Clin. exp. Immunol. (1989) 75, 493–500

Antigenic relationships between the surface-exposed, secreted and somatic materials of the nematode parasites *Ascaris lumbricoides*, *Ascaris suum*, and *Toxocara canis*

M. W. KENNEDY, F. QURESHI, E. M. FRASER, M. R. HASWELL-ELKINS,* D. B. ELKINS* & H. V. SMITH† *Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Bearsden, Glasgow, *Department of Pure and Applied Biology, Imperial College of Science and Technology, London and †Scottish Parasite Diagnostic Laboratory, Department of Bacteriology, Stobhill General Hospital, Springburn, Glasgow*

(Accepted for publication 1 December 1988)

SUMMARY

The cosmopolitan nematode parasites *Ascaris lumbricoides*, *Ascaris suum*, and *Toxocara canis* are closely related phylogenetically, and are all pathogenic to man. In the case of the latter, the antigens released by the tissue-invasive parasitic larvae *in vitro* ('excretory/secretory' or 'ES' antigens) are routinely used for serodiagnostic purposes. Here we have found, using radioimmunoprecipitation with defined rabbit antiserum, and SDS-PAGE, that there is a significant antigenic similarity between the secreted and somatic antigens of the three nematodes, and have characterized cross-reactive components. Among these is a 14 kD internal protein which has a homologue in all three parasites. This molecule is the subject of an IgG antibody response in *Ascaris* infection, but there is no measurable response to it in toxocariasis. Lastly, using quantitative immunofluorescence, the antigens exposed on the surface of intact, living, larvae were found to be cross-reactive or specific depending on the developmental stage of the parasites. This means that the surface of tissue-invasive *Ascaris* larvae bears stage-specific epitopes.

Keywords *Toxocara canis* *Ascaris lumbricoides* *Ascaris suum* surface antigens parasitic nematodes visceral larva migrans

INTRODUCTION

Of the pathogenic parasitic helminths to which man is exposed, there are many species which are phylogenetically and antigenically closely related. Their antigenic inter-relationships bedevil serodiagnosis as well as the elucidation of their immunobiology in humans. Among these parasites are the ascarid nematodes, for one of which, *Ascaris lumbricoides*, man is the definitive host. Both the porcine homologue, *Ascaris suum*, and the roundworm of dogs, *Toxocara canis*, are infective and pathogenic to humans.

T. canis gives rise to a persistent, insidious, and chronic disease in man, which involves parasitic invasion of the soft tissues of the body, including the brain and the eye. Ocular infection has resulted in confusion with a common neoplasm of childhood, retinoblastoma, and consequent enucleation (Glickman & Schantz, 1981; Searl *et al.*, 1981). Reports of ocular toxocariasis have mainly come from developed countries, and its impact in other areas is not known, although likely to be significant.

The prevalence of individuals seropositive for toxocariasis has been estimated in several studies to be between 2–3% of the adult population in developed countries (Glickman & Schantz, 1981). These estimates are almost certainly underestimates of the true prevalence since they are often unable to take into account the difference between adults and children in seroprevalence rates; toxocariasis is usually acquired in childhood, and any serum antibody response might decay in later life. Support for this comes from the few studies carried out in children, in which seropositivity rates as high as 7·1% and 14·3% have been found in European schoolchildren (Josephs, Bhinder & Thompson, 1981; Van Knapen, *et al.*, 1983), and 23·1% in children in North Carolina, USA (Worley *et al.*, 1984). Moreover, a study in the Caribbean found a convex age-seropositivity curve for toxocariasis, peaking in the first decade of life at 60%, and decaying with increasing age (Bundy *et al.*, 1987).

In most areas in which *A. lumbricoides* is endemic, exposure to *A. suum* and *T. canis* is likely to be sufficiently common to confuse serodiagnosis and obscure our understanding of patho-

Correspondence: Dr Malcolm Kennedy, Wellcome Laboratories for Experimental Parasitology, University of Glasgow, Bearsden, Glasgow G61 1QH, Scotland, UK.

logy and acquired immunity. We have examined antigenic homology between these parasites, using secreted, somatic and surface-exposed antigens in radioimmunoprecipitation and SDS-PAGE analysis as well as immunofluorescence. The former combination is now widely used in the the characterization of antigenic homologies, and we used the latter to examine the host-parasite interface directly. One particular note of concern is the degree of heterologous reactivity which we found with the excretory/secretory materials (ES) of *T. canis*, which is the antigen source of choice in the serodiagnosis of toxocariasis in man (Ogilvie & de Savigny, 1982).

Lastly, we concentrated on the 14 kD internal proteins of *Ascaris* and *Toxocara*, which are probably homologous, and against which there were contrasting responses in toxocariasis and ascariasis.

MATERIALS AND METHODS

Parasite materials: in-vitro-released and somatic

Adult *Ascaris suum* and *Ascaris lumbricoides* worms were obtained from infected pigs and humans, respectively, and the in-vitro-released products of the tissue-invasive larval stages were prepared as described previously (Kennedy *et al.*, 1987c). The culture supernatants are conventionally termed 'excretory/secretory' (ES) materials. The products of two larval stages of *Ascaris* were examined, namely the infective and the pathogenic lung-stage larvae. The first of these is in its second stage of development when infective, was artificially hatched from fully embryonated eggs of the parasite before culture, and its product is abbreviated 'L2 ES'. The lung-stage larvae are in transition from the third to fourth larval stages, were obtained from the lungs of infected rabbits, and their culture supernatant is termed 'L3/4 ES'. The in-vitro-released materials of the canine ascarid infective to man, *Toxocara canis*, were prepared following similar established methods (Maizels, de Savigny & Ogilvie, 1984). The perienteric fluids of adult *A. suum* and *T. canis* worms were collected on ice, microfuged at 13 000 *g* for 10 min and stored at −70°C until used.

