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Parasitology

The role of glycosylated epitopes in the serodiagnosis of *Strongyloides stercoralis* infection☆,☆☆,★

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ABSTRACT

Carbohydrates of pathogen antigens have been disrupted by periodate oxidation, in order to reduce nonspecific bindings and improve serodiagnosis of parasite infections. In the present study, the enzyme-linked immunosorbent assay (ELISA) was carried out with filariform larvae antigen treated, or not treated, with sodium metaperiodate. Groups of sera from patients with *Strongyloides stercoralis* infection, with other intestinal parasites and a normal control, were used. The oxidation of *Strongyloides stercoralis* glycosylated epitopes reduced the seroreactivity of sera from patients with *S. stercoralis* infection as demonstrated by ELISA, with a decrease in sera optical densities. The number of cross-reactions of IgG and IgE-ELISAs increased by 12% and 16%, respectively, after antigen treatment with metaperiodate. This was more often observed in patients infected with *Schistosoma mansoni* and hookworm. Moreover, the IgG depletion from sera tested by IgE-ELISA led to the detection of previous false-negative samples from *S. stercoralis*–infected patients.

1. Introduction

Strongyloides stercoralis is widely distributed in tropical and subtropical regions, and it is estimated that approximately 35 million people are infected worldwide, with frequencies above 6% in Brazil (De Bona and Basso, 2008; Inês et al., 2011; Kothbary et al., 1999; Oliveira et al., 2002; Olsen et al., 2009). *S. stercoralis* infection in immunocompetent patients usually results in asymptomatic chronic disease. Nevertheless, hyperinfection and dissemination can occur in high-risk groups, such as patients undergoing glucocorticoid therapy; those with hematologic malignancy; those co-infected with human T-lymphotropic virus type I (HTLV-1) and HIV; and those suffering from chronic alcohol abuse (reviewed by Keiser and Nutman, 2004). The diagnosis of *S. stercoralis* infection usually relies on the detection of larvae in stool samples. The majority of cases involve a chronic infection with an intermittent and small larvae load in the feces, and a decrease in the sensitivity of parasitologic tests. It has been recommended that at least 4 negative results for stool examinations are required to rule out the *Strongyloides stercoralis* infection (Dreyer et al., 1996; Liu and Weller, 1993; Roxby et al., 2009; Uparanukraw et al., 1999). Stool culture in agar plate has been shown to be a more sensitive diagnostic tool than the Baermann–Moraes technique, although it is more laborious and time consuming (Inês et al., 2011; Jongwutiwes et al., 1999; Koga et al., 1992). To bypass the limitations of parasitologic methods, an enzyme-linked immunosorbent assay (ELISA), to detect IgG anti–*S. stercoralis*, has been used. Nevertheless, the cross-reactivity with other intestinal helminths overestimates the true prevalence of *S. stercoralis* infection and represents a great limitation to serologic assays (Costa-Cruz et al., 2003; Dreyer et al., 1996; Uparanukraw et al., 1999). The demonstration of specific IgE response in human strongyloidiasis by ELISA has been used for diagnosis, since the cross-reactivities with other helminthes are lower than those reactions with specific IgG. In spite of this, IgE anti–*S. stercoralis* may not be detected by ELISA due to the presence of excessive amounts of circulating IgG which produces a competitive inhibition of IgE binding sites (Costa-Cruz et al., 2003; Leoratti, 2004). Moreover, patients under steroid therapy or coinfected with *S. stercoralis* and HTLV may have lower circulating specific IgE (Machado et al., 2011; Porto et al., 2001).

