IMMUNOLOGY OF MALARIA

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Malaria is still a major threat to human lives in developing countries including many parts of Southeast Asia. The present control programme has failed for many reasons, including resistance of the malarial parasites to commonly used drugs, changes of the habit of the vectors, population movement and administrative failure. It has been suggested that an alternative control measure has to be integrated into the current control programme, for example the development of an effective vaccine. Production of a malaria vaccine is deemed possible after the successful introduction of the continuous *in vitro* culture of *Plasmodium falciparum* by Trager and Jensen in 1976. Though good progress has been made in the improvement of the techniques of cultivation of malarial parasites and in the methods of preparing pure merozoites free from host cell contaminants, a considerable period of time is required before a safe inexpensive malaria vaccine would become available for field operation. As a necessary step prior to the vaccine trial in man, research has to be expended to understand the nature of the host immune mechanism in the fight against malaria. This review is not meant to be an extensive search of literature on malaria immunology, but would rather give highlights in some aspects related to the defense mechanisms against malaria.

RESISTANCE TO MALARIA

Innate resistance (Non-specific resistance)

Innate resistance to malaria appears to be related to factors (inherited or otherwise) associated with red blood cells and the presence of the natural killer (NK) cells. The factors associated with red blood cells are:

(a) **Receptor:** It is generally accepted that receptor for *P. vivax* and *P. knowlesi* is associated with the Duffy blood group of Fy^a or Fy^b (Miller *et al.*, 1977a). Exposure of American blacks with Duffy group negative to the bites of vivax-infected mosquitos failed to produce malaria, whereas infection of African blacks with Duffy group possitive produced infection (Miller *et al.*, 1976). In addition, Duffy negative red blood cells were not infected *in vitro* by the Malaysian strain of *P. knowlesi* (Miller *et al.*, 1975). The mechanism of resistance of Duffy negative red blood cells to *P. knowlesi* infection has recently been elucidated (Miller *et al.*, 1979). Cytochalasin treated *P. knowlesi* merozoites attached to Duffy-negative human red blood cells even though these red blood cells were resistant to invasion by the parasite. The attachment to these red blood cells differed from susceptible red blood cells in that there was no junction formation. Therefore, the Duffy associated antigen appeared to be involved in junction formation, not in initial attachment (Miller *et al.*, 1979). Duffy negative red blood cells have normal susceptibility to *P. falciparum* (Miller *et al.*, 1977b). Only En (a") erythrocytes had a reduced susceptibility (50% of control). In addition, treatment with trypsin or neuraminidase reduced susceptibility of red blood cells to *P. falciparum* (Miller *et al.*, 1977b).

(b) **Haemoglobin type:** The sickle cell gene produces relative but not absolute resistance in African children to *P. falciparum* (Allison, 1954; 1964). This gene is believed to protect

the heterologous children from lethal falciparum malaria. *In vitro* studies showed that in high (18%) oxygen atmosphere, there was no apparent sickling of cells and the growth and multiplication of *P. falciparum* was identical in normal (AA), haemoglobin S homozygous (SS) and haemoglobin S heterozygous (SA) erythrocytes (Friedman, 1979a). Cultures under low (1-5%) oxygen showed clear inhibition of growth. The SS red cells killed and lysed most or all of the intracellular parasites, but in SA red cells the parasites were killed primarily at the large ring stage (Friedman, 1979a). Electron microscopic study showed that parasites in SS cells appeared to be disrupted by intrusions of needle like deoxy HbS aggregates: disintegration of cytoplasm and membrane followed. In SA red cells, the parasites were generally not disrupted but there was extensive vacuolization (Friedman, 1979 b).

(c) **Enzymes:** It has been documented that African children with Glucose 6 phosphate dehydrogenase (G6PD) deficiency has lower average *P. falciparum* count that in other children (Allison, 1960), and a hypothesis has been advanced that acquisition of G6PD deficiency gene would confer survival advantage. The study from the Faculty of Tropical Medicine showed that there was no significant difference between the infection rate in children 1-3 years of age with normal G6PD and G6PD deficiency, but the parasite density in G6PD deficient children was relatively less suggesting that G6PD deficiency may confer protection against falciparum malaria. *In vitro* study showed that the growth of *P. falciparum* in G6PD deficient erythrocytes was significantly less than that of the control only under the environment of high (30%) oxygen tension, and this growth inhibitory effect could be reverted in the presence of dithiothreitol (Friedman, 1978b).

