

ANTIBODY SECRETIONS IN MALARIOUS INDIVIDUALS
(IMMUNOREGULATION IN MALARIA)

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OBJECTIVE : To examine the synthesis and secretion of total IgM, IgG and IgA during 12 days of *in vitro* culture, by peripheral blood mononuclear cells from malarious Thais.

BACKGROUND : Patients with malaria present with a number of immunological abnormalities. The major focus of *in vitro* studies to date have been directed at T cell and Fc receptor bearing cell functions by peripheral blood mononuclear cells (MNC). In particular there have been careful studies examining such areas as mitogen induced blast transformation; changes in the percentages and absolute number of lymphocyte subpopulations; general functional capabilities in antibody induced, lectin induced, and spontaneous cell mediated cytotoxicity; and the presence of cold-reactive antibodies.

The presence of autoantibodies is often taken as an indication of an alteration in immunoregulation. Immunoglobulins may be involved in mediating or inducing damage in the malarious individuals. Furthermore, the presence of antibodies in the sera of malarious individuals may not necessarily indicate antibody production as a result of specific immunization but may be the result of polyclonal activation induced by ligands acting non-specifically on T and/or B cells. Thus, studies on the nature of antibody synthesis and secretion by MNC from malarious patients are of importance because of the ability of *in vitro* antibody production to serve as a uniquely sensitive indication system for studying immunoregulatory pathways as well as delineating functional and quantitative alterations in both the humoral and cellular components of the immune response during malarial infection.

METHODS : *Isolation of peripheral blood MNC* ; Peripheral blood MNC were obtained from freshly drawn blood of healthy volunteers or patients with malaria by fractionation over Ficoll-Hypaque gradients according to the method of Boyum (1) with modifications as previously described by MacDermott et al. (2, 3).

Blastogenic response and membrane fluorescence of MNC ; Peripheral blood MNC are assayed for their response to pokeweed mitogen (PWM). The proliferative

response was investigated as previously described (4). Briefly, 5×10^4 cells in 0.1 ml of media were pipetted into round-bottom microtiter plates (Costar, Cambridge, MA) which contained 0.05 ml of either RPMI 1640 with 20% pooled human serum and antibiotics alone or with PWM final concentration 1:100; GIBCO, Grand Island, NY). After five days of incubation, the cultures were pulsed for four hours with 0.2 μCi methyl- (^3H) thymidine (specific activity 46 Ci/mmol, New England Nuclear, Bethesda, MD), processed using a MASH II harvester (Microbiological), and radioactivity counted.

Surface immunoglobulin bearing (SIg^+) cells are detected by membrane fluorescence as previously described (5, 6). In brief, cells (2.5×10^5) were washed three times in M199 (GIBCO, Grand Island, NY) containing 10% FCS. The final pellet was resuspended in .02 ml of polyvalent fluoresceinated (Fab')₂ -rabbit anti-human (Fab')₂ (Kallestad Laboratories, Chaska, MN) and incubated for 30 minutes at 37°C, resuspending every five minutes. Cells were then washed three times with media, resuspended in glycerol-PBS buffer and read with a Zeiss Photomicroscope III. Cells were counted under phase contrast and the percentage showing membrane fluorescence determined.

In vitro antibody synthesis and secretion during 12 days cultures ; In order to study the transition of B cells into immunoglobulin secreting plasma cells, peripheral blood MNC are cultured *in vitro* with or without PWM using modifications of previously published techniques (7-9).

For the present study, we utilize a solid phase system, with commercially obtained rabbit anti-IgA, IgM, or IgG covalently bound to a cross linked polyacrylamide bead (BioRad, Richmond CA). Specificity was tested with purified immunoglobulins with no cross reactivity detected. The IgG, IgA, and IgM synthesized and secreted into the media were measured by separate solid phase radioimmunoassays for each immunoglobulin class with specific rabbit anti-immunoassays and ^{125}I -labelled immunoglobulins. The assays were performed in 96 well, round bottomed microtiter plates. To each well was added 10 μ of culture supernatant or standard, 50 μ of radio-labelled IgG, IgA, or IgM (25,000 CPM), and 50 μ of appropriate solid phase antisera. After overnight incubation at 25°C, the wells were resuspended and harvested on glass fiber filter strips using a Bellco microharvester (Vineland, NJ). Individual discs were then counted using a gamma counter (Beckman, Palo Alto, CA).

RESULTS : We are presently standardizing our antibody synthesis assay and do not have conclusive or significant data to report.

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