Antisera

The rabbit serum against infection with the two species of *Ascaris*, and *Toxocara*, and the specific antiserum to the SDS-PAGE-purified 14 kD component of the perienteric fluid of *A. suum* adult worms were all produced as before (Kennedy *et al.*, 1987c). Antiserum to the ES of *T. canis* was raised in a rabbit as previously described (Smith *et al.*, 1981) and antiserum to *Ascaris* body fluid (ABF) was produced by injecting 1 mg protein in Freund's complete adjuvant intramuscularly on two occasions 7 days apart, followed 2 months later with 1 mg protein in Freund's incomplete adjuvant in multiple sites on the back skin, and blood was taken 12, 14, and 16 days later. We did not use sera from mice infected with *Ascaris* for any of the experiments, unlike other workers (Boyce, Branstetter & Kazacos, 1988), because we find that the parasite antigens recognized depends on the number of infections and strain of mouse used (Kennedy *et al.*, 1987a).

Radioiodination, immunoprecipitation and SDS-PAGE

These techniques were all as previously described (Kennedy & Qureshi, 1986).

Immunofluorescence

Living parasitic larvae from 5-day (*Ascaris* L2), 7-day (*Ascaris* L3/4), and up to 3-month (*T. canis* L2), cultures were washed in ice-cooled PBS/0·1% NaN_3 to remove any excretory/secretory products. The parasites were then incubated in test serum at various dilutions for 30 min on ice, and bound antibody detected with FITC-conjugated donkey anti-rabbit IgG (whole molecule) (Scottish Antibody Production Unit, Carluke, Scotland, product number S076-201) at a final dilution of 1:30, for 30 min on ice. To inhibit the shedding of surface materials, which commonly occurs in nematodes, all subsequent washing and incubation steps were carried out with ice-cooled buffers in the presence of 0·1% NaN_3. Parasites were viewed under ultraviolet light, in the presence of an anti-quenching agent ('Citifluor AF3', Citifluor Ltd., London), and the fluorescence quantified by photon counting using a Leitz MPV Compact 2 microscope photometer linked to an Epson PX 4 computer. The photometer field diaphragm was set to measure a rectangular area, in the range 230 to 555 μm^2 using a Leitz NPL Fluotar ×40 objective. The areas selected for measurement were chosen to be away from edge fluorescence, and the area measured was kept constant in any one experiment. The fluorescence of one of the brightest specimens was used to standardize the photometer at the arbitrary value of 650. The values given are the mean for at least 25 estimations per data point, and are background subtracted. Standard deviations are not included, for clarity, but were in all cases less than 10%.

RESULTS

The starting point for this work was the observation that humans living in an endemic area react differentially to the ES antigens of both the infective and lung stages of *Ascaris* (M. Haswell-Elkins *et al.*, submitted). There are clearly many factors which could contribute to this, including intensity of infection, frequency of exposure, genetic constitution of the hosts, and cross-reactions with other infecting agents. We already know that the major histocompatibility complex has a dominant role in restricting antigen recognition in infection with *Ascaris* in rodents, and that there are sequential responses to distinct secreted antigens (Kennedy *et al.*, 1987a). We will concentrate here on the potential for cross-reactions to contribute to the effect, following on from our previous finding of antigenic similarities between *Ascaris* and *Toxocara* (Kennedy, *et al.*, 1987c).

Antigenic similarity between the ES of Ascaris *and* Toxocara

Taking the ES of *Ascaris* first, Fig. 1A illustrates the extent of heterologous recognition of the secretions of the infective stage (L2) of the human parasite, *A. lumbricoides*. The SDS-PAGE profile of the products of this larva are virtually indistinguishable from those of the pig parasite, with the exception of the 17 kD component which we have only found in the secretions of the human-specific worm (Kennedy *et al.*, 1987c). Anti-*T. canis* serum precipitated several *Ascaris* ES components, with the notable exception of the 14 kD molecule. This component appears to be identical to that found in abundance in the perienteric fluid of adult *Ascaris* worms, since an antiserum specific for the 14 kD only precipitated one 14 kD component from the ES (track f). We cannot eliminate the possibility that the 14 kD might have arisen in the ES from dead or moulting

