Malaria parasite interactions with the human host

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Received : 29-08-03
Review completed : 02-10-03
Accepted : 30-11-03
PubMed ID : 15047996
J Postgrad Med 2004;50:30-34

ABSTRACT

The interaction between the malaria parasite and the human host involves a number of interactions that result in the parasite evading the human immune system. Since the stages of the malaria lifecycle are complex, this allows the use of various immune evasion strategies by the malaria parasite and has major implications in the development of a vaccine for malaria endemic areas. The present review highlights key host:parasite interactions. Plasmodia puts selection pressure on human gene frequencies, and studies into host genetic factors such as the Duffy blood group and sickle cell anaemia offer insight into the host- parasite relationship. In addition, parasite interactions with the different effector arms of the immune system can result in altered peptide ligand (APL) antagonism which alters the immune response from a pro- to an anti-inflammatory T cell response. Recent insights into the interaction between professional antigen presenting cells, dendritic cells (DCs), and malaria parasites is discussed in detail.

KEY WORDS: Malaria, genetics, dendritic cells, altered peptide ligand antagonism, vaccines

The malaria parasite is a prevalent human pathogen with at least 300 million acute cases of malaria each year globally and more than a million deaths. A deeper understanding of the nature and regulation of protective immune mechanisms against this parasite will facilitate the development of much needed vaccines. Persistence of the asexual erythrocytic (blood) stages following natural recovery from the acute phase of the infection is common in malaria infections.[1] An important reason for the persistence of malaria infections within populations is the ability of the parasites to undergo repeated antigenic variation.[2,3] However, during the acute, and to a lesser extent the chronic phase of blood stage infection, there is also significant suppression of the immune response to heterologous antigens,[4-6] as well as general immunosuppression, such as impairment of antigen presenting cell (APC) function.[7] There is evidence of the suppression and evasion of parasite-specific responses during acute malaria: mechanisms which include clonal antigenic variation and altered peptide ligand (APL) antagonism.[3] Despite extensive efforts in vaccine development and design, there is still no effective vaccine available for use in malaria endemic areas. Rodent models have largely facilitated the understanding of the effects of blood stage malaria infection on the development of immune responses. Considered collectively, studies to date indicate that generating protective immunity via vaccination is a realistic goal, but also pose questions about host: parasite immune inter-phase.

The malaria parasite has a complex lifecycle, involving humans and Anopheles mosquitoes (Figure 1). The human stages develop after an infected female Anopheles mosquito injects *sporozoites* (10 to 100 during the blood meal) into the human.[8,9] These migrate to the liver (within 30 minutes), where those not blocked by antibodies penetrate into the liver, and begin dividing within hepatocytes.[10] During this time, cytotoxic T cells (CTLs) and IFN gamma-producing cells can promote elimination of intracellular parasites. This replication lasts from 2-10 days, and merozoites develop within hepatocytes. These cells then rupture, and merozoites enter the blood and invade erythrocytes. Each hepatocyte releases tens of thousands of merozoites.[11] These events comprise the pre-erythrocytic (liver) stage of malaria. After merozoites have invaded host erythrocytes they mature and continue to divide asexually to become schizonts, rupturing 48 hours later.[12] Each intraerythrocytic expansion-burst-infection cycle results in 20-30 new

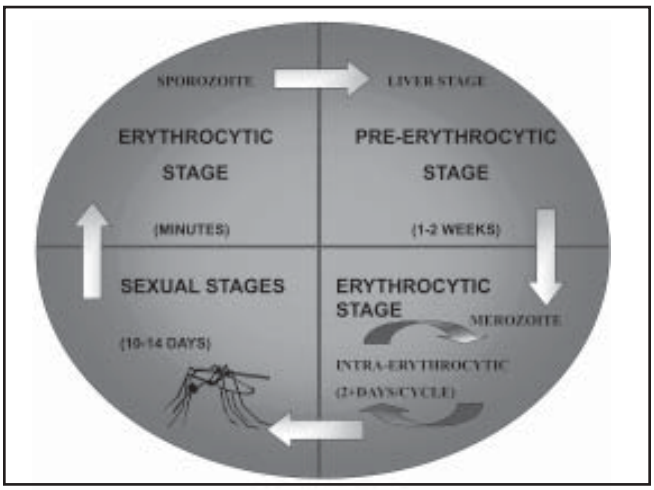

Figure 1: The timing and kinetics of the liver, blood and sexual stages of the human malaria parasite *P. falciparum*

merozoites.[12] Murine strains of malaria complete pre and intraerythrocytic development faster than human strains, and produce less merozoites per schizont. Rodent malaria models indicate blood-stage protection can be antibody-mediated, but IFN gamma production and T cell proliferation in response to blood-stage antigens are also associated with protection.[13] A sexual form of blood stage parasite is responsible for the infection of the salivary glands of new mosquito vectors.

