

## MECHANISMS OF PROTECTIVE IMMUNITY AGAINST ASEXUAL BLOOD STAGES OF *PLASMODIUM FALCIPARUM* IN THE EXPERIMENTAL HOST *SAIMIRI*

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*In the Saimiri monkey, an experimental host for human malaria, acquired protection against Plasmodium falciparum blood stages depends on the IgG antibody populations developed. In vivo protective anti-falciparum activity of IgG antibodies is correlated with the in vitro opsonizing activity promoting phagocytosis of parasitized red blood cells. In contrast, non protective antibodies inhibit this mechanism by competing at the target level. A similar phenomenon can be observed in human infection. Anti-cytoadherent and anti-rosette antibodies developed by Saimiri and humans prevent the development of physiopathological events like cerebral malaria which can also occur in this experimental host. Furthermore, transfer to protective human anti-falciparum IgG antibodies into infected Saimiri monkeys exerts an anti parasite activity as efficient as that observed when it is transferred into acute falciparum malaria patients, making the Saimiri an even more attractive host. Studies on the role of immunocompetent cells in the protective immune response are still in their infancy, however the existence of a restricted polymorphism of MHC II class molecules in the Saimiri confers additional theoretical and practical importance to this model.*

Key words: malaria – protective immunity – IgG antibodies – *Saimiri*

A question which periodically arises when working with human malaria, is the use of experimental human malaria animal models and more particularly, the relevance of primate models due to their close phylogenetic relationship with humans. Interactions between the malaria parasite and the host with special attention to immune mechanisms have been addressed for many years. Particularly well documented is the important role played by both human and Saimiri IgG class antibodies developed in response to a *falciparum* infection (Cohen et al., 1961; Cohen & McGregor 1963; Edozien et al., 1962; Bouharoun-Tayoun et al., 1990; Groux & Gysin 1990; Gysin et al. 1982, 1987; Michel et al., 1983; Fandeur et al., 1984; Croux et al., 1990; Gysin et al., 1992). *Saimiri* and *Aotus*, both susceptible new world monkeys sustain the development of asexual blood stages *falciparum* infections (Geiman & Meagher 1967; Rossan et al., 1972; Schmidt, 1978; Campbell et al., 1980; Gysin et al., 1980; Gysin & Fandeur, 1983) and are recommended experimental hosts for malaria related studies (Memorandum W.H.O., 1988). These two monkeys species have been and probably will still be, used for the identification and evaluation of presumed *falciparum* vaccine candidates

(Gysin et al., 1982a; Perrin et al., 1984; Dubois et al., 1984; Jendoubi et al., 1984; Collins, 1986, 1988, 1991; Patarroyo et al., 1987; Postal et al., 1988). Basic aspects related *stricto sensu*, to cell mediated mechanisms and regulations had until recently never been addressed for a primate hosts and are still fragmentary. (Garraud et al., 1989, 1990, 1991). The reasons that we discuss malarial immunological aspects solely for the squirrel monkey have been determined by the continuous disponibility in our breeding colony of *Saimiri sciureus* of karyotype 14-7 and Guiana phenotype. As far as we know, similar immunological aspects had never been explored in *Aotus* monkeys. Additionally, experiences in *Aotus* monkeys can hardly be conducted repetitively on a homogenous basis with regard to karyo- and phenotypes and for some physiopathological aspects, like cerebral malaria, it is not the appropriate host (Aikawa et al., 1990).

### DESTRUCTIVE ANTI-FALCIPARUM ANTIBODY DEPENDENT PROTECTION

Malaria related immunological studies in the *Saimiri*, have focused for instance almost exclusively on the antibody mediated immune

response. Antibodies developed by this host in response to a *falciparum* infection have been analyzed by evaluating almost exclusively, their capacity for *in vivo* and *in vitro* physical eliminating of asexual blood stage parasites. (Gysin et al., 1982a, b; Michel et al., 1983; Fandeur et al., 1984; Gysin et al., 1987; Bouharoun-Tayoun et al., 1990; Groux et al., 1990). Also diverse aspects of interactions between antibodies and physiopathological events generated by *falciparum*-infection, had been neglected in primate hosts and only recently have been reconsidered in the global anti-malarial strategy.

We started some 13 years ago to use squirrel monkeys of karyotype 14-7 and Guiana phenotype due to circumstantial reasons and today as we will see, we have the conviction that this host is different in some regards from the *Aotus* monkey.

