ANTIBODY RESPONSES IN HUMAN GNATHOSTOMIASIS

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INTRODUCTION

Human gnathostomiasis in Thailand is caused by *Gnathostoma spinigerum*. Currently the diagnosis of the disease in the non-parasitologically proven cases is only presumptive based on clinical features including eosinophilia in the peripheral blood, intermittent migratory swelling, itching and pain and the history of consuming raw or half-cooked meat (Daengsvang, 1985). Serological diagnosis of human gnathostomiasis has not been routinely used, and this is probably due to the complex and cross-reactive nature of the antigens of *Gnathostoma* with other parasites (Punyagupta and Pacheco, 1961; Kasemsuth *et al.*, 1981; Tada *et al.*, 1987). Moreover, relatively little is known of the specific antigens which induce the host's antibody responses during the course of infection. Recent studies by the enzyme-linked immunosorbent assay (ELISA) have shown that patients with gnathostomiasis had serum IgG as well as IgE responses against the crude somatic extract of advanced third stage larvae of *G. spinigerum* (L3G) (Suntharasamai *et al.*, 1985; Dharmkrong-at *et al.*, 1986; Soesatyo *et al.*, 1987). However, the specific antigens inducing the antibody responses in these patients have not been defined. The present study was aimed at characterization of the antibody responses against the various L3G antigens by using the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) and Western blot analysis with sera from parasitologically confirmed gnathostomiasis patients and those from normal healthy individuals.

MATERIALS AND METHODS

Patients: Sera were collected from 4 male patients with parasitologically confirmed gnathostomiasis. Advanced third stage larvae of *G. spinigerum* were recovered from three patients; one of each from the eye ball (Gn001, 35 years old, Chiang Mai), skin of buttock (Gn002, 39 years old, Lopburi), and skin of abdomen (Gn004, 19 years old, Nakhon Nayok). A young adult *G. spinigerum* worm was also recovered from the skin of abdomen of another patient (Gn003, 10 years old, Bangkok). These patients were negative for other parasitic infection by stool, blood and sputum examinations.

Controls: Sera were obtained from 18 healthy individuals with no previous history of intermittent cutaneous migratory swelling and their stool, blood and sputum examinations were negative for any parasitic infection at the time of blood collection. Their age range was from 10 to 52 years with the mean of 30 years.

Antigen preparation: L3G were obtained from livers of naturally infected fresh water eels purchased from local vendors in Bangkok. Living larvae were individually identified under a dissecting microscope using the morphological criteria described by Daengsvang (1986). They were washed in 0.9% NaCl until freed of blood and tissue debris, pooled, lyophilized and kept at -20°C. A crude water extract of the larvae was prepared by grinding the lyophilized worms with a small amount of alumina (Sigma Chemical Company, Mo., U.S.A.) in distilled water. The preparation was sonicated for 10 minutes at 4°C and centrifuged at 10,000 g for 20 minutes at 4°C. The protein content of the collected supernatant was determined by the method of Lowry *et al.*, (1951) using bovine serum albumin (BSA) as a standard. The antigen extract was kept in aliquots at −20°C until use.

SDS−PAGE and Western blot analysis: The electrophoresis was performed in a vertical slab gel apparatus using the method of Laemmli and Favre (1973). A 4% acrylamide stacking gel and a 10% acrylamide separating gel were used. Samples containing approximately 30 μg of the L3G water extract antigen were boiled at 100°C for 3 minutes in sodium dodecyl sulfate (SDS) and 2-mercaptoethanol before loading onto the gel. The separated protein bands were visualized by staining with Coomassie brilliant blue R (Sigma). The molecular weights (M.W.) of unknown proteins were estimated by comparing the relative migration ratios against those of M.W. standards (Phamacia Fine Chemicals, Uppsala, Sweden). For Western blot analysis, the SDS−PAGE resolved components were electroblotted onto a 0.45 μm nitrocellulose membrane (Bio-Rad Laboratory, Calif., U.S.A.) according to the method of Towbin *et al.*, (1979) using a Bio-Rad Transblot apparatus. After blotting the unreacted sites on the membrane were blocked by soaking the strips in phosphate-buffered saline (PBS) containing 1% gelatin, 2% BSA and 0.04% NaN_3 at 26°C for 1 hour. The strips were allowed to react with various sera diluted in PBS containing 0.5% gelatin, 1% BSA and 0.04% NaN_3 at 26°C for 1 hour with gentle rocking. The blots were washed five times with PBS containing 0.5% gelatin and 0.05% Tween 20 before incubated with ^{125}I−labeled protein A ($1-5 \times 10^5$ cpm/ml in PBS containing 3% gelatin and 0.04% NaN_3) at 26°C for 30 minutes. The protein A (Phamacia Fine Chemicals) was labeled with ^{125}I (sodium salt, Amersham International plc., Buckinghamshire, England) by the iodogen method (Markwell and Fox, 1978). The strips were washed as above, dried, and autoradiographed onto the Kodak X−Omat RP films (Eastman Kodak Co., NY, U.S.A.) with light intensifying screens at −70°C.