Parasite / Host Genetic Interactions

In addition to host-driven genetic selection acting on malaria parasite populations, *Plasmodium* exerts selection pressure on human gene frequencies. Where malaria is endemic, it is reasonable to assume that infection contributes positively to the allele frequency of variants associated with protection. The first such alleles identified affect the RBC structure or function. The gene conferring the Duffy blood group is the most striking example; people of this blood type are completely immune to *Plasmodium vivax* blood stage infection, as they lack the relevant receptor on RBC membranes.[14] Sickle cell anaemia allele, severely deleterious in the homozygote, is associated with malaria protection in the heterozygote, possibly due to abnormal RBC shape. Along with RBC-related genes, various genes affecting components of the immune system have been associated with protection from malaria. Inducible nitric oxide synthase 2 (iNOS2) is an enzyme that modulates nitric oxide (NO) production, ultimately affecting malarial immunity. Protective variant alleles associated with high NO production have been identified in multiple African populations.[15,16] Fc gamma receptor II (Fc gamma RII) facilitates monocyte binding to the IgG subclasses. A polymorphism restricting the affinity of Fc gamma RII to IgG1 and IgG3 has been investigated in Western Kenyans, and correlates with *P. falciparum* immunity.[17] Severe malaria, which can lead to neurological sequelae and death may involve CD36-mediated sequestration of parasitised erythrocytes.[18] Heterozygosity for an allelic variant of CD36 is associated with protection from severe malaria in Africans. Importantly, MHC Class I B53 (MHC-B53), and II DQB1*0501 and DRB1*1302 alleles are associated with protective clinical responses in African populations.[19,20] MHC-B53 is very common in African populations and presumably, this association has contributed to the high frequency observed in these populations.

Parasite Interaction
Altered Peptide Ligand (APL) Antagonism

Natural immunity to malaria takes years to acquire, at least partly due to a very effective immune evasion strategy mediated by naturally occurring variants of the same antigenic epitopes, capable of inhibiting memory T cells (Figure 2). This so-called 'altered peptide ligand' (APL) antagonism affects specific cell lysis and lymphocyte proliferation, as well as cytokine production. Naturally exposed individuals have cytotoxic T lymphocytes (CTLs) specific for pre-erythrocytic stage antigens, and CD4 T cells as well as antibodies specific for both erythrocytic and pre-erythrocytic stage antigens.[11,20-24] The most abundant protein on the sporozoite coat is the circumsporozoite (CS) protein, which participates in the parasites binding to the liver cells. Antibodies against CS protein can block liver cell infection *in vivo*[25,26] and *in vitro*.[27] CTLs against CS protein alone can confer complete protection in mice[26,27] suggesting that it is an important target for generating liver stage immunity. In addition to B cell and CTL epitopes, the CS protein also contains CD4 T cell epitopes and thus could theoretically induce a broad range of effector mechanisms.

Many of the T cell epitopes in the CS protein are polymorphic, with the immuno-dominant CD4 T cell epitope of CS (Th2R, aa 326-347) containing the most known sequence variability.[22] Fourteen variants have been observed,[28] with 9 co-existing in The Gambia.[28,29] Interestingly, the same variants can be found in widely different geographical regions, which may represent a convergent evolution.[28,30-32] Two naturally occurring APL variants of this epitope have been shown to inhibit proliferation and IFN gamma production from T cells reactive to the index (vaccine) variant.[33] The mechanism of this antagonism appears two-fold. One APL variant is able to promote a switch towards IL-10 production when co-presented on the same or a separate antigen presenting cell (APC) with the IFN gamma-inducing vaccine variant.[33] This IL-10 then switches off proliferation and IFN gamma production; these can be restored by neutralising IL-10 activity *in vitro* with monoclonal antibodies.[33] The other variant can similarly impair proliferation and IFN gamma production when co-presented on the same APC with the index variant, but is unable to do so if presented on a separate APC.[33] The mechanism of suppression by this second variant is still being investigated.

