INFLUENCE OF HUMAN T-CELL LYMPHOCYTOTROPIC VIRUS TYPE 1 INFECTION ON SEROLOGIC AND SKIN TESTS FOR STRONGYLOIDIASIS

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Abstract. The aim of this study was to determine whether human T-cell lymphocytotropic virus type 1 (HTLV-1) infection may affect the levels of parasite-specific immunoglobulin (Ig) G and IgE and the positivity of the skin test for strongyloidiasis. Participants included 67 patients with strongyloidiasis (40 without HTLV-1 infection and 27 coinfected with HTLV-1). We determined IgG and IgE levels by enzyme-linked immunosorbent assay, and the immediate hypersensitivity skin test was performed with the metabolic Strongyloides stercoralis antigen. Specific IgE levels and the size of skin reactions in patients without HTLV-1 were higher ($P < 0.01$) than those observed in patients coinfected with HTLV-1. Additionally, 89% of patients without HTLV-1 had specific IgE and 92.5% had positive skin tests; however, these values were significantly reduced ($P < 0.01$) in patients coinfected with HTLV-1 (44% and 59%, respectively). These data show that HTLV-1 infection decreases the sensitivity of detection of S. stercoralis-specific IgE, the size of the immediate hypersensitivity reaction, and the sensitivity of these tests in the diagnosis of strongyloidiasis.

INTRODUCTION

Although stool examination is the most direct and simplest test for diagnosis of strongyloidiasis, the diagnostic value is compromised by the following: irregular and scanty output of larvae by intestinal adult females; failure to detect acute infection; and occasional negative results in some patients with severe disease. Immunodiagnostic tests such as an assay for specific immunoglobulin (Ig) G antibody and an immediate hypersensitivity skin test can also be used and are especially useful for epidemiologic studies. Parasite-specific IgE antibodies have also been documented in patients with strongyloidiasis. Because this isotype is involved in defense against helminths, interest in characterizing the IgE response has recently increased. Considerable evidence has recently accumulated concerning certain complications of strongyloidiasis, such as development of severe disease, nonresponse to therapy, or both in patients concurrently infected with human lymphocytotropic virus type 1 (HTLV-1). Some patients infected with HTLV-1 produce high levels of interferon gamma and reduced total serum IgE levels, which may account for the more severe disease and impaired response to treatment of strongyloidiasis in patients infected with both agents. The aim of the present study was to determine whether coinfection with HTLV-1 decreases the humoral immune responses to Strongyloides stercoralis antigen, specifically parasite-specific IgG and IgE, and the immediate hypersensitivity skin test.

MATERIALS AND METHODS

Patients. Patients were recruited into the study from 3 sources: referrals of HTLV-1–positive seroreactors from blood banks, residents from a rural endemic area near Salvador with positive fecal examination for S. stercoralis infection, and 3 patients admitted at Hospital Santo Antonio due to severe strongyloidiasis. A clinical history was taken and physical examination performed. Laboratory analyses included serology (IgG and IgE) for S. stercoralis; serology for HTLV-1, and immediate hypersensitivity skin test for S. stercoralis. The criterion for a diagnosis of strongyloidiasis was a positive fecal examination for larvae by the Baermann concentration technique. Initial screening by direct fecal smears was not done because of low sensitivity of the method, especially in chronic infections. Protocols, including consent forms written in Portuguese, were approved by ethical review committees of the University Hospital in Salvador, Brazil, as well as of the National Institutes of Health in the United States. All patients provided written informed consent.

Preparation of antigens. Antigens for the skin test and serology were prepared from infective larval stages (L3) of the parasites recovered originally as rhabditiform larvae from fecal specimens of infected monkeys and allowed to develop at 25°C in carbon cultures for 7–10 days. Larvae were separated from the charcoal by the Baermann procedure and washed repeatedly by centrifugation. They were then exposed to 0.25% sodium hypochlorite for 3–5 min for surface sterilization, followed by multiple cycles of centrifugation in RPMI medium (Gibco, Grand Island, NY) containing 100 μg/mL gentamicin. The somatic antigen for the enzyme-linked immunosorbent assay (ELISA) was prepared from the soluble supernatant of sonicated larvae. Metabolic antigen for skin testing was prepared from 24- and 48-hr harvests of larval cultures incubated at 33°C. After separation of larvae by centrifugation and Millipore filtration, these fluids were pooled and lyophilized, reconstituted in distilled water, and dialyzed with phosphate-buffered saline (PBS) at pH 7.2. An equal volume of RPMI medium containing 100 μg/mL gentamicin was lyophilized and treated exactly as the antigen to provide a diluent control preparation. The antigen and control were then treated with 1:4,000 formalin at 37°C for 14 days and checked for sterility and endotoxin content before storage in multidose vials in the presence of 0.4% phenol at 4°C. Additional details concerning the skin test antigen are provided elsewhere.

