Immune activation and induction of memory: lessons learned from controlled human malaria infection with *Plasmodium falciparum*

ANJA SCHOLZEN* *and* ROBERT W. SAUERWEIN*

Department of Medical Microbiology, Radboud university medical center, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

(*Received 4 April 2015; revised 28 May 2015; accepted 31 May 2015; first published online 7 January 2016*)

SUMMARY

Controlled human malaria infections (CHMIs) are a powerful tool to assess the efficacy of drugs and/or vaccine candidates, but also to study anti-malarial immune responses at well-defined time points after infection. In this review, we discuss the insights that CHMI trials have provided into early immune activation and regulation during acute infection, and the capacity to induce and maintain immunological memory. Importantly, these studies show that a single infection is sufficient to induce long-lasting parasite-specific T- and B-cell memory responses, and suggest that blood-stage induced regulatory responses can limit inflammation both in ongoing and potentially future infections. As future perspective of investigation in CHMIs, we discuss the role of innate cell subsets, the interplay between innate and adaptive immune activation and the potential modulation of these responses after natural pre-exposure.

Key words: Malaria, *Plasmodium falciparum*, controlled human infection, sporozoite, blood stage, immune memory, activation, regulation, innate immunity, adaptive immune response.

INTRODUCTION

Malaria remains one of the most widespread and mortality-causing human infectious diseases worldwide (WHO, 2013), despite major and partially successful control efforts (Feachem *et al*. 2010). A vaccine effectively preventing infection, disease and/or transmission would be an important tool in control and eradication of the mosquito-transmitted parasite, but to date remains elusive (Riley and Stewart, 2013). One major hurdle remains our incomplete understanding of the development, regulation and maintenance of immunity to malaria (Struik and Riley, 2004; Erdman *et al*. 2008; Langhorne *et al*. 2008).

The vast majority of research into anti-malarial immune responses has been conducted using either murine models of malaria infection (Stephens *et al*. 2012) or samples derived from naturally exposed individuals. Murine models allow a careful dissection of immune responses in all organs, at well-defined stages of infection, and in the context of different disease manifestations depending on the combination of parasite strain and murine host (Lovegrove *et al*. 2006; Nduati *et al*. 2010; Nganou-Makamdop *et al*. 2012; Frevert *et al*. 2014; Gun *et al*. 2014). At the same time, however, these murine models present non-natural pathogen–host combinations (Druilhe *et al*. 2002), and conclusions from murine models are often not transferable to the human situation (Mestas and Hughes, 2004). One major limitation of field-based studies is the unknown timing of exposure, which precludes analysis of immune responses at defined time points after infection. Other potential confounders, especially for people residing in rather than just travelling into malaria endemic areas, are the influence of unknown previous exposure as well as the multitude of potential co-infections or morbidities.

A third approach to complement investigations of anti-malarial immune responses in humans is the deliberate exposure of human subjects to *Plasmodium* parasites. Infection with *Plasmodium* parasites was used in the 1920s–1960s as a tool to treat syphilis, prior to the availability of antibiotics (Snounou and Perignon, 2013). Retrospective analysis of those patients has provided first evidence for the fact that anti-parasite and anti-disease immunity (tolerance) develop within a single infection (Collins and Jeffery, 1999*c*). This acquired immunity reduced parasitaemia and clinical symptoms in a repeated exposure to both homologous and heterologous parasite strains, but less so to different *Plasmodium* species (Collins and Jeffery, 1999*a*, *b*; Molineaux *et al*. 2002; Collins *et al*. 2004). In recent decades, deliberate exposure of volunteers in so-called controlled human malaria infections (CHMIs) (Fig. 1) has become an indispensable tool in clinical malaria

* Corresponding authors. Department of Medical Microbiology, Radboud university medical center, Route 268, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands. Tel: +31 24 3614306. Fax: +31 24 3614666. E-mail: anja.scholzen@radboudumc.nl; robert.sauerwein@radboudumc.nl

