

# Detection of specific IgE antibodies in parasite diseases

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## Abstract

Activation of Th1 or Th2 cells is associated with production of specific immunoglobulin isotypes, offering the opportunity to use antibody measurement for evaluation of T cell function. Schistosomiasis and visceral leishmaniasis are diseases associated with Th2 activation. However, an IgE response is not always detected in these patients. In the present study we evaluated specific IgE antibodies to *S. mansoni* and *L. chagasi* antigens by ELISA after depletion of serum IgG with protein G immobilized on Sepharose beads or RF-absorbent (purified sheep IgG antibodies anti-human IgG). In schistosomiasis patients, specific IgE to SWAP antigen was demonstrable in only 10 of 21 patients (48%) (mean absorbance  $\pm$  SD =  $0.102 \pm 0.195$ ) when unabsorbed serum was used. Depletion of IgG with protein G increased the number of specific IgE-positive tests to 13 (62%) and the use of RF-absorbent increased the number of positive results to 20 (95%) (mean absorbances  $\pm$  SD =  $0.303 \pm 0.455$  and  $0.374 \pm 0.477$ , respectively). Specific IgE anti-*L. chagasi* antibodies were not detected in unabsorbed serum from visceral leishmaniasis patients. When IgG was depleted with protein G, IgE antibodies were detected in only 3 (11%) of 27 patients, and the use of RF-absorbent permitted the detection of this isotype in all 27 visceral leishmaniasis sera tested (mean absorbance  $\pm$  SD =  $0.104 \pm 0.03$ ). These data show that the presence of IgG antibodies may prevent the detection of a specific IgE response in these parasite diseases. RF-absorbent, a reagent that blocks IgG-binding sites and also removes rheumatoid factor, was more efficient than protein G for the demonstration of specific IgE antibodies.

IgE production is dependent on IL-4, a cytokine involved in differentiation of CD4<sup>+</sup> Th2 cells (1). Since IL-4 is also one of the main cytokines secreted by Th2 cells, determination of total IgE or antigen-specific IgE has been used to determine the occurrence of Th2 activation in several clinical conditions

including autoimmune and parasite diseases (2,3). Although the IgE isotype is mainly associated with allergic reactions classified as type I hypersensitivity (4), its relationship to infectious diseases such as helminthiasis (5) and more recently with protozoan and viral infections has been demonstrated (3,5).

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Research supported by PRONEX,  
FINEP and NIH grant AI-30639.  
E.M. Carvalho is the recipient  
of a CNPq fellowship.

Received July 15, 1998  
Accepted May 25, 1999

## Key words

- IgE antibodies
- Immunoassays
- Schistosomiasis mansoni
- Visceral leishmaniasis
- Kala-azar

In these conditions, the demonstration of specific IgE antibodies has been used for the diagnosis of toxoplasmosis (6) and total IgE production has been used as a marker of disease severity in malaria and HIV infection (7-10).

Although assays to detect allergen-specific IgE antibodies in sera have been standardized, these procedures cannot be applied to detect antigen-specific IgE antibodies in patients with diseases associated with polyclonal B cell activation and hypergammaglobulinemia. In such cases, false-negative results may occur due to IgG antibody competition for the same epitopes. In the present study we tested the efficacy of previous depletion of IgG with protein G or purified sheep IgG anti-human IgG in immunoassays to facilitate the demonstration of antigen-specific IgE antibodies in serum of patients with schistosomiasis and visceral leishmaniasis.

*Schistosoma mansoni* soluble antigens were obtained from adult worms of the Puerto Rico strain. Briefly, adult worms were disintegrated with a sonicator and centrifuged at 100,000 g for 45 min to obtain clear supernatants corresponding to the soluble antigen (SWAP). Protein concentration was determined by the method of Bradford (11).