Skin test. The test was performed by intradermal injection into the forearm of 0.1 mL of metabolic antigen containing...
3.5 μg/mL protein with a 27-gauge needle and a 1.0-mL allergy syringe. An equal volume of the control preparation was injected intradermally 2–3 cm from the antigen site. After 15 min, the reactions were measured by blotting an outline of the urticarial wheal onto moist filter paper (Whatman No. 1), allowing the paper to dry, cutting out the reactions, weighing them to the nearest 0.1 mg on an analytical balance, and converting the weights to areas in square millimeters from a nomogram of weights of known areas. The values for final skin test reactions were areas greater than the reactions to the control preparation. A difference in area of 35 mm² was considered to be a positive reaction. Itching, as expected with any immediate hypersensitivity test, was the only complaint in some patients and did not require use of medication. No systemic reaction to the skin test was observed.

Parasite-specific IgG assays. Strongyloides stercoralis–specific serum IgG was measured by ELISA with microtiter plates (Linbro, Flow Laboratories Inc., McLean, VA). Plates were coated overnight with S. stercoralis L3 antigen (2.5 μg/mL) and blocked with 0.25% bovine serum albumin in PBS. After 5 washes in PBS containing 0.05% Tween 20 (PBST), 2-fold dilutions of test sera, starting at 1:32, were added (100 μL/well) and incubated for 30 min at 37°C. The wells were washed 5 times with PBST and then incubated with 100 μL of goat anti-human IgG phosphatase conjugate at 1:1,000 as described above. The wells were washed again, and the color was developed for 30 min with 4-nitrophenyl phosphate. Absorbances were measured at 405 nm with an ELISA reader. Optical density values that exceeded the mean plus 2 standard deviations of values obtained with negative control sera were considered positive.

Parasite-specific IgE assays. Strongyloides stercoralis–specific serum IgE was measured by ELISA on microtiter plates (Immulon 2; Dynatech Laboratories, Chantilly, VA) as previously described.4 Sera were first depleted of IgG by incubation with Gamma-Bind G Sepharose (Pharmacia Biotech, Uppsala, Sweden) before reaction with antigen overnight at 4°C. IgE was detected with goat anti-human IgE conjugated to alkaline phosphatase (Sigma, St. Louis, MO); the substrate was p-nitrophenyl phosphate (Sigma), and the results were expressed as international units. Values ≥ 6.0 IU were considered positive. This was the minimum value that could be detected on the curve obtained with a pool of positive control sera.

Serology for HTLV-1. Serology for HTLV-1 was performed in the Blood Center by ELISA (Cambridge Biotech, Worcester, MA). Only ELISAs that could be confirmed by Western blot (HTLV Blot 2.4, Genelabs, Science Park Drive, Singapore) were considered positive.

Statistical analysis. Fisher's exact test was used to compare the proportions. Correlations between IgG and IgE were analyzed by Spearman's rank coefficient of correlation (rs). The Spearman rank sum test was used to compare means.

RESULTS

Patients were divided into 2 groups on the basis of serology for HTLV-1. Group 1 had 40 people with negative serology for HTLV-1, and Group 2 had 27 patients with positive serology for HTLV-1. The sex ratio (M:F) of the 40 HTLV-1–negative and of the 27 HTLV-1–positive patients was 1.5:1 and 1.7:1, respectively. The mean age of Groups 1 and 2 was 35 ± 10 years and 23 ± 13 years, respectively. The 2 groups were comparable regarding nutritional status, and all patients denied use of drugs and had negative serology for human immunodeficiency virus. The majority of patients was asymptomatic for strongyloidiasis. Three patients required hospitalization due to severe strongyloidiasis characterized by diarrhea, dehydration, and hypoalbuminemia.

The distribution of IgE levels, expressed in international units, and the results of the skin tests, expressed in square millimeters, in patients with strongyloidiasis without HTLV-1 infection and in those coinfected with HTLV-1 are shown in Figure 1. The mean IgE in patients without HTLV-1 infection was 250 ± 438 IU compared with 74 ± 94 IU in patients with strongyloidiasis coinfected with HTLV-1 (P < 0.01). In contrast, both groups had similar levels of IgG: 0.187 ± 0.094 and 0.194 ± 0.086, respectively (P = 0.54). Although there was a direct correlation between IgG and IgE levels in patients with strongyloidiasis without HTLV-1 (P < 0.01; rs = 0.39), there was no correlation between these isotypes in the group with concomitant HTLV-1 and S. stercoralis infection (P = 0.59; rs = 0.19). Skin test responses in patients with S. stercoralis without HTLV-1 were higher than those observed in patients coinfected with S. stercoralis and HTLV-1 (136 ± 75 mm² versus 74 ± 66 mm²; P < 0.01).