Squirrel monkeys were used by our laboratory mainly for the evaluation of vaccine candidates, eliciting interest in the understanding of the possible immune mechanisms developed by this experimental host for the build up of a protective immune response after experiencing a drug controlled *falciparum* infection. It is clear that the different immune mechanisms responsible for the acquired protection against *falciparum* are still not fully understood. However, several data suggest that IgG class antibodies are playing a cardinal role in immune protection developed by both humans and *Saimiri* in response to a *falciparum* infection. (Cohen et al., 1961, 1963; Edozien et al., 1962; Bouharoun-Tayoun et al., 1990; Groux & Gysin, 1990; Gysin et al., 1982a, b; Michel et al., 1983; Gysin et al., 1987; Groux et al., 1990) Experiences of passive transfer with intact IgG (Gysin et al., 1982, 1987), but not (Fab'2) anti-*falciparum* antibodies (Fandeur et al., 1984) showed that it was possible to confer protection to naive and non immune recipient monkeys. Moreover, it was shown that antibodies inhibitory for the *in vitro* the development of the parasite are not obligatorily protective antibodies *in vivo* (Fandeur et al., 1984).

The development of mouse monoclonal antibodies specific for *Saimiri* Ig allowed by passive transfer experiments into naive recipient monkeys, the differentiation and the functional characterization of protective anti-*falciparum* antibodies (Gysin et al., 1987). Protective antibodies were shown to be species

specific, but not strain restricted and consistently associated with an *in vitro* opsonizing activity via the gFcRIII receptor on circulating monocytes (Groux et al., 1990a). In contrast, non protective anti-*falciparum* antibodies are not opsonizing and compete at the target *in vitro* with the protective opsonizing antibodies level when coincubated with parasitized erythrocytes (Groux et al., 1990a). Thus, it follows that protection in this host not only depends on the recognition of target epitopes exposed on the surface of infected erythrocytes, but also on the quantitative and qualitative fluctuation of the two antibody populations (Groux et al., 1990a).

Recently, Bouharoun-Tayoun et al. (1990) presented data indicating that transfer of anti-*falciparum* IgG extracts from African people having a strong premunition against the parasite are capable of diminishing significantly the parasitaemia in Thai patients suffering from acute *falciparum* malaria. Antibodies from the same human IgG preparation are also capable of diminishing a 13-25% parasitaemia of the Palo-Alto (FUP-1) strain to 3,7-0,01% in splenectomized squirrel monkeys, following 5 consecutive daily intravenous injections of 12,5 mg IgG. For almost 13 years the Palo-Alto (FUP-1) strain has been exclusively maintained by transfer of asexual blood stages into both splenectomized and intact *Saimiri* monkeys. This has apparently not altered the expression of essential target epitopes recognized by the protective human antibodies. These observations suggest that epitopes recognized by the protective antibodies expressed on wild strains in Africa are conserved between the wild strains in Thailand and the monkey adapted Palo-Alto (FUP-1). Furthermore, the expression of surface antigens on parasitized erythrocytes which are modulated by both antibodies and the spleen are apparently not essential for the antibody dependent protection. In this context, the fact that *falciparum* strains as IPC/RAY (from French Guiana) and IPC/TARCY (a strain from the Republic Central Africa also adapted to the squirrel monkey) elicit cross-protective antibodies, independent of the strain which initiated the antibody response and whether the animals were intact or splenectomized enforce the existence of target epitopes of limited polymorphism. Moreover, earlier data which compared the antibody kinetics in response to a primo drug controlled *falciparum* infection in intact versus splenectomized squirrel monkeys, revealed to quantitative or qualitative differ-



ences (Gysin et al., 1982b). This is in agreement with today results.

The observation that human IgG are functionally capable of protecting a squirrel monkeys supposes that the involved Fc structure of the IgG molecule and a given immunocompetent cell have to be identical, or extremely similar in the two hosts. Indeed, opsonization *via* the fGcRIII receptor on human phagocytes follows an identical schema as that described for the squirrel monkey model (Groux & Gysin, 1990).

In human, IgG 1 and 3 are opsonizing antibodies and compete *in vitro* at the target level with non opsonizing IgG2 and 4 antibodies (Groux & Gysin, 1990; Groux et al., 1990).

In light of these overall data, it can be assumed that the tandem of squirrel monkey and *falciparum* (laboratory strains) is a representative model for the identification and immunological evaluation of potential *falciparum* vaccine candidates.

#### ANTIBODIES PREVENT PHYSIOPATHOLOGICAL EVENTS GENERATED BY A *FALCIPARUM* INFECTION

The main objective in designing an anti-axsexual blood stage malaria vaccine is based on the use of target epitopes sufficiently well recognized by the immune system to elicit the development of a cell- and antibody mediated immune response capable of eliminating the parasite. If a vaccine only achieves an efficient, but not sterile immunity and there remains a low level parasitaemia this could have potential physiopathological affects which could include cerebral malaria (Del Giudice et al., 1988).

