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Dendritic cells in *Plasmodium* infection

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Infection with *Plasmodium* parasites (malaria) contributes greatly to morbidity and mortality in affected areas. Interaction of the protozoan with the immune system has a critical role in the pathogenesis of the disease, but may also hold a key to containing parasite numbers through specific immune responses, which vaccine development aims to harness. A central player in the generation of such immune responses is the dendritic cell. However, *Plasmodium* parasites appear to have profound activating and suppressing effects on dendritic cell function, which may enhance immunopathology or facilitate the parasite's survival by depressing beneficial immunity. Furthermore, immune responses to other infections and vaccines may be impaired. A greater understanding of the effects of the parasite on dendritic cells will contribute to insight and potential defeat of this infectious disease.

The morbidity and mortality caused by *Plasmodium* infection makes it one of the most significant infectious diseases. There are an estimated 500 million cases of malaria annually, and of the 1 million fatalities the majority are children under 5 years in sub-Saharan Africa. Globally, disease inflicts a massive socioeconomic burden on the numerous affected regions in tropical and subtropical countries.

The complex life cycle of the protozoan parasite has provided a range of subject areas for research that attempts to understand better the biology of the pathogen and how it interacts with the immune system. Host responses against the blood stage of the parasite, both the innate and adaptive arms of the immune response, have been shown to make a major contribution to disease etiology [1]. The dendritic cell (DC) is a central player in the immune system, critically linking the innate and adaptive arms and thus dictating not only the generation of antiparasite T cells and antibodies, but also the quality (cytokine or antibody profile) of these responses. Thus, the interaction between the parasite and DCs, the subject of this article, is critical to the generation of parasite-specific adaptive immune responses. Such responses may impart protection from infection or disease when generated by natural exposure or by vaccination [2]; or conversely, may contribute to adverse immunopathology. It is important to bear in mind that studies on DC function in conjunction with *Plasmodium* have examined responses of differing DC types (myeloid and plasmacytoid), of human or murine origin, infected with different *Plasmodium* species, both *in vivo* and *in vitro* [3].

However, despite disparate data, a picture begins to emerge that may reconcile the wide range of heterogeneous studies.

Background on dendritic cells

DCs are mononuclear leukocytes that originate in the bone marrow. Their precursors circulate in the blood and localize to most tissue types where they differentiate into phagocytic cells (immature DCs or related cells) that capture antigens in their vicinity [4]. They are also able to recognize a wide range of molecules, particularly those derived from pathogens that bind sets of receptors including scavenger receptors and the Toll-like receptor (TLR) family [5]. In this way they are able to sense a broad range of situations in the host that require a reaction, sample associated antigens and instigate an appropriate immune response, such as cell-mediated immune responses for intracellular pathogens or regulatory responses for self/harmless antigens. Indeed, DCs have a unique ability amongst antigen-presenting cells (APCs) to prime naive T cells. Once they have ingested foreign antigen and been stimulated, DCs mature and change their functions from antigen uptake in tissues, to antigen processing and presentation, together with migration to local lymph nodes through the expression of homing molecules (e.g., chemokine receptor CCR7). Complex antigen-processing pathways allow DCs to present antigen-derived peptides both by major histocompatibility complex (MHC) class I molecules to CD8⁺ T cells and by MHC class II molecules to CD4⁺ T cells, both molecules of which are upregulated upon activation. Their activation also gives rise to expression of costimulatory molecules,

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chiefly CD80 and CD86, which are critical to priming naive T cells, together with the secretion of cytokines that promote cell-mediated immunity, such as IL-12 or type I interferons.

Subtypes of DC exist based on myeloid or plasmacytoid characteristics (mDCs and pDCs, respectively). Human pDCs are characterized by expression of high levels of IL-3 receptor (CD123) and the ability to secrete large amounts of IFN- α , whilst mDCs are characterized by expression of CD11c, CD1c or CD83 and notably secrete IL-12. Murine mDCs are also CD11c⁺ but express various combinations of CD4 and CD8 α . Murine pDCs possess low levels of CD11c and are B220⁺. Differential TLR expression by DCs indicates further functional heterogeneity. Typically, pDCs possess TLR7 and 9 which are involved in the recognition of bacterial DNA CpG motifs and viral-derived dsRNA, whereas mDCs recognize bacterial and fungal structural or membrane molecules (e.g., lipopolysaccharides) via TLR2 and 4. Certain types of DC are also able to present native antigen to naive B cells [6], thus instigating their activation to produce specific antibodies.

