Br. J. exp. Path. (1973) **54**, 198

THE EFFECT OF MALARIA ON THE RELATIVE AFFINITY OF MOUSE ANTIPROTEIN ANTIBODY

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Received for publication November 23, 1972

Summary.—Inbred mice infected with *Plasmodium berghei yoellii* produce antibody of significantly lower relative affinity to human serum transferrin than do uninfected animals of the same strain. The levels of antibody produced were similar in both groups. The possibility that the infection resulted in partial immunosuppression is discussed.

EXPERIMENTS on inbred strains of mice selected on the basis of their susceptibility to lymphocytic choriomeningitis virus-induced chronic soluble complex disease have suggested that the production of antibody with low affinity for antigen may be involved in the processes leading to such disease (Soothill and Steward, 1971). Low affinity antibody is poor at immune elimination of antigen (Alpers, Steward and Soothill, 1972) and in the case of chronic infections resulting in the release of large amounts of antigen together with the production of low affinity antibody, antigen excess soluble complexes would be formed. In view of these findings and the growing interest in malarial renal immunopathology, we have investigated the effect of malarial infection on the affinity of antibody in inbred mice.

MATERIALS AND METHODS

Mice.—Two-month old inbred Simpson mice, maintained at the Institute of Child Health, were used in these experiments.

Infections.—Fourteen female Simpson mice were infected with the rodent malaria parasite *Plasmodium berghei yoelii* by the intraperitoneal injection of 1×10^6 parasitized cells from a heavily infected donor mouse. The parasitaemia was followed daily by examination of Giemsa stained blood films. After immunization with antigen, the malarial infections were controlled by maintaining the mice on a milk diet for 3 days.

Immunization.—The infected mice and a group of 25 controls were given 2 intraperitoneal injections of 1 mg of human serum transferrin (HST) in 0·1 ml saline one week apart. The mean parasitaemia in the infected animals at the time of the first injection was 2% and at the second injection 8·9%. This immunization schedule was designed to ensure that the antigen was present at the time of peak parasitaemia only. The mice were exsanguinated under anaesthesia 2 weeks after the last injection and serum obtained.

Antibody measurements.—Antibody–antigen interactions are reversible and at equilibrium the binding energy or affinity can be represented by an equilibrium constant K:

$$K = \frac{k_a}{k_d} = \frac{[\text{AbAg}]}{[\text{Ab}][\text{Ag}]}$$

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where k_a = association constant; k_d = dissociation constant
[AbAg] bound antibody
[Ab] free antibody
[Ag] free antigen.

High affinity antibody has less tendency to dissociate from the antigen than does low affinity antibody and thus, at equilibrium, more antigen is bound with high than with low affinity antibody.

The levels and quality of antibody produced (expressed as relative affinity, K_R) in each serum were determined by previously described methods (Steward and Petty, 1972*a*, *b*).

Gel filtration.—In order to determine the major immunoglobulin class (*i.e.* 19S or 7S) of antibody produced, serum pools (800 μl) from infected and control animals with detectable anti-HST antibody were fractionated on a column of Sephadex G200 (60 × 2·0 cm) in 0·1 mol/l phosphate buffer pH 6·8. The first peak (19S) and second peak (7S) fractions were pooled and concentrated. Anti-HST antibody was detected by reacting ^{125}I-labelled HST with the concentrated fractions and precipitating the globulin-bound radioactivity with 50% saturated ammonium sulphate.

RESULTS

All infected mice developed fulminating parasitaemia which was effectively controlled by the milk diet.

The levels of antibody produced by both infected and control groups were very similar. However, the number of animals responding to the immunization procedure by the production of detectable antibody was higher in the infected groups (79%) than in the control group (40%) (Table). As can be seen from the

TABLE.—*The Immune Response to Human Serum Transferrin in Malarial Infected and Uninfected Mice*

	Malaria infected	Control
Number of mice in group	14	25
Number of mice producing antibody	11 (79%)	10 (40%)
Mean antibody level (p mol binding sites per ml)	311	258
Antibody relative affinity (K_R L/M)	$3·9 \times 10^5$	$1·9 \times 10^6$

figure, the malarial infected mice produced antibody to HST of lower relative affinity than the control animals given the same immunizations. These results were highly significant by Student's *t*-test ($P < 0·001$). Similar results were obtained in a second experiment carried out as described above. The ratios of 7S : 19S binding of [^{125}I]HST by concentrated fractions from Sephadex G200 gel filtration of pooled sera were very similar in the infected and uninfected groups.

DISCUSSION

Previous studies of rodents infected with malaria have shown diminished antibody responses to some antigens (sheep erythrocytes, human gamma-globulin) injected during the phase of acute parasitaemia, but no effect on the response to other antigens (keyhole limpet haemocyanin and HSA in adjuvant) (Salaman, Wedderburn and Bruce-Chwatt, 1969; Greenwood, Playfair and Torrigiani, 1971*a*; Voller, Gall and Manawadu, 1972). Infected mice, however, show normal rejection of skin homografts and other cell-mediated responses (Greenwood *et al.*, 1971*a*). Malarial infection has other effects on the immune systems of experi-

