STRONGYLOIDES STERCORALIS: ANTIGENIC ANALYSIS OF INFECTIVE LARVAE AND ADULT WORMS

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Abstract—Northern C. and Grove D. I. 1990. Strongyloides stercoralis: antigenic analysis of infective larvae and adult worms. International Journal for Parasitology 20: 381–387. The protein composition of Strongyloides stercoralis infective larvae and adult worms solubilized sequentially in water, sodium deoxycholate and sodium dodecyl sulphate (SDS) and their excretory/secretory products were analysed by one- and two-dimensional SDS-polyacrylamide electrophoresis. These extracts were demonstrated to be complex mixtures containing many proteins, some of which were common and others which were stage-specific. Western blot analysis of these antigens with infected human sera showed most sero-reactivity against larval antigens, whilst normal human sera were unreactive. These data identify immunogenic antigens which may be available for detection in an antigen assay.

INDEX KEY WORDS: Strongyloides stercoralis; infective larvae; adult worms; antigens; stage-specificity; Western blots; human sera.

INTRODUCTION

Strongyloides stercoralis is an intestinal parasite of humans that continues to elude complete understanding and accurate diagnosis. Infection often persists undetected with the worm reinfecting the host over a period of many years by a process of autoinfection. The parasite cycles between a systemic phase where infective larvae penetrate the tissues in their migration throughout the body and an intestinal phase in which the adult worm resides in the intestinal wall. An understanding of any antigenic differences between these stages may shed light on the parasites and on protective host responses to them.

People infected with S. stercoralis produce specific antilarval IgA, IgE, IgG and IgM antibodies. These antibodies have been measured in various diagnostic assays including the enzyme-linked immunosorbent assay (ELISA) (Carroll, Karthigasu & Grove, 1981; Genta, Frei & Linke, 1987), the immunofluorescent antibody assay (IFA) (Grove & Blair, 1981), the radioallergosorbent test (RAST) (Genta, Douce & Walzer, 1986) and with skin tests (Neva, 1986). The problem for accurate diagnosis, however, lies in the fact that the level of antibody measured does not necessarily differentiate between past and present infections (Grove, 1982). This difficulty would be solved by the development of an assay for parasite antigens in host serum or urine.

In order to approach both objectives it is first necessary to characterize the antigenic profiles of tissue-penetrating infective larvae and adult worms within the intestinal mucosa. In this study, we have compared the protein composition of infective larvae and adult worms. We employed a sequential solubilization technique in the preparation of parasite material for immunochemical analysis in order to maximize the number of antigens for study. The antibodies produced against these antigens by normal and infected individuals were then identified with Western blots.

MATERIALS AND METHODS

Animals and parasite. Parasites were recovered originally from a man who had been infected while a prisoner-of-war in Southeast Asia 35 years previously (Grove, 1980). The parasite has since been maintained in mongrel dogs obtained from the local pound. The animals were washed, treated with anthelmintics bunnidine hydrochloride and pyrantel pamoate, and their faeces examined to ensure they were free of helminth eggs. The methods for preparation of larvae, infection of animals, and quantitation of larvae in the faeces have been described previously (Grove & Northern, 1982). Worm burdens were increased by immunosuppressing dogs with prednisolone plus or minus azathioprine (Grove, Heenan & Northern, 1983).

Preparation of antigen. Infective larvae were obtained from cultures of dog faeces, then washed three times by...
overnight at 4°C. The supernatant fluid was centrifuged at 5000 g for 30 min at 4°C, then the supernatant fluid of this step was respun at 100,000 g for 60 min at 4°C. The final supernatant fluid was filtered through a 0.22-μm Millex GV low protein-binding filter (Millipore Corp., Bedford, U.S.A.); this is designated as ‘water-soluble fraction’. The two pellet preparations were combined and resuspended in fresh APB. They were then homogenized in a Braun® homogenizer; equal volumes of worm suspension and glass beads, 0.45–0.50 mm in diameter, were placed in a bottle which was placed in a rotating cylinder cooled with rapidly flowing CO₂ for 2 min. The homogenate was allowed to stand overnight at 4°C. The supernatant fluid was centrifuged at 9000 g for 2 min. The homogenate was allowed to stand overnight at 4°C. The supernatant fluid was centrifuged at 11,000 g for 5 min and was loaded onto the gels. These were stained with silver as described by Poehling & Neuhoff (1981). Relative molecular weights (Mᵣ) of sample polypeptides were calculated from the mobility of molecular weight standards co-electrophoresed in a separate well of each gel. Molecular weight estimates of 94 or above are extrapolations and only serve as approximate guides.