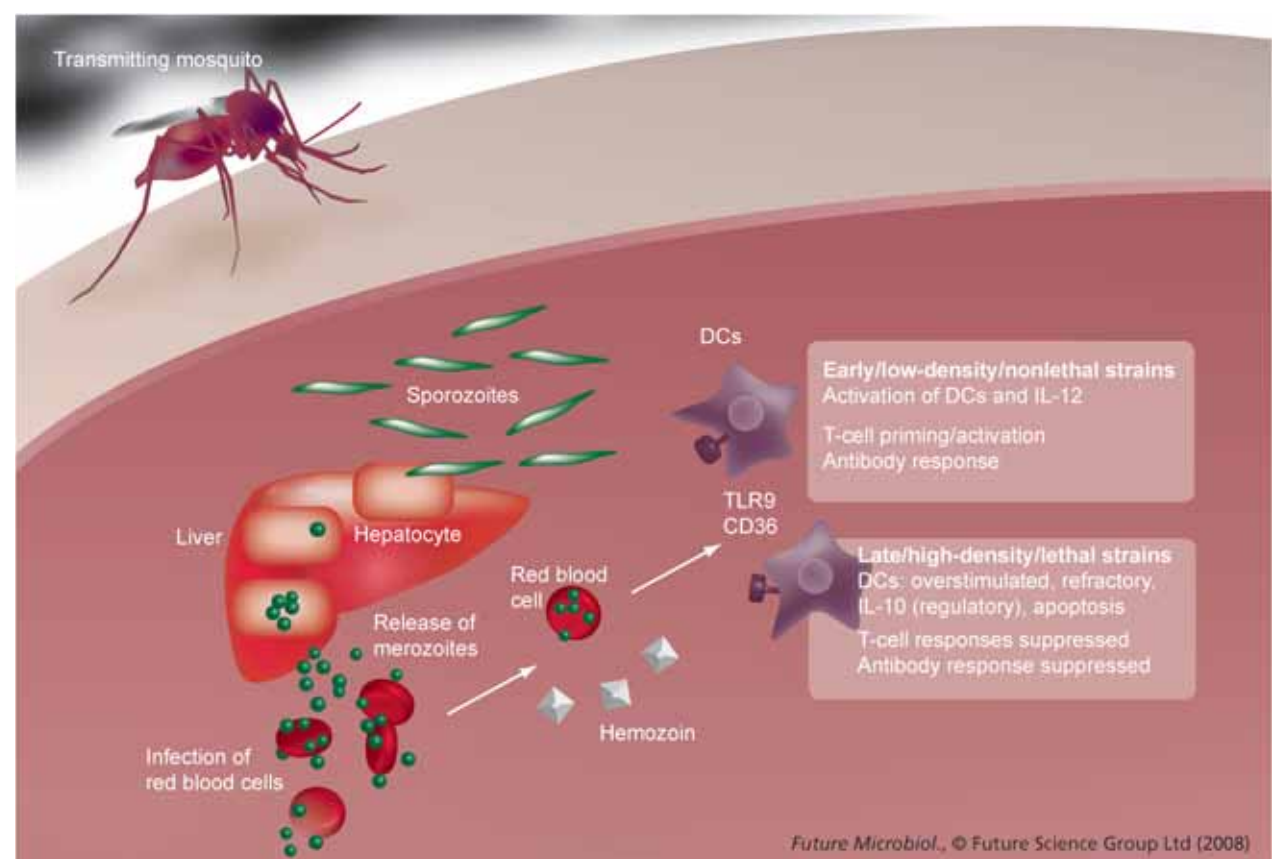
Malaria life cycle

Human hosts become infected when a female anophelene mosquito carrying plasmodial species takes a blood meal. Out of four major species to infect man, *Plasmodium falciparum* presents with the most severe and life-threatening diseases and receives most attention in clinical and basic human research. There are also mouse-specific *Plasmodium* species used to model infection. Sporozoites that exit from the mosquito salivary gland during feeding are injected (a mean of 123 sporozoites, as shown in mouse models [7]) into the host's dermis from which they migrate into the circulation via blood or lymph [8] and home to the liver within minutes (Figure 1). Within hepatocytes the parasite replicates asexually (and asymptotically) into the merozoite stage, typically over a 7-day period for *P. falciparum*, and amplifies in number from a single sporozoite to tens of thousands of merozoites. Hepatocytes release phagocyte-resistant vesicles containing merozoite progeny into the bloodstream [9], during which the initial effects on the immune system, chiefly an inflammatory response and fever, may be observed owing to released plasmodial molecules (e.g., glycerophosphatidyl inositol anchors [10]). Merozoites subsequently infect red blood cells (RBCs) giving rise to schizonts over 48-hour cycles for *P. falciparum*