For cerebral malaria, which is the most severe form of a *falciparum* infection, two main mechanisms have been incriminated: (i) the phenomenon of cytoadherence which is thought to be responsible for sequestration of mature blood stage parasite in cerebral microvessels (Oquendo et al., 1989; Howard et al., 1990; Udeinya, 1990; Aikawa et al., 1990; Pongponran et al., 1991) and (ii) by the same blood stages the spontaneous formation of rosettes with normal erythrocytes of the host engendering probably together with the sequestration a cascade of biochemical events responsible for the gravity of cerebral malaria and its possible

fatal outcome (Udomsangpetch et al., 1989; Handunetti et al., 1989; Carlson, et al., 1990; Wahlgren et al., 1991).

In the squirrel monkey, strains such as Palo-Alto (FUP-1) and a clone, MHB11, derived thereof, together with isolate IPC/RAY (Gysin et al., 1980; Hommel et al., 1983; Groux et al., 1990) can provoke cerebral malaria in some animals (Gysin et al. 1992). Both sequestration and spontaneous formation of rosettes occur in this host. The receptors/ligands on the surface of parasitized erythrocytes involved in the phenomenon of rosetting seem to be very similar, if not identical, to those described in the human system (Carlson et al., 1990).

Antibodies may play an important preventive role against mechanisms that promote physiopathological events. In monkeys partially protection against *falciparum* can occur as shown by the occurrence of self-cured transient parasitaemia and this partial protection can be conferred to recipient monkeys by passive IgG transfer. These antibodies can both inhibit cytoadherence of infected erythrocytes to C32 and transfected COS cells and dissociate rosettes (Gysin et al., 1992). The sustain the *in vivo* observations and demonstrate once more, the preserved expression of functionally distinct, but as yet not identified receptors on parasitized *Saimiri* erythrocytes and the conserved ligands on normal erythrocytes involved in the rosetting phenomenon.

Cerebral malaria as it is commonly defined does not exist in the *Aotus* monkey (Aikawa et al., 1990) and the capacity of spontaneous rosetting by schizonts with normal *Aotus* erythrocytes seems also to absent when using *Saimiri* adapted or human rosetting positive phenotype isolates. Data also suggest that in squirrel monkeys the build up of anti-physiopathological event antibodies in response to a *primo* drug controlled *falciparum*-infection, are preceding those developed by the host for the physical elimination of the parasite.

Immunoregulation of this very efficient antibody dependent protective schema against *falciparum* in the *Saimiri* host implies of course immunocompetent cells. Cellular immunology for this host and primates in general, is still in its infancy. One of the most striking recent observations on this subject is the absence of

mixed lymphocyte reaction (MLR) between *Saimiri* monkeys of karyotype 14-7 and Guiana phenotype, which suggests a limited MHC class II polymorphism (Garraud et al., 1990). Passive PMBC transfer may be performed between animals of the same background without an allogeneic reaction, opening interesting opportunities for the understanding of immune cell-mediated mechanisms developed by this host against a malaria parasite.

#### DISCUSSION

If immune mechanisms participating in the destruction of *P. falciparum* blood stages and the suppression of associated physiopathological events are not fully understood, it seems however likely, that IgG antibodies play a determinant role in acquired protection. In the *Saimiri* this antibody dependent protection is consistently associated with an *in vitro* opsonizing activity via the gFcRIII receptor on circulating monocytes and probably also resident phagocytes. Non protective and non opsonizing anti-*falciparum* antibodies compete with the protective ones at the target level. The quantitative and qualitative balance between the two antibody populations is determinant for the protection.

The capacity of protective African human anti-*falciparum* IgG antibodies to exercise also a anti-parasitic effect for the *Saimiri* long term-adapted Palo-Alto (FUP-1) strain, consolidates the relevance of this experimental host for studies aimed at the identification and immunological evaluation of *falciparum* vaccine candidates. *In vitro* the opsonizing activity of protective antibodies are species specific, but not strain restricted and the observation is in agreement with the cross-acting protection generated *in vivo* by different *falciparum* strains, but not different malaria species, adapted to the *Saimiri* monkey.

The observation that *Saimiri*, in contrast to *Aotus* monkeys are also prone to cerebral malaria makes this host even suitable for studying physiopathological events promoted by *falciparum* infection.

The capacity of *Saimiri* to develop antibodies capable *in vitro*, of dissociating rosettes as well as cytoadherence of mature parasites opens additional opportunities for immunological and pharmacological malaria related studies in this host.

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