Two-dimensional electrophoresis (2DE). 2DE was performed using a modification of the method of O’Farrell, Goodman & O’Farrell (1977) as described earlier (Northern & Grove, 1987). Samples were prepared in lysis buffer, 9.5 M-urea (Biorad), 2% Nonidet P40 (Sigma), 2% Pharmalyte 3–10 (Pharmacia Fine Chemicals) and 5% 2-mercaptoethanol. The first dimension was separated by non-equilibrium pH gradient gel electrophoresis (NEPHGE) on a 0.7-mm thick slab gel as described by Ferreira & Eichinger (1981). The second dimension was separated by SDS-PAGE as before and the gels were stained with silver.

Western blot analysis. A pool of sera from patients with parasitologically proven strongyloidiasis was obtained. These sera were confirmed to have high antibody titres by ELISA against S. stercoralis antigen (Carroll et al., 1981). Western blot analysis was undertaken as described earlier (Northern & Grove, 1988). In brief, proteins and prestained molecular weight standards (Bethesda Research Laboratories, MD, U.S.A.) separated by SDS-PAGE were transferred to nitrocellulose paper (Biorad) by the method of Towbin, Staehlin & Gordon (1979). Immunoreactive bands were detected using alkaline phosphate-conjugated antibodies (My Lien Dao, 1985). The nitrocellulose was blocked with 0.5% Tween 20 in 20 mM-Tris, 500 mM-NaCl, pH 7.5 (TTBS) for 30 min at room temperature in order to saturate unused protein binding sites. It was then incubated overnight in normal or pooled immune human serum diluted 1 in 250 with TTBS. The nitrocellulose was washed twice in Tris-buffered saline (TBS) (20 mM-Tris, 500 mM-NaCl, pH 7.5) and placed in 10 mM-EDTA to inactivate any endogenous alkaline phosphatase activity. It was then washed three times in TBS and incubated with alkaline phosphatase-conjugated anti-human immunoglobulin (Silenus Laboratories, Hawthorn, Australia) diluted 1:500 in TTBS for 1 h. The nitrocellulose was washed three times in TTBS and then rinsed in substrate buffer (0.06 M-sodium borate pH 9.7 containing 1.2 mg ml⁻¹ magnesium sulphate). Immune complexes were visualized after staining with O-dianisidine (0.25 mg ml⁻¹) and N-naphthyd acid phosphate (0.25 mg ml⁻¹) in substrate buffer. Following enzyme staining, the nitrocellulose was washed in a solution of methanol, acetic acid and water (5:1:5; v/v/v) followed by a final rinse in water.

RESULTS

One-dimensional electrophoresis

The SDS–PAGE profiles of infective larval water-, DOC- and SDS-soluble preparations and ES proteins are illustrated in Fig. 1. A large number of bands were resolved for each preparation with differences noted according to the method of solubilization. In the water-soluble larval extract major bands were resolved at 16, 17, 18, 28 and 59 mol. wt. Major DOC-soluble bands had mol. wts of 31, 36, 59 and 97, while the SDS-soluble bands were resolved at 18, 31, 36, 50, 55 and 62 mol. wt. The ES preparation yielded a dominant band of mol. wt 52 with additional minor components. The SDS–PAGE profiles of adult worms are illustrated in Fig. 2. Five preparations were analysed: water-, DOC-,
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revealed major components at 33, 44, 60 and two bands greater than 94 mol. wt. The sequential SDS-soluble extract had major bands at 30, 33, 37, 66, 78 and 83 while the SDS-total-soluble extract contained dominant bands at 21, 24, 26 and 66 mol. wt. The ES extract had major bands at 16, 33 and 71 mol. wt.

**Two-dimensional electrophoresis**

Greater discrimination of these differences was provided by 2DE. Many proteins were visualized in the water-soluble fraction of infective larvae (Fig. 3a). A number of additional spots were seen in the sequential DOC-solubilized preparation (Fig. 3b). In the third preparation in which the pellet was finally solubilized in SDS a further number of proteins were demonstrated (Fig. 3c). It was not possible to make direct comparisons between the infective larval and adult worm somatic components as the paucity of adult worms did not permit sequential solubilization of this stage of the parasite. Nevertheless increased discrimination of proteins was obtained when 2DE was performed on adult worm material extracted directly with SDS (Fig. 4a).

A smaller set of proteins was isolated from larval excretory/secretory products (Fig. 3d). The pattern of distribution of proteins in the ES products derived from adult worms (Fig. 4b) was quite different from that seen with larvae thus indicating differences in the stage-specificities of the parasite.

