Serodiagnosis of human strongyloidiasis by an enzyme-linked immunosorbent assay

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Summary

The sensitivity and specificity of an enzyme-linked immunosorbent assay (ELISA) for the serodiagnosis of strongyloidiasis has been investigated. 45 men with long-standing strongyloidiasis were compared with the same number of age- and sex-matched control subjects. The ELISA detected antibody in 84% of patients with parasitologically proven strongyloidiasis. When the technique was compared with an indirect immunofluorescent assay (IFA), a high correlation coefficient was obtained. Specificity was demonstrated by observing a marked fall in optical density of pooled positive serum after prior incubation with Strongyloides ratti soluble antigen but not after incubation with antigens derived from Ascaris suum or Dirofilaria immitis. The test is simple and offers a useful method for the diagnosis of strongyloidiasis. In these patients it was more reliable than a single parasitological examination of faeces or duodenal contents.

Introduction

Strongyloidiasis is one of the major human intestinal nematode infections. A diagnosis is usually made when larvae are found in the stool, in duodenal fluid or, sometimes, in the tissue of infected persons. Unfortunately, parasitological confirmation of a suspected infection is often time-consuming as the worms are frequently absent from such specimens or are present in very small numbers (Grove, 1980). Consequently, a simple serodiagnostic assay would be an important advance. Grove & Blair (1981) have described the sensitivity and specificity of an indirect immunofluorescent assay (IFA) for Strongyloides antibodies. That assay, however, requires costly equipment and reagents which may not be readily available in many endemic areas. The enzyme-linked immunosorbent assay (ELISA), first described by Engvall & Perlmann (1971), has a wide application in detecting antibodies to parasitic infections (Voller et al., 1976a, 1978; Ruttenberg & Van Knapen, 1977). This system has the advantage of being economical and not requiring sophisticated equipment for its performance (Voller et al., 1976b).

This paper describes modifications to the ELISA, reports the findings in patients with strongyloidiasis and control subjects, and compares the results of the enzyme-linked assay with those given by the immunofluorescent technique.

Materials and Methods

Patients

Sera were obtained from 45 Australian ex-servicemen who had been prisoners-of-war in South East Asia during the Second World War and in whom a diagnosis of strongyloidiasis had been confirmed. The clinical characteristics and parasitic infections in these men have been described previously (Grove, 1980). Sera were obtained from 45 age-matched Australian veterans who had not been exposed to S. stercoralis; they had not served overseas or lived in the northern parts of Australia or elsewhere in the tropics.

Antigens

Soluble antigens were prepared from infective larvae of S. ratti, adult Dirofilaria immitis (kindly supplied by Dr. J. Dunsmore, School of Veterinary Studies, Murdoch University, W.A.) and adult Ascaris suum (obtained from the local abattoir). Filariform larvae of a homogenic strain of S. ratti were obtained from experimentally infected Sprague Dawley rats; the techniques for the isolation of larvae have been described elsewhere (Dawkins et al., 1980). Bacterial contaminants were removed by repeatedly washing the filariform larvae trapped on 8.0 μm Millipore filters (Millipore Corporation, Bedford, Massachusetts, USA) with sterile 0.9% saline. Worms were ground in Pyrex tissue grinders incubated at 4°C overnight, then centrifuged for one hour at 30,000 g.

The protein content of the supernatant was determined (Lowry et al., 1951) and adjusted to 1 mg/ml using 0.1 M phosphate buffer (PB) pH 7.2. The antigens were aliquoted and stored at −20°C.

Antigen carriers

Kayline TUS 96 polystyrene plates (Kayline Plastics, Camden Park, South Australia) were used as antigen carriers after preliminary investigations showed that they gave the most satisfactory results.

Reagents

Goat anti-human IgG was labelled with alkaline phosphatase (Sigma, St. Louis, Missouri, USA) as described by Engvall & Perlmann (1971).

Enzyme-linked immunosorbent assay

The method of Voller et al. (1976a) was used with the following modifications: Antigen 10 μg/ml was coated on microplates for one hour at 37°C
then overnight at 4°C. Test sera were diluted in 0·1 M PB pH 7·2 containing 0·2% Tween 20 and 20% foetal calf serum (FCS) to 1:1280 as preliminary investigations showed this to be the most discriminating dilution. Sera were then incubated for one hour at 37°C. Conjugate was diluted in PB Tween 20% FCS and incubated for one hour at 37°C. The substrate, p-nitrophenyl phosphate (3mg/ml), was incubated at 37°C for 15 min, then at room temperature until reference positive sera read 1·0 at 405 nm using the Titretek-Multiskan reader (Flow Laboratories, Rockville, Maryland, USA).

Immunofluorescent antibody assay

Immunofluorescent antibodies were measured as described elsewhere (Grove & Blair, 1981). In brief, living infective larvae of S. ratti were incubated with serial dilutions of test serum, washed, then reincubated with fluoresceinated sheep anti-human globulin, rewashed and the antibody titre determined by observation of cuticular fluorescence.

