Antigenemia in Patients with Mediterranean Visceral Leishmaniasis
dedicated to the detection of circulating studies (1, 2, 8) using ELISA and Western blot analyses of antibodies is generally concomitantly performed as an aid for diagnosis. Nevertheless, anti-
Leishmania infantum
leishmaniasis (MVL) due to

L. infantum
(3) previously developed for measuring parasite burdens in BALB/c mice experimentally infected with

L. infantum
promastigotes (a sample of 106 promastigotes corresponded to 0.1 ml of assay buffer) was dispensed into the wells and the plates were incubated for 2 h. Bound-enzyme activity was revealed with a chromogenic substrate as described previously (3). The threshold assay sensitivity was 0.02 µg/ml of Leishmania antigens, corresponding to 5,000 parasites/ml. The method was validated with a panel of cryoconserved serum samples obtained from the collection of the parasitology-mycology department of the Centre Hospitalier Universitaire de Nice. The analyzed samples included (i) control samples from an area where Leishmania is not endemic (Reims, France), (ii) samples from asymptomatic contacts of infected patients, diagnosed on the basis of positive results from Western blotting against 14- and 18-kDa Leishmania antigens and/or positive skin tests (6, 7), from an area of endemicity (Nice, France), (iii) samples from immunocompetent or HIV-coinfected patients (23 males aged 22 to 75 years and 26 females aged 18 to 81 years) from an area of endemicity (Nice, France) with patent MVL diagnosed on the basis of parasite detection by PCR or direct examination, and (iv) samples from patients with African trypanosomiasis or acute malaria (these samples were a gift from B. Bouteille, Limoges, France). All samples were previously tested at a 1/500 dilution for the presence of anti-
Leishmania antibodies by classical ELISA using Leishmania antigen-coated plates (4). CLAs (Fig. 1) were undetectable in 13 control serum samples from an area where Leishmania is not endemic, as well as in samples from 19 healthy contacts from an area of endemicity, 2 (10.5%) in the latter group being antibody positive by ELISA using crude Leishmania antigens. In contrast, at the time of diagnosis, CLAs (range, 0.03 to 4 µg/ml) were detected in 23 (53%) of 44 immunocompetent patients with MVL and higher levels (range, 0.2 to 20 µg/ml) were detected in 4 (80%) of 5 patients coinfected with HIV (Fig. 1). Interestingly, two of these four coinfected patients with detectable CLAs (Fig. 1) were negative by antibody ELISA. In addition (Fig. 1), serum samples from acute malaria patients or individuals with Afri-
can trypanosomiasis, which showed cross-reacting anti-*L. infantum* antibodies upon ELISA analysis in 18 and 50% of cases, respectively, gave CLA values close to background levels. Therefore, for the panel of sera studied, direct detection of CLAs by ELISA exhibited overall sensitivity of 55.1% and specificity of 100% for the diagnosis of MVL. Furthermore, monitoring of antigenemia in immunocompetent MVL patients receiving successful liposomal amphotericin B (AmBisome) chemotherapy (Fig. 2) indicated that in all studied cases, CLAs were completely cleared from circulation by day 25 but that antibody levels decreased only slowly during this period. Consequently, antigenemia decline measured by direct ELISA following chemotherapy is a more sensitive indicator of therapeutic efficacy than antibody decrease, at least in immunocompetent MVL patients. Finally, molecular sieving of serum samples from immunocompetent MVL patients by using a Sephacryl S-200 column (data not shown) indicated that a great proportion of CLAs occurred in the free form in patients at the time of diagnosis and showed for the first time the minimal frequency of CLAs in a serum panel from MVL patients coinfected with human immunodeficiency virus type-1. Am. J. Trop. Med. Hyg. 65:589–596.


