Evaluation of Cellular Immune Response During Chronic Schistosomiasis in Humans by the Leukocyte Aggregation Test and the Leukocyte Migration Inhibition Test

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Cellular immune response was evaluated in 31 patients with chronic *Schistosoma haematobium* and *Schistosoma mansoni* infections and in 15 healthy normal persons by using *S. mansoni* soluble worm and egg antigens. Although the leukocyte migration inhibition test demonstrated false-positive reactions, the specificity of the leukocyte aggregation test was confirmed by the negativity of all of the controls. Among the patients, only 10% were positive for the leukocyte aggregation test. This low cellular reactivity was in contrast to markedly elevated specific humoral response determined by an enzyme-linked immunosorbent assay for immunoglobulin G and paper allergosorbent test for immunoglobulin E with soluble worm antigen. These results confirm that the cellular immune reactivity to schistosome antigen as demonstrated by the leukocyte aggregation test is either minimal or absent in chronically infected patients.

On the basis of the well-known aggregating activity of lymphokines (15), we have recently developed a new technique for the in vitro evaluation of cell-mediated immune response in humans, involving the measurement of light transmission of cell suspensions (25). The leukocyte aggregation test (LAT) was found to be more sensitive and reliable than measurement of migration inhibition of the leukocytes for the detection of cell-mediated hypersensitivity with penicillin and purified protein derivative of tuberculosis antigen in humans (14, 26).

In human chronic schistosomiasis, most of the studies concerning cellular immune response have been realized in vitro by using the lymphocyte transformation test with conflicting results, either negative (6, 21) or positive (18, 23), whereas the leukocyte migration inhibition test (LMIT) has been rarely used (30, 31). We attempted to evaluate cell-mediated immunity with the LAT and the LMIT.

Tests were performed in 31 adult patients (12 women and 19 men), of whom 23 were infected with *Schistosoma haematobium* and 8 were infected with *Schistosoma mansoni*. All of them had viable parasites in their urine or stool. Clinical symptoms were mild, and no acute form was observed. Microscopic hematuria was found in 11 (47.8%) of the *S. haematobium*-infected patients, and most of the patients with *S. mansoni* infection had no clinical symptoms. Eosinophilia was either normal or slightly increased (with a maximum of 2,059/mm3). An associated filariasis was found in seven patients which was found to be not correlated to the eosinophilia and specific tests. Other parasitic infestations were absent.

All of the patients, who had been living in France for several months, originate from endemic areas of Africa. In view of this, schistosomiasis was considered to be of the chronic form. The controls for this study comprised 15 normal healthy European subjects without parasitic infection who had not stayed in endemic areas.

Since different studies have shown extensive cross-reactivity between the two African species of schistosome (4, 17), only *S. mansoni* antigens were prepared. Both schistosome soluble egg antigen and soluble worm antigen were used. Crude saline extracts were prepared by successive freezing and thawing of adult worms, and purified eggs (2) were collected from infected hamsters. After centrifugation (60 min, 5,000 × g, 4°C) the supernatant was dialyzed against distilled water and lyophilized. Protein estimation was done by the Lowry et al. technique (16).

Schistosome-specific serum immunoglobulin E (IgE) was measured by immunoenzymatic techniques performed with commercial kits (Phadezym radioallergosorbent technique for specific IgE; Pharmacia Fine Chemicals). Results are expressed in classes of positivity ranging from 0 (absence of specific IgE) to 4. Because weak, nonspecific reactions were observed in controls, only classes 3 and 4 were considered significant.

An enzyme-linked immunosorbent assay was performed with soluble worm antigen for specific antischistosome serum IgG determination. This was carried out in a Microelisa rigid polystyrene tray (Dynatech Laboratories, Inc.) as previously described (7) using peroxidase-labeled anti-human IgG (Pasteur) and orthodianisidine as the substrate. The soluble worm antigen was diluted to a protein concentration of 5 μg/ml in carbonate buffer pH 9.6 (the concentration defined as optimal by previous titration with positive and negative sera). Sera were tested in duplicate at a single dilution of 1/100. With this technique, a serum having an optical density at 400 nm of more than 0.3 was considered significantly positive (7).