Parasitology (2016), **143**, 224–235.
doi:10.1017/S0031182015000761

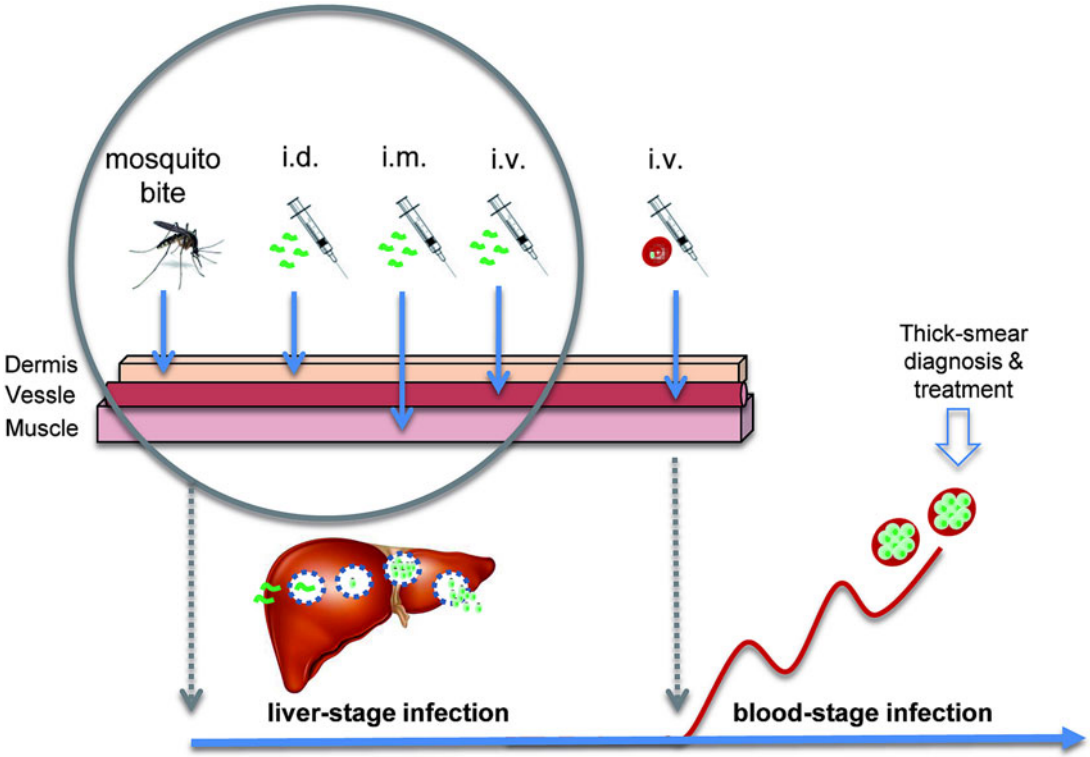

Fig. 1. CHMI is initiated by inoculation of *Plasmodium* sporozoites via the mosquito vector, injection of cryopreserved sporozoites via different routes of inoculation, or by intravenous injection of blood-stage parasites. Following a week of clinically silent replication in the liver, blood-stage parasites are monitored at least daily by quantitative polymerase chain reaction (qPCR) or microscopy. When a certain threshold of parasitaemia is reached, volunteers are drug-treated to eliminate parasites. CHMI, controlled human malaria infection.

research. CHMI can be initiated by inoculation of *Plasmodium* sporozoites via the mosquito vector; this system has been set up for *Plasmodium falciparum* in multiple centres in Europe and the USA (Chulay *et al*. 1986; Herrington *et al*. 1988; Verhage *et al*. 2005; Epstein *et al*. 2007; Talley *et al*. 2014) and recently also for *Plasmodium vivax* in Columbia (Herrera *et al*. 2009, 2011). More recently, cryopreserved *P. falciparum* sporozoites have become available for parenteral injection into dermis, muscle (Roestenberg *et al*. 2013; Sheehy *et al*. 2013) or directly into the circulation (Clinicaltrials.gov identified NCT01624961). Alternatively, asexual blood-stage parasites can be inoculated by intravenous injection (Rzepczyk *et al*. 1996; McCarthy *et al*. 2011). The primary outcome of most CHMI trials is success of blood-stage infection and, following on from this, assessment of drug or vaccine efficacy (Sauerwein *et al*. 2011; Duncan and Draper, 2012; Engwerda *et al*. 2012). Additionally, those studies provide unique opportunities to gain insights into primary immune responses, with distinct advantages over similar investigations in natural infections (Box 1). CHMI enables longitudinal analysis of samples at defined time points before, during and after infection in a well characterized, small cohort. This allows a detailed dissection of early immune activation, regulation and priming. In this review, these findings are discussed in view of both (i) the contribution of different cell types to immune activation and regulation during and beyond acute infection and (ii) the induction and capacity to maintain parasite-specific immune memory.