RESULTS

SDS−PAGE analysis of L3G crude somatic extract revealed a complex pattern comprising of more than 40 protein bands with the relative M.W. ranging from 13 kilodaltons (kd) to 150 kd (Fig. 1A). The predominant protein bands were 150, 135, 94, 72, 49, 47, 35 and 15 kd. The optimum serum dilution for use in the Western blot analysis was determined by reacting the electroblotted L3G components with Gn001 serum diluted 1:50, 1:100, 1:200, 1:400 and 1:800 before probing the antigen-antibody complex by ^{125}I-protein A. The autoradiograms of the serum reactivities are shown in Fig. 1B−1F. Approximately 20 bands of L3G antigens were found with the predominant bands at 150, 135, 120, 94, 84, 82, 72, 55, 54, 49, 43, 38, 35, 32 and

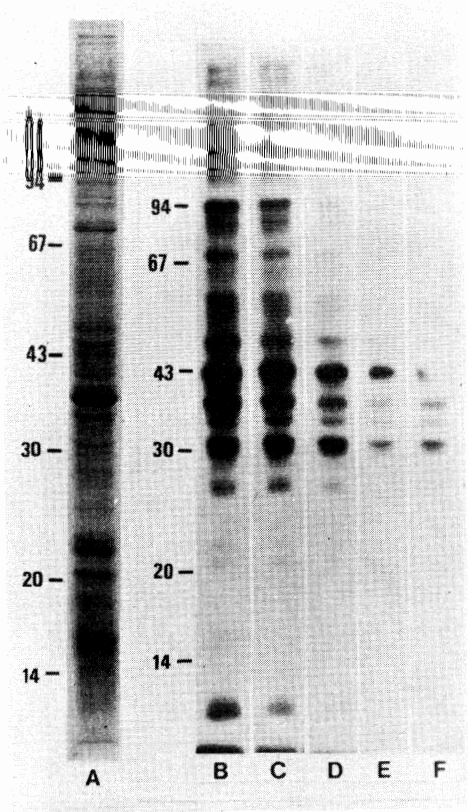

Fig. 1–Protein staining of crude aqueous somatic L3G extract after SDS–PAGE (A) and reactivities of Gn001 serum against the electroblotted L3G antigens at the dilution of 1:50 (B), 1:100 (C), 1:200 (D), 1:400 (E), and 1:800 (F). Numbers on left indicate M.W. standards in kd.

28 kd. A serum dilution of 1:100 which gave the optimum reactivity was chosen for further study. All four sera obtained from the patients with gnathostomiasis gave an identical pattern of reactivities against the parasites except that Gn003 did not give reactivities against the major L3G antigens of 35, 32, and 28 kd (Fig. 2). In contrast, further testing of 18 healthy adult sera against the L3G antigens showed that all of these sera gave low to relatively strong reactivities with the single antigen of approximately 38 kd while some of them had, in addition, reactivities against the antigens of 49 and 43 kd (Fig. 3).

DISCUSSION

At present relatively few studies on human antibody responses against *Gnathostoma spinigerum* antigens have been done. Previous studies by ELISA using the crude somatic L3G antigens have shown that patients with gnathostomiasis had increased specific IgG and IgE antibody responses against the parasite (Suntharasamai *et al.*, 1985; Dharmkrong-at *et al.*, 1986; Soesatyo *et al.*, 1987). However, a considerable cross-reactivity of the antigens with sera from patients with other helminthic infections has also been found, thus, limiting the use of such assay for routine immunodiagnosis of human gnathostomiasis. The purpose of the present

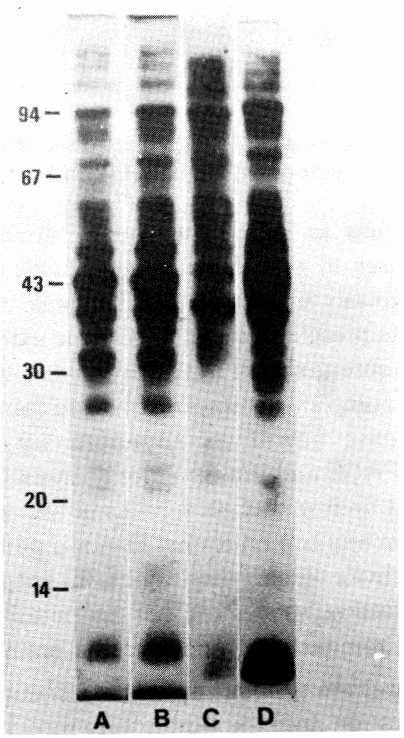

Fig. 2–Reactivities of Gn001 (A), Gn002 (B), Gn003 (C) and Gn004 (D) sera against the electroblotted L3G antigens. Numbers on left indicate M.W. in kd.

