REVIEW

IMMUNITY TO THE SIMIAN MALARIAS IN THEIR NATURAL HOSTS - SOME PROBLEMS AWAITING INVESTIGATION

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The lack of progress in worldwide malaria control in the last fifteen years has prompted a considerable expansion in research, particularly in the immunology of malaria, with the ultimate goal being the development of a malaria vaccine. For obvious reasons, the major focus has been on human malaria, especially *Plasmodium falciparum,* but the practical limitations of working on the immunology of human parasites has encouraged research on animal models and the rodent malarias have played a prominent role.

Work on the immunology of simian malaria, most especially of the natural host-parasite combinations, has been limited, for a variety of reasons. One practical advantage of working on *P. falciparum* is the comparative ease with which erythrocytic stages can be maintained *in vitro,* and for which there is always a readily available supply of human red cells. In contrast, a commonly used simian malaria, *P. knowlesi,* has a 24 hour life cycle and a synchronous, rapid growth rate, both of which put considerable stress on *in vitro* maintenance systems (Butcher, 1979; Wickham *et al,* 1980). Apart from the outbred nature of the host animals, which restricts the types of experiments possible, considerations such as the expense of keeping monkeys in captivity and the pressure to reduce primate use have restrained research. This is regretable in some respects because the complexity of immune reponses to malaria makes it imperative that no source of information, particularly in primates, is neglected. Furthermore, *P. falciparum* is more closely related to avian malarias than to either primate or rodent malarias, unlike *P. vivax* which is related to Asian primate malarias (Waters *et al,* 1990).

A feature of simian malarias that makes them unusually interesting is the relative lack of serious pathology apparently experienced by their hosts (Coatney *et al,* 1971; Garnham, 1966), although this conclusion is subject to certain limitations (see below). A series of studies by Desowitz and co-workers in the 1960s was done on *P. coatneyi* (Desowitz *et al,* 1967) and *P. inui* (Desowitz *et al,* 1968a) using "traditional" measurements such as liver enzyme function etc, but no equivalent study has been made with the more recently described immunopathological features of malaria in mind (see below). The relatively mild illness suffered by simian hosts is in contrast to the severe illness caused by human malarias, and to a lesser extent rodent malarias. In general, simian hosts also experience lower parasite rates (see below) than humans and rodents.

It has been suggested by one author (Wheatley, 1980) that malaria may have contributed to the speciation of macaques in Southeast Asia, a point of general biological interest that gives added incentive for studying these malarias in their natural hosts. Furthermore, although the difference between the rhesus and other macaques in response to malaria is striking (see below), hardly any *comparative* study of the mechanisms of resistance to malaria in these animals has been performed.

This review will be largely restricted to the Asian monkeys and their natural parasites and only brief mention will made of experimental data from South American monkeys infected with human malaria or African monkeys given Asian parasites.

The main hosts and parasites are summarized in Table 1. There is a marked lack of information on the distribution of the various species of malaria between different hosts. Obviously, most observations on wild caught animals leave many questions unanswered, especially whether young animals are more seriously affected than mature adults.

Table 1

Parasitemias and mortality in different hosts.

Parasite	Nat Host[1]	Peak para[2]	Mort[3]	Other Hosts[4]
P. cynomolgi	M. fasc	$< 5 \times 10^4$	0	M. nem; M. rad M. cycl; M. sin
P. fieldi	M. nem	3×10^2	0	M. fasc; M. rad
P. coatneyi	M. fasc	8×10^2	0	M. nem
	M. spec	5×10^3	0	
P. fragile	M. fasc	1×10^2	0	M. rad; M. sin
P. knowlesi	M. fasc	5×10^4	0[5]	M. nem

1 = Natural host species: M. spec = *M. speciosa;* M. nem = *M. nemestrina;* M. fasc = *M. fascicularis* M. rad = *M. radiata;* M. cyclo = *M. cyclopsis;*
2 = Peak para = peak parasitemia - parasites per µl blood.
3 = Mort = mortality
4 = Other hosts = other species of host into which the parasite will go.
5 = Some sub-species (?) of this monkey may exhibit mortality (see Schmidt *et al,* 1977).
Data based mainly on Coatney *et al* (1971).

Table 2

Simian malarias in the Rhesus monkey - *M. mulatta.*

Parasite	Peak parasitemia[1]	Mortality
P. cynomolgi	3×10^5	0
P. fieldi	7×10^3	0
P. coatneyi	1×10^5	< 40%
P. fragile	2×10^5	< 30%
P. knowlesi	$< 5 \times 10^6$	< 100%

1 = Peak parasitemia = parasites per µl blood
Data based mainly on Coatney *et al* (1971).

