Malaria, monocytes, macrophages and myeloid dendritic cells: sticking of infected erythrocytes switches off host cells
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The adhesive phenotypes expressed by Plasmodium-falciparum-infected erythrocytes were previously thought simply to permit sequestration of parasites in the peripheral circulation. Recent work has illuminated how falciparum-infected erythrocytes may modulate the function of monocytes, macrophages and myeloid dendritic cells through the action of haemozoin from digested haemoglobin and through adhesion of infected cells to their surface.

Introduction
The vast majority of morbidity and mortality from malaria is caused by infection with Plasmodium falciparum. Falciparum malaria is a major public health problem throughout the tropical world where climatic conditions permit replication of the parasite in Anopheles mosquitoes. In such regions, malaria is responsible for 1–3 million deaths per year, mainly in children [1*].

Human infection begins with the injection of sporozoites from Anopheles mosquitoes. After replication within hepatocytes, merozoites are released into the circulation, and enter and multiply within erythrocytes. The parasites can multiply approximately eight-fold every two days [2*]. Within a short time a high proportion of erythrocytes may be infected, comprising up to several grams of foreign matter including glucose-6-phosphate dehydrogenase, hyaluronic acid (HA), or rosette uninfected erythrocytes or clump platelets [3,4] (Figures 1,2). These adhesive phenotypes are collectively responsible for the phenomenon of sequestration of infected blood cells in the post-capillary venules and are associated with the development of severe clinical disease including coma particular to falciparum malaria [5,6]. Adhesion is mediated, at least in part, by a family of parasite-encoded antigens (P. falciparum membrane protein 1 [PfEMP-1]) on the surface of infected erythrocytes [7]. These antigens contain functionally conserved adhesive domains that are nevertheless highly variable antigenically. Rapid, mutually exclusive expression of different variant PfEMP-1 proteins not only allows the appearance of heterogeneous adhesive phenotypes within a clone but also permits evasion of clinically protective humoral immune responses [8–10].

In endemic areas, infection with P. falciparum results in a range of outcomes from asymptomatic infection, through mild disease, severe disease and death. Immunity is exposure-related and therefore age-related and occurs rapidly to severe non-cerebral disease, more slowly to mild disease and probably never to asymptomatic infection [11,12]. The prevalence of infection within a population is still rising in those age groups in which the incidence of severe disease is already declining [13]. High levels of TNF-α, IFN-γ, IL-6 and IL-1 are more frequently observed in children suffering from severe malarial disease than those suffering from mild disease or asymptotically infected [14–17]. These observations have led to the hypothesis that infections early in life result in inflammatory immune responses effective against the parasite but are also associated with immunopathology. With increasing exposure, immune deviation and immuno-regulation may reduce immunopathology during infection, with the exception of cerebral malaria.

Animal models and indirect evidence from clinical observations suggest phagocytosis of infected red blood cells by splenic macrophages is a critical component of host defence mechanisms against blood-stage parasites. Other innate immune responses, including the role of NK cells and neutrophils, are less well defined. Specific host effector responses against the malaria parasites include cytotoxic lymphocytes recognising intra-hepatic parasites, and antibodies against parasite molecules; these antibodies inhibit invasion of erythrocytes, stimulate antibody-dependant cytotoxicity or recognise the adhesive proteins (PfEMP-1) expressed on the surface of erythrocytes [18]. It is clear that many of the adaptive immune responses would have
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to be primed by antigen-presenting cells, amongst which myeloid dendritic cells (DCs) are probably important because of their ability to stimulate naïve T cells [19].

It is clearly in the interest of the parasite, and more formally a source of selective advantage for strains of falciparum parasites, to develop methods to inhibit immune and inflammatory responses, potentially harmful to the parasite and the host. However, until recently the specific mechanisms of modulation of host cell responses have been poorly understood.

It has been assumed that the sole advantage of adhesion of infected erythrocytes to endothelium for malaria parasites was simply avoidance of splenic phagocytes. In this review, we will concentrate on the recent studies demonstrating a direct modulation of leukocytes by malaria parasites and the significance of these findings for the parasite, and their unwilling and sometimes unfortunate human host.

