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A MONOCLONAL ANTIBODY-BASED ENZYME IMMUNOASSAY TO DETECT SPECIFICITIES IN MALARIAL ANTISERA: A PRELIMINARY REPORT

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Serological techniques as applied to a variety of parasitic diseases serve three main purposes: (i) to detect the presence of current infection, (ii) to detect past experience with the parasite, and (iii) to assess levels of protective immunity (Morrow, 1981). The contribution of serological methods for the study of malaria has been limited to the second of the above purposes in the form of seroepidemiological surveys. Although serological techniques can be ancillary in the establishment of the diagnosis of current infection, it is only through direct methods of demonstration of the parasite in the peripheral blood that definitive diagnosis can be made. Most serological tests are based on the detection of all antibody specificities present in the serum that can be expressed through binding to an antigen mixture presented in either soluble or particulate form. Therefore, reactivity will be a function of the additive binding activities of all individual antibody species to their target antigens available in the mixture. If these single specificities could be detected amongst the polyclonal serological reactivity in a simple test, one would be able to pinpoint peculiarities of the immune response in individual patients and correlate those findings with disease or epidemiological attributes.

The advent of hybridoma technology has presented a practical means of producing monoclonal antibodies (McAbs) to defined antigenic determinants. These McAbs could be used as probes to detect serological specificities in specimens from patients as described above.

We report herein preliminary results with the development of a competitive-binding enzyme immunoassay (CBEIA) using McAbs of serologically defined specificity that allows the quantitation of the fraction of the overall assay reactivity which is attributable to the target activity of the McAb. The assay was used to define the specificity profile for ten McAbs in collections of human malarial antisera from different geographical regions.

A solid phase enzyme immunoassay for detection of antibody reactivity (IgG class) to malarial antigen was used as test design. Polystyrene flat bottom 96-well microtitration plates were sensitized with antigen extracts from *Plasmodium falciparum* blood forms cultured *in vitro* (Trager & Jensen, 1976). The antigen mixture was obtained by centrifugation of sonicated saponin-treated suspensions of schizont-rich culture preparations. Inhibition of binding of antimalarial human antibodies present in serum specimens to the coated plates was determined after preincubation of the antigen with McAbs.

Each individual test combination of McAb and serum consisted of four duplicate wells as follows:

A. The reaction product of serum to the McAb-treated, antigen-coated solid phase.

B. The reaction product of serum on an antigen-coated well which had been post-treated with an unrelated McAb with respect to the antigen.

C. The background reaction product of the assay diluent (phosphate-buffered saline pH 7.2 with 1% bovine serum albumin) on McAb-treated, antigen-coated wells.

D. The background reactivity of diluent on antigen-coated wells which had been post-treated with unrelated McAb.

The fraction of the overall reactivity which had been suppressed by the McAb was designated the attributable decrement in reactivity (ADR) and was calculated as follows: $ADR = [(B - D) - (A - C)] \times 100$. In the formula, A, B, C, and D represent the absorbance values obtained in the CBEIA.

Interpretation of ADR values with respect to the significance of suppressed reactivities was based on the statistical analysis of control sera from normal individuals. As such, discriminant ADR values for all McAbs were defined as levels above those seen as baseline, inhibited non-specific reactivities in normal controls.

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Five batteries of antisera (240 specimens), collected in different endemic malarious areas, differed with respect to frequencies and degree of inhibitions by the various McAbs. Three of these batteries had been originated from previous serosurveys in *P. falciparum* endemic areas in Africa and Oceania. Another serum panel had been obtained in a malaria endemic Amazon area which had been previously shown as to be free of *P. falciparum* (Sulzer et al., 1978). A fifth battery was comprised of specimens from clinically suspected cases of malaria in nonimmune persons having acquired the disease when traveling outside the U.S. The lowest frequencies of inhibition were observed in the *P. vivax* and *P. malariae* battery from South America. The highest inhibition frequencies for all McAbs were observed in a battery of specimens representative of primary immune responses to malaria parasites. Of the 10 McAbs used, four produced significantly greater inhibition than the other six antibodies when tested with all batteries of sera. Degree of inhibition of binding by those four McAbs in the CBEIA was also highly correlated with total anti-*P. falciparum* reactivity in individual sera, which indicates that their target antigens represented dominant serological specificities among all sera. One McAb suppressed the anti-malarial reactivity exclusively of specimens from the battery with presumable primary responses to malaria.

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