Information on the main biological features of these malarias is outlined in the books by Coatney *et al* (1971) and Garnham (1966). Since these volumes were written, it has been demonstrated that *P. cynomolgi,* like *P. vivax,* has a hypnozoite stage (Bray and Garnham, 1982).

Parasitemias and mortality in different simian host-parasite combinations.

A significant feature of the information summarized in Table 1 and 2 is the comparatively high parasitemias and mortality experienced by rhesus monkeys (*Macaca mulatta)* (Table 2). This presumably reflects their lack of immune responsiveness to malaria and is in marked contrast to other species of macaques, most particularly *M. fascicularis* (see below), with which it will interbreed (Wheatly, 1980). It would be very interesting to know the functional basis of these differences in immunity.

Unfortunately, we have little data on the possible differences between adults and juveniles in their responses to parasites for the various malarias. Information on parasitemias etc with respect to sub-species of the different hosts is also limited, although it is thought that *M. fascicularis* from different areas exhibits quite marked variation in susceptibility to *P. knowlesi* (Schmidt *et al,* 1977).

It is important to point out that the rhesus monkey has been used for experimental purposes more frequently than other species precisely because it has little naturally occurring malaria and therefore lacks any acquired immunity. Also, in most laboratories it is more convenient to maintain parasites by blood rather than mosquito passage and blood passage strains may be considerably more virulent than mosquito passaged parasites. Even a rhesus monkey may survive mosquito passaged strains of *P. knowlesi,* whereas blood passage usually leads to infections with extremely high parasitemias and higher mortality rates (Richards *et al,* 1977).

As with the other malarias, an individual host may have several concomitant malaria infections. *M. fascicularis* is host to at least four species (Table 1). The limited information available (reviewed in Richie, 1988) indicates the pattern of infection follows the classic course of a wave of dominant parasitemia by one parasite being followed by a second parasite. Interestingly, gametocyte infectivity for mosquitos for both dominant and secondary parasite may occur at the same time. The mechanisms underlying these alternating waves of parasites are unknown.

Red cell factors in simian hosts

The ease with which simian malarias are transfered from one species of host to another (Coatney *et al,* 1971; Garnham, 1966) indicates there is little restriction of merozoite invasion by red cell surface glycoproteins. This view is reinforced by infection of human and *Aotus* red cells with *P. knowlesi in vitro* (Butcher *et al,* 1973), the use of simian malarias in malarial therapy of neurosyphyllis (Cuica *et al,* 1934), occasional zoonotic or laboratory-acquired infections (Coatney *et al,* 1971) and the susceptibility of African monkeys to these parasites (Coatney *et al,* 1971; Garnham, 1966). The red cell receptor for *P. knowlesi* appears to involve the Duffy antigen (Mason *et al,* 1977) but information on the receptor requirements of merozoites of other parasite species is lacking. In limited studies on *P. knowlesi, in vitro* growth was as efficient in *M. fascicularis* red cells as in *M. mulatta* (Butcher *et al*,1973), but there were marked differences in parasitemias *in vivo,* suggesting that for this parasite, as probably for others, immune mechanisms are primarily responsible for the control of parasite replication in the natural hosts. However, this does not necessarily exclude a possible secondary contribution from innate red cell factors equivalent to those described in humans - the hemaglobinopathies, enzyme deficiencies, membrane defects, etc.

Immune mechanisms

With the exception of *P. knowlesi* in the rhesus and in *M. fascicularis* monkeys of Philippine origin (Schmidt *et al,* 1977), macaques control their malaria parasites with relatively little trouble and parasitemias rise only to low levels (Table 1).

In line with other parasite models, complete immunity normally requires an intact spleen, but again the rhesus proves to be an oddity. Its own naturally occurring parasite is *P. inui.* Once immunity is established to this parasite, the host remains chronically infected but following splenectomy it is completely cleared (Wyler *et al,*1977), suggesting the spleen can have an immunosuppressive as well as a protective role.

If the spleen is removed from rhesus vaccinated against *P. knowlesi* they will survive a challenge given immediately after the splenectomy, but a challenge a month later is likely to prove fatal (Butcher *et al,* 1978). It was concluded from these experiments that while the spleen may be a source of effector cells it is not the main site of parasite killing. This is consistent with the fact that splenectomized monkeys are often able to control their parasites, albeit at higher parasitemias than in intact animals (Coatney *et al,* 1971).