EARLY IMMUNE ACTIVATION AND REGULATION AFTER PRIMARY INFECTION

Clinically silent, liver-stage infection in mice has thus far only been shown to initiate local innate type I interferon-driven responses (Liehl *et al*. 2014; Miller *et al*. 2014). In contrast, circulating blood-stage parasites produce ligands such as glycosylphosphatidylinositol anchors, DNA or hemozoin, bound to host fibrinogen or parasite DNA, that activate a multitude of toll-like receptors (TLRs) and other pattern recognition receptors and initiate a systemic type II interferon-directed pro-inflammatory response (Gun *et al*. 2014). This initial reaction to the parasite has a dual function: it mediates the immediate response to the parasite to control infection, and directs the subsequent adaptive response.

Early innate cytokine production

Serum cytokine profiling and transcriptional analysis at defined time points during primary CHMI has

https://doi.org/10.1017/S0031182015000761 Published online by Cambridge University Press

Box 1. Immunological evaluation after CHMIs compared with natural infections

Advantages

- **Host population are healthy individuals who undergo pre-infection screening, which allows detailed knowledge of potential pre-exposure (e.g. based on travel history, but also serology) and co-morbidities during CHMI.**
- **Fit-for-purpose model to focus investigation on particular life-cycle stages by inoculation via the natural route or with defined doses of sporozoites or blood-stage parasites.**
- **Single infection with well characterized parasite clone/strain over fixed period of time; no multiplicity of infection.**
- **Polymerase chain reaction (PCR) monitoring allows correlation of immunological outcomes with parasite load.**
- **Availability of baseline/pre-infection samples and ability to sample blood at defined time points during and post-infection, including liver-stage.**

Limitations

- **Restricted to adults, while in endemic countries malaria is first encountered during childhood.**
- **Limited blood-stage exposure due to obligatory early drug treatment at relatively low parasitaemia compared with endemic situation.**
- **Current inoculation protocols result in different dynamics of infection than during a natural exposure (once off exposure to multiple infectious mosquito bites *vs* continuous or infrequent exposure to less bites).**

consistently shown increased levels of the type II interferon IFNγ shortly after the onset blood-stage infection and prior to development of symptoms (Fig. 2a) (Harpaz *et al*. 1992; Hermsen *et al*. 2003; Walther *et al*. 2005; Ockenhouse *et al*. 2006; Bijker *et al*. 2013; Elias *et al*. 2014; Scholzen *et al*. 2014). The IFNγ-induced chemokine CXCL9 has been detected in the vast majority of volunteers examined, and correlated with IFNγ production (Bijker *et al*. 2013; Elias *et al*. 2014). In contrast, secretion or transcription of other cytokines including IL-1α, IL-1β, TNFα, IL-6, IL-8, IL-12p40, IL-12p70, TGFβ and IL-10 have been detected only in a fraction of volunteers (Harpaz *et al*. 1992; Hermsen *et al*. 2003; Walther *et al*. 2005; Ockenhouse *et al*. 2006; De Mast *et al*. 2008; de Mast *et al*. 2009*b*; Elias *et al*. 2014). This suggests heterogeneity in the innate responses to the malaria parasite, in line with inter-individual differences in the ability of naïve individuals to control *Plasmodium* blood-stage infections, as evidenced in malariotherapy studies conducted in the 1940–1960s (Molineaux *et al*. 2002). Walther *et al*. showed that malaria-naïve volunteers can respond to CHMI in two different manners: (i) rapid pro-inflammatory cytokine production in association with more rapid parasite control but linked with more severe clinical symptoms or (ii) early immune-suppressive TGFβ production associated with weaker parasite control but fewer clinical symptoms (Fig. 2b) (Walther *et al*. 2005, 2006). In addition, acute blood-stage during primary CHMI has been shown to modulate responsiveness to TLR ligands and increase inflammatory responsiveness to the parasite itself (McCall *et al*. 2007). In this study, TLR priming during CHMI correlated with fever and levels of the inflammation-induced, liver-derived C-reactive protein (CRP) (McCall *et al*. 2007). These combined findings suggest that some volunteers appear to be more susceptible in exacerbating their inflammatory response to the parasite.

