascaris* infection, and our preliminary work on sera from individuals living in endemic areas has reinforced this view. The problems of cross-reactivity remain to be solved, of course, but these are not unique to ES, and other sources of antigen lack its clear advantages.

The complex serology of ascarid infection seems likely to be both a mirror and a stumbling block to an understanding of the immunobiology of human ascariasis. Certainly, for this group of nematodes at least, the early hopes of straightforward species-specific serodiagnosis, using ES products, seem likely to be frustrated. On the other hand, the close homology between the antigens

of *A. suum* and *A. lumbricoides* justifies the continued use of the former for both pure and applied work on the human disease.

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