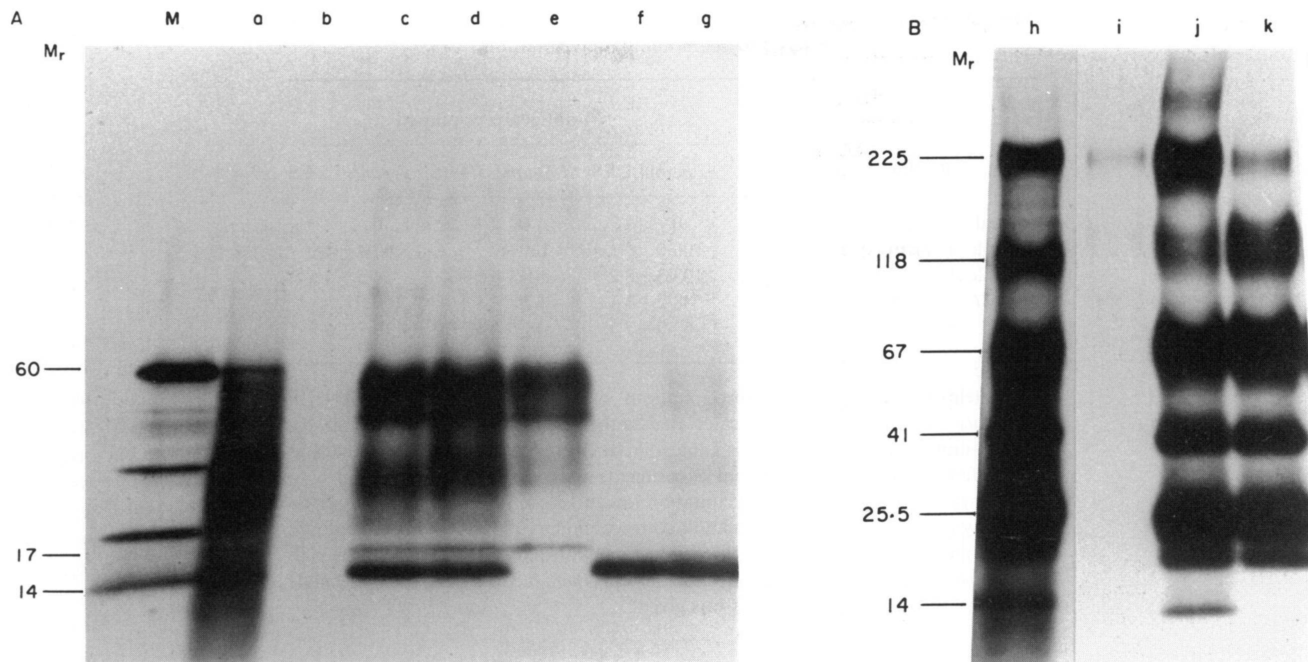

Fig. 1. Cross-recognition of *Ascaris* antigens by anti-*Toxocara* infection sera. Panel A: radioiodinated ES antigen of *Ascaris lumbricoides* infective larvae (L2) (a), immunoprecipitated with normal rabbit serum (b), or rabbits infected with *A. suum* (c), *A. lumbricoides* (d), *T. canis* (e), or immunized with the purified 14 kD molecule from *A. suum* (f), or whole body fluid from *A. suum* (g). Panel B: Radio-iodinated ES from the lung-stage larvae (L3/4) of *A. suum* (h), immunoprecipitated with normal rabbit serum (i), and serum from rabbits infected with *A. suum* (j), or *T. canis* (k). 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 (Pharmacia 17-0446-01) were run in track M. Relative mobilities (M_r) of the major antigen components are given in kilodaltons (kD), as calculated from a standard curve constructed using commercial protein mixtures (Pharmacia 17-0446-01 and Boehringer Mannheim 750 115).

parasites in the culture, rather than as a true secretion of the larva. An indication that this was unlikely, however, came from the antigen recognition of an antiserum to the whole perienteric fluid of the parasite. This only faintly precipitated any ES component other than the 14 kD (track g). The slight degree of precipitation of the higher molecular weight molecules might testify to some contaminating internal components, but could also be due to cross-reactions with internal precursors of secreted antigens.

When the antigens released by the later larval stages of *Ascaris* (L3/4) were examined (Fig. 1B), the degree of cross-recognition was similar to that with the product of the infective larva, and, as before, the anti-*T. canis* serum failed to recognize the 14 kD molecule.

Reciprocity in the cross-reactions was demonstrated using *T. canis* ES as target antigen (Fig. 2A). Here, the ascariasis sera precipitated several *T. canis* ES molecules, and there was an unexpected difference between the specificity of the *A. suum* and the *A. lumbricoides* infection sera in the recognition of the 32 kD molecule. One point of note is the lack of precipitation of the *T. canis* ES 400 kD component by both ascariasis sera, which was consistent from experiment to experiment.

T. canis larvae are very stable during culture *in vitro*, and, being under developmental arrest, do not moult. This offered an opportunity to look at the cross-reactivity using an antiserum to the purified ES of a single developmental stage. The experiment illustrated in Fig. 2A established the credentials of the anti-*T. canis* ES serum in reaction with homologous antigen, and Fig. 2B confirmed the substantial degree of epitopes held in common with the ES of *Ascaris* L3/4 ES. One point of interest is the apparent difference between the recognition profiles of anti-*T. canis* infection serum and the antiserum raised to the purified ES; only the former recognized the 25·5 kD molecule in *A. suum* L3/4 ES. The quantity of radio-iodine incorporated into the immunoprecipitates used in the above gel analyses was recorded, and emphasized the degree of cross-recognition (Table 1).

The common 14 kD component, and non-responsiveness to it in toxocariasis.