Innate immune cell activation during CHMI

The cellular basis of these differences in innate responses might relate to heterogeneity in distribution of innate immune cell subsets such as monocytes, natural killer (NK)- and γδT-cells. Rapid production of IFNγ during CHMI (Harpaz *et al*. 1992; Hermsen *et al*. 2003; Walther *et al*. 2005; Ockenhouse *et al*. 2006; Bijker *et al*. 2013; Elias *et al*. 2014; Scholzen *et al*. 2014) is likely linked to NK- and γδT-cells, which are also the prime producers of IFNγ in response to *P. falciparum*-infected red blood cells (PfRBCs) *in vitro* (Fig. 2c) (Korbel *et al*. 2005; D'Ombrain *et al*. 2007; Teirlinck *et al*. 2011, 2013; Obiero *et al*. 2015). Another feature of *in vitro* PfRBC-stimulated NK cells is de-granulation and granzyme production (Korbel *et al*. 2005). And while a sequential increase in plasma granyzmes A and B has also been shown during CHMI (Hermsen *et al*. 2003), the source of these cytotoxic mediatos *in vivo* remains to be determined.

γδT-cells are activated during blood-stage infection after CHMI, as shown by their sequential up-regulation of the activation markers CD69 and human leukocyte antigen (HLA)-DR, as well as CD45RO, which marks transition to a memory phenotype (Rzepczyk *et al*. 1996). At least *in vitro*, NK- and γδT-cells are not activated uniformly; instead activation appears to be linked to individual subsets and is influenced by differences in

https://doi.org/10.1017/S0031182015000761 Published online by Cambridge University Press