Modulation of macrophages

Macrophages phagocytose malaria-infected erythrocytes. It is intriguing that macrophages may not only ingest intact infected erythrocytes but also extract parasites from recently infected erythrocytes, leaving the erythrocytes to continue to circulate [20,21]. However, these crucial defences are undermined and manipulated during malaria infection.

Macrophage function is reduced in malaria infection [22].

In an original series of studies, Paulo Arese and colleagues
13-eicosatetraenoic acid) [27, 28•]. These lipoperoxides (HETE is the abbreviation for 15-hydroxy-6, 8, 11, acid — including 4-hydroxynoenal and 15(R, S)-HETE endo-peroxides — formed by peroxidation of arachadonic by haemozoin is the generation of biologically active inhibition of macrophage function.

More recently, an interesting and initially surprising series of observations from Kevin Kain and colleagues has provided another mechanism for modulation of macrophage function. They observed that infected erythrocytes expressing the appropriate variant antigen (PfEMP-1) were phagocytosed by macrophages via CD36 without increasing or priming for secretion the pro-inflammatory cytokine, TNF-α [35••] (Figure 3b). This group has more recently shown that proliferation-activated receptor γ-retinoic acid X receptor agonists and 9-cis-retinoic acid — a metabolite of vitamin A — increase the levels of CD36 expression on macrophages and the phagocytosis of infected erythrocytes while reducing the secretion of pro-inflammatory cytokines [36,37*].

The apparent anti-inflammatory effect of adhesion of infected erythrocytes may have a profound effect on the outcome of malaria infection. These observations suggest the inhibition of phagocytosis and inhibition of other inflammatory responses, mediated by adhesion of infected erythrocytes to monocytes, may facilitate survival of both parasite and host.

Modulation of dendritic cells

It is reasonable to assume that DCs play a pivotal role in initiating immune responses to malaria. We observed that falciparum-infected erythrocytes could bind to the surface of myeloid DCs in vitro and profoundly modulate the maturation and function of DCs. Infected erythrocytes could inhibit the normal upregulation of MHC class II molecules, adhesion molecules (e.g., ICAM-1) and co-stimulatory molecules (CD83 and CD86) on DCs after stimulation with lipopolysaccharide (LPS). The ability of DCs to stimulate not only allogenic but also antigen-specific primary and secondary T-cell responses was profoundly reduced [38**,39] (Figure 3c).

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The interaction of monocytes and myeloid DCs with infected erythrocytes and haemozoin. Transmission electron microphotographs of (a) a monocyte with numerous ingested particles of haemozoin; (b) a monocyte with adherent falciparum-infected erythrocytes (the ruffling of the monocyte membrane can be seen between the infected erythrocytes); and (c) two falciparum-infected erythrocytes adhering to a myeloid DC.

The close apposition between the plasma membrane of the DC and the infected erythrocyte can be clearly seen. Pictures are included courtesy of David Ferguson (Nuffield Department of Clinical Laboratory Sciences), Dominic Kwiatkowski (Nuffield Department of Paediatrics) and Tony Berendt (Nuffield Department of Clinical Medicine), University of Oxford, and are reproduced with permission from David Ferguson.
heterodimers). Ligation of CD36 and/or CD51 mimics the inhibition of DCs by infected erythrocytes and by apoptotic cells. It was striking that these molecules are not only receptors for infected erythrocytes but are also involved in the recognition of apoptotic cells by phagocytes.

In our experiments it was apparent that apoptotic cells also inhibit the maturation and function of DCs. Moreover, infected erythrocytes, ligation of CD36 and/or CD51 or apoptotic cells — including apoptotic DCs — reduced the secretion of IL-12; by contrast modulated DCs secrete IL-10. Monocytes and macrophages are also modulated by falciparum-infected erythrocytes and by apoptotic cells. When activated by LPS or IFN-γ, macrophages secrete inflammatory cytokines including TNF-α. Exposure to falciparum-infected erythrocytes or ligation of CD36 does not induce TNF-α secretion. However, after ingestion of apoptotic cells, secretion of the anti-inflammatory cytokines IL-10 and transforming growth factor β (TGF-β) is enhanced. Extensive functional impairment of macrophage function may also follow ingestion of haemozoin from infected erythrocytes (not shown).