The 14 kD molecule has similar physicochemical characteristics to a major allergen of *Ascaris* (Ambler *et al.*, 1973a, b; J. F. Christie, E. M. Fraser & M. W. Kennedy, unpublished). It was important, then, to establish whether or not *T. canis* produced a homologous protein, and, if so, to better characterize the response to it in toxocariasis. We first characterized the total protein content of the body fluids of both *T. canis* and *A. suum* by SDS-PAGE and stained for protein with Coomassie blue (Fig. 3). Most of the proteins had apparent molecular masses of less than 200 kD, but, in non-reduced material, molecules of up to about 400 kD are present. One of these migrated in approximately the same position as the 340 kD marker, and

Table 1. Cross-reactions between the antigens of *A. suum*, *A. lumbricoides* and *T. canis*, measured by radio-immunoprecipitation

Rabbit serum	^{125}I-antigen precipitated		
	A. suum L2 ES	*A. suum* L3/4 ES	*T. canis* L2 ES
Normal	0	0	0
Multiple *A. suum* infections	100·0	100·0	42·4±4·1
Multiple *A. lumbricoides* infections	122·3±8·2	81·3±6·9	54·5±4·5
Single *T. canis* infection	38·2±3·1	67·7±4·4	100·0
Anti-*T. canis* ES	62·9±7·7	48·5±4·2	110·7±6·9

Protein-A-mediated immunoprecipitations were carried out as described in *Materials and Methods*, and then calculated as the fraction of the TCA-precipitable input ct/min per assay tube. For this Table, the data were background-subtracted, and results from at least three different experiments were pooled and expressed as a percentage of the homologous precipitation (mean±s.e.). Hence the normal serum reactivity is taken as 0% and the homologous reaction as 100%. *Ascaris* infection in the rabbit is self-limiting, and so multiple infections with 3000 viable eggs were given to the rabbits. In contrast, *T. canis* infection is a chronic infection, and so only a single infection initiated with 20 000 eggs was given.

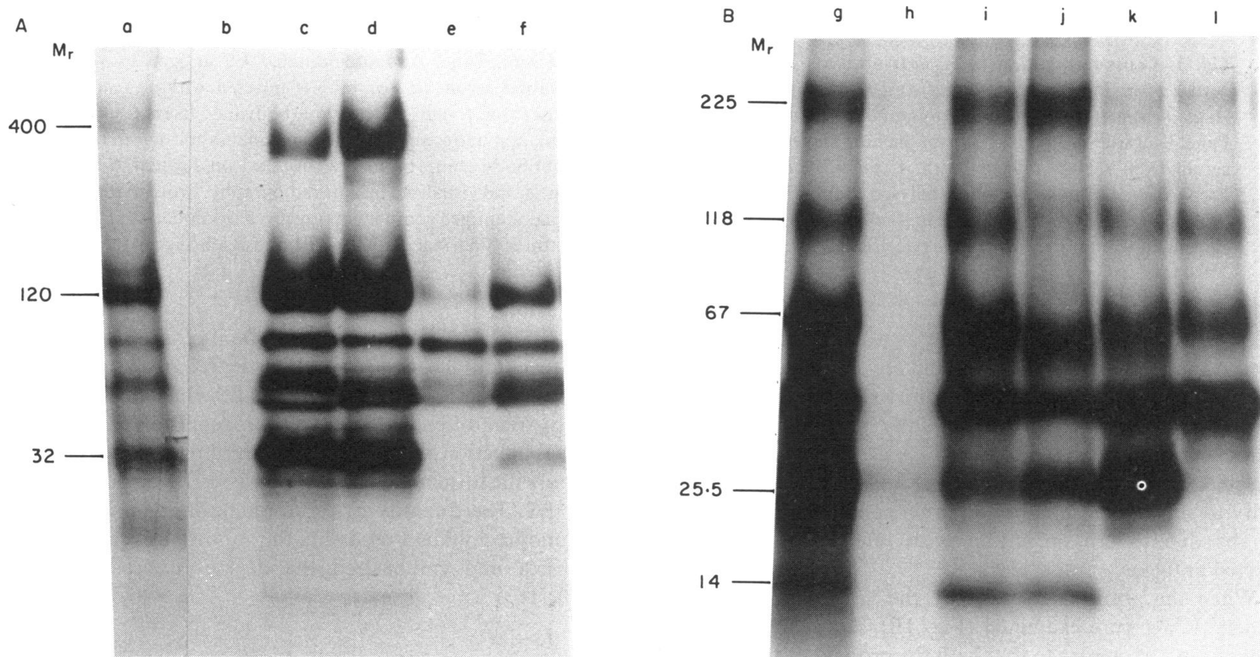

Fig. 2. Heterologous recognition of *T. canis* ES and reactivity of anti-*T. canis* ES serum on *A. suum* L3/4 ES. Panel A: *T. canis* ES (a), immunoprecipitated with normal rabbit serum (b), serum from a rabbit infected with *T. canis* (c), immunized with purified *T. canis* ES in adjuvant (d), or infected with *A. suum* (e), or *A. lumbricoides* (f). Electrophoresis was carried out under reducing conditions. Panel B: *A. suum* L3/4 ES (g), immunoprecipitated with normal rabbit serum (h), or from rabbits infected with *A. lumbricoides* (i), *A. suum* (j), *T. canis* (k), or immunized with *T. canis* ES (l). Electrophoresis was carried out under non-reducing conditions.

might represent Da.S1, an allergen of *Ascaris* previously described as 360 kD in mass (subunits 140 kD and 220 kD) (O'Donnell & Mitchell, 1978).

