

## IgE responses in human gnathostomiasis

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### Abstract

Specific IgE antibody levels in the serum of patients with proven gnathostomiasis and in those with intermittent cutaneous migratory swellings were determined by the enzyme-linked immunosorbent assay (ELISA) using aqueous extracts of *Gnathostoma spinigerum* infective larvae as antigen. There was not only an elevation of specific IgE antibody levels but also a marked increase of total IgE in the serum of these patients. The mean levels of specific IgE antibody in both groups of patients were significantly higher than that of healthy controls ( $P < 0.01$ ). Only one of the 50 serum specimens tested had an ELISA reading that fell within the mean + 2 standard deviations of the control group, suggesting that the method would be useful with a high degree of reliability in confirming a clinical diagnosis of gnathostomiasis in humans. Compared with healthy controls, there was almost a 10-fold rise ( $P < 0.01$ ) in the total IgE in the serum of these patients, indicating that *G. spinigerum* infection potentiates IgE production similarly to many other nematode parasites.

### Introduction

Elevation of parasite-specific and total IgE levels has been reported in several helminth infections in man and animals (JOHANSSON *et al.*, 1968; RADERMECKER *et al.*, 1974; HUSSAIN *et al.*, 1980; PFISTER *et al.*, 1983; KORENAGA *et al.*, 1986; REINER & ZAHNER, 1986). A considerable proportion of the IgE produced is not specific for the parasites but most probably results from a polyclonal stimulation of the IgE response by parasite antigens. Specific IgE response in the serum may occur before (ROUSSEAU-PREVOST *et al.*, 1978), simultaneously with (PFISTER *et al.*, 1983), or after (JARRETT *et al.*, 1976) the increase in total IgE. The stimulation of IgE production is more obvious with helminth infections, particularly those having a tissue migratory phase (RADERMECKER *et al.*, 1974).

*Gnathostoma spinigerum* is a nematode of dogs and cats that can cause severe and even fatal infection in humans (DAENGSVANG, 1980; SUNTHARASAMAI, 1983). The disease has been reported in Thailand and other southeast Asian countries, and sporadically in many other countries. Man is an accidental host, acquiring the disease by consuming poorly cooked intermediate hosts, e.g. eels, frogs, fishes and snakes. Following ingestion, the parasite may migrate to any tissue of the body. The most common outcome is intermittent cutaneous migratory swellings that may persist for many years. The migration of the parasite within the central nervous system results in intracranial haemorrhage or eosinophilic meningoencephalitis, or both, which can be fatal if not properly handled. The host-parasite relationship is still poorly understood. We previously showed a rise of specific IgG antibody in such patients and suggested that it might be used to support a clinical diagnosis of gnathostomiasis (DHARMKONG-AT *et al.*, 1986).

Because in humans the nematode undergoes tissue migration which causes persistent swelling, information regarding IgE antibody response may help to understand further the host-parasite relationship, thus leading to an improved management of the patients. The purpose of this study was to analyse the IgE status of patients with migratory swellings and to compare it with that of proven gnathostomiasis cases.

### Materials and Methods

#### Specimens

Sera were obtained from patients attending the Outpatients Clinic of the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. Stool examinations for intestinal parasitic infections together with blood and urine analyses were routinely performed. Only those in whose stools no intestinal parasites were detected were considered for this study. Of these, 46 patients had intermittent cutaneous migratory swelling (MS) consistent with infection by *G. spinigerum*. Many of these patients experienced repeated swellings before coming in for diagnosis and treatment. 4 additional patients from the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, with parasitologically proven gnathostomiasis and from whom *G. spinigerum* larvae were recovered, were also available for study. Healthy adults with no previous history of gnathostomiasis and whose stools were negative for intestinal parasites at the time of study served as healthy controls.

#### Antigen preparation

Third-stage larvae of *G. spinigerum* were obtained by liver digest of infected eels available from local markets. A somatic aqueous extract was prepared and standardized as previously described (DHARMKONG-AT *et al.*, 1986).

#### IgE antibody determination

IgE antibody reactive with *G. spinigerum* antigen was quantitated by enzyme-linked immunosorbent assay (ELISA) essentially as described for the IgG antibody determination by DHARMKONG-AT *et al.* (1986). The optimal antigen concentration for coating microtitre plates (Immulon II, Dynatech Laboratories, Alexandria, Virginia, USA), was determined by checkerboard titration to be 5 µg/ml. Serum

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was serially diluted 5-fold, starting from 1:5. Horseradish peroxidase-conjugated rabbit anti-human IgE was obtained from Dakopatts (Copenhagen, Denmark). All conditions and conjugate dilution were optimized as described previously. IgE antibody titres for each specimen were calculated using the end point criteria described by BANCHUIN *et al.* (1985).

#### Quantitation of total IgE

IgE levels in the serum of the patients and healthy controls were quantitated by ELISA using rabbit anti-human IgE (Dakopatts) diluted 1:100 in ELISA buffer to coat the microtitre plates. The concentration of IgE in unknown samples was read from a standard curve calibrated with IgE standards (Behringwerke, Federal Republic of Germany).