Is DC function impaired during malaria infection? The number of CD83+ peripheral blood DCs was not significantly different in healthy children and in children with malaria. However, the percentage of HLA-DR+ DCs in peripheral blood was significantly reduced in children with malaria [42]. These data gave only a gross indication of the function of DCs in falciparum malaria as they did not distinguish between myeloid and plasmacytoid DCs nor did they examine the kinetics of DC turnover and their capacity to stimulate T cells. Furthermore, the relative effects of malaria pigment and adhesion of
infected erythrocytes on myeloid cell function have not been established.

**Animal models**

As the story of modulation of host cell function by falciparum-infected cells and their products has unfolded, there has been some interest to see whether similar effects are observed in animal models of malaria. These models are somewhat limited by the peculiar host-restriction of malaria parasites to primates, rodents, birds and lizards. The rodent malaria *P. chabaudi chabaudi* in laboratory mice represents a useful model of the early inflammatory response in human malaria. Furthermore, the parasite may sequestrate in heart, lung, liver and spleen, but not the brain of mice and so provide a model for severe non-cerebral malaria [43–45].

Do erythrocytes infected with this rodent malaria parasite inhibit the function of murine myeloid DCs? It appears not. After exposure to *P. c. chabaudi*-infected erythrocytes, murine DCs mature normally and stimulate T-cell responses. Furthermore, they produce TNF-α (over 2 hours), IFN-γ and IL-12 (over 10 hours) [46•]. These data contrast to observations of the downregulation of IL-12 but upregulation of IL-10 secretion by human myeloid DCs after exposure to erythrocytes infected by the falciparum parasites [40•].

By contrast, it has been recently reported that murine macrophages, having ingested apoptotic cells, not only downregulate secretion of IL-12, but also show a reduced capacity to stimulate antigen-specific T-cell responses [47•]. Together, these data suggest that at the cellular level parallels exist between the modulation of human and murine myeloid DC function by apoptotic cells but not by malaria-infected erythrocytes. In humans, definition of the receptors used by phagocytes to recognise and ingest apoptotic cells or falciparum-infected erythrocytes are well characterised. Although *chabaudi*-infected erythrocytes express variant antigens [48], can cytoadhere [43] and may bind to CD36 [44], it remains to be seen whether these characteristics are mediated by the same protein (families) or similar properties of different protein (families). Further studies on the molecular interaction of *P. c. chabaudi*-infected erythrocytes or indeed apoptotic cells with murine macrophages or DCs are awaited with interest.

**Conclusions**

How do these observations of the modulation of macrophage and DC function by falciparum-infected erythrocytes explain the pathophysiology of malaria and what hypotheses do they generate for future studies?

In the older literature, impairment of immune responses to vaccine antigens has been described in children vaccinated during malaria infection [49,50]. Furthermore, the association of Burkitt’s lymphoma — where unchecked Epstein–Barr virus (EBV)-derived proliferation of B lymphocytes leads to malignant transformation — with malaria-endemic areas suggests suppression of immune responses by malaria infection [51,52]. We now have evidence from *in vitro* studies that the immunosuppressive effect(s) may be caused by pigment and by adhesion of infected erythrocytes to antigen-presenting cells.

The role of pigment and the lipoperoxides generated by the catalytic effect of pigment in the outcome of malaria infection is not clear. However, the association of pigment-containing monocytes and neutrophils in the peripheral blood with the severity of clinical disease is consistent with a causal relationship. If this effect is established then inhibiting this pathway of cellular damage may allow amelioration of malaria morbidity and mortality.