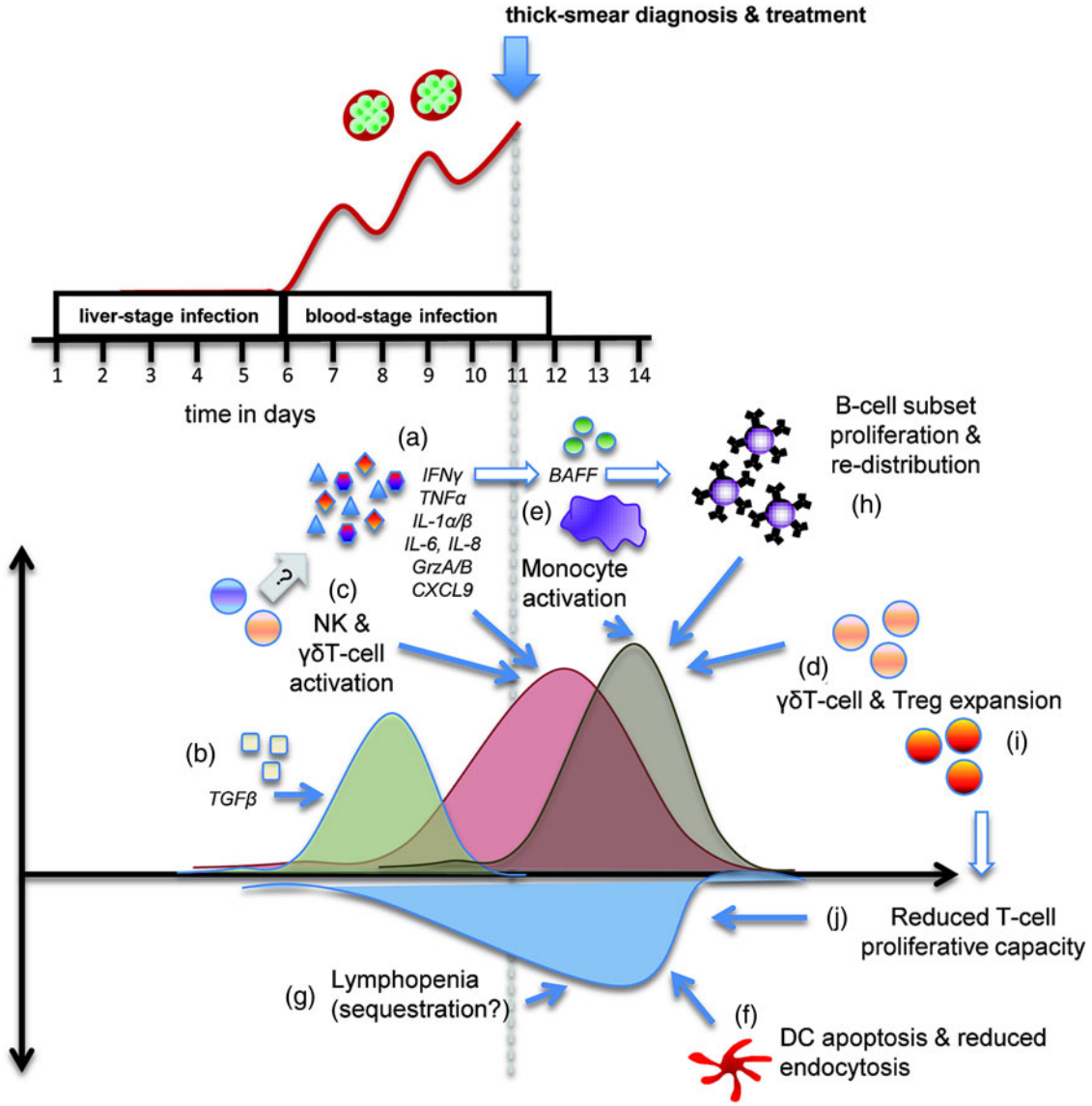

Fig. 2. Early immune activation during a primary acute blood-stage infection. In the days after emergence from the liver, blood-stage parasite exposure during CHMI results in a sequence of events of immune activation. Pro-inflammatory cytokines are released (a); likely by innate cells including NK-cells, γδT-cells (c) and monocytes. Pro-inflammatory cytokine release can be preceded by release of TGFβ (b), which limits the ensuing pro-inflammatory response. Cytokines such as IFNγ activate monocytes, which release BAFF (e). Elevated BAFF levels are associated with B-cell re-distribution (h). While absolute lymphocyte numbers decrease during acute infection (g), individual B-cell subsets such as plasma blasts, classical and atypical MBC (h), γδT-cells (d) and Tregs (i) proliferate and expand. Treg activity appears to contribute to the reduced proliferative capacity of T-cells during acute infection (j). DCs show transiently enhanced apoptosis and reduced endocytic capacity (f).BAFF, B-cell activating factor; CHMI, controlled human malaria infection; DCs, dendritic cells; MBC, memory B-cells, Tregs, regulatory T-cells

NK receptor expression or polymorphisms of killer Ig-like receptors (Artavanis-Tsakonas *et al*. 2003; Korbel *et al*. 2005; D'Ombrain *et al*. 2007; McCall *et al*. 2010; Obiero *et al*. 2015). Differential activation of individual NK- or γδT-cells subsets *in vivo* during CHMI, however, has not been examined to date. Equally unexplored is the contribution of so-called innate lymphoid cells, which can direct innate and adaptive responses, to the early inflammatory response during CHMI (Artis and Spits, 2015).

Of note, activation of the innate compartment not only occurs during acute infection, but also appears to extend beyond a primary CHMI, illustrated by sustained expansion of the γδT-cell compartment several weeks after drug cure (Rzepczyk *et al*. 1996; Teirlinck *et al*. 2011; Obiero *et al*. 2015), and even up to over a year after a single infection (Fig. 2d) (Teirlinck *et al*. 2011). Such as expansion of the γδT-cell compartment has also been shown after recovery from a single naturally acquired infection in travellers to malaria endemic areas (Martini *et al*. 2003). It remains unclear whether this expansion is polyclonal or restricted to specific γδT-cell subsets. That expansion is not only observed after blood-

https://doi.org/10.1017/S0031182015000761 Published online by Cambridge University Press

stage infection induced by inoculation with asexual parasite (Rzepczyk *et al*. 1996) or fully infectious sporozoites (Teirlinck *et al*. 2011; Obiero *et al*. 2015), but also after (repeated) injection of irradiated sporozoites (Seder *et al*. 2013) suggests that both life-cycle stages contribute to γδT-cell expansion. Moreover, both γδT- and NK-cells show memory-like enhanced IFNγ responses upon re-stimulation with PfRBC *in vitro* at several weeks after parasite clearance (Fig. 3a) (McCall *et al*. 2010; Teirlinck *et al*. 2011; Obiero *et al*. 2015) At least in NK-cells, this seems to depend on enhanced secretion of T-cell derived cytokines such as IL-2 (Horowitz *et al*. 2010; McCall *et al*. 2010).