*Leishmania chagasi* antigens were prepared from 10<sup>9</sup> stationary phase promastigotes grown in LIT medium supplemented with 10% fetal calf serum, RPMI 1640 (Gibco, Grand Island, NY, USA) and gentamicin. The promastigotes were washed 3 times with phosphate-buffered saline (PBS) and lysed with 6 mM CHAPS (3-[(3-cholanidopropyl)-dimethyl-ammonio]-1 propane sulfonate) in 50 mM Tris-HCl buffer containing 150 mM NaCl, pH 7.5. After centrifugation at 13,000 g for 5 min the protein content was measured by the method of Bradford (11) and stored at -20°C until use.

Twenty-one sera from patients with schistosomiasis and 27 sera from patients with

visceral leishmaniasis were screened for the presence of specific IgE antibodies. Patients with visceral leishmaniasis were admitted to Hospital Universitário Prof. Edgard Santos or Hospital Santo Antônio (Salvador, BA, Brazil). They presented with the classical clinical picture of visceral leishmaniasis and the diagnosis was confirmed by demonstration of amastigote forms in material obtained from bone marrow or spleen aspiration and stained with Giemsa. IgG antibodies were also detected by ELISA in all patients (12). The schistosomiasis patients were from the endemic area of Caatinga do Moura, Bahia, and had *S. mansoni* eggs in their stools. They suffered from chronic schistosomiasis in its intestinal or hepato-intestinal form.

IgE antibodies against *S. mansoni* were investigated by indirect ELISA performed on wells of polystyrene microtiter plates previously coated with 4 µg protein of SWAP. The tests were performed with unabsorbed sera diluted 1:10 in PBS containing 0.05% Tween. In order to eliminate competition from IgG antibodies, immunoassays were also performed with 100 µl of serum diluted 1:10 in PBS containing 25% Sepharose-protein G (Pharmacia, Uppsala, Sweden) or purified sheep IgG anti-human immunoglobulin IgG (RF-absorbent; Behring Diagnostics, Marburg, Germany) previously reconstituted to 1.5 ml. After centrifugation to remove the Sepharose beads or immunoprecipitates, all primary reactions were developed with 100 µl of the supernatants for 18 h at 4°C, whereas secondary reactions were carried out with 100 µl of peroxidase-conjugated mouse monoclonal anti-human IgE (Medix Biotech, Inc., Miami, FL, USA) for 1 h at 37°C. The immunoassays were developed with 100 µl of sodium acetate buffer containing TMB (3,3',5,5'-tetramethylbenzidine) and H<sub>2</sub>O<sub>2</sub> for 30 min at room temperature, and the absorbances of the reactions were measured at 450-600 nm with an ELISA reader after the addition of 50 µl of 2 N HCl.

Anti-leishmania-specific IgE in sera from

visceral leishmaniasis and control subjects were also investigated by indirect ELISA performed as described above, using 500 ng of *L. chagasi* antigen, and patient sera were diluted 1:6 in PBS containing protein G or RF-absorbent. Specific leishmania antigens recognized by the IgE antibodies from patient sera were identified by Western immunoblotting (13). For these experiments, 500  $\mu$ l of visceral leishmaniasis or control sera was previously depleted of human IgG by diluting the sera 1:5 in PBS with 25% (v/v) protein G Sepharose.

The cut-offs of the immunoassays were determined using the mean plus 3 SD of the absorbance obtained with serum from 10 healthy individuals. Specificity and sensitivity were determined as recommended by Ferreira and Ávila (14). The computer program GRAPHPAD was used for statistical analysis.

IgE antibodies anti-*S. mansoni* were detected in only 10 (48%) of 21 patients when unabsorbed sera were used in the immu-

noassays. Depletion of IgG by treatment with protein G beads increased the number of positive sera to 13 (62%) and the use of RF-absorbent allowed the detection of specific IgE antibodies in 20 (95%) of 21 positive sera (Figure 1). Mean absorbance ( $\pm$  SD) was  $0.108 \pm 0.195$  when the unabsorbed sera were used. These values were  $0.303 \pm 0.455$  in sera depleted of IgG using protein G and  $0.374 \pm 0.477$  in sera pre-absorbed with RF-absorbent ( $P < 0.01$ ).

