A Modified Immunofluorescent Antibody Technique for the Serodiagnosis of Human Toxocaral Larva Migrans

P. VIENS, H. STRYKOWSKI, R. RICHARDS and S. SONEA

A fluorescent antibody technique for the serological diagnosis of toxocarial visceral larva migrans in man is described, using cryostat sections of either second-stage larvae or whole adult worms embedded in frozen guinea-pig muscle. Larval antigens provide more sensitive and specific antigenic material than whole worm. Under the conditions encountered in local sero-epidemiological surveys, the test is believed to be specific.

Since observations by Beaver in 1952 on Toxocara canis visceral larva migrans (VLM) infestation in man (1), relatively few studies have been undertaken to investigate the extent of the problem as a public health threat. This is due, in part, to difficulties in isolating the parasites in suspected lesions, and, in part, to the amorphism of the clinical picture of the disease (2). When man swallows infective eggs, these hatch in the bowel, and the larvae burrow their way through the mucosa into the portal circulation. The larvae will be trapped whenever the diameter of the vessel becomes too small; they then emerge from the blood vessels, and through tissue migration, will cause host-reactive granulomata to be formed in the liver, lungs, brain (3) and retina (4). It is only in severe hepatic involvement that biopsy material might reveal the causative agent, such techniques becoming largely unreliable in moderate or light hepatic infestations (5).

Material and Methods

Antigen

Second-stage Toxocara canis larvae were obtained as follows: naturally- or experimentally-infected puppies were sacrificed and adult worms recovered. Male worms were used as whole-worm antigen (WA) (see below). Fertilized female worms (as checked by the presence of fertilized eggs obtained by uterine syringe puncture) provided eggs. These were collected by squeezing the middle-third portion of the worm into an Erlenmeyer-flask containing 0.1N H2SO4. Embryonation was carried out at room temperature under aerobic conditions with constant agitation of the flask for a minimum period of one month. From 60 to 75% of the eggs thus developed into second-stage larvae. Eggs were decoated in household bleach for 30 minutes, then washed several times with phosphate buffered-saline (PBS) at pH 7.2. Several attempts were made to mechanically disrupt the egg shells with indifferent results. It was finally found that gentle homogenization in a glass homogenizer resulted in partial release of embryonated larvae, and the whole resulting mixture (unembryonated eggs, intact and ruptured embryonated eggs, liberated free larvae and empty egg-shells) was washed three times in saline. This constituted the larval antigen (LA).

Ascaris adult worm wrapped in thin layers of guinea-pig abdominal muscle was also used in cross-reaction controlled experiments (AA).
Embedding of antigen in guinea-pig muscle

Adult *T. canis* male worms (WA) were tightly wrapped in thin layers of guinea-pig abdominal muscle (12), frozen to -20°C and cryostat-sectioned in 6 micron-thick layers onto circular areas delimited by silicone-spraying of glass slides (fluoroglide “film bonding grade”, Resistaloy Ltd., Montreal), masked with a home-made device (Commar Ltd. Ste-Geneviève-de-Pierrefonds, Québec) (Fig. 1).

Thrice-washed larval antigen (LA) were embedded as follows: guinea-pig muscle was first tightly wrapped around a 2-mm-diameter stainless-steel rod and deep-frozen. A current was then applied to both ends of the rod for a few seconds, thus loosening the frozen muscle tubes. These were sectioned in 1 cm lengths which were then water-sealed on petri dishes at -70°C: the washed larval sediment was cast in, the resulting “sausage” being cryostat-sectioned when needed as described above.

Antigen slides were kept at -20°C. Unused “sausages” were maintained at -20°C or -70°C. One-cm-long wrapped worm (WA) or one drop of sedimented larvae could provide for close to 1500 antigenic test surfaces. This technique proved most economical, and provided easy-to-store material.

Antisera

Serum from a single proven case of human visceral larva migrans was available to us through the courtesy of Dr. Shapiro, Montreal Children’s Hospital. Since monkey globulins were known to cross-react with anti-human globulin fluoresceine-conjugated serum (anti-Hum FITC) (Ambroise-Thomas, personal communication), we orally infected two Rhesus monkeys with bi-weekly doses of 1000-3000 infected toxocaral larvae included in their fruit meals. Monkeys were bled at intervals and the level of fluorescent antibodies (FA) determined. They were exsanguinated after 35 days, when the FA titer of one of them reached 1:640. Sera from young normal infants were used as negative controls. Sera from *Ascaris*- and other nematode-infected

Fig. 1 Preparation of slides for the toxocaral fluorescent antibody test.