**Western blots**

The soluble extracts of infective larvae and adult worms were analysed by Western blots with a pool of normal, uninfected human sera and a pool of immune sera from individuals with parasitologically proven strongyloidiasis. Figure 5 shows the profile of reactivity of normal sera to the antigenic mixtures and Fig. 6 illustrates the same profile of antigens reacted with immune sera. The normal serum profile is notable for its lack of reactivity against virtually all proteins. A single band in the larval DOC-soluble preparation with mol. wt 38 is faintly detected. In contrast, the immune serum profile identifies a number of antigenic bands in both the larval and adult worm preparations. The larval water-soluble preparation has major reactive bands with mol. wt 38, 48 and 58 as do the DOC-soluble preparation and the SDS-soluble preparation, the latter also having an additional band at 27 mol. wt. The ES products comprise many but fainter reactive bands; the major ones have mol. wts 11, 14, 38, 48 and 58 and six bands with a mol. wt greater than 75. In contrast to the larval preparations the adult worm preparations are characterized by a paucity of strongly reactive components. The adult SDS-total-soluble preparation has two major reactive bands, one with mol. wt 38 and the other a band at greater than 130. The adult ES preparation shows only a number of faintly staining bands, the strongest being with mol. wt 58.
Fig. 3. Two-dimensional electrophoretic profiles of *S. stercoralis* infective larvae. Samples were analysed by NEPHGE in the first dimension and SDS-PAGE in the second dimension. Water-soluble fraction (panel A), DOC-soluble fraction (panel B), SDS-soluble fraction (panel C) and ES products (panel D). Acidic and basic regions of the gels are indicated. Molecular weight standards are shown on the right.
DISCUSSION

In this study we have shown that the antigenic profiles of the tissue penetrating infective larvae and mucosal-dwelling adult worms differ. In addition, we have demonstrated that both the somatic extracts and excretory/secretory products are complex mixtures containing many proteins which can be separated in high resolution electrophoresis systems.

The method of sequential solubilization of the worms yielded different sets of proteins reflecting differing solubilities in the detergents used. Although exposed to strong anionic detergents such as sodium deoxycholate and sodium dodecyl sulphate the proteins retained their antigenicity when utilized in a Western blot assay.

We have made similar observations in the comparison of Strongyloides ratti infective larvae and adult worms (Northern & Grove, 1987) as have others with different nematodes such as Trichinella spiralis, Nematodirus dubius and Dipetalonema viteae (Phillip, Parkhouse & Ogilvie, 1980; Pritchard, Maizels, Behnke & Appleby, 1984; Baschong, 1985).
infective larvae on the basis of immunoprecipitation reactions with human sera and purified larval ES proteins bound to a column of human IgG from immune human sera. They demonstrated a number of bands with mol. wts 12, 25, 30, 33, 35, 40, 50, 54, 60, 90 and 240. We also demonstrated antigenic bands at some of these weights although direct comparison is difficult.

Our interest has been to study both infective larvae and adult worms in order to compare the antigenic profiles of the two stages of the parasite. We have shown that both the somatic extracts and the excretory/secretory products of two developmental stages of *S. stercoralis* are highly complex but that common and stage-specific proteins could be demonstrated. The use of 2DE permitted greater discrimination of the various protein bands found by SDS–PAGE.

Furthermore we have shown with Western blots utilizing sera obtained from infected humans that many of these compounds are immunogenic. The patients were suffering from strongyloidiasis and had no other concomitant parasitic infections. Most sero-reactivity was directed against larval antigens. Similar major bands were seen in the water-, DOC- and SDS-soluble extracts reflecting their increasing solubility in stronger detergents. When adult worm somatic antigens were extracted using the strongest detergent, SDS, very few antigenic bands were identified. A plethora of antigenic bands was shown in the metabolic products produced by infective larvae. In contrast, only a small number of bands were seen in the metabolic products derived from adult worms. The difference in sero-reactivity between infective larvae and adult worms is not surprising when the physical locations of these stages of the parasite are considered. Infective larvae migrate through the tissues and are in close proximity to the cells of the immune system. On the other hand, adult worms live in tunnels in the small intestine epithelium and do not penetrate the basement lamina (Grove, Warton, Yu, Northern & Papadimitriou, 1987). They are therefore relatively isolated from immunoreactive cells.

These studies provide a framework for the identification and separation of those antigens which are most relevant to the generation of host-protective immune responses and which may be made amenable to detection in an antigen assay.

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REFERENCES


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