The distinct similarities between the somatic materials of the two species of parasite was particularly striking in the 14 kD region. Radio-immunoprecipitation of parasite body fluids with homologous and heterologous antisera showed that the anti-*Ascaris* serum reacted with the 14 kD from both preparations, but the toxocariasis serum reacted with neither (Fig. 4). The antigenic homology between the 14 kD from *Ascaris* and *Toxocara* was confirmed in an experiment using the anti-*Ascaris* 14 kD serum in which only the 14 kD from *T. canis* somatic materials was precipitated (not shown). A similar analysis showed that the 14 kD was also present as a major component in homogenates of the infective larva of *T. canis*, and, as before, this was precipitated by anti-*Ascaris* infection serum, anti-ABF

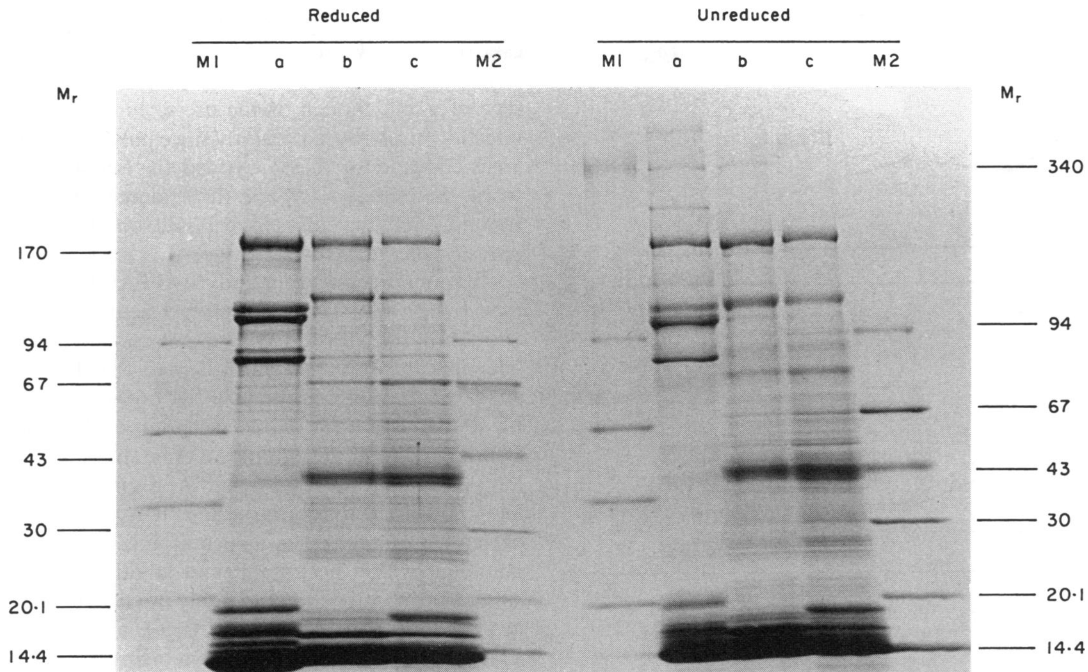

Fig. 3. SDS-PAGE profiles of the perienteric fluid of *A. suum* and *T. canis*. Perienteric fluid of *T. canis* adult female (a), *A. suum* adult female (b), and male (c), run under reducing and non-reducing conditions, and stained for protein with Coomassie blue. M1: molecular mass standard proteins; apparent molecular mass in kD as given by the manufacturers: 340 (170 when reduced), 97·4, 55·4, 36·5, 20·1. M2: molecular mass standard proteins; apparent molecular mass in kD: 94, 67, 43, 30, 20·1 and 14·4.

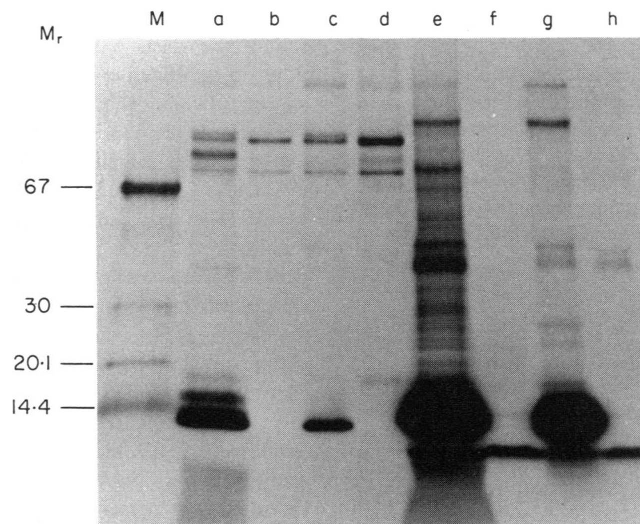

Fig. 4. Failure of *T. canis* infection to elicit an antibody response to the 14 kD molecule of both *T. canis* and *A. suum*. Radio-iodinated perienteric fluid of adult female *T. canis* (a–d), and *A. suum* (e–h), immunoprecipitated with normal rabbit serum (b & f), serum from a rabbit infected with *A. suum* (c & g), or *T. canis* (d & h). Tracks (a) and (e) show the profiles of the antigen preparations used for the immunoprecipitations.

and anti-14 kD serum, but not by anti-*T. canis* antiserum (not shown).