Acetone-cleaned slides are masked with a special device (A) and silicone-sprayed (B). Three-well slides are used for helminth antigens, eight-well slides for protozoa (C).
## TABLE I

<table>
<thead>
<tr>
<th>ANTISERA</th>
<th>ANTAGENS</th>
<th>ADULT Toxocara (WA)</th>
<th>LARVAL Toxocara (LA)</th>
<th>ADULT Ascaris (AA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monkey #1 *</td>
<td></td>
<td>40 **</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td>Monkey #2 *</td>
<td></td>
<td>640</td>
<td>1280</td>
<td>160</td>
</tr>
<tr>
<td>VLM Human case</td>
<td></td>
<td>320</td>
<td>640</td>
<td>160</td>
</tr>
<tr>
<td>Ascaridosis **</td>
<td></td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Ascaridosis ****</td>
<td></td>
<td>160</td>
<td>20</td>
<td>160</td>
</tr>
<tr>
<td>Ankylostomiasis and Trichuriasis *****</td>
<td></td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Trichinosis (4 cases) (&gt; 1:160) ******</td>
<td></td>
<td>40</td>
<td>40</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Trichinosis (2 cases) (&lt; 1:160) ******</td>
<td></td>
<td>40</td>
<td>20</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&lt;20</td>
</tr>
</tbody>
</table>

* Infected orally with 1 x 10^3 infected T. canis larvae bi-weekly and bled after 35 days
** Reciprocal of the last dilution giving a positive reaction
*** Mean of five human cases of more than 6 months duration
**** Serum from a case of pulmonary eosinophilia proved later to be ascaridosis
***** Two human cases of more than one year's duration
****** Homologous anti-trichinella FA titration

1. Second-stage toxocaral larvae provide a more sensitive and a more specific antigen than whole adult worm.
2. The test is specific when performed with sera (diluted 1:40) of patients with long-standing ascariasis, ankylostomiasis and trichuriasis. In acute trichinosis, and migrative phases of ascaris larvae, cross-reactions were observed up to a serum dilution of 1:40; therefore, 1:80 was selected as the lowest specific dilution.

### Discussion

The use of guinea-pig muscle-embedded *T. canis* larvae provides a sensitive, specific and easy-to-store antigen for the serological diagnosis of VLM in man. Provided that care is exercised in determining the conditions of specificity, and particularly the starting patient’s serum dilution (1:80 in our hands), the test is specific and sensitive.

Ascaridosis, especially of recent infestation, might result in some cross-reactions. It appears however that this could be overcome by absorption of serum with ascaris extract (11), and we do not feel that this would constitute a major handicap, in view of the low incidence of native ascariasis in our population. False-positive reactions due to clinical trichinosis represent only a theoretical problem since the correct etiology can be secured by the history, course of the disease, specific anti-trichinella serology and muscle biopsy. The technique, as described here, works well in the serodiagnostic laboratory we are organizing at the Hotel-Dieu Hospital in Montreal (Viens et al, manuscript in preparation). It can be used by any FAT-using laboratory, to which antigen slides can be sent for local use, thereby permitting decentralization of the technique. The ease of manipulation enables a single technician to screen up to 200 sera in one working day.

Studies are on their way to apply this technique in a serological survey of various population groups in the Montreal area: retarded children institutions, eye clinic attendants, asthmatic patients, unexplained eosinophilias, etc.
Acknowledgements

This work was carried out with the aid of a National Health Grant (No. 605-1021-28) from the Department of National Health and Welfare, Ottawa, Canada.

Una modification de la technique des anticorps fluorescents a été utilisée pour le diagnostic sérologique de la toxocarase (larva migrans viscérale), au moyen des coupes en congélation de vers adultes ou de cobaye congelé. L’antigène larvaire est plus sensible et plus spécifique que le ver adulte. Cette technique assure une spécificité suffisante dans les circonstances où se dérouleraient les enquêtes de dépistage.

REFERENCES


Cope Schwenger, Injuries and Injury Control, continued from page 233