It was observed that American blacks had reduced levels of pyridoxal kinase (Chern and Beutler, 1975) and Negro soldiers seldom acquired resistant falciparum malaria in Vietnam (Hall and Canfield, 1972). Furthermore, Nigerian children with severe falciparum infections had higher levels of this enzyme than uninfected matched controls (Martin *et al.*, 1977). It was proposed that low level of this enzyme might account for milder course of falciparum malaria in black patients (Neva, 1977).

The natural killer (NK) cells belong to a lymphocyte population which are capable of lysing certain tumour cell lines (Herberman *et al.*, 1975). It was shown that inbred mice genetically resistant to *P. berghei* had high NK cell activities whereas those genetically susceptible to this murine malaria had low NK cell activity (Eugui and Allison, 1979).

Measure to increase non-specific resistance

The following measures have been reported to increase non-specific resistance to malaria:-

(1) BCG: Mice previously inoculated intrat venously with BCG are strongly resistant to challenge with *P. vinckei* which produces lethal infections in unprotected mice (Clark *et al.*, 1976). BCG treated mice produced a transient, low parasitaemia when infected with *P. vinckei* but the recovered mice were completely immune to challenge with *P. vinckei* at anytime thereafter.

(2) Live *Brucella abortus*, strain 19 (Herod *et al.*, 1977).

(3) killed *Corynebacterium parvum* (Clark *et al.*, 1977).

(4) Endotoxin (Clark, 1978).

It has been speculated that stimulation by agents mentioned above would lead to stimulation of macrophages which then cause inhibition of growth and death of murine malaria by 2 main mechanisms:-

Firstly by production of type 1 interferon which in turn stimulates production of NK cells which kill the malarial parasites in red blood cells. This idea is strengthened by the recent finding of Sauvaget *et al.*, (1978) that both death rate and percentage of parasitized erythrocytes in mice infected by *P. berghei* were enhanced by injections of anti-interferon globulins. Secondly, by activation of macrophages to release faclors including the tumour necrotic factor (TNF) which inactivate rapidly dividing cells such as malarial parasites and tumour cells (Clark, I.A. 1979; pers comm.).

Acquired Resistance

Evidence has been accumulated showing that both specific malarial antibody and cell-mediated immunity play part in malarial immunity. In patients with malaria, specific antibody has been demonstrated by several serological tests including precipitation (McGregor *et al.*, 1966) indirect haemagglutination (Farshey and Kagan, 1972; Meuwissen, 1974), immunofluorescence (Ambrois Thomas, 1974). ELISA (Voller *et al.*, 1975) and merozoite invasion inhibition test (Cohen *et al.*, 1969; Mitchell *et al.*, 1976), nevertheless much of the specific antibody formed during infection do not have a protective function (Cohen *et al.*, 1977 a).

Acquired immunity in malaria is directed mainly against the asexual parasite cycle in the blood. Circulating gametocytes of *P. falciparum* are apparently unaffected by immune serum. Immunity to the erythrocytic stage of infection does not modify the exoerythrocytic development of malaria parasites in man (Garnham, 1970).

The protective effect of the antibody has been demonstrated in the passive transfer of the antibody. It was demonstrated that immune Gambian gamma globulin (IgG) given intramuscularly for 3 consecutive days in children with falciparum malaria with a total dosage of 1.2-2.5 g/child resulted in the fall in parasitaemia and progressive alleviation of clinical illness (Cohen and McGregor, 1963). The protection was of limited duration lasting no more than 12 weeks (Cohen and McGregor 1963).

Though the passive transfer study shows that malarial antibody is protective, it does not permit determination of the mechanism of action of protective antibody. The protection afforded by the antibody is likely to be mediated by 2 mechanisms, namely by direct action on the merozoites thus preventing re-entry into the red blood cells and by synergistic action with the cells.

The ability of the antibody to inhibit the merozoite invasion was first demonstrated by Cohen *et al.*, (1969) that the cyclic proliferation of *P. knowlesi* maintained *in vitro* was inhibited by immune sera from rhesus monkeys. Parasite growth was assessed by incorporation of tritiated leucine into parasite protein. Immune serum had no effect upon growth of intracellular parasites but inhibited the cycle of development that followed schizogony. This effect was species specific, dose dependant, complement independent and mediated by IgG and IgM (Mitchell *et al.*, 1976). Immune human sera were found also to inhibit invasion of merozoites of *P. falciparum* (Phillips *et al.*, 1972). The validity of the merozoite invasion inhibition as a test for immunity to malaria did not gain support from the work of Miller *et al.*, (1977c) who found only poor correlation between the merozoite invasion inhibition and functional immunity in rhesus monkey after repeated infection with *P. knowlesi*. More work is needed in order to establish conclusively that this test has correlation with immunity in patients or residents in the endemic areas after natural exposure to infected mosquito bites.