The failure of *T. canis*-infected rabbit serum antibody to react with the 14 kD was consistent from one rabbit to another, and anti-14 kD antibodies have not been detected by these methods even in animals infected for prolonged periods (up to 100 days).

Surface antigens

The surfaces of parasitic nematodes are known to be highly antigenic, and, for *T. canis*, it is known to contribute to 'ES' by antigen shedding (Smith *et al.*, 1981; Maizels *et al.*, 1984). However, exposure of epitopes on the surface of living parasites is much more restricted than for ES or detergent-released surface antigens, and an epitope common between two species

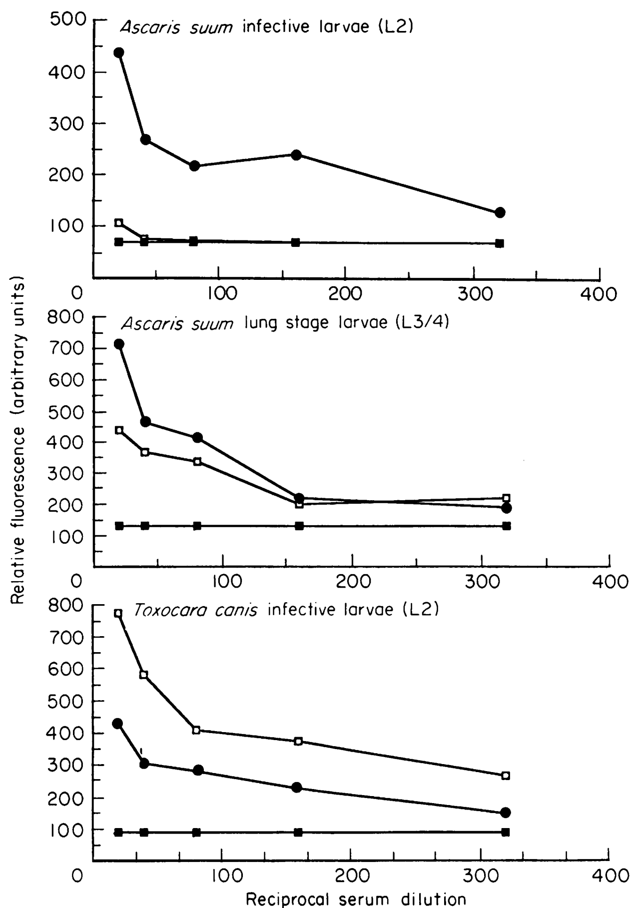

Fig. 5. Antibody binding to the surfaces of the parasitic larvae of *A. suum* and *T. canis*. Parasitic larvae were probed using normal rabbit serum (■), rabbit serum raised to infection with *T. canis* (□), or to multiple infections with *A. suum* (●). The normal rabbit serum was kept at a constant dilution of 1/20 throughout, and plotted as a baseline. Bound antibody was detected with a fluoresceinated second layer antibody, and quantified by photon counting as described in *Materials and Methods*.

of parasite might be exposed on one, but not on the other (Kennedy *et al.*, 1987b; Maizels *et al.*, 1987). There is no published information on epitope exposure on *Ascaris* larvae, but the possibility was that they were similarly restricted, thereby limiting cross-reactivity. To test this we used intact larvae, and measured homologous and heterologous antibody binding by quantitative fluorescence in an indirect immuno-fluorescence assay. As illustrated in Fig. 5, the recognition of surface antigens on the infective larva of *A. suum* was indeed specific, with only background binding by the anti-*T. canis* serum. This specificity broke down, however, when the targets were lung stage of *A. suum* or the infective stage of *T. canis*.

We also took this opportunity to establish whether the 14 kD was present on the surface of *Ascaris* larvae, but failed to find it (not shown).

DISCUSSION

There has been recent interest in the literature on cross-reactivity between *Toxocara* and *Ascaris*, and means by which it can be circumvented (reviewed by Glickmann, Schantz & Grieve, 1986; Lynch *et al.*, 1988). A major problem with this type of work, though, is the use of human sera of uncertain infection histories, particularly since polyparasitism abounds in areas endemic for *Ascaris*. It was the intention of the present work, therefore, to use, and three sources of antigen: secreted, somatic, and the antigens exposed on the surface of living parasitic larvae.

Immunoprecipitation and SDS-PAGE analysis revealed that there was significant cross-reactivity between the ES antigens of the parasites, using both sera raised in the context of infection and raised to purified toxocaral ES. This reactivity appeared to extend to the majority of the components of each ES source, with the notable exception of the 14 kD molecule. This is present in the culture supernatant of both infective and lung larval stages of *Ascaris*, and is presumably actively secreted and/or released during moulting. It is not present in readily detectable quantities in toxocaral ES, but was found to be abundant within both adult and larval *T. canis*. How, then, should antibody to the 14 kD only be elicited in ascariasis? The most obvious possibility is that *Ascaris* secretes the antigen, but *T. canis* does not. Also, *T. canis* remains under developmental arrest in non-canid hosts, unlike *Ascaris*, providing no opportunity for the release of internal components during moulting. Parasite load is probably not a contributory factor, since no anti-14 kD response was detected in rabbits given greater numbers of *T. canis* eggs than of *Ascaris*. Given the chronicity of toxocariasis and the apparent lack of killing of larvae during its course, the answer might lie merely in subthreshold exposure of the immune system to the 14 kD. It might be predicted, then, that toxocariasis patients would seroconvert if they were to develop an effective immune response to the larvae, or upon successful chemotherapy.