Specificity of antibody

Pooled positive serum was incubated for one hour at 37°C with equal volumes of S. ratti, D. immitis and A. suum antigen solutions and then antibody levels were measured by ELISA.

Results

Measurement of Strongyloides ELISA antibodies in patients

The results obtained in 45 parasitologically proven patients and in 45 unexposed subjects are shown in Fig. 1. The highest optical density observed in control subjects was 0·34. Seven of 45 (16%) patients with proven strongyloidiasis had an optical density <0·34.

Correlation with immunofluorescent antibody titre

Fig. 2 compares the results obtained by ELISA with the IFA titles in the 45 patients with proven strongyloidiasis. A high correlation was found between the two techniques (r = 0·89, p<0·001).

Specificity of antibody

The effects of prior incubation with S. ratti, D. immitis and A. suum soluble antigens on the optical density obtained by ELISA with pooled immune serum are shown in Table I. No significant change was observed after incubation with D. immitis and A. suum antigens but a dramatic fall was found with prior incubation with S. ratti antigen (P<0·001, "t" test). The optical density of serum after incubation with S. ratti was not significantly different from the value obtained in normal control serum.

<table>
<thead>
<tr>
<th>Optical Density</th>
<th>S.D.</th>
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<tbody>
<tr>
<td>Pooled immune serum (a) unabsorbed</td>
<td>1·04 0·09</td>
</tr>
<tr>
<td>(b) absorbed with</td>
<td></td>
</tr>
<tr>
<td>(i) S. ratti</td>
<td>0·21 0·03</td>
</tr>
<tr>
<td>(ii) A. suum</td>
<td>1·06 0·06</td>
</tr>
<tr>
<td>(iii) D. immitis</td>
<td>1·04 0·03</td>
</tr>
<tr>
<td>Normal control serum</td>
<td>0·28 0·03</td>
</tr>
</tbody>
</table>

Discussion

The ex-servicemen described in this study have provided a rare opportunity to assess the serological response to Strongyloides infection in persons without concurrent infection with other gastrointestinal
helminths. Many of these veterans were infected with hookworm, *Ascaris lumbricoides* and *Trichuris trichiura* during the Second World War, but since these helminths cannot multiply, those infections have subsided spontaneously. In contrast, *S. stercoralis* infection has persisted for 35 years. This eliminates the possibility of any recent production of antibodies against these other intestinal helminths which may cross-react with *Strongyloides*.

This study has shown that the ELISA provides a useful means of diagnosing strongyloidiasis using a serological technique. Although 16% of patients gave false negative readings this is still considerably better than the results of parasitological investigations. For example, larvae were found in the first microscopical examination of faeces in only 66% of these men (Grove, 1980). The reasons for the failure to detect antibody in this small proportion of persons are uncertain, but Voller et al. (1980) have also noted that the ELISA is not very efficient in its ability to discriminate between sera that are negative or have low antibody content.

A valid serodiagnostic assay must have both acceptable sensitivity and negligible cross-reactivity with other parasites. In order to determine the specificity of the assay, pooled sera from *Strongyloides* patients were incubated with an antigen extract of another intestinal nematode, *A. suum*. In contrast to the findings when *S. ratti* antigen extract was used, no change in optical density was found, confirming the absence of significant cross-reactivity between these two parasites. It has been claimed that the cross-reactivity between the tissue nematode *D. immitis* and *Strongyloides* is sufficiently marked to enable the former parasite to be used as an antigen source in the serodiagnosis of strongyloidiasis (Kanani & Rees, 1979). This possibility has been examined by incubating sera from *Strongyloides* patients with *D. immitis* antigen extract; again, no change in optical density was noted, indicating that there is no significant cross-reactivity. Thus, it seems likely that the ELISA which we have described has both reasonable sensitivity and specificity.

Further confirmation of the validity of the ELISA is provided by a comparison with the results obtained by immunofluorescence. A high correlation between the two techniques was found. Unfortunately, ELISA does not seem to be as sensitive as the immunofluorescent assay because the proportion of false negative results was 16% compared with a rate of 4% noted when the same patients were tested by immunofluorescence (Grove & Blair, 1981). It is possible that further modification to the technique may improve the sensitivity of the ELISA, for example, by the use of metabolic products as an antigen source (de Savigny et al., 1979).

Attributes in favour of the ELISA are that it is simple to perform and does not require sophisticated equipment or expensive reagents. Furthermore optimal results were obtained with immunofluorescence only when living *S. ratti* larvae were used. In contrast, the ELISA uses an antigen extract which can be prepared and standardized in central locations, distributed to diagnostic laboratories, and kept frozen until required (Anon., 1976; de Maar, 1979). Thus, ELISA may provide a useful tool for the serological diagnosis of strongyloidiasis, both in individual patients and in epidemiological investigations. This serodiagnostic test does not require the collection of faeces or duodenal fluid and is much less unpleasant than faecal microscopy, a procedure which sometimes needs to be repeated many times.

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**References**


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