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# MECHANISMS OF IMMUNE PROTECTION IN THE ASEXUAL BLOOD STAGE INFECTION BY *PLASMODIUM FALCIPARUM:* ANALYSIS BY *IN VITRO* AND *EX-VIVO* ASSAYS

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*Mechanisms of immune protection against the asexual blood stage infection by* Plasmodium falciparum *are reviewed. Recent studies of two independent lines of research developed at the Institut Pasteur, in humans and primate infections clearly indicate an obligatory interaction of antibodies and effector cells to express the anti-parasitic effect.*

Key words: malaria – protective immunity – IgG isotypes – protective antibodies

The immune protective effect of antibodies against blood stages malaria parasites was demonstrated first by passive transfer of immune serum in the rhesus monkey infected with *Plasmodium knowlesi,* (Coggeshall & Kumm, 1937). This was later confirmed and extended to Plasmodia of birds, rodents and primates (reviewed in Kreier & Green, 1980).

With the human parasite *Plasmodium falciparum,* the pioneer work of Cohen, Mc Gregor and Carrington (1961) showed that IgG purified from sera of gambian adults, clinically immune to *P. falciparum,* was able to considerably reduce the parasitemia when transfused to infected children from Gambia and East Africa.

Chronic malaria infection by *P. falciparum* in human adults is associated with an immune status characterized by hyper-gamaglobulinemia. However, only a minor fraction of the seric IgG corresponds to specific malaria antibodies. Non-specific antibodies include heterophyle and auto-antibodies against red blood cells and other self-antigens. Among specific malaria antibodies, again, only a minor fraction correspond to protective antibodies (reviewed in Cohen & Deans, 1988).

In endemic areas with high levels of transmission, immunity to blood infection develops slowly in children, after many clinical episodes in a two-stage process (reviewed in Mc Gregor & Wilson, 1988): (1) the acquired resistance to severe clinical symptoms, which starts to be apparent in children of three, or even two years old, becomes clearly established at five to seven years age; (2) the acquired ability to control all symptoms and parasite multiplication in the blood takes more time to develop and is fully expressed only in adults. Adults usually show very low levels of parasitemia, not associated with clinical symptoms; a situation defined as premunition (Sergent & Perrot, 1935).

The slowness with which anti-parasite mechanisms are consolidated has been taken as evidence of polymorphic antigenic structure of *P. falciparum* strains circulating in a given transmission area. This has been confirmed by serological (McBride et al., 1982) and molecular (Anders & Smythe, 1989) analytical studies. However, two important elements must be remembered (1) an effective protective immunity finally develops in all highly exposed individuals; (2) the protective immunity is mediated by antibodies which are active against parasite strains of different geographic areas (Cohen et al., 1961).

These two positive elements indicate the viability of the aim to develop vaccines against the asexual blood stages of *P. falciparum.* This depends basically, however, on a better understanding of the mechanisms involved in acquired immunity and of the reasons for their slow development in natural conditions. Many

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questions remain partially unanswered: Which antigens present genetic polymorphism and to what extent? What is the role of other immune-evasion mechanisms? What is the origin of B and T cell polyclonal activation? Is tolerance developed by children born from infected mothers? Which are the target antigens of the protective immune responses? Do protective antibodies act by blocking (neutralizing) parasite functions, or depend on complement or/and interaction with effector cells?

In the present review we will concentrate our attention on the analysis of this last question, namely: mechanisms by which antibodies are protective in *P. falciparum* infection. Recent studies in two independent lines of research developed at the Pasteur Institute (humans and primate infections) clearly indicate an obligatory interaction of antibodies and effector cells to express anti-parasitic effects.

## BLOCKING ACTION OF MALARIA ANTIBODIES

To elucidate the nature of the protective action of immune IgG, observed in passive transfer experiments, Cohen and co-workers have studied the effect of immune serum of rhesus monkey upon the *in vitro* growth of *P. knowlesi* short term cultures. An inhibitory effect was observed after the onset of schizogony that prevents the second cycle of parasite development. Inhibition was not complement dependent and was interpreted as resulting from merozoite neutralisation by antibodies, as in viral infections (Cohen & Butcher, 1969). With the development of the *in vitro* culture technique for *P. falciparum* (Trager & Jensen, 1976) studies could be performed with the human parasite, using immune sera (or purified antibodies) from naturally infected adult humans and experimentally infected primates. The experiments performed in several laboratories (Mitchel et al., 1976; Wilson & Phillips, 1976; Chulay et al., 1981; Brown et al., 1982; Wahlin et al., 1984) have essentially led to a generalization of the original interpretation of Cohen and co-workers (Cohen, 1979). As a consequence, the search of parasite antigens related to protection was concentrated on those which are targets of blocking (neutralizing) antibodies. The isolation of monoclonal antibodies able to inhibit erythrocyte invasion by merozoites, and the identification of the corresponding target antigen (Perrin et al., 1981) were then considered as the main approach for defining protective antigens. However, our studies on experimental *P. falciparum* infection in the *Saimiri* monkey and in natural infections of man led to entirely different interpretations. These will be described in the following paragraph.