Specific IgE antibodies anti-*L. chagasi* were not detected when whole serum from visceral leishmaniasis patients was used in the ELISA. After IgG depletion with protein G, IgE antibodies were detected in 3 (11%) of 27 sera and the use of RF-absorbent increased the detection of IgE antibodies to all sera tested (100%) (Figure 2). Mean absorbance ( $\pm$  SD) was  $0.104 \pm 0.03$  when sera depleted of IgG with RF-absorbent were used in the ELISA. Western immunoblotting was performed with sera from three patients with visceral leishmaniasis. The IgE antibodies

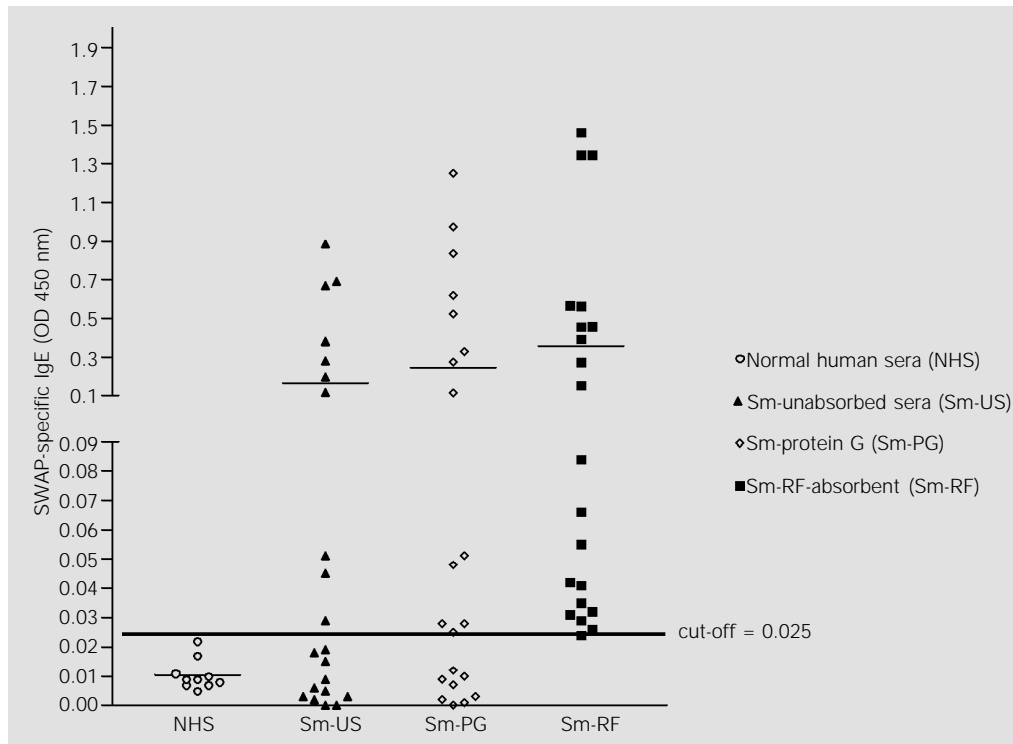
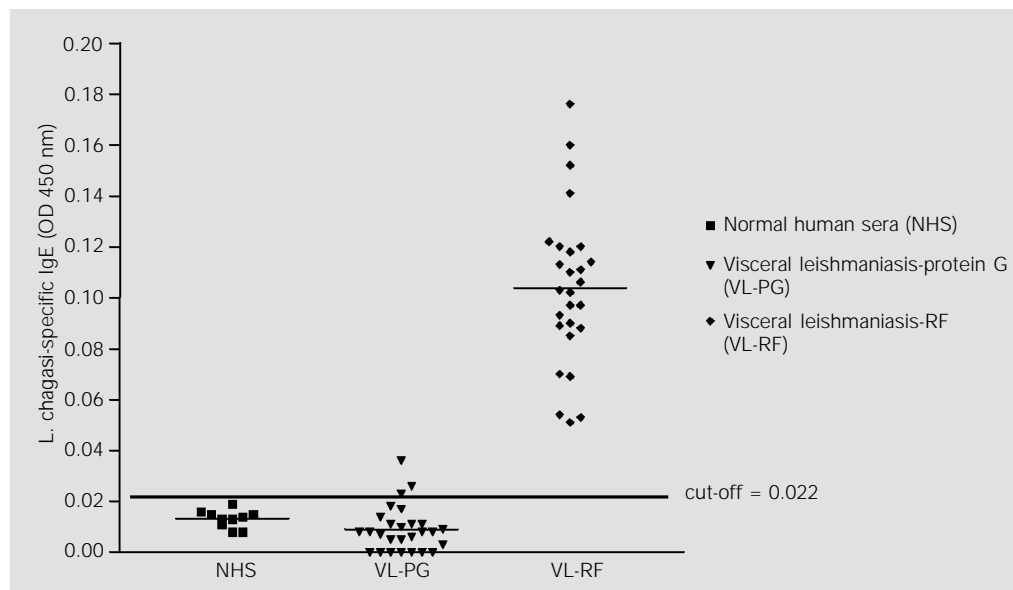


