LONGITUDINAL STUDY OF THE ANTIBODY RESPONSE TO RECOMBINANT ENTAMOEBA HISTOLYTICA ANTIGENS IN PATIENTS WITH AMEBIC LIVER ABSCESS

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Abstract. Serology is a critical component in the diagnosis of amebic liver abscess. However, in areas endemic for amebiasis there is a high background level of seropositivity for amebiasis (owing to previous infection with Entamoeba histolytica), which may complicate the interpretation of a positive serologic test result. Recently, we reported that serologic tests based on recombinant E. histolytica antigens might offer improved diagnosis of current invasive amebiasis because they apparently differentiated active infection from past exposure to the parasite. To confirm this finding, we have performed a longitudinal study on 20 patients with amebic liver abscess by examining their seroreactivity over time with recombinant versions of two major E. histolytica proteins, the serine rich E. histolytica protein (SREHP), and the 170-kD subunit of the galactose-specific adhesin. We found that more than 50% of the patients examined had become seronegative by one or both recombinant tests within 180 days of their diagnosis of amebic liver abscess. In the case of the recombinant SREHP-based tests, 12 patients had become seronegative 90 days after presentation. In contrast, all patients remained seropositive by a standard conventional test, an indirect hemagglutination test, at more than six months after presentation. Our study shows that patients lose seroreactivity with the recombinant SREHP or 170-kD antigen-based tests more rapidly than with a conventional serologic test; this may make them useful for the serologic diagnosis of amebiasis in endemic areas.

The intestinal protozoan parasite Entamoeba histolytica remains a major cause of morbidity and mortality worldwide. Much of the mortality seen with amebiasis comes from amebic liver abscess, the most common extraintestinal manifestation of the disease. The diagnosis of amebic liver abscess is based on a triad of 1) clinical symptoms (fever, abdominal pain), 2) a space-occupying lesion in the liver, and 3) positive amebic serology. Current serologic tests for amebiasis, such as the widely used indirect hemagglutinin (IHA) test, are highly sensitive and specific tests for amebic infection. However, seropositivity can persist for years after infection. This is borne out by seroepidemiologic studies in areas endemic for amebiasis, in which between 6% and 20% of healthy subjects will have a positive serologic test result for amebiasis, probably secondary to prior infection with E. histolytica. A high background level of seropositivity in endemic areas can limit the usefulness of serologic tests to diagnose acute (current) invasive amebiasis. Antigen detection tests may provide an effective approach to differentiating current from past amebic infection, but none are yet marketed for clinical use.

One approach to reducing background seropositivity is to identify individual amebic antigens that may be associated with a more short-lived antibody response. Recently, we have described serologic tests for amebiasis based on recombinant versions of two major E. histolytica surface proteins, the serine-rich E. histolytica protein (SREHP) and the 170-kD subunit of the galactose-specific adhesin of E. histolytica. In retrospective studies, we found that serologic tests using recombinant SREHP or 170-kD fusion proteins had better positive predictive values for acute invasive amebiasis than a conventional test (agar gel diffusion) in a selected population from an endemic area. The recombinant SREHP-based test was less sensitive (80%) than the conventional test (90–100%), but the sensitivity of immunoblotting using the 170-kD antigen (90%) was close to that of agar gel diffusion. We have now extended our longitudinal study on seroreactivity to recombinant versions of the SREHP and 170-kD antigens in a group of patients with amebic liver abscess. Herein we show that a significant proportion of these patients lose seroreactivity with recombinant SREHP and 170-kD proteins within 120 days of their presentation with amebic liver abscess.

POPULATION, MATERIALS, AND METHODS

Study population. Patients (n = 20) with amebic liver abscess (diagnosed as described previously) seen at the King George IV Hospital in Durban, South Africa who were willing to return for additional serum collections were enrolled in this study. Informed consent was obtained from all individuals and this protocol was approved by the Human Studies Committees of the University of Natal Medical School and Washington University School of Medicine. All patients were treated with metronidazole at the time of diagnosis, and were documented by stool examination and/or stool culture to have cleared amebic intestinal infection. No patient had clinical or laboratory (stool culture) evidence for disease recurrence or new infection during the follow-up period. A total of 108 serum samples from the 20 patients were obtained periodically after therapy, and were analyzed in this study. Since there was variability in the timing of serum collection and length of follow-up for each patient, sera were grouped into those collected within one week of presentation with amebic liver abscess (available from all 20 patients), those collected in the ensuing 8–30 days (20 patients), those collected 31–90 days following presentation (20 patients), samples collected 91–120 days following presentation (16 patients), samples collected 121–180 days following presentation (14 patients), and samples collected 181 days or more following presentation (nine patients).

Antigens. A recombinant SREHP/maltose binding protein (MBP) fusion protein, which contains amino acids 10 to 222 of the native SREHP molecule fused to the MBP molecule, was purified from bacteria using affinity chromatography...
with a monoclonal antibody against SREHP as previously described. The MBP molecule was also expressed and purified by affinity chromatography on amylose resin using the manufacturer’s instructions (New England Biolabs, Beverly, MA). The 170-kD recombinant protein was used was the 170CR/glutathione-S-transferase (GST) fusion protein. This fusion protein contains amino acids 649 to 1202 of the 1,202 amino acid 170-kD protein. We have previously shown that this fusion protein (which can be expressed at high levels) appears to be equivalent to a fusion protein containing the entire 170-kD antigen as a target antigen for serodiagnosis using Western blotting. The 170CR/GST fusion protein was purified from bacterial lysates on glutathione-Sepharose using the manufacturer’s protocol (Pharmacia LKB Biotechnology, Piscataway, NJ). The GST protein alone was also expressed and purified on glutathione-Sepharose as described.