(but differing for other species), which release further merozoites together with other molecules and metabolites (e.g., hemozoin, a malarial by-product of RBC pigment). Hemozoin, together with molecules expressed by infected RBCs (iRBCs), further stimulate inflammatory immune responses, notably TNF- α and acute-phase proteins. Serious sequelae include the sequestration of iRBC in the blood vessels in the brain contributing to cerebral malaria, which is fatal in approximately 20% of cases. Finally, a proportion of the merozoites develop into gametocytes, which are ingested by a feeding mosquito, in the gut of which they combine to give rise to ookinetes. Sporozoites ultimately develop, which migrate to the salivary gland where they can be injected into the host and start the cycle again.

Immune response against malaria

There is much evidence that immunity to *Plasmodium* infection generated by natural exposure, or through vaccination, is able to mediate a significant reduction in disease incidence, despite the large array of target antigens and their genetic variability [2,11]. However, immune responses are also responsible for infection-associated pathology [1,12], and require careful tuning between beneficial and pathological responses to allow survival of the host. The stage of the life cycle is critical when considering immune responses. The liver stage does not generate overt systemic inflammatory responses nor disease because a limited number of hepatocytes are infected, and liver-stage antigen is relatively limiting. Antibody responses against sporozoites can reduce hepatocyte infection, under certain circumstances such as vaccination [13], rather than natural immunity. Importantly, we and others have been able to demonstrate IFN- γ T-cell responses generated by natural exposure that confer a degree of resistance to infection [14,15]. Furthermore, vaccinations with viral vectors encoding liver-stage antigens generate protective IFN- γ CD4⁺ and CD8⁺ T-cell responses [16]. Although T-cell responses against the blood stage of infection have shown some association with protection in a vaccination study [17], antibody stage antibodies appear to be the main responses that confer protection in endemic regions [18–20], and are the aim of numerous vaccine approaches [2]. The success of malaria vaccines in such areas depends on vaccinees possessing a fully functioning immune system, in particular their DCs. Immunity to other infections and vaccines may also be impaired.

Figure 1. Effect of *Plasmodium* on dendritic cells.

Mosquitoes transmit sporozoites which home to liver cells and develop into vast numbers of merozoites. Upon release these infect red blood cells. Early during infection when parasite density is low, DCs become activated (via TLR9) and can generate T cells and associated immune responses. Later during infection when parasite densities are high, DCs may be overstimulated via CD36 or TLR9, or due to high levels of TNF- α , and may be refractory to activation, 'regulatory' and/or may produce IL-10. They also may become apoptotic. T cells and associated immune responses are suppressed or reduced.

DC: Dendritic cell; TLR: Toll-like receptor.

Dendritic cells & malaria

There is disparate and often contradictory data on responses of DCs to *Plasmodium* parasites (Table 1), and their clear description benefits from describing human and mouse responses separately.

Despite early suggestions that malaria infection might suppress immune responses [21], particularly APC function [22], the first demonstration of parasites influencing human DC function came in 1999. Our study showed that malaria iRBCs were able to bind to human mDCs *in vitro* and inhibit maturation and their ability to stimulate T cells [23]. These findings were taken further to show that this process involved the binding of iRBC to CD36 on DCs [24], which is a multiligand scavenger receptor known to bind apoptotic cells, against which immune responses are often not desired. More recent *in vitro* studies have shown additional suppressive effects not involving parasite binding to

CD36 but implicating chondroitin sulphate-A, and also noncontact mechanisms [25]. Furthermore, this study showed that high doses of parasites (100 iRBC:1 DC) were suppressive and caused DC apoptosis, whilst low doses (10:1) activated DCs. The physiological relevance of these doses remains unclear. Hemozoin, a byproduct of malarial infection of RBCs, was shown to inhibit maturation of human mDCs [26], whilst pDCs responded to schizont extract by producing IFN- α , upregulating CD86 and induced $\gamma\delta$ T-cells that secrete IFN- γ rather than conventional T cells [27]. *In vivo*, children suffering with acute malaria infection show reduced expression of HLA-DR (MHC class II) on mDCs and increased frequencies of BDCA-3-expressing DCs, possibly an IL-10-suppressed phenotype [28,29]. Both these findings suggest depressed DC activity.

Table 1. Key studies on Plasmodium effects on dendritic cells.