In addition to decreased IgE and skin test responses in the group with strongyloidiasis associated with HTLV-1 infection, the frequency of positive tests was lower in these patients (Table 1). Although 33 (82.5%) of 40 patients with strongyloidiasis without HTLV-1 infection had elevated IgE levels, only 15 (55.5%) of 27 patients had detectable IgE (P < 0.01) when coinfected with HTLV-1 and S. stercoralis. The number of people with positive skin tests (37 of 40, 92.5%) in the group without HTLV-1 infection was also higher (P < 0.01) than the number of patients who had strongyloidiasis associated with HTLV-1 (16 of 27, 59%). In both patient groups, there was a direct correlation between the area of the skin test and IgE levels (P < 0.01). It is of interest that the 3 patients with evidence of severe strongyloidiasis were in the group with HTLV-1 infection. Additionally, although these patients had IgG antibodies against S. stercoralis, they did not have detectable parasite-specific IgE antibodies in their serum, and their skin tests with S. stercoralis antigen were negative.

DISCUSSION

Infection with HTLV-1 and strongyloidiasis are highly prevalent in Salvador, a city located in northeastern Brazil.11,12 Although several reports have described the association of these 2 infections, little is known about changes in the immune response to either agent during concomitant infection. In the present study, we show that although the specific IgG levels did not differ in the 2 groups, coinfected people had decreased IgG and skin test reactions to Strongyloides antigens.

Peripheral blood mononuclear cells from HTLV-1-infected patients, including asymptomatic carriers of the virus, express activation markers such as interleukin-2 receptors and...
Figure 1. Comparison of parasite-specific serum immunoglobulin E levels and measures of the skin test reactivity in patients with strongyloidiasis coinfected or not with human T-cell lymphocytotropic virus type 1 (HTLV-1).

Table 1: Influence of HTLV-1 infection on IgG and IgE serum antibody responses and skin test reactivity to Strongyloides stercoralis antigen.

<table>
<thead>
<tr>
<th>Test</th>
<th>HTLV+ positive (n = 27)</th>
<th>HTLV- negative (n = 40)</th>
<th>P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE</td>
<td>15 (55.5%)</td>
<td>33 (82.5%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IgG</td>
<td>24 (88.8%)</td>
<td>34 (85.0%)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Positive skin test</td>
<td>16 (59.0%)</td>
<td>37 (92.5%)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* HTLV-1 = human T-cell lymphocytotropic virus type 1; Ig = immunoglobulin.
† Fisher’s exact test.

human leukocyte class II antigens. In addition, they demonstrate spontaneous proliferation and interferon gamma secretion in culture. 9,13,14 Although several reports have shown that patients coinfected with HTLV-1 and S. stercoralis have decreased total IgE synthesis, 9,13,16 only one report shows that reduction in parasite-specific IgE may occur in such patients. 17

Here, we confirm that infection with HTLV-1 significantly reduces the sensitivity of a parasite-specific IgE test for strongyloidiasis. Additionally, we document that coinfection with HTLV-1 reduces the sensitivity of the immediate hypersensitivity skin test for diagnosis of strongyloidiasis. Our previous observation that coinfection of S. stercoralis with HTLV-1 decreases total IgE levels and the current data showing no correlation between IgG and IgE absorbances in patients coinfected with S. stercoralis and HTLV-1 suggest that an impairment in the IgG-IgE switch may occur in these patients. This could lead to a reduction in parasite-specific IgE molecules linked to mast cells, resulting in lower or absent skin reactivity to S. stercoralis antigen.

The majority of patients in the present study had asymptomatic or mild strongyloidiasis. As noted in Results, the 3 patients with severe disease were also infected with HTLV-1. The finding that all 3 patients had no detectable IgE or skin test responses to S. stercoralis indicates a strong association between disseminated strongyloidiasis, absence of parasite-specific IgE antibodies, and HTLV-1 infection. Thus, it is possible that HTLV-1 infection may change the clinical course of strongyloidiasis by suppressing parasite-specific IgE. In reviewing infectious complications associated with HTLV-1 infection, Marsh 18 concluded that HTLV-1 did not appear to predispose to primary infection with S. stercoralis but that it may alter the host’s immune response and affect the clinical outcome of the parasite infection. In experimental models of helminthic infection, mast cell activation is associated with parasite killing or parasite expulsion from the intestinal lumen. 19,20 Studies are under way to determine how HTLV-1 may affect parasite killing or parasite elimination in the intestine.

Although concomitant infection with HTLV-1 and S. stercoralis decreases the sensitivity of the parasite-specific IgE antibody test and the immediate hypersensitivity skin test for diagnosis of strongyloidiasis, IgG absorbances against parasite antigens were not reduced in patients coinfected with HTLV-1. Therefore, in patients with severe strongyloidiasis coinfected with HTLV-1, the IgG ELISA may still be useful for diagnosis of strongyloidiasis.

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