### Results

#### Specific IgE antibody response to *Gnathostoma antigen*

The levels of IgE antibody to *Gnathostoma* antigens in patients with parasitologically proven gnathostomiasis and in those with migratory swellings (MS) were markedly higher than the background levels in healthy controls (Figure). The ELISA values, i.e., optical density (OD) readings at 492 nm of a 1:5 serum dilution, for both groups of patients were significantly higher than those of controls (Duncan's multiple range test,  $P < 0.01$ ). Only one of the 46 patients with migratory swellings had an OD reading below the value for the mean + 2 standard deviations of the control group. The mean antibody titre for the MS group was 1:98; for the gnathostomiasis group it was 1:589. All 12 healthy controls had antibody titres below 1:5, the lowest dilution tested.

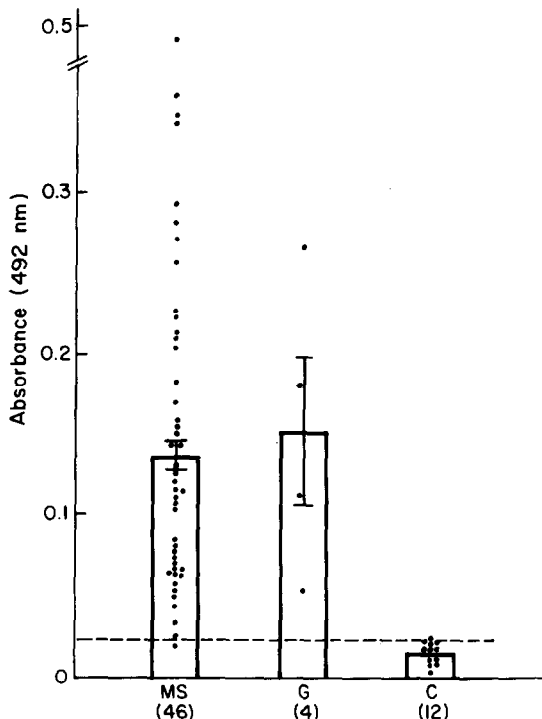


Figure. Optical density values (mean  $\pm$  standard error of the mean) of the ELISA reaction for IgE antibody in patients with intermittent cutaneous migratory swellings (MS) and in patients with proven *G. spinigerum* infection (G), compared with those of healthy controls (C). Also shown is the mean + 2 standard deviations value for the normal control group (----).

Table. Total IgE in sera from patients with migratory swellings, patients with proven gnathostomiasis, and healthy controls.

| Clinical manifestations | Number | Serum IgE (iu/ml)<br>(mean $\pm$ S.E.) |
|-------------------------|--------|----------------------------------------|
| Migratory swellings     | 46     | 3736 $\pm$ 455                         |
| Proven gnathostomiasis  | 4      | 3662 $\pm$ 763                         |
| Healthy (control)       | 12     | 443 $\pm$ 102                          |

#### Total serum IgE levels

Total serum IgE levels in both groups of patients were also markedly elevated compared with those of healthy controls. The mean levels for both groups of patients were indistinguishable from one another and both were significantly higher than that of the control group (Table).

There was only a weak correlation between specific IgE antibody titres and total IgE levels in the serum of patients with migratory swellings (Spearman's rank correlation,  $R = 0.43$ ). A similar finding was noted with the 4 patients with proven gnathostomiasis.

### Discussion

This study demonstrated that an infection caused by *G. spinigerum* not only stimulated specific IgE antibody response in the patients but also potentiated IgE response to other antigens. This observation is not entirely unexpected as both specific and non-specific IgE production in man and animals have been noted in many other helminth infections (JOHANSSON *et al.*, 1968; RADERMECKER *et al.*, 1974; HUSSAIN *et al.*, 1980; JARRETT, 1972). Nevertheless, the response noted with this tissue nematode infection is of special interest because most gnathostomiasis patients are believed to have been invaded by only one small migrating parasite (DAENGSVANG, 1980) and it is amazing that such an infection can stimulate so strong an IgE response. We previously demonstrated a significant specific IgG antibody response in similar patients (DHARMKRONG-AT *et al.*, 1986); the mean IgG antibody titre was almost two orders of magnitude higher than the mean IgE titre, and in no patient did we find a higher IgE response (unpublished observations). There was only a weak correlation between IgG and IgE antibody titres in either group of patients (unpublished observations).

There is no information regarding the role of IgE antibody in this parasitic disease. Nothing is known about the effect of the immune response on the pathophysiology of the disease (DAENGSVANG, 1980). It is difficult to say if either class of antibodies plays a significant role in protection against reinfection.

The diagnosis of gnathostomiasis in man is only presumptive unless the parasite can be recovered and identified. The cutaneous hypersensitivity reaction has given variable results which are difficult to interpret (DAENGSVANG, 1980). The provisional diagnosis is currently based on clinical manifestations and eosinophilia. A history of consuming improperly cooked fish or other intermediate hosts is sometimes helpful. We suggested in a previous study that the presence of a specific IgG antibody response is reliable and useful in supporting the clinical diagnosis of gnathostomiasis in patients with intermittent cutaneous migratory swellings (DHARMKRONG-AT *et al.*

*al.*, 1986). However, a considerable degree of cross reactivity was noted. With the limited information available on other parasitic diseases, some improvement in specificity was noted with the IgE antibody measurement (data not shown). However, the method needs to be improved if it is to be used as an immunodiagnostic method for human gnathostomiasis. Our preliminary data with a mouse model show the presence of components with immunodiagnostic potential in the crude extract. Work is now in progress to identify and purify this antigen, and to assess its value.

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