The at-first-sight counter-intuitive effect of adhesion to CD36 and/or TSP on host cell function makes sense of the pattern of adhesive phenotypes expressed by *P. falciparum*-infected erythrocytes. The vast majority of isolates from those suffering from malaria can bind to CD36 and to TSP [53–56] (Figure 2). Furthermore, a functional CD36-binding domain is conserved in the majority of variant antigens that can be expressed on the surface of infected erythrocytes, although the sequence of this domain is highly variable.

These data support the importance of adhesion to CD36 for the success of and survival of parasite strains.

What is the significance of the adhesion of infected erythrocytes to CD36 for the host? Infected erythrocytes with the highest affinity for CD36 binding are more frequently found in isolates from patients with mild disease compared with isolates from patients with severe disease [55,56]. These observations suggest that adhesion of infected erythrocytes to CD36 may reduce the pro-inflammatory response to the parasite and so influence the outcome of severe disease (Figure 4).

It is tempting to speculate that the other common adhesive phenotypes of falciparum-infected erythrocytes, such as adhesion to ICAM-1, CR1 (C35) and CD31, may also modulate host cell function (Figure 2). Indeed, the pattern of adhesive phenotypes expressed by infected erythrocytes, once thought to be extensive, is in fact quite restricted given the large number of potential host molecules expressed on endothelium and leukocytes.

These insights of the modulation of host-cell function by cytoadherence of malaria parasites also suggest that adhesive phenotypes expressed by other pathogens may also represent ‘switching’ and not just ‘sticking’. Many other pathogens do adhere to αvβ3 integrins including: two picornaviruses (coxackie A9 and foot and mouth virus); *Neisseria meningitidis*; *Mycobacterium avium* and *M. intracellulare*, and finally the spirochete *Borrelia burgdorferi* [57–61]. The immuomodulatory effects of these specific host-cell interactions have not been described.

In summary, studies of the modulation of the immune system by malaria parasites have revealed pathways of
immune modulation by apoptotic cells which may be important for the maintenance of peripheral tolerance to self. It appears that malaria parasites use this physiological pathway to subvert innate and acquired immune responses against the parasite (Figure 4). The underlying mechanisms of modulation of myeloid DCs and of macrophages and subsequently of T-cell function remain to be elucidated. One would predict these mechanisms will be of continued biological interest and potentially of medical importance.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
•• of outstanding interest


parasitized by *Plasmodium falciparum* may explain malaria protection in G6PD deficiency. *Blood* 1998, 92:2527-2534. The authors show that malaria-infection erythrocytes from G6PD-deficient people are recognised and ingested early in the growth cycle, when little pigment has accumulated. This trait is known to protect people from severe malaria. These in vitro data support the hypothesis that haemoglobinopathies may protect people from severe malaria by facilitating early clearance of relatively non-toxic forms of infected erythrocytes.

35. McGlynn JD, Serghides L, Kapus A, Rotstein OD, Kain KC: Nonopsonic macrophage/microbicidal phagocytosis of *Plasmodium falciparum*-parasitized erythrocytes: a role for CD36 in malarial clearance. *Blood* 2000, 96:3231-3240. The authors show that non-opsonised infected erythrocytes are recognised by CD36 on macrophages. Phagocytosis of infected erythrocytes by this route does not induce TNF-α secretion. These data suggest that this route of parasite clearance does not elicit an inflammatory response and as such would not contribute to severe clinical disease.


37. Serghides L, Kain KC: Mechanism of protection induced by vitamin A in falciparum malaria. *Lancet* 2002, 359:1404-1406. The authors show that metabolites of vitamin A, which is known to provide some protection from malaria disease, may increase expression of CD36 on the surface of macrophages and permit increased phagocytosis of infected erythrocytes without secretion of inflammatory cytokines.


The authors show that murine DCs that have ingested apoptotic cells secrete IL-10 and not IL-12 and have a reduced capacity to stimulate T-cell responses. These data are consistent at the cellular level with observation in vitro of inhibition of human DC maturation and function after co-culture of apoptotic cells.