The synergistic action of malarial antibody and cells can play an important role in protective immunity. This is suggested by the finding that immune spleen cells conferred greater protection than serum when passively transferred to rats challenged with *P. berghei* (Phillips *et al.*, 1970). Similarly, the antimalarial action of passively transferred immune serum in rat was greatly diminished by previous splenectomy of the normal recipients (Golenser *et al.*, 1975). Rhesus monkeys vaccinated with merzoites in Freund's complete adjuvent and known to be immune became susceptible to *P. knowlesi* 6 weeks after splenectomy eventhough merozoite inhibitory antibody levels were unchanged (Butcher *et al.*, 1978). These findings clearly indicate that the mere presence of merozoite inhibitory antibody is insufficient for the expression of protective immunity which requires cell components from the spleen.

The nature of the cells synergistically acting in concert with the antibody has not been precisely determined. The phagocytic activity of macrophages has long been recognized in malaria and the role of specific antibody in promoting macrophage ingestion of parasites has been demonstrated *in vitro* (Brown *et al.*, 1970; Brown and Hill, 1974). The role of K cells has not been clearly demonstrated, but increased K cell activity in the spleen of mice infected with *P. chabaudi* was found 6 to 15 days but not 3 days after infection (McDonald and Phillips, 1978). Recently it was shown that lymphocytes from uninfected immune adult Gambians as well as lymphocytes from Gambians infected with *P. falciparum* were capable of killing *P. falciparum in vitro* (Brown and Smalley, 1980). However, the precise nature of the lymphocytes has not been determined.

Cell-mediated immunity

T cells are essential for protective immunity against malaria. Studies on *P. berghei* and *P. yoelii* infections in thymectomised or in congenitally athymic rodents showed that recovery from infection was thymus dependent. Rats with *P. berghei* infection had a higher parasitaemia and a higher mortality rate, and low grade resolving *P. yoelii* infections were converted into fatal infections with fulminating parasitaemia (Brown and Allison 1968; Clark and Allison, 1974). Reduced resistance was demonstrated also in rodents, which received antithymocyte serum (Spira *et al.*, 1970). Nevertheless, this evidence are not sufficient to rule out the role of the antibody because of the established fact of T and B cell co-operation in the immune responses against most soluble protein antigens. Demonstration of a protective effect afforded by the passive transfer of immune spleen cells (Stechschulte, 1969), could not be taken as evidence for cell-mediated immunity since such cells were capable of producing specific malarial antibody (Phillips, 1970). In an attempt to demonstrate definitely the role of CMI in protective immunity, variable results ensued. Brown *et al.*, (1973, 1974) treated *P. berghei*-infected rats with antithymocyte serum, the ability of lymphoid cells to transfer immunity was reduced, whereas removal of immunoglobulin-secreting B cells with an anti-Ig column did not reduce the capability of cells to transfer immunity. On the other hand, treatment of mice immune to *P. yoelii* with cyclophosphamide, which affects mainly B cells, reduced the ability of these mice to confer protection (Jayawardena *et al.*, 1975). Anti-theta serum treatment *in vitro* of immune cells did not impair the ability of these cells to transfer immunity effectively to either intact or T-cell deprived recipients (Jayawardena *et. al.*, 1975).

Vaccination against Malaria

Four approaches of immunization against malaria are known, namely vaccination with sporozoites, with parasitized erythrocytes or

parasite fractions, with merozoites and with gametocytes.

Immunization with sporozoites has been thoroughly dealt with in a recent review by Nussenweig, (1977) and only important points will be highlighted.

In rodent malaria effective protection is afforded by repeated intravenous immunization with irradiated sporozoites (Spitalny *et al.*, 1972), or through the repeated bites of infected irradiated mosquitoes (Vanderberg *et al.*, 1970). Alternative method is by giving suppressive drug treatment to the animal host after infection with viable sporozoites (Verhave *et al.*, 1974). A small number of irradiated sporozoites (1.5×10^5 parasites/mouse) administered intravenously in 3-5 doses produces protective immunity.

Sporozoite immunization elicits complete resistance, i.e. there is virtually no detectable parasite development upon sporozoite challenge. Protection is strictly stage specific, i.e. protective immunity developed is afforded only against sporozoite challenge and not against challenge with the erythrocytic stage (Nussenzweig *et al.*, 1969).