Previous studies have shown that a specific antibody reactivity with glycoproteins of pathogen antigens was improved after periodate oxidation of their glycosylated epitopes at acid pH (Alarcón de Noya et al., 2000; Albuquerque et al., 2005; Kouguchi et al., 2011). The disruption of carbohydrates from *Schistosoma mansoni* egg soluble antigen increased the specificity of schistosomiasis immunodiagnosis

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(Alarcón de Noya et al., 2000; Noya et al., 2002) and allowed the discrimination between the chronic and acute forms of paracoccidioidomycosis by using a carbohydrate-modified antigenic preparation to test the reactivity towards IgG and IgM (Ferreira et al., 2008). On the other hand, with visceral leishmaniasis, the metaperiodate treatment of *Leishmania infantum/chagasi* antigens resulted in a reduction of the seroreactivity by ELISA (Atta et al., 2004).

In the present study, we describe, for the first time to our knowledge, an ELISA for detection of *Strongyloides stercoralis* IgG and IgE antibodies using parasite soluble antigens treated with sodium metaperiodate to remove the glycosylated molecules. Furthermore, the efficacy of previous depletion of IgG from the sera of *S. stercoralis*–infected patients for detection of IgE reactivity was evaluated.

2. Materials and methods

2.1. Patients and sera samples

The present study was carried out from November 2009 to December 2010 on individuals seen at the Clinical Analysis Laboratory of Pharmacia College, Federal University of Bahia, Brazil. Daily, around 2–3 sera samples from patients were randomly selected according to the coproparasitologic results of spontaneous sedimentation, Baermann–Moraes method, and agar plate culture methods. A total of 100 sera samples from parasitized individuals consisting of 50 from *S. stercoralis* monoinfected patients and 50 with other intestinal parasites, including hookworm (10), *Ascaris lumbricoides* (9) *Schistosoma mansoni* (10), *Trichuris trichiura* (10), *Enterobius vermicularis* (2), and *Trichostrongylus* sp. (9) were used to perform the ELISA for detection of *Strongyloides*-specific IgG and IgE. As negative controls, 34 sera samples from newborns of mothers with negative parasitologic test results and 14 from healthy adults, who were members of the laboratory staff, were used. False IgE-negative results from patients shedding *S. stercoralis* larvae in feces were evaluated in 10 IgG-depleted samples.

This study was approved by the Committee of Ethics in Research of the Gonçalo Moniz Institute, Oswaldo Cruz Foundation, and an informed consent for participation was obtained from each patient during delivery of clinical specimens.

2.2. Strongyloides stercoralis soluble antigen

Strongyloides stercoralis soluble antigen (SsAg) was prepared as described previously (Arakaki et al., 1990). Briefly, *S. stercoralis* filariform larvae were obtained from feces culture of hyperinfected patients. Larvae were washed 5 times in 0.15 mol/L of phosphate-buffered saline (PBS, pH 7.2) by centrifuging for 7 min at 1.8 × *g*. Parasites were suspended for 5 min in 0.25% sodium hypochlorite and then washed 5 times in PBS as described above. The larvae were suspended in PBS with protease inhibitors (5 mmol/L EDTA, 1 mmol/L phenyl-methylsulfonylfluoride [Sigma], 0.05 mmol/L TPCK/TLCK, 1 μg/mL leupeptin) and sonicated in an ice bath for a duration of 9 cycles of 80 s at 40 kHz (Branson Sonifier Cell Disruptor, Branson Instruments, Danbury, CT, USA). The larvae homogenate was then centrifuged at 11,000 × *g* for 30 min at 4 °C. The supernatant with soluble antigen was analyzed for protein content according to Lowry et al. (1951), divided into aliquots, and stored at −70 °C until use.

2.3. IgG-depleted sera

IgG depletion from human serum was carried with rheumatoid factor (RF) absorbent according to the manufacturer's instructions (Siemens, Marburg, Germany). Briefly, sera samples were diluted 1:4 in PBS–0.05% Tween-20 and 1% BSA followed by a new 1:2 dilution in RF absorbent. Samples were well mixed, incubated for 18 h at 4 °C, and then centrifuged for 5 min at 1.8 × *g*. The supernatant was used to perform the immunoassay to detect specific IgE.