## PROTECTIVE ANTIBODIES IN THE EXPERIMENTAL INFECTION OF *SAIMIRI*

The infection of the squirrel monkey (*Saimiri sciureus*) with adapted strains of *P. falciparum* produced an antibody response similar to that observed in the primary infection of humans (Gysin et al., 1982). When the infection is controled by chemotherapy, the animal develops resistance to reinfection by the homologous strain of parasite and this resistance increases with the number of parasite re-inoculations. It was shown that the resistance is mediated by antibody, since passive transfert of sera, or purified IgG, from resistant animals provide protection against the infection in naive infected monkeys (Gysin et al., 1982).

In subsequent experiments it was shown that the protective effect of passive transfer did not correlate with the *in vitro* inhibition of the parasite (Fandeur et al., 1984). Indeed, some IgG preparations obtained from immune monkeys were able to clear the infections in naive receptors after transfer of total doses of IgG lower than 5 mg. Considering the blood volume of *Saimiri* monkeys this amount would provide, at best, a seric level of 0.1 mg/ml of IgG from the donor in the recipient animal. The same IgG preparation was, however, completely devoided of inhibitory activity against the parasite in culture in concentrations 20 fold higher (2mg/ml).

The F(ab')2 fragments prepared from protective IgG lost the protective effect of the intact molecule. This indicates a need for the antibody's Fc region and suggested that the antiparasitic effect results from antibody/effector cell interactions.

## OPSONIC ANTIBODIES AND IMMUNO-PROTECTION IN *SAIMIRI* INFECTIONS

The presence of opsonic antibodies able to mediate phagocytosis of infected red blood cells and merozoites has been frequently described in many experimental malaria models, particularly in rodent malaria (reviewed in Kreier & Green, 1980; Cohen & Deans, 1988). However, with a few exceptions (Celada et al.,

1982) no relation was observed between immunoprotection and opsonic antibodies.

A clear correlation between protection and opsonic activity of anti *P. falciparum* antibodies was demonstrated by Khusmith et al. (1982) using sera, or purified IgG from chronically infected humans. The phagocytic activity, directed mainly to free merozoites, was observed with monocytes from malaria patients, or healthy subjects (Khusmith & Druilhe, 1983; Lunel et al., 1989). In the primate model, Michel et al. (1983) also observed a clear correlation between protective and opsonic activity, where the phagocytosis was directed essentially to the infected red blood cells (trophos and schizonts). These studies had a further development after the identification in the *Saimiri* monkey of two IgG populations, one able and the other unable to confer protection by passive transfer (Gysin et al., 1987). It was shown that the protective IgG population (recognized by the anti-Ig mAb 3A2/G6) is cytophilic and mediates phagocytosis of infected red blood cells (IRBC) by normal *Saimiri* monocytes, while the non-protective IgG (recognized by the mAb 3E4/H8) is non opsonizing and non cytophylic. Moreover, in competition experiments, it was observed that increasing amounts of the no opsonizing antibody progressively inhibited phagocytosis of IRBC. This observation indicates that both IgG populations recognize the same target antigens (Groux et al., 1990).

Two specific Fc receptors could be demonstrated on the surface of *Saimiri* monocytes, using mAb reagents specific to humans Fc receptors: Fc RI and Fc RIII. Both receptors are functional in phagocytosis of IRBC, since anti-receptor mAb inhibits phagocytosis (Groux et al., 1990). In humans, adhering macrophages express both Fc receptors at the surface, while monocytes normally express only the Fc RI receptor. This could be one reason for the more rapid development of protective immunity in the primate model, when compared to the natural human condition. In humans, however, the presence of opsonic antibodies has been demonstrated. High titers of phagocytic activity were observed in sera of clinically protected humans, while acutely infected europeans showed no opsonic antibodies in sera (Groux & Gysin, 1990). Opsonic antibodies belong to the cytophylic class and are represented by isotypes IgG1 and IgG3. In competition experiments, using fractionated IgGs prepared from sera of clinically immune donors, phagocytosis of IRBC by IgG1 and IgG3 antibodies is inhibited by IgG2 and IgG4 from the same donor (Groux & Gysin, 1990).