It would seem unlikely, then, that infection with *T. canis* will explain the heterogeneity of human responsiveness to the 14 kD in *Ascaris* endemic areas (M. Haswell-Elkins, D. B. Elkins & M. W. Kennedy, unpublished). Other likely factors would include recency or intensity of exposure to infection, or to genetic differences between hosts. If the latter were the case, then this finding has wider implications; our unpublished amino acid and isoelectric focusing analysis would suggest that the 14 kD is similar or identical to Allergen A, a major allergen of *Ascaris* (Ambler *et al.*, 1973a, b), and the purified antigen is highly active in passive cutaneous anaphylaxis assays in infected rats (L. A. Tomlinson, J. F. Christie & M. W. Kennedy, unpublished). This antigen could, therefore, have direct relevance to the life-threatening hypersensitivity responses associated with the pulmonary stages of *Ascaris* infection in humans, and it would seem important to establish whether host variability in responses to this molecule is related to immunopathology.

SDS-PAGE combined with immunoprecipitation or immunoblotting are increasingly used to characterize antigens of complex transmissible agents, and antigenic similarity between them. The main advantages of radio-immunoprecipitation are that it can provide both quantitative and qualitative characterization of antibody responses, with very small quantities of parasite material. It could be argued, though, that this type of analysis is over-sensitive in the detection of antigenic similarities; incorporation of a given molecule into an immunoprecipitate could occur with only one antibody-epitope interaction per antigen

molecule. Immunoblotting might circumvent the problem, since it is more quantitative of antibody binding to individual antigen components. However, this technique requires considerably more antigen than radio-immunoprecipitation, and this would present a considerable limitation to work on the ES of parasitic nematodes.

Apart from obvious relevance to serological applications, the characterization of antigen homology should have wider implications for an understanding of the principles of cross-reactivity between closely related helminth parasites in general. Among nematodes, this is arguably most advanced for *T. canis* in which monoclonal antibodies to its ES have been found to recognize several distinct components of the antigen preparation. All but one of these monoclonals also recognize ES components of a related parasite of cats, *Toxocara cati* (Kennedy *et al.*, 1987b), and the species-specific epitope was found to be a carbohydrate determinant (Maizels *et al.*, 1987). Despite the extensive cross-reaction between *T. canis* and *Ascaris*, we have found that none of the above monoclonals react with *Ascaris* ES. Also with respect to carbohydrate epitopes, it is known that *T. canis* larvae bear human ABO blood group determinants on their surfaces (Smith, Kusel & Girdwood, 1983), but nothing is known of this in *Ascaris*.

The anti-*T. canis* ES monoclonals also showed that there is restricted epitope exposure on the surface of the living larvae of the parasite (Kennedy *et al.*, 1987b; Maizels *et al.*, 1987); all of the MoAbs immunoprecipitate surface-iodinated and detergent-solubilized surface antigens, but only two of them bind to intact parasites. It was this observation which prompted us to examine the *Ascaris*/*T. canis* cross-reaction by immunofluorescence in the hope that common epitopes might be less in evidence. We did indeed find that only homologous serum antibody bound to the surface of the infective stage of *A. suum* (Fig. 5), but the reverse was not true; anti-*Ascaris* antibody bound to the surface of infective *T. canis* larvae. This was in spite of the fact that the infective stage of *Ascaris* is the development equivalent of the inciting stage in *T. canis* infection. The specificity also broke down with the lung stage larvae of *Ascaris*, and we do not know how it should be that only the later stages of *Ascaris* should cross-react. It does mean, however, that the surface of *Ascaris* larvae bears stage-specific epitopes.

Our finding of extensive antigenic similarities between *Ascaris* and *Toxocara* would seem to preclude simple, specific serodiagnosis for these parasites. On the other hand, it remains to be seen how far the present results relate to the specificity achievable with more commonly used assays, ELISA in particular. Cross-reaction problems in ELISA have forced previous workers to the lengths of blocking heterophile antibodies with excess worm antigens to achieve specificity (Lynch *et al.*, 1988). Reports differ as to the extent of this cross-reactivity in human serology, but there is a need to establish the full spectrum of antibody responses against these parasites in areas of the world in which polyparasitisms abound, and in which there can be substantial differences in the parasite load of different individuals in the same environment.

ACKNOWLEDGMENTS

We thank The Wellcome Trust, the Medical Research Council (U.K.), the Scottish Home and Health Department, and the World Health Organisation for grants to MWK, and the Rockefeller Foundation and the National Science Foundation (U.S.A.) for supporting M.H.-E. and D.B.E., respectively. We are also indebted to Fiona Seath, Anne MacIntosh and David McLaughlin for their help at the Wellcome Laboratories, and to Dr Sukumar in India.

REFERENCES

AMBLER, J., CROFT, A.R., DOE, J.E., GEMMELL, D.K., MILLER, J.N. & ORR, T.S.C. (1973a) Biological techniques for studying the allergenic components of nematodes. II. The characterisation of the allergen released by *Ascaris suum* maintained in saline. *J. Immunol. Meth.* **2,** 315.