The hypergammaglobulinemia normally associated with chronic malaria has been reported for simian malarias (Desowitz *et al,* 1968b) but infections of *P. knowlesi* in the rhesus monkey were an exception to this rule (Butcher, 1970). In the light of ideas that multiple cross-reacting epitopes are responsible for hypergammaglobulinemia (Anders and Smythe, 1989), the absence of this in the rhesus is interesting but problemmatical.

Extensive work on *P. knowlesi* in the rhesus by Coggeshall and co-workers in the 1930s (summarized in Coggeshall, 1943) demonstrated clearly for the first time in malaria that antibody was protective when passively transferred to non-immune hosts in sufficiently large volumes or at sufficiently high titer. Later work demonstrated a role for IgG (Butcher *et al,* 1970) although it

seemed to be less effective than the whole serum used by Coggeshall (1943). The classic experiments of Cohen and MacGregor in human malaria (Cohen *et al,* 1961) followed on from the work of Coggeshall and his co-workers.

In a short series of experiments, employing single cycles of parasite replication *in vitro,* it was shown that sera from non-immune (UK born) *M. fascicularis* inhibited growth of *P. knowlesi* (a natural parasite of this monkey) as early as 10 days after a first infection (Cohen *et al,* 1977). Significantly, this inhibition was common to several antigenic variants (Cohen *et al,* 1977). On the other hand sera from immune animals lacked this inhibitory activity (Butcher, unpublished data). Sera from highly susceptible *M. mulatta* were unable to inhibit parasites until day 30, and then only in a variant specific manner, almost certainly mediated by antibody (Butcher and Cohen, 1972). The natural host may have an ability to produce a non-variant specific anti-merozoite antibody early in infection or, alternatively, generates a non-specific, non-antibody factor, perhaps equivalent to that observed in mice at high parasitemias (Butcher and Clark, 1990) but which subsequently wanes. In *M. fascicularis,* the inhibitory factor is generated without the concomitant illness observed in mice. Nevertheless, these protective mechanisms can be overwhelmed by injections of very large doses of *P. knowlesi* and the natural host dies like a rhesus with very high parasitemia (Butcher, unpublished data).

In culture experiments with *P. knowlesi,* no inhibition of growth of the intraerythrocytic parasites by immune sera or IgG could be detected (Butcher, 1989). In contrast, trophozoites and schizonts of *P. fragile* were said to be killed by immune IgG from tocque monkeys *(M. sinica)* (Handunnetti *et al,* 1987). It is difficult to reconcile these results and to explain the findings with the tocque monkey sera without invoking some parasite-induced increase in permeability of the tocque monkey red cells or possibly prior damage in the spleen. There are no reports of other parasites, such as *P. falciparum* being damaged by antibody in the absence of leukocytes so the *P. fragile* result is unusual and surprising - it would appear to be strongly to the disadvantage of the parasite to be so vulnerable for much of its development. A further curious finding of Handunnetti *et al* (1987) was that antigenic variants appeared in sequence, again an observation not reported with any other malaria. However, in spite of the observed antigenic variation, monkeys were able to control their infections. The significance of these data on *P. fragile* remains obscure. *In vitro* inhibition of *P. fragile* has also been observed by sera from immune rhesus monkeys, but no information on parasite stage specificity was given (Guo *et al,* 1984). Variant-specific opsonization of *P. knowlesi* - infected red cells was observed in immunized rhesus (Brown *et al,* 1970).

Intraerythrocytic parasite death with the appearance of morphologically damaged parasites (crisis forms) is not uncommonly seen in the natural host of *P. knowlesi* (Butcher, unpublished data) and suggests some role for non-specific immunity. On the other hand, although antigenic variation can be shown to occur *in vivo* in this host, as with *P. fragile,* there is no suggestion that new variants evade the host's dominance of the duel between host and parasite - major recrudescences of new variants do not appear.

Similarly, in *P. knowlesi*-vaccinated animals concomitantly infected with a second parasite *(P. cynomolgi bastanellii),* crisis forms of both species of parasite can be present at the same time following a large parasite challenge with *P. knowlesi,* but a species specific immunity to the other parasite still had to develop (Butcher *et al,* 1978). Indeed, there is only very limited cross-immunity between the different species of simian malaria (Voller and Rossan, 1969), as one might expect in parasites inhabiting common hosts (Richie, 1988). Although spleen cells from infected *M. fascicularis* were able to inhibit parasites *in vitro* (Langhorne *et al,* 1977), indicating possible involvement of non-antibody mediated mechanisms, an interaction of both specific and non-specific mechanisms is presumably required to maintain host resistance. We lack any detailed information on these various mechanisms.