**Western blotting.** Western blotting of the recombinant proteins with human serum was performed as described previously. Approximately 10 μg of recombinant protein/well was used for each run. Human serum samples were used at a 1:500 dilution for all experiments because this dilution was found to give maximum sensitivity and specificity results in previous studies. Autoradiographs were exposed for 16 hr. Blots were considered positive if serum bound to the recombinant SREHP/MBP or 170CR/GST proteins and did not bind to the recombinant MBP or GST fusion partners. No serum sample in the study bound to the MBP or GST proteins.

**Indirect hemagglutination test.** All serum samples were also analyzed using the amebiasis IHA test according to the manufacturer’s instructions (Behring Diagnostics Inc., Somerville, NJ). A positive IHA test result was defined as a titer $\geq 1:128$ (manufacturer’s suggested cut-off for active or recent infection).

**RESULTS**

The number of patients seropositive in either the recombinant SREHP/MBP-based test, the 170CR/GST-based test, or the IHA test over time is summarized in Figure 1. Serum samples obtained within the first week of presentation with amebic liver abscess were reactive with recombinant SREHP/MBP in 14 of 20 patients (70%), with recombinant 170CR/GST in 17 of 20 patients (85%), and by IHA test with a titer $> 1:128$ in all 20 patients. These results are consistent with the findings from previous studies, in which seropositivity with recombinant SREHP and seropositivity with recombinant 170CR was seen in 80% and 90%, respectively, of the patients presenting with amebic liver abscess.

When serum samples obtained from each patient 8–30 days after presentation were examined, three of the 20 patients who had been seroreactive with SREHP were now seronegative (11 of 20 remaining seropositive), while two of the 17 individuals who had been seroreactive with the recombinant 170CR/GST protein were now seronegative (15 of 20 remaining seropositive). All patients remained positive by IHA with titers $\geq 1:512$ in 19 of 20 patients (1:128 in the remaining patient). Thirty-one to 90 days after presentation with amebic liver abscess, two additional patients were no longer seropositive in the 170CR/GST-based test (13 of 20 remaining seropositive), while three patients were no longer seropositive for SREHP/MBP (8 of 20 remaining seropositive). All patients remained seropositive by the IHA test (titers $\geq 1:512$).

Serum samples were available from 16 patients at the 91–120 day point. Among those individuals, only three were seropositive for SREHP/MBP, while 10 of 16 were seropositive for 170CR/GST, and all 16 were seropositive by the IHA test. At the 121–180 time point, only one of 14 individuals tested remained seropositive for SREHP/MBP, while none of 14 remained seropositive for 170CR/GST, and all 14 were seropositive by the IHA test. Finally, in the nine individuals from whom serum samples were obtained at time points greater than 180 days past presentation, none remained seropositive for SREHP, while only three of nine remained seropositive for 170CR/GST. All patients remained seropositive by the IHA test.

An analysis of the serologic reactivity of the individual patients shows that among the 14 individuals seropositive for SREHP/MBP within the first week after presentation, 13 of 14 had become seronegative by the time of their last serum collection. This value was significantly different ($P \leq 0.001$) from the results seen with the IHA test, in which all patients remained seropositive at the time of their last serum collection. Six of the 13 patients who became seronegative with the SREHP/MBP-based test were seronegative within 90 days of their presentation with amebic liver abscess. Similarly, of the 17 patients seropositive for the 170CR/GST protein, 11 of 17 had become seronegative by the time of their last serum collection. This result was significantly different ($P \leq 0.006$) from the results seen with IHA test.

**DISCUSSION**

Individuals with amebic liver abscess may remain seropositive for amebiasis by conventional serologic tests for years following their acute infection. Previously, we have shown that serologic tests based on recombinant E. histolytica antigens may be more specific for current amebic infection than conventional tests. This finding suggests that antibodies that recognize the recombinant antigens are present for a shorter period of time than antibodies to the anti-
gens present in the amebic lysates used in conventional tests, or it may reflect a reduced sensitivity of the recombinant antigen-based tests compared with current tests. We have examined this by assessing seroreactivity to two recombinant amebic antigens and comparing them with the conventional IHA test in a longitudinal serologic study of patients with amebic liver abscess. While all patients remained seropositive (tested as long as two years after presentation with amebic liver abscess) by the IHA test, more than half of the patents had become seronegative by one or both of the recombinant antigen-based tests by the time of their last serum collection. Especially striking was how rapidly some individuals became seronegative with the SREHP-based test, with a significant number of them becoming seronegative within 90 days of their initial diagnosis.

These results confirm that tests based on either the 170CR or SREHP recombinant proteins might offer some advantages for the serologic diagnosis of amebiasis in regions with a high background level of seropositivity by conventional tests. In this study with a limited number of patients, we found no statistically significant difference between the two recombinant antigen tests in the results at initial testing (patients seropositive within the first seven days of presentation), but the SREHP-based test was less sensitive than IHA (P < 0.05), and our previous studies have suggested that the SREHP-based test is less sensitive for the diagnosis of amebiasis than the recombinant 170 kD antigen-based test.

This disadvantage may be offset by the trend seen in this study for patients to become seronegative more rapidly with the SREHP-based test. The SREHP-based test could have utility in confirming a positive conventional serologic test result in individuals in whom the clinical presentation is not suggestive of amebiasis, while the 170-kD antigen-based test deserves consideration as a standalone serologic test for invasive amebiasis.

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