2.4. Enzyme-linked immunosorbent assay for IgG and IgE detection

Indirect ELISA for specific IgG and IgE was conducted as previously described by Van Doorn et al. (2007) with some modifications. Briefly, the microtiter plates (Corning Costar polystyrene EIA/RIA plates, Corning) were coated with 10 μg/mL of *S. stercoralis* antigen in 0.06 mol/L carbonate–bicarbonate buffer (pH 9.6), incubated overnight at 4 °C, and washed 3 times with PBS containing 0.05% Tween-20 (PBS-T). To test the role of glycosylated epitopes, the SsAg was treated with sodium metaperiodate (MSsAg) according to Woodward et al. (1985). Soon thereafter, SsAg-coated plates, as described above, were washed with 50 mmol/L sodium acetate buffer (pH 4.5) and then glycosylated epitopes were oxidated by the addition of 100 μL of 10 mmol/L sodium metaperiodate in 50 m mmol/L sodium acetate buffer (pH 4.5). Plates were then incubated for 1 h at room temperature in the absence of light, washed 3 times with PBS (pH 7.2), and incubated with 100 μL of 50 mmol/L sodium borohydride in PBS for 30 min at room temperature, followed by rinsing with 50 mmol/L sodium acetate buffer (pH 4.5). MSsAg- and SsAg-coated plates were blocked with 100 μL PBS-T plus 5% w/v skim milk (PBS-T-Milk) or with PBS-T plus 1% w/v bovine serum albumin (PBS-T-BSA) for 1 h at 37 °C for *S. stercoralis* IgG and IgE detection by ELISA, respectively. After blocking, wells were washed as described previously.

Assays for specific IgG antibodies were performed with sera samples diluted at 1:100. Plates coated with MSsAg or SsAg were incubated in duplicate with 100 μL of sera per well for 1 h at 37 °C. Thereafter, 100 μL of conjugated anti-human IgG linked to horseradish peroxidase (Sigma-Aldrich, St. Louis, MO, USA) diluted at 1:4000 was added and incubated in the same conditions. The reaction was revealed by the addition of 100 μL of substrate (100 μL of 0.051 mol/L citrate–phosphate buffer [pH 5.0] containing 0.0037 mol/L *p*-phenylenediamine and 0.04% hydrogen peroxide 30 volumes) and incubated for a period of 20 min in the absence of light, followed by the addition of 20 μL of 8N sulfuric acid to stop the reaction. The absorbance (Abs) was measured at 450–630 nm with a microplate reader (Awareness Technology, USA).

Assays for specific IgE antibodies were carried out using whole or IgG-depleted sera as discussed in Section 2.3. To each well coated with MSsAg or SsAg, 100 μL of 1:8 dilution of sera samples was added (depleted or undepleted of IgG) and incubated for 1 h at room temperature. The wells were washed 3 times with PBS-T, and 100 μL of biotinylated goat anti-human IgE (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA) diluted 1:2000 was added. After incubation for 1 h at room temperature, the wells were washed as described

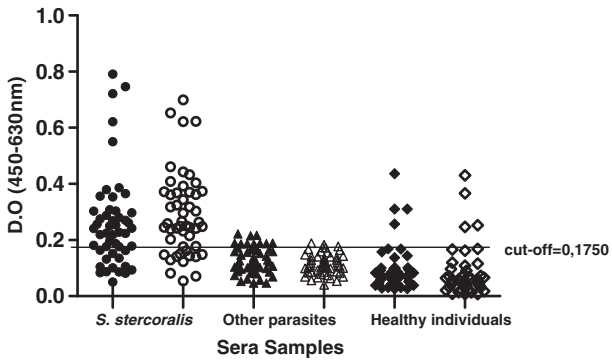

Fig. 1. Indirect ELISA to detect IgG anti–*S. stercoralis* using *S. stercoralis* soluble antigens treated with sodium metaperiodate (black symbols) and without treatment (hollow symbols). Sera samples from infected patients with *S. stercoralis* ($n = 50$) or with other intestinal parasites ($n = 50$) and from healthy individuals (n = 48) were tested. The cut-off was calculated by the ROC curve.