AMBLER, J., MILLER, J.N., JOHNSON, P. & ORR T.S.C. (1973b) Characterisation of an allergen extracted from *Ascaris suum*. Determination of the molecular weight, isoelectric point, amino acid and carbohydrate content of the native allergen. *Immunochemistry*, **10,** 815.

BOYCE, W.M., BRANSTETTER, B.A. & KAZACOS, K.R. (1988) Comparative analysis of larval excretory-secretory antigens of *Baylisascaris procyonis*, *Toxocara canis* and *Ascaris suum* by Western blotting and enzyme immunoassay. *Int. J. Parasitol.* **18,** 109.

BUNDY, D.A.P., THOMPSON, D.E., ROBERTSON, B.D. & COOPER, E.S. (1987) Age-relationships of *Toxocara canis* seropositivity and geohelminth infection prevalence in two communities in St. Lucia, West Indies. *Trop. Med. Parasitol.* **38,** 309.

GLICKMAN, L.T. & SCHANTZ, P.M. (1981) Epidemiology and pathogenesis of zoonotic toxocariasis. *Epidemiol. Rev.* **3,** 230.

GLICKMAN, L.T., SCHANTZ, P.M. & GRIEVE, R.B. (1986) Toxocariasis. In: *Immunodiagnosis of Parasitic Diseases, Volume 1, Helminthic Diseases* (eds. K. H. Walls & P. M. Schantz) pp. 201–231. Academic Press, Inc., Orlando and London.

JOSEPHS, D.S., BHINDER, P. & THOMPSON, A.R. (1981) The prevalence of *Toxocara* infection in a child population. *Public Health* **95,** 273.

KENNEDY M.W. & QURESHI, F. (1986) Stage-specific secreted antigens of the parasitic larval stages of the nematode *Ascaris*. *Immunology*, **58,** 515.

KENNEDY, M.W., GORDON, A.M.S., TOMLINSON, L.A. & QURESHI, F. (1987a) Genetic (major histocompatibility complex?) control of the antibody repertoire to the secreted antigens of *Ascaris*. *Parasite Immunol.* **9,** 269.

KENNEDY, M.W., MAIZELS, R.M., MEGHJI, M., YOUNG, L., QURESHI, F. & SMITH, H.V. (1987b) Species-specific and common epitopes on the secreted and surface antigens of *Toxocara canis* and *Toxocara cati* infective larvae. *Parasite Immunol.* **9,** 407.

KENNEDY, M.W. & QURESHI F., HASWELL-ELKINS, M. & ELKINS, D.B. (1987c) Homology and heterology between the secreted antigens of the parasitic larval stages of *Ascaris lumbricoides* and *Ascaris suum*. *Clin. exp. Immunol.* **67,** 20.

LYNCH, N.R., WILKES, L.K., HODGEN, A.N. & TURNER, K.J. (1988) Specificity of *Toxocara* ELISA in tropical populations. *Parasite Immunol.* **10,** 323.

MAIZELS, R.M., DE SAVIGNY, D. & OGILVIE, B.M. (1984) Characterisation of surface and excretory-secretory antigens of *Toxocara canis* infective larvae. *Parasite Immunology*, **6,** 23.

MAIZELS, R.M., KENNEDY, M.W., MEGHJI M., ROBERTSON, B.D. & SMITH H.V. (1987) Shared carbohydrate epitopes on distinct surface and secreted antigens of the parasitic nematode *Toxocara canis*. *J. Immunol.* **139,** 207.

O'DONNELL, I.J. & 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 a passive cutaneous anaphylaxis test. *Aust. J. Biol. Sci.* **31,** 459.

OGILVIE, B.M. & DE SAVIGNY, D. (1982) Immune response to nematodes. In: *Immunology of Parasitic Infections* (eds. S. Cohen & K. S. Warren) p. 715. Blackwell Scientific Publications, Oxford.

SEARL, S.S., MOAZED K., ALBERT, D.M. & MARCUS, L.C. (1981) Ocular toxocariasis presenting as leukocoria in a patient with low ELISA titer to *Toxocara canis*. *Ophthalmology*, **88,** 1302.

SMITH, H.V., QUINN, R., KUSEL, J.R. & GIRDWOOD, R.W.A. (1981) The effect of temperature and antimetabolites on antibody binding to the outer surface of second stage *Toxocara canis* larvae. *Mol. Biochem. Parasitol.* **4,** 183.

SMITH, H.V., KUSEL, J.R. & GIRDWOOD, R.W.A. (1983) The production of human A and B blood group substances by *in vitro* maintained second stage *Toxocara canis* larvae: their presence on the outer larval surfaces and in their excretions/secretions. *Clin. exp. Immunol.* **54,** 625.

VAN KNAPEN, F., VAN LEUSDEN, J., POLDERMAN, A.M. & FRANCHIMONT, J.H. (1983) Visceral larva migrans: Examinations by means of enzyme-linked immunosorbent assay of human sera for antibodies to excretory-secretory antigens of the second-stage larvae of *Toxocara canis*. *Z. Parasitenknd.* **69,** 113.

WORLEY, G., GREEN, J.A., FROTHINGHAM, T.E., STURNER, R.A., WALLS, K.W., PAKALNIS, V.A. & ELLIS, G.S. (1984) *Toxocara canis* infection: clinical and epidemiological associations with seropositivity in kindergarten children. *J. Infec. Dis.* **149,** 591.