CS-specific CTL have been found only infrequently and at low levels in naturally exposed humans.[34] Moreover, 3 out of 4 known human CTL CS epitopes are polymorphic.[21] This ob-

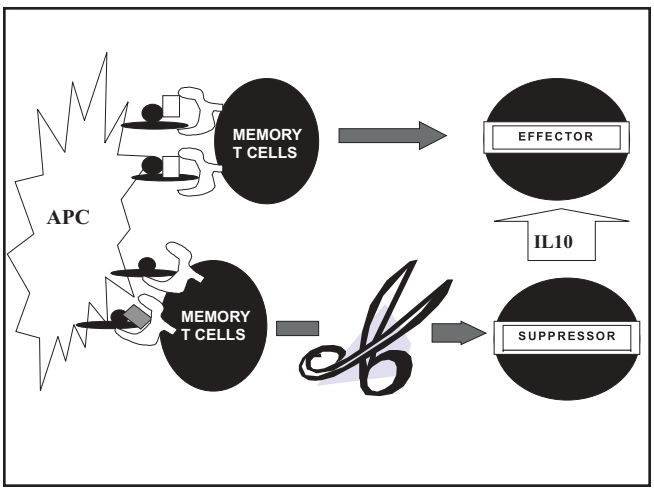

Figure 2: Altered peptide ligands of cytotoxic T cell epitopes prevent adequate T cell activation by simultaneous or sequential presentation of a closely related variant epitope, which provides an altered activation signal to the T cell, resulting in the inactivation of some of its effector functions. This results in a bias towards Th2 type cytokines, for example the immunosuppressive cytokine IL-10, instead of Th1 type cytokines that promote malaria protection through cytotoxic T lymphocytes

servation stands in contrast to the CTL associated epitopes of other proteins present during the liver stage, many of which are highly conserved.[21] The two most polymorphic CS CTL epitopes are the ones found within the helper epitope regions TH2R and TH3R, and which we will call TC2R and TC3R. Most TC2R variants can bind HLA-A2 and some TC3R variants bind HLA-B35,[21] both of which are very common in West Africa, where *P. falciparum* malaria is endemic.[35] The two HLA-B35 binding natural variant epitopes have been found to be not only mutually antagonistic *in vitro*,[36] but also to be able to prevent the priming of specific memory T cells from naive precursors.[33] Preliminary studies suggest that the TC2R region will have similar potent APL antagonistic variants, although the pattern of distribution in the population is likely to be complex, given the large number of variants in this region. This potent antagonistic activity at two levels, memory/effector and naive/memory may explain why parasites bearing these antagonistic variants are found more frequently together (as co-infections) in West Africa, and more frequently in HLA-B35 individuals than in individuals with other HLA types.[36]

Impairment of Dendritic Cell Function

Dendritic cells (DC) are usually the first cells of the immune system to encounter foreign organisms. Their activation in the face of an infectious pathogen, and their subsequent interaction with other cells of the immune system is a major component of the immune response.[37] For example, Tascon et al (2000) showed that the interaction between *Mycobacterium tuberculosis* and a DC cell line (tsDC) results in an increase in surface expression of CD80 and CD86 and the secretion of cytokines in culture, such as IL-1 which promotes the pro-inflammatory activity of macrophages, and IL-12 which promotes Th1 type T cell differentiation and effector function.[38] This results in induction of antigen-specific Th1 CD4 cell responses capable of protecting mice against subsequent experimental tuberculosis challenge.[38] Some pathogens such as *Leishmania major* infect DCs directly; in this case the Langerhans cells that resident in the skin. Infected DCs migrate and present antigen to T cells in draining lymph nodes resulting in the activation of antigen-specific CD8 and CD4 T cells and immunity.[39,40]

It is reasonable to assume that DCs also play a critical role in initiating immune responses to malaria. Urban et al (1999) first showed that *P. falciparum* infected erythrocytes could prevent up-regulation of MHC Class II and co-stimulatory molecules CD83 and CD86 on human DCs in response to liposaccharide (LPS). In addition to affecting maturation, this impeded their ability to induce antigen-specific primary and secondary T cell responses.[13,41] CD36 and CD51 were identified as the receptors on DCs responsible for this inhibitory effect.[42] These same receptors were found to mediate the inhibitory effect of macrophage function by decreasing TNF alpha (TNFα) and IL-1 secretion during malaria infection.[43] It is interesting that these molecules are responsible for the recognition of apoptotic cells by phagocytic cells, which can also have suppressive effects on DC function and maturation.[44] Interaction of infected erythrocytes with DCs also results in decreased IL-12 secretion by DCs, which would otherwise promote adequate T cell effector proliferation and differentiation towards a Th1 phenotype. An increase in IL-10 is also observed during infection, which could potentially directly suppress the stimulatory function of DCs as well as promote the generation of anti-inflammatory suppressor T cells.[45-47]