Effect on DCs	Mechanism	Plasmodium species	Form of stimulus	Host species	In vivo/in vitro	DC type	Laboratory/ref.
Suppression	CD36	Pf	iRBC	Human	In vitro	mDC	Oxford [21,22]
Suppression	High dose	Pf	iRBC	Human	In vitro	mDC	Melbourne [23]
Activation	Low dose						
Suppression	No DC maturation	Pf	HZ	Human	In vitro	mDC	Turin [24]
Activation	TLR9	Pf	iRBC extracts	Human	In vitro	pDC	Bangkok [25]
Activation	DC:NK cross-talk	Pf	iRBC	Human	In vivo	mDC	London [51]
Activation	TLR9 MyD88	Pf	HZ	Mouse	In vitro	mDC pDC	Osaka [32]
Activation	Phagocytosis of iRBC, IL-12	Pch	iRBC	Mouse	In vitro In vivo	mDC	Montreal [28]
Suppression	T cell: DC clustering	Pch	HZ	Mouse	In vivo	mDC	Glasgow [30,31]
Suppression	DC apoptosis Th1 to Th2	Pch	iRBC	Mouse	In vivo	mDC	London [38]
Suppression	Regulatory DC	Py	iRBC	Mouse	In vivo	mDC	New York [46]
Suppression	Lethal strains, TNF- α	Py	iRBC	Mouse	In vivo	mDC	Brisbane [43,45]
Activation	Nonlethal strains						

DC: Dendritic cell; HZ: Hemozoin; iRBC: Infected red blood cells; mDC: Myeloid dendritic cell; NK: Natural killer cell; Pch: Plasmodium chabaudi; pDC: Plasmacytoid dendritic cell; Pf: Plasmodium falciparum; Py: Plasmodium yoelii; TLR: Toll-like receptor.

In mouse studies, using murine-compatible *Plasmodium* species, iRBCs (schizonts or lysates thereof) were able to stimulate murine mDCs to produce IL-12 and elicit T-cell activation [30], as well as DC surface expression of CD40 and CD86 and migration to T-cells zones in lymphoid tissue *in vivo* [31]. In contrast with such activation, another group showed hemozoin to impair murine DC function in lymphoid tissue, particularly DC:T-cell clustering, and generated poorly functional T cells [32,33]. Conversely, another study showed that purified hemozoin enhanced murine DC maturation and IgG2a generation, an effect that seemed to be dependent on TLR9 and MyD88 signaling [34]. It has been more recently suggested that it is malarial DNA bound to hemozoin, and acting via TLR9, that activates DCs [35]. A specific role for DCs [36], and of MyD88 [37], in proinflammatory responses and disease has also recently been demonstrated, as well as the cAMP regulation of IL-6 in a transcriptome profile of DCs stimulated with *Plasmodium* [38].

The qualitative nature of the antimalaria adaptive immune response is essentially a result of the interaction of DCs with malaria. The first encounter that DCs may have with the parasite is following the injection of sporozoites by mosquitoes into the dermis where they can be taken up by local DCs and prime protective T cells in draining lymph nodes [39]. However, the most profound effects on DCs occur during the blood stage of infection. Murine studies highlight that a particular DC subtype (CD8⁻) is most efficient in priming CD4⁺ T cells during such acute infection [40], whereas CD8⁺ DCs cross-present and prime CD8⁺ T cells early during infection. Cross-presentation is abrogated as parasitemia develops owing to a systemic inflammatory response [41]. Others reported that presentation of malarial antigens to CD8⁺ T cells may induce their cell cycle arrest, associated with a partial defect in DC function [42,43]. However, co-administration of a DC-specific chemokine, DC-CK1, may circumvent such a phenomenon [44].

The capacity of murine splenic CD11c⁺ DCs to become activated and to present antigen to T cells is believed to change over the course of infection [25,40,42], and this observation may reconcile some of the heterogeneous findings that showed either activation or suppression of DC function associated with malaria. In simple terms, early on in infection when parasite density is relatively low, IL-12-mediated mechanisms induce IFN- γ -producing CD4⁺ T cells. This DC