There is hardly any information on cell-mediated immunity to malaria in simian hosts although delayed hypersensitivity (DH) to parasite extracts, frequently a feature of rodent malaria, is absent in rhesus monkeys rendered immune to *P. knowlesi* by infection (Butcher *et al,* 1978). Results from the *in vitro* equivalent test for DH with *P. falciparum* antigen - the monocyte procoagulant assay, suggests that humans are similar to monkeys in

this respect (Butcher, 1990). DH does become apparent in rhesus monkeys, however, after immunization with *P. knowlesi* in Freund's Complete Adjuvant (Phillips *et al,* 1970; Butcher *et al,* 1978) and correlates with thymidine incorporation of spleen and lymph node cells (Phillips *et al,* 1970).

In a limited series of experiments, injection of recombinant human interferon gamma had an inhibitory effect on the development of exoerythrocytic parasites of *P. cynomolgi,* but no effect on the erythrocytic stages (Maheshwari *et al,* 1986).

There may be a role for non-antibody mediated resistance in the blood feeds taken up by mosquitos in the transmission of malaria in some simians (Mendis *et al,* 1990), but the precise nature of these factors remains to be determined.

Pathology

Unlike the acute and feverish episodes characteristic of even low parasitemias in infections in human malarias, initial infections in monkeys seem to be well tolerated (Coatney *et al,* 1971; Garnham, 1966). This suggests that fever-inducing cytokines such as TNF and IL-1 (Clark *et al,* 1989) are either not produced in the initial stages of infection or that these responses are in some way negated. In contrast, even minor parasitemias of *P. vivax* in humans, for example, can induce high levels of TNF (Butcher *et al,* 1990). Clark has pointed out that monkeys, like mice but unlike humans, are relatively resistant to endotoxin (Clark, 1978). As both human and rodent malarias are now known to have endotoxin-like molecules (Taverne *et al,* 1990), this unresponsiveness may reflect macrophage insensitivity to such molecules. However, in the apparent absence of the marked non-specific macrophage activation that in mice and humans probably contributes to illness and the control of parasite replication [through a variety of mechanisms (Butcher, (1989)], parasite levels in simians are nevertheless prevented from rising to high levels.

Rhesus monkeys immunized inadequately by the use of parasites in Freund's incomplete adjuvant exhibited signs of illness in the absence of high parasite levels following challenge with *P. knowlesi* (Butcher *et al,* 1978); interestingly, these symptoms were associated with the appearance of crisis form parasites in the blood, suggestive evidence for the involvement of cell-mediated responses (Clark *et al,* 1989). Crisis forms are rarely seen in human malaria infections but sera from some cerebral malaria patients may induce their formation *in vitro* (Butcher *et al,* 1985). As already noted (see above) in the natural host of *P. knowlesi* crisis forms may be seen in the absence of any illness of the host.

Modern interest in the role of sequestered *P. falciparum* parasites in the causation of cerebral malaria has led to research on possible equivalents in simian malarias. *P. coatneyi* sequesters in the brain, but interestingly, this appears to be relatively harmless to the host animals (Aikawa, personal communication). This suggests that sequestration alone is not enough to cause cerebral malaria and leaves open a significant role for other factors such as cytokines, although precisely how these influence events is a matter of considerable debate. Clark has argued that cytokines, such as TNF and IL-1, may have a direct effect on neurological mechanisms (Clark *et al,* 1989). A useful contribution to answering questions of this nature could, perhaps, come from more research on simian models.

Conclusion

It is clear from this brief review that our knowledge of the immunology of simian malaria is very limited and is strongly biased towards experimental infections of *P. knowlesi* in the rhesus, an extremely susceptible host which can be infected with as few as 10 parasites. Experimental work on immune mechanisms in more natural host-parasite combinations has been very limited. An intriguing question conerns the absence of immunopathology. How has selection operated to establish such a satisfactory balance between host and parasite, especially as there is a suggestion that certain human populations have evolved an almost as satisfactory balance with their malaria parasites as the Asian macaques (Rosenburg *et al,* 1990)? Futhermore, how do several different species of parasite manage to coexist in the same host and at low levels? Our inability to provide answers to these question indicates how much remains to be discovered about malaria.

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