Pioneering *in vitro* human studies provoked the generation of a number of animal models investigating the functions of DCs during malaria infection *in vivo*. There are, however, definite differences between murine and human DCs, and species of rodent malaria differ from *Plasmodia* species that infect humans. Although there are less than a handful published studies, there are already inconsistencies in the literature on the effect of DC function after interaction with malaria parasites. Seixas and colleagues reported that GM-CSF grown bone marrow derived DCs (BM-DCs) up-regulate surface expression of MHC II, CD40 and CD86 after exposure to *P. chabaudi* infected erythrocytes. Their ability to stimulate T cell responses was maintained, and increased production of TNFα, IL-12 and IFN gamma was evident.?[48] Luyendyk and colleagues focused on analysing splenic $CD11b^+$ and $CD11c^+$ DC subtypes after acute infection with *P. yoelii* infected mice. They found that MHC II and CD80 molecules were up-regulated, and levels of CD86 were maintained.[49] These studies are in direct contradiction to what is seen in human DC studies, and to a recent study in mice by Ocana-Morgner and colleagues, who showed that GM-CSF grown BM-DCs and parasitised erythrocytes from *P. yoelii* inhibit DC maturation *in vitro*.[50] Thus, there is disagreement in the literature on the role of DCs in protective immunity to blood-stage malaria. Since the *Plasmodia* parasites have been shown to impair human[41,50] and murine DC maturation[50] *in vitro*, it has been suggested that DCs do not prime protective immunity during infection. However, a recent publication by Bruna-Romero and colleagues showed that DCs presenting *P. yoelii* artificial (recombinant) sporozoite antigens can induce protective immunity against liver-stage malaria in mice and stimulate CD8 and CD4 T cell responses.[51] Our laboratory has addressed directly the hypothesis that DCs can prime blood-stage malaria immunity, and found that this is possible despite potential impaired maturation (Pouniotis, In Press) as measured by impaired up-regulation of co-stimulatory molecules and specific inhibition of CD8 antigen-specific responses (Pouniotis et al, *submitted*).

Host-parasite Interactions and Implications for Malaria Vaccine Development

Presently there is no malaria vaccine available to travellers or individuals living in malaria endemic areas. A recent promising vaccine to undergo clinical trials in Africa is the *P. falciparum* CS protein construct with recombinant hepatitis particles (RTS,S). Previously, this vaccine induced 50-60% pre-erythrocytic protection against homologous *P. falciparum* challenge in naïve volunteers.[52,53] However, in Gambian adults, this vaccine did not induce similar patterns of immunity and protection was short-lived.[54,55] It could be argued that this vaccine, targeting the highly polymorphic CS antigen, worked as expected, since a heterogenous population of parasites exist

Table 1: Summary of human malaria vaccines in field trials

Vaccine candidate	Human population	Immunogenecitystudies	Challenge studies	Refs
Liver stage				
NYVAC-Pf7	Naïve adults	Low antibody responses Cellular response in >90%	1/35 protection	57
PfCSP	Naïve adults	Antigen-specific CD8+ T cells in 11/17		58
	Naïve adults	IFN gamma 14/14 Antigen-specific CD8 T cells 13/14		59
RTS,S	Naïve adults	T cell antigen-specific lymphoproliferation and IFN gamma secretion	18/41 protective efficacy	52
	Naïve adults		71% 9 weeks post-immunisation	54
	Gambian adults		0% 15 weeks post-immunisation	55
DNA-MVA vaccine (Prime boost)	Gambian adults	>1000 specific TRAP producing cells per million	Heterologous (TRAP) challenge strain	56
Blood stage				
MSP-1 (19)	Gambian adults	14/40 serum antibody responses		60
	Naïve adults	antigen-specific IFN gamma, IL-4		61
	Gambian & Kenyan adults	and IL-10 producing T cells		
MSP-1 MSP-2	Naïve adults	Low antibodies, higher T cells responses		62,63
RESAAdjuvant (SEPPIC)	Adult males in endemic area of PNG	Increases in total IgG and T cell stimulation assay all low to MSP-1 and RESA		64

in endemic areas. McConkey and colleagues recently reported highly encouraging results on the induction of substantial *P. falciparum* specific CD4 and CD8 T cell levels in naive humans using Prime-boost.[56] Such protocols, if combined with high selectivity in antigen and variant/conserved epitope inclusion, may help overcome the problems of variant-specific immune evasion. Indeed, the most potent vaccine for malaria endemic areas may prove to be a multi-antigen, multi-stage combination. Recent malaria vaccine candidates tested in field trials are summarised in Table 1.

However, identification of new and conserved antigens alone may not be sufficient for a vaccine that is successful in malaria endemic areas, as acute malaria infection is associated with T cell immunosuppression with a variety of generalised immunological changes, some of which are discussed above. An additional problem common to all vaccines is that in the field, they may preferentially re-stimulate pre-existing immunity induced by past infection with a bias towards a specific, and not necessarily protective, cytokine secretion pattern. It is hoped that as new vaccine approaches and understanding of the mechanisms of immune evasion at the host: parasite interphase progress, elements of its design will provide an effective vaccine for use in malaria endemic populations.

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