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Research Note

ELISA AND SERODIAGNOSIS OF *PLASMODIUM FALCIPARUM:* A NOTE ON THE SPECIFICITY OF CRUDE SOLUBLE MALARIAL ANTIGEN

ERLINDA SANCHEZ* & HILDA A. PEREZ

Instituto Venezolano de Investigaciones Científicas, Centro de Microbiología, Apdo 21827, Caracas, 1010A, Venezuela

During preliminary studies performed in our laboratory using the ELISA for the serodiagnosis of *Plasmodium falciparum* a relatively high response to non-infected red blood cells (RBC) was observed. The obvious solution to this problem rely upon the use of purified antigens. However, methods described for the fractionation of *P. falciparum* blood-stage antigens (P. H. Jakobsen et al., 1987, *J. Clin. Microbiol., 25:* 2075-2079; P. H. Jakobsen et al., 1987, *Parasitol. Res., 73:* 518-523; G. Jaureguiberry et al., 1988, *J. Chromatogr., 25:* 385-396) are difficult to adapt to the production of antigen for diagnostic purposes. In this note the authors report a simple, cheap, rapid and efficient method for the remotion of RBC antigens from crude extracts of the blood stages of *P. falciparum.* The ELISA was carried out as described by A. Voller et al. (1974, *Bull. WHO., 51:* 209-211). In order to obtain antigen, *P. falciparum* taken directly from the blood of infected patients was cultured using the candle jar method (W. Trager & J. B. Jensen, 1976, *Science, 193:* 673-675), harvested at 7% parasitemia and processed as described by A. Voller et al. (loc. cit.). Briefly, infected cells were disrupted by sonication and the supernatant obtained after centrifugation at 12,000xg was used to sensitize Costar (USA) microtiter plates. Optimal dilutions for antigen, test sera and conjugates (Protein A-peroxidase) were determined by previous checker-board titrations, chromogenic substrate was ABTS (Sigma) and OD were determined at 405 nm. Serum samples were as follow: twelve controls from subjects without history of malaria nor residence in endemic areas (laboratory staff), 16 sera from individuals with diagnosis of *P. falciparum* malaria established on the basis of a direct observation of parasites on blood smears and a reference pool of 7 positive sera from patients with *P. falciparum* infection and known IFA titers ($\geqslant$ 1:640). Specificity was controlled by using material obtained from non-infected RBC as antigen. Microwells were coated with an amount of RBC equivalent to that used in the *P. falciparum* antigen.

Results showed that albeit the reactivity of the reference positive pool to *P. falciparum* sera was significantly higher than that to RBC control antigens there was a significant level of reactivity to RBC which could not be attributed to either endogenous peroxidase like-activity in the absorbed antigen or nonspecific binding of the conjugate. Further examination of both malaria and control sera with the control RBC antigen revealed that most of positive and some control sera exhibited a significant reactivity against RBC which strongly diminished following individual absorption of sera with a AB+ non-infected erytrocytes. The procedure, although effective, probed to be tedious and time-consuming mainly when assaying a large amount of samples. Experiments were therefore designed to remove RBC "antigens" from the crude preparation of soluble malarial antigen. A rabbit antiserum against AB+ RBC was prepared and the antibodies used to coat a polystyrene petri dish (M. G. Mage et al., 1977, *J. Immunol. Methods, 5:* 47-56). Briefly, selected sera with a hemagglutination titer against non infected AB+ cells higher than 1:20,000 were appropriately diluted in PBS and 2 ml were added into a 60 mm ϕ petri dish. Coating was carried out overnight at 4°C and after drawing off the antiserum dilution the dish was washed thrice with cold PBS. Following the final washing the dish was tapped on tissue paper. An appropriated dilution of the soluble malarial antigen was then added and after 1 hr incubation at room temperature the absorbed malarial antigen was carefuly removed. Comparison of

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the reactivity against absorbed and non-absorbed malarial antigen of control (0.29 ± 0.01 EEM vs 0.198 ± 0.03 EEM, n = 10) and patients sera (0.82 ± 0.02 vs 0.58 ± 0.04 EEM, n = 10) revealed that host cell contaminants had been efficiently removed. Moreover, for most sera the reactivity against the absorbed *P. falciparum* malarial antigen was similar to the difference calculated after substracting the absorbance value against RBC from that determined in the well coated with non-absorbed malarial antigen. In subsequent assays all the crude preparations of soluble malarial antigen were therefore affinity absorbed with rabbit antibodies against AB+ RBC prior to coating of ELISA microplates. We believe that the procedure herein described is a simple and efficient mean to remove "host cell antigens" from crude preparations of malarial antigen. It is worth of mention that cross reactions with RBC antigens is one of the difficulties observed when using the ELISA test for serological studies of malaria (M. Wahlgren et al., 1983, *Clin. Experimental Immunol., 54:* 127-134; P. Demedts et al., 1987, *Am. J. Trop. Med. Hyg., 36:* 257-263). Improvement of malarial antigen, therefore, is of great interest for serological surveys in endemic areas. We agree, however, that the technical solution to this problem will probably come from the identification of relevant antigens using current techniques of immunology and genetic engineer and further production of the required antigens by organic synthesis.

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