The technique of Soborg and Bendixen (27) was used for the LMIT; the LAT was performed as previously described (14), with some modifications. Briefly, peripheral blood leukocytes were isolated by dextran, and 1-ml samples of cell suspensions (3 × 108 cells per ml) in Ham medium with or without antigen (1, 10, 50, 100, and 200 μg) was incubated at 37°C for 18 to 20 h in stoppered tubes. Cultures with the addition of fetal calf serum were gassed. No antibiotic was added. The tubes were gently inverted, and the cell suspension was transferred into the glass cuvette of the aggregometer for absorbance reading.

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The relative absorbance of the cell suspension was immediately measured. The results are expressed as the difference in absorbance, quantified in centimeters on graph paper, between samples cultured with and without antigen. A 1-cm difference in absorbance between treated and control samples is equivalent to 10% difference in light transmission between a leukocyte suspension of 3 × 10⁶ cells per ml and a tube with medium only. Aggregation more than 2 cm was thus considered positive (14). The assay were done in triplicate.

Schistosome-specific serum antibodies determined by the enzyme-linked immunosorbent assay and RAST showed that specific IgGs were present in 26 patients (19 S. haematobium and 7 S. mansoni) and that increased concentrations of specific IgE (classes 3 and 4) were noted in 16 sera 9 S. haematobium and 7 S. mansoni (Table 1).

With the LMIT, 46.6% of the controls were positive at several concentrations of both antigen (Table 2), making the positive data on patients unreliable. As indicated by us previously for the penicillin antigen system, it is possible that the LMIT often yields false-positive results (14). Moreover, cytotoxic antibodies (19), aggregated globulins (12), and antigen-antibody complexes (13) have been shown to be responsible for the inhibition of migration, and their role in false-positive LMIT results in schistosomiasis remains to be elucidated. On the other hand, the specificity of the LAT can be assessed in this study by the complete negativity of the reaction in the controls. In the 31 chronically infected patients, only 15% significant positive reactions were observed (two S. haematobium and three S. mansoni patients). No significant differences were noticed with either soluble egg antigen or soluble worm antigen at five different concentrations each. In view of both the high sensitivity of the LAT test as has been demonstrated with different antigen preparations (14,25) and the highly positive humoral tests with the same antigen preparation, it is difficult to consider the 84% negative results as false-negative.

Thus, the dissociation between high production of specific antibodies and low cellular reactivity can be interpreted in relation to the experimental observations in chronically infected mice. In this model, there is a diminished granulomatous hypersensitivity response, characterized by a reduction in lymphokine production, and a minimal or absent cell-mediated immune response, accompanied by simultaneous increase in specific serum antibodies (1,5).

In humans with active schistosome infections, the lack of antigen-induced lymphokine production has been well shown (10). A variety of regulatory phenomena has been proposed, including adherent suppressor cell (20,28) and suppressive serum factor (6,22,29). The role of parasite antigens in the induction of this suppressive response, directly or via different immunological mechanisms, has been established in humans (11,24) and in animals (3,8,9). However, the humoral response does not seem to be affected by this modulation in chronic schistosomiasis.

It is tempting to suggest that in our patients, an absence of specific leukocyte aggregation as demonstrated by LAT could be due to the absence of delayed hypersensitivity.

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**LITERATURE CITED**


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### TABLE 1. Percentage of positive specific serum IgG and IgE antischistosomal antibody titers

<table>
<thead>
<tr>
<th>Patient group</th>
<th>No. of subjects</th>
<th>% Positive (no. positive)</th>
<th>IgG ELISA</th>
<th>IgE RAST*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schistosomiasis</td>
<td>23</td>
<td>82.6 (19)</td>
<td>39.1 (9)</td>
<td></td>
</tr>
<tr>
<td>(S. haematobium)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>8</td>
<td>87.5 (7)</td>
<td>87.5 (7)</td>
<td></td>
</tr>
<tr>
<td>(S. mansoni)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* ELISA, Enzyme-linked immunosorbent assay. ** RAST, Radioallergosorbent technique.

### TABLE 2. Comparison between LAT and LMIT

<table>
<thead>
<tr>
<th>Patient group</th>
<th>No. of subjects</th>
<th>% Positive (no. positive)</th>
<th>LAT</th>
<th>LMIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schistosomiasis</td>
<td>23</td>
<td>8.7 (2)</td>
<td>43.5 (10)</td>
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</tr>
<tr>
<td>(S. haematobium)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>8</td>
<td>37.5 (3)</td>
<td>62.5 (5)</td>
<td></td>
</tr>
<tr>
<td>(S. mansoni)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>0</td>
<td>46.6 (7)</td>
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