phenotype may be maintained over the entire course of infection with nonlethal strains of *Plasmodium* [45], although it is unclear how lethal and nonlethal strains differ with respect to the immune response they generate, and the influence of dose. Later, when parasite densities are peaking, DCs may become refractory to TLR and other signaling events, possibly due to overstimulation or due to TNF- α [46,47], thus abrogating IL-12 and TNF- α secretion, and as capacity to produce IL-10 increases. While secretion of IL-10 may reduce inflammatory responses, a desirable effect when linked to clinical immunity [1], specific immune responses to malaria antigens as well as to other infections and vaccines may be reduced. Most recently, *Plasmodium yoelii* infection of mice has shown a prevalence of 'regulatory' CD11c^{low}, CD45RB^{high} DCs in the spleen, overtaking conventional CD11c^{high} DCs, which induce IL-10-secreting T cells [48]. Such DCs may also secrete TGF- β and prostaglandin PGE2 and IL-10 [49], thus inhibiting generation of T cells; against liver stage in this study. The suppression of DC function by iRBCs mirrors that produced by endotoxin LPS [48], and may be part of a negative feedback or tolerance mechanism occurring at high parasite dose.

A human study that supports the dose theory showed that very low doses of iRBC generated T-cell responses that were protective against subsequent blood-stage challenge [17]. Of further relevance, we have recently demonstrated that malarial parasitemia in Kenyan volunteers during vaccination trials with viral vectors encoding liver-stage antigens gave rise to reduced T-cell responses [50]. We speculate that such an effect may be in part via parasite-induced suppression of DCs. A possibility for vaccination, therefore, is to administer a course of antimalarial drugs prior to and during the vaccination regimen in trials in endemic regions.

The generation of antigen-specific regulatory T cells (FOXP3⁺ and TGF- β ⁺) following experimental malarial infection in naive individuals suggests that modulated DCs are involved in priming of T cells, although suppressive in nature and possibly promoting parasitemia [51]. We have recently identified deficiencies in blood stage-specific T-cell memory development during similar infection [Todryk S, Walther M, Bejon P *et al.*, Manuscript in preparation].

Last, DCs have recently been implicated in intricate cross-talk with natural killer cells to produce IFN- γ in response to iRBCs and to maintain DC maturity [52].

Conclusion & future perspective

DCs are at the heart of the immune system, sensing and initiating immune responses to 'dangerous' infectious microbes, or harmless/self antigens, which are ultimately critical for the survival of the host. For the complex disease that is malaria, it is essential that immune responses are generated within the correct boundaries so as to be protective against parasitemia without causing immunopathology or disease. The plasticity of DC phenotypes and function enables the existence of such immunity, through parasite recognition, and is affected by parasite dose and previous exposure. Low-dose immunization may be pursued in vaccination [17] or through intermittent preventive treatment with antimalarial drugs [53], which may allow the generation of immunity by keeping down parasite numbers, thus avoiding high-dose suppression. Furthermore, drugs could be administered in conjunction with vaccine trials to avoid immune suppression by high malarial parasite numbers.

A picture is beginning to form as to how DCs may orchestrate antimalaria responses, but differences in the way humans and mice recognize and respond to stimuli, such as DNA vaccines [54], means that murine findings may not always apply directly to humans. In addition, *in vitro* studies, whilst being valuable reductionist approaches, breaking down the DC–malaria interaction, may

not reveal the real *in vivo* multifactorial situation. Further clinical studies are therefore required in humans, examining DCs and associated immune responses in both experimental and natural infection, in order to provide more clues for manipulation of such responses in prophylactic and therapeutic immune-based intervention. The complicated nature and expense of such studies mean that it may take several years before knowledge of the interaction between DCs and *Plasmodium* impacts directly on clinical developments.

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Executive summary

Introduction

- There are 500 million cases of malaria a year, with 1 million of these being fatal.
- The immune response contributes to disease etiology.
- Dendritic cells are central players in immune responses.

Background on dendritic cells

- Dendritic cells (DCs) are phagocytic antigen-presenting cells.
- DCs are sentinels of immune system detecting pathogens.
- Myeloid and plasmacytoid types of DCs exist.
- DCs direct the generation of specific or adaptive immune responses.

Immune response against malaria

- Immune responses may be protective or may mediate disease.
- Antibodies and T-cell responses are targets for vaccines.
- Functional DCs are required.

Dendritic cells & malaria

- DCs are suppressed by infected red blood cells via CD36.
- DCs are activated and suppressed by pigment byproduct hemozoin.
- Low parasite dose causes DC activation, and high parasite dose causes DC suppression.
- High parasite dose may cause a 'regulatory' DC phenotype and DC apoptosis.

Conclusion & future perspective

- DC flexibility allows immune activation and suppression required for host survival.
- Parasite density controlled by drugs may enhance natural immunity or that generated by vaccines.
- Despite their obvious value, we should avoid over-reliance on *in vitro* and murine studies.
- It will take some time before appropriate human studies can impact on clinical developments.

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