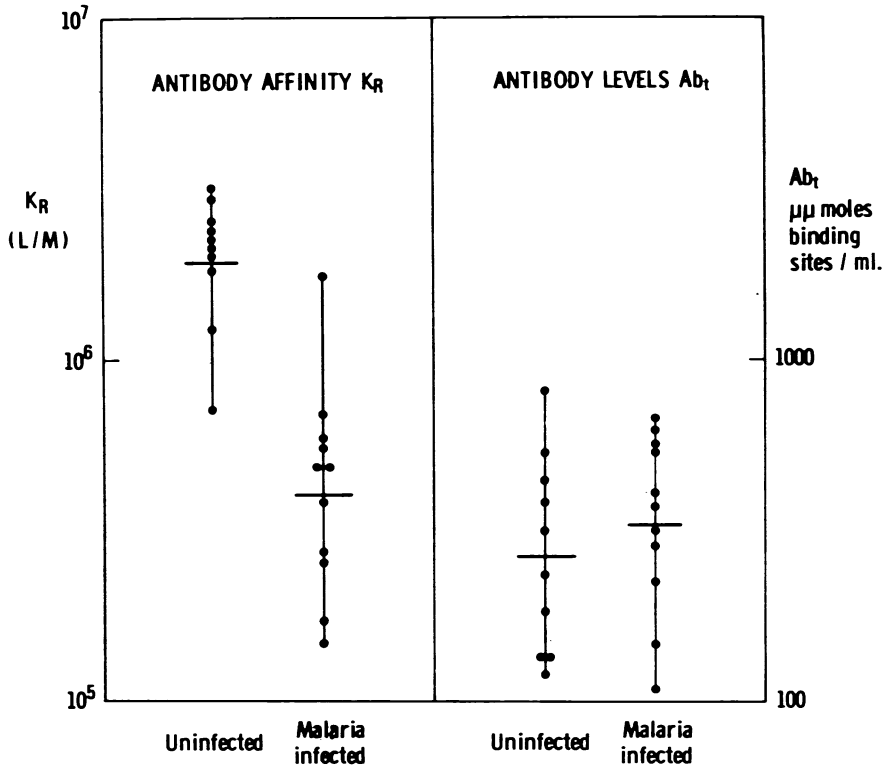

FIG.—Comparison of levels and relative affinity of antibody to human serum transferrin produced in malarial infected and uninfected mice.

mental animals, in that it appears to suppress the autoimmune haemolytic anaemia of NZB mice and the nephritis of the NZB/W F_1 hybrid mice, all mice surviving for one year (Greenwood and Voller, 1970*a*, *b*). A recent report, however (Whelton *et al.*, 1972), suggests that such infection results in only partial suppression of the disease in NZB/W F_1 mice. The frequent association of *Plasmodium malariae* infection with nephrotic syndrome of children in the tropics (Gilles and Hendrickse, 1963) and the suggestion that immune complexes of malarial antigen and antibody are involved in this disease (Soothill and Hendrickse, 1967; Hendrickse *et al.*, 1972) raises the important question of the nature of the effect of the infection on the immune response of the host.

In experiments reported here, we have shown that the immune response to HST injected in saline at the time of peak parasitaemia is altered compared with that of uninfected animals. Our data suggest that the levels of antibody in both infected and uninfected animals are similar but the number of animals producing detectable antibody was higher in the infected group. However, antibody quality, expressed as relative affinity (K_R) is significantly lower ($P < 0{\cdot}001$) in the infected group compared with uninfected control animals. The finding of increased numbers of animals making detectable antibody coupled with a decrease in the affinity of the antibody produced is difficult to explain. It is possible that

such differences in K_R may be due to changes in class or subclass of antibody in the two groups of animals, but our limited data do not indicate differences in major (*i.e.* " 19S " or " 7S ") immunoglobulin class of anti-HST antibody produced.

It has been suggested (Greenwood *et al.*, 1971*a*) that malarial infection results in a disturbance of macrophage function. It is possible that such infection in our mice resulted in impaired antigen processing by macrophages, perhaps resulting in the antigen-sensitive cells bearing higher affinity receptors being rendered unresponsive by non-macrophage associated antigen more readily than those with lower affinity receptors. Indeed, recent experiments have shown that mice normally producing high relative affinity antibody to HST make antibody of significantly lower affinity when their macrophages are blocked by carbon (Passwell Steward and Soothill, 1973). However, the clearance of carbon and ^{51}Cr-labelled sheep erythrocytes from the peripheral blood of malarial infected mice is enhanced at the time of maximal parasitaemia (Greenwood *et al.*, 1971*b*). Thus, the observed effects of the infection on the immune response cannot be due simply to reticuloendothelial blockade. Malarial infection may interfere with the transport of immune complexes into germinal centres (Greenwood *et al.*, 1971*b*) and this phenomenon may underlie, in part, the depression of antibody affinity observed in these experiments.

Mice of the NZB and NZB/W F_1 strains produce low affinity antibody, particularly when young animals are immunized (Petty and Steward, 1972). It is thus possible that the suppression of the spontaneous nephritis in NZB/W F_1 mice when infected with malaria (Greenwood and Voller, 1970*a*) may result from the production of antibody of even lower affinity to the antigen(s) involved in the immune complex nephritis, to a level below that necessary for pathogenic immune complex formation.

In view of the suggestion that genetic predisposition to the production of antibody of low affinity may underlie much soluble immune complex disease (Soothill and Steward, 1971; Petty, Steward and Soothill, 1972), it is possible that the nephritis seen in humans in association with malaria may be occurring in individuals genetically programmed to make low affinity antibody to malarial antigens. In addition, the data reported here indicate that individuals capable of making high affinity antibody may, when infected with malarial parasites, produce low affinity antibody to the malarial antigens, which would also potentiate the process of failure of immune elimination, persistence of antigen and formation of soluble immune complexes which localize in the kidney. Thus, the immune complex nephritis seen in association with malaria may be explained, at least in part, on the basis of partial immune suppression resulting in low affinity antibody production as a consequence of the infection.

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