Protective sporozoite antigen is acquired at a late stage of morphogenesis, i.e. at the time of their migration to the mosquito's salivary glands, being absent in oocyst sporozoites (Vanderberg *et al.*, 1972). Part of this protection is antibody mediated, since transfer of immune serum from vaccinated animal accelerates significantly the rate of sporozoite clearance from the circulation and a considerable reduction of the number of erythrocytic stages (Nussenzweig *et al.*, 1972). This protective effect is transient in nature, since all immune serum recipients eventually developed fatal patent infection, whereas most of the actively immunized animals remained negative despite large challenge dose.

Immune serum has two effects on the sporozoites in the absence of complement, namely sporozoite neutralizing activity (SNA) and circumsporozoite precipitation (CSP) (Vanderberg *et al.*, 1969). Nevertheless, protective immunity and the presence of CSP could be dissociated. Immunization with a single does of irradiated sporozoites afforded some degree of protection at a time (7 days after immunization) when no CSP antibody is detectable in the blood (Spitalny *et al.*, 1973). Likewise was the sporozoite immunization in the splenectomised animal which resulted in some degree of protection in the absence of CSP (Spitalny *et al.*, 1976).

T cells play an important role in the sporozoite induced immunity. Immunization of T cell deficient mice with irradiated sporozoites of *P. berghei* did not confer protection, whereas immunization of B cell deficient mice conferred protection, though CSP and SNA were absent (Chen *et al.*, 1977).

The protective protein antigen with a molecular weight of 44,000 (Pb 44) has been demonstrated to be present on the surface of the *P. berghei* sporozoite. Monoclonal antibody against Pb 44 has been shown to have both CSP and SNA activity (Yoshida *et al.*, 1980).

In simian malaria the result is in essence similar to that in the rodent malaria including species specificity and extensive cross reactivity among different strains of the same species (Nussenzweig and Chen, 1974), production of CSP and SNA. Nevertheless, time required for full protection to develop takes several months (Chen, 1974).

In man two groups of investigators were successful in immunization of human volunteers with bites from irradiated infected mosquitoes (Clyde *et al.*, 1973a, b; Clyde *et al.*, 1975; Rieckmann *et al.*, 1974). Immunity is species specific lasting about 3 months for *P. falciparum* and 6 months for *P. vivax* (Clyde *et al.*, 1975).

Immunization with parasitized erythrocytes or parasite fractions could be accomplished by: (a) active infection; (b) attenuated parasites; (c) irradiated parasitized erythrocytes; (d) parasitized erythrocytes in adjuvant; and (e) parasite fractions. (WHO, 1975).

All these procedures did not afford as good protection as immunization by the merozoites, and accordingly will not be discussed here. Nevertheless, it should be mentioned that the histidine rich protein from *P. lophurae* could confer protection in the immunization schedule which does not require Freund's complete adjuvant (Kilejian, 1978).

Immunization with merozoites has been reviewed by Cohen, (1977), The results could be summarised as follows :-

Merozoites (10^8-10^9) emulsified in FCA were administered intramuscularly on 2 occasions at intervals of 2-5 weeks. The animal had a uniform survival after challenge with either homologous or heterologous strains. Such challenge sometimes produced no detectable parasitaemia or produced a transient infection (1-12 days) of low intensity (maximum parasitaemia 1.5%). After initial challenge, the immunized animal developed immunity lasting for several months, and that protection is species specific.

Successful vaccination by mature segmenters of *P. falciparum* or merozoites incorporated in Freund's complete adjuvant has been demonstrated in aortus monkey (Mitchell *et al.*, 1977; Siddiqui, 1977). The undesirable effect of Freund's complete adjuvant has been obviated by using other adjuvant, the muramyl dipeptide (MDP) (Reese, 1978) or 6-0 stearoyl MDP (Siddiqui *et al.*, 1978). Similar to *P. knowlesi* infection in the rhesus monkey, immunity is conferred not only against homologous strain but also against the heterologous strain (Siddiqui *et al.*, 1979).

Immunization with gametocytes was aimed at blocking the transmission of the gametocytes in the mosquitoes, but it has no effect in the protection of the host from the erythrocytic stage. Successful immunization has been reported in the *P. gallinaceum*/chicken system (Gwadz, 1976) and in the *P. knowlesi*/rhesus monkey system, (Gwadz and Green, 1978). It was found that single immunization with 10^7 micro-gametocytes in Freund's complete adjuvant suppressed significantly the infectivity in mosquitoes, whereas multiple intravenous administration of gametocytes and trophozoites had no suppressive effect. Immunity was afforded against both homologous and heterologous strain; Immunity was mediated solely by the anti-gamete antibody, which exerted its effect through immobilization of the micro-gametes, and the spleen was not involved in the development of the transmission blocking immunity.

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