## PROTECTIVE ANTIBODIES IN HUMAN INFECTION

We have recently re-assessed the protection achieved by parasite transfer of IgG (Bouharoun-Tayoun et al., 1990). The IgG used were purified from sera of adults (19 to 25 years old) from an endemic area of Ivory Coast. The receivers were Thai children (7 to 13 years old) at the Hospital of Tropical Medicine in Bangkok, Thailand. The 8 recipient children had previously experienced *P. falciparum* infections showing a R1 resistance type to quinine treatment. The treatment with immune IgG was introduced when the parasitemia started to increase i.e., at low level of parasitemia, thus giving a security period of at least 48 hours in case of need of chemotherapy.

Passive transfer of IgG was performed with different therapeutic schedules and produced, in all cases, disappearance of fever and a drop in parasitemia of 26 to 1200 fold. In some cases, when parasitemia started to increase again, 15 days after the treatment, the administration of a second dose of the same IgG preparation produced a new important drop (500 to 1200 fold) in parasite counts, showing that no resistant population of parasites had been selected by the first treatment.

The parasite strains from the recipient individuals were isolated and adapted to cultures. The african IgG preparation showing a strong protective effect against all isolates (when used in passive transfer) had no inhibitory effect against the same parasites when analyzed in cultures. On the contrary, a stimulatory effect in parasite growth was observed when the IgG preparation was added (Bouharoun-Tayoun et al., 1990).

## ANTIBODY DEPENDENT CELLULAR INHIBITORY EFFECT

In the experiment of Ig transfer, the protective IgG pool had by itself no inhibitory effect on the parasite in culture. However, when used in conjunction with monocytes, immune IgG had a strong inhibitory effect (Khusmith & Druilhe, 1983; Bouharoun et al., 1990). Inhibition does not affect reinvasion, but intracellular development of the parasite. The toxic

effect on the parasite does not depend on direct contact between the effector and the target cell (the infected red blood cell). It is not, therefore, an effect of the ADCC type and has been called ADCI for antibody dependent cellular inhibitory effect (Lune & Druilhe, 1989). The effector cells are monocytes activated by immunocomplexes formed by cytophilic antibodies, but the "toxic" product secreted by the activated monocyte has not been identified yet.

The comparison of the antibody response to malarial antigens in protected and non-protected individuals in a hyperendemic area in West Africa has been recently performed analyzing the dominant isotypes (Bouharoun-Tayoun & Druilhe, 1992).

It was observed that in adults, showing a high level of premunition, IgG1 and IgG3 antibodies predominate in response to a series of antigens (cytophilic antibodies). In contrast, antibodies of the IgG2 class, which is non cytophilic, were dominant in individuals recovering from a first infection. In children an imbalance was observed concerning mostly IgM antibodies. Both of these IgG2 and IgM antibodies were able to block *in vitro* the ADCI promoting effect of IgG1 from immune adults. These results suggest that protective and non protective antibodies can compete for the same epitope. This imbanlanced isotype response appears as one of the important phenomena to explain the slowness in the acquisition of protective immunity in the humans communities of endemic areas.

## HUMAN PROTECTIVE ANTIBODIES ARE EFFECTIVE IN THE *SAIMIRI* INFECTION

As described above, the protective effect of cytophilic antibodies against *P. falciparum* depends on the interaction with monocytes (macrophages), both in the human natural infection and the squirrel monkey experimental infection. Since the Fc receptors of *Saimiri's* monocytes are recognized by human specific reagents it could be expected that human protective IgG would be active against the *Saimiri* infection. The african IgG protective pool, indeed showed a remarquable protective effect in the blood infection of the squirrel monkey (Gysin et al., 1992). This result opens new possibilities for the analysis of the protective role of parasite antigens using immunoabsorbants. A significant protective effect have already been obtained with antibodies eluted from immunoabsorbants prepared with a family of membrane associated schizonts antigens (Kuhn et al., 1991).

## *IN VITRO* ASSAYS AND FUNCTIONAL ANALYSIS OF PARASITE ANTIGENS

Two independent lines of evidence obtained with squirrel monkeys (Gysin & co-workers) and immune humans (Druilhe & co-workers) point out the importance of cytophilic antibodies from isotypes IgG1 and IgG3 in immune-protection against *P. falciparum* infection.

Two *in vitro* assays were defined on this basis to assess for the potential value of a vaccine candidate antigen:

In the *phagocytosis inhibition assay* (Gavoille et al., 1992) a candidate molecule (peptide, recombinant protein or purified parasite product) is added to the culture medium, in the presence of infected RBC, protective antibodies and monocytes (macrophages). The *specific inhibitory activity,* measured using the necessary controls, indicates that the molecule is a possible target of opsonic antibodies.

In the *ADCI assay,* the candidate molecule is added together with protective antibodies and monocytes to a culture and the *specific growth inhibition* is evaluated. An indirect approach can also be used, namely the analysis by ELISA, or Western blot of a preferential binding of cytophilic antibodies of IgG1 of IgG3 isotypes from sera of a protected adults.

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