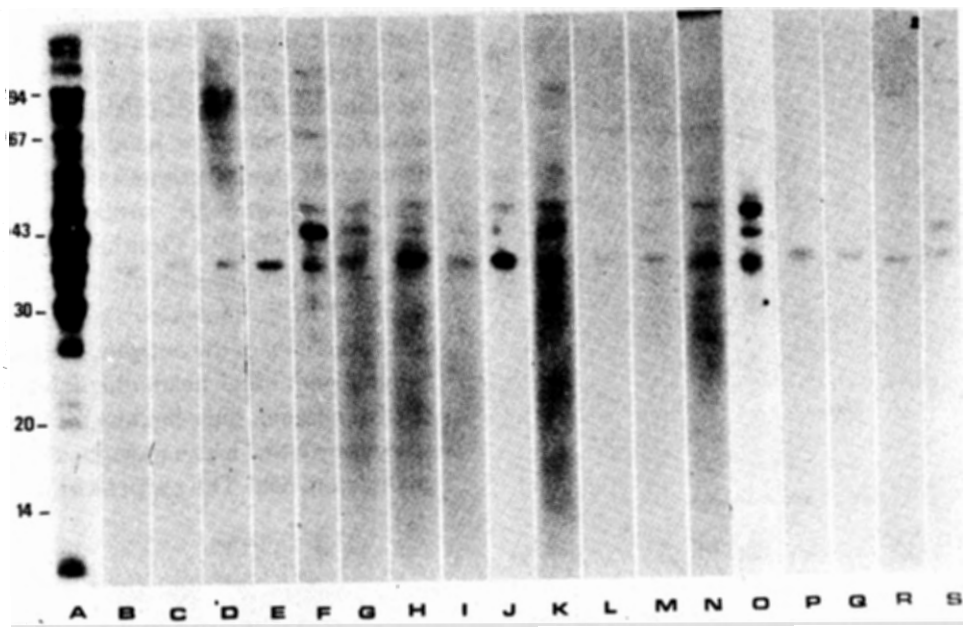

Fig. 3–Reactivities of Gn001 serum (A) and 18 healthy control sera (B–S) against the electroblotted L3G antigens. Numbers on left indicate M.W. in kd.

study was to define the specific antibody responses in sera from patients with gnathostomiasis against various antigenic components present in the L3G somatic extract. It was observed that the L3G antigens were highly complex, comprising of more than 20 antigenic bands as revealed by the SDS–PAGE and Western blot analysis. The present finding that all 4 patients with gnathostomiasis had an almost identical pattern of reactivity against the major L3G antigens is interesting; suggesting that the parasite is highly immunogenic and that the antibody responses are systemic and characteristic for the infection since the worms were recovered from different tissues from these patients. A minor difference in the antibody responses found in one patient (Gn003) was probably due to the different time of blood collection during the course of infection among these patients. This could be clarified if a serial blood sample collection was made from the patients.

It was also found in the present study that all of sera from healthy individuals with negative parasitological finding were reactive against L3G antigen of M.W. 38 kd and, in certain individuals, the antigens of M.W. 49 and 43 kd. However, the degree of the cross-reactivities in these sera was much lower in extent than from patients with gnathostomiasis. This finding is consistent with the previous findings of the low background IgG ELISA reactivity in the sera of healthy individuals (Suntharasamai *et al.*, 1985; Dharmkrong-at *et al.*, 1986). The nature and origin of the antigen(s) inducing the cross-reacting antibody were not known and await

further investigation.

SDS-PAGE and Western blot analysis have been widely used for characterization of the host's antibody responses against antigens from varying sources, e.g., bacteria (Nachamkin and Hart, 1985), protozoa (Taylor and Wenmen, 1987), and helminths (Ruppel *et al.*, 1985) and also used as a confirmed immunodiagnosis of the acquired immunodeficiency syndrome (Sarangadharan *et al.*, 1984). Results of the present study show consistent and characteristic serum antibody responses against L3G antigens in gnathostomiasis cases which indicate the possibility of using the SDS-PAGE and Western blot analysis as a tool for a specific or a confirmed immunodiagnosis of human gnathostomiasis. Work is now in progress to define the specificity of the serum antibody reponses against the parasite in presumptive cases of gnathostomiasis, and those with other parasitic infections.

SUMMARY

Sera from 4 patients with parasitologically confirmed gnathostomiasis and from 18 healthy individuals were studied by SDS-PAGE and Western blot analysis using radioiodinated protein A to detect antibody responses against crude aqueous somatic extract of advanced third stage larvae of *Gnathostoma spinigerum* (L3G). It was found that the L3G extract was highly complex, comprising of more than 40 polypeptides among which more than 20 components were antigenic in human. The relative M.W. of the proteins ranged from 13 kd to 150 kd with the major antigenic bands at 150, 135, 120, 94, 84, 82, 72, 55, 54, 49, 43, 38, 35, 32 and 28 kd. All 4 sera from gnathostomiasis patients gave almost an identical pattern of reactivities against the L3G antigens whereas sera from the normal individuals gave much lower reactivities against the L3G antigen of M.W. 38 kd and, in certain individuals, those of 49 and 43 kd. The present findings suggest that the serum antibody response against the parasite is specific and may be useful in a specific or a confirmed immunodiagnosis of human gnathostomiasis.

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