Figure 1 - Detection of specific IgE antibodies anti-*S. mansoni* (Sm) by ELISA with unabsorbed sera and sera treated with protein G or RF-absorbent from 21 *S. mansoni* patients. The cut-off is the mean  $\pm$  3 SD of 10 sera from healthy subjects.

Figure 2 - Detection of specific IgE antibodies anti-*L. chagasi* by ELISA in sera from 27 visceral leishmaniasis patients pretreated with protein G or RF-absorbent. The cut-off is the mean  $\pm$  3 SD of 10 sera from healthy subjects.



predominantly recognized antigens with molecular masses of 96.67 and 46 kDa. Due to the limited amount of sera available, Western immunoblotting was also performed with pooled sera from 5 other patients and a similar antigen recognition pattern was obtained.

The present study describes a simple method to detect specific IgE antibodies in patients with two different parasite diseases, both of them associated with hyperglobulinemia and evidence of Th2 activation (15-17). In schistosomiasis there is an increase in IgE production and higher levels of specific IgE against parasite antigens and IgE:IgG4 ratios are significantly associated with a lower intensity of re-infection (18,19). In a previous study on schistosomiasis conducted in Africa, IgE antibodies were detected directly in unabsorbed sera (20). The present study shows that the sensitivity of detection of SWAP-specific IgE is low when unabsorbed sera are used, whereas the use of RF-absorbent facilitated the detection of IgE antibodies in up to 95% of the schistosomiasis patients.

Visceral leishmaniasis is associated with high mRNA for IL-4 and Th2 activation. Since this cytokine signals B cells to produce IgE (1) and previous studies have shown

high levels of total IgE in visceral leishmaniasis, we determined the levels of *L. chagasi*-specific IgE in patients with this disease. When unabsorbed sera were used, ELISA failed to detect *L. chagasi*-specific IgE, whereas the inhibition of IgG by RF-absorbent permitted the detection of IgE in all patients tested.

Depletion of IgG antibodies by protein G has been previously used to rule out the blocking effect of IgG in serological tests (18). In the present study, protein G and RF-absorbent were compared and RF-absorbent was found to increase the sensitivity of the test, facilitating detection of specific IgE antibodies in the majority of schistosomiasis patients and in all visceral leishmaniasis sera tested. Two reasons might explain the relatively low effectiveness of protein G. First, in both conditions studied there are very high IgG levels and even when using protein G the sera from patients with visceral leishmaniasis continue to contain up to 60% IgG. Second, IgG antibodies against IgE are detected in situations where polyclonal activation is observed (21). In such case, the use of protein G may deplete the IgE that is complexed with the IgG, decreasing the sensitiv-

ity of detection of IgE antibodies. RF-absorbent is not very effective in removing IgG. Its main function is to block the IgG-binding sites, efficiently reducing the reaction of IgG with the antigens and allowing the detection of IgE antibodies.

## Acknowledgments

We thank Mr. Antônio de Souza and Aurinha Barbosa for assistance with the field work, and Elbe M. Silva for help in preparing the manuscript.

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