Monocytes come in different 'flavours' (Mitchell *et al*. 2014), and all the three main monocytes subsets become activated after CHMI, as evidenced by enhanced BAFF production. (Fig. 2e) (Scholzen *et al*. 2014). Monocyte activation could be mediated directly by the parasite via pattern recognition receptors, induced cytokines such as IFNγ, but also by anaphylotoxins such as C5a resulting from complement activation (Conroy *et al*. 2009), as observed in CHMI (Roestenberg *et al*. 2007). Monocytes are the main TGFβ-producing PBMC subset during CHMI and TGFβ-positive monocytes are particularly increased in those individuals with a more balanced, less inflammatory response (Walther *et al*. 2005). It remains to be determined which monocyte subset is responsible for this regulatory response, and which subset mediates pro-inflammatory priming as proposed previously (McCall *et al*. 2007).

Monocytes as well as dendritic cells (DCs) are important mediators for both innate and adaptive immune responses. All monocytes subsets as well as blood dendritic cell antigen (BDCA)-1+ DCs increase B-cell activating factor (BAFF) expression during CHMI (Fig. 2e), suggesting that they contribute to directing B-cell activation or homeostasis during *P. falciparum* infection (Scholzen *et al*. 2014). On the other hand, circulating DCs showed increased apoptosis and decreased endocytic activity during early blood-stage infection (Fig. 2f) (Woodberry *et al*. 2012). This could indicate impaired DC function as suggested from murine and *in vitro* models and field work (Wykes and Good, 2008). Of note, reduced endocytic activity, at least when determined *in vitro*, is a typical consequence of DC maturation (Liu and Roche, 2015), and as an important mechanism to prevent immunopathology, DCs increase their susceptible to major histocompatibility complex (MHC) class II and type I interferon-driven apoptosis upon activation and maturation (Bertho *et al*. 2002; Kushwah and Hu, 2010; Fuertes Marraco *et al*. 2011). Therefore, apoptosis and reduced endocytosis during CHMI might also simply be a consequence of parasite-induced DC maturation. Successful parasite-induced DC activation would also be in line with

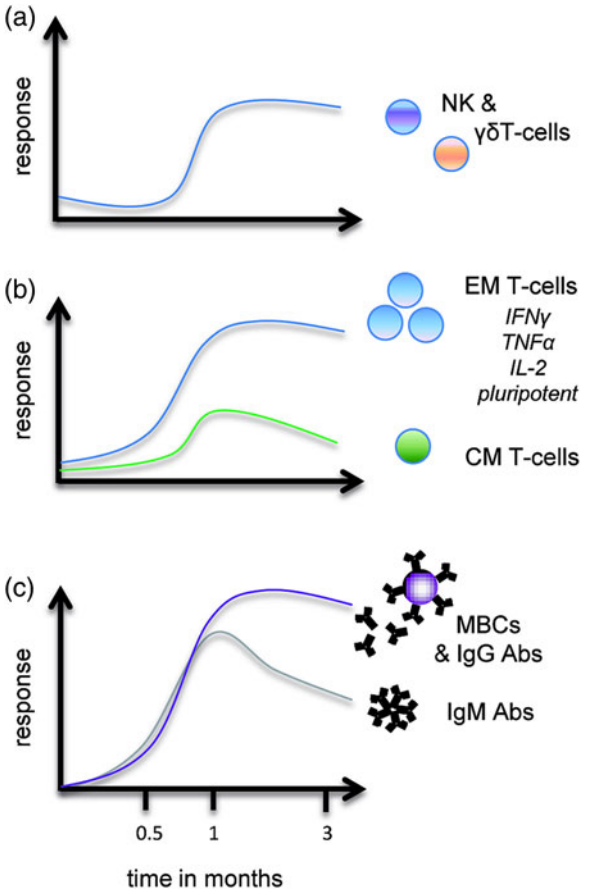

Fig. 3. Maintenance of parasite-specific memory responses after a resolved, primary CHMI. Following a single controlled *P. falciparum* infection, immune memory is induced and maintained in multiple compartments. This includes memory-like enhanced IFNγ production by innate lymphocytes such as NK- and γδT-cells (a). Single- and pluripotent EM T-cells are induced at a greater magnitude and maintained more stable than CM T-cell responses (b). Parasite-specific antibodies and MBC are also readily induced, and IgG responses are more stable than IgM responses (c).CM, central memory; CHMI, controlled human malaria infection; EM, effector memory; IFN, *interferon;* MBC, memory B-cells.

the successful priming of parasite-specific T-cell responses, as outlined below (Walther *et al*. 2005; Todryk *et al*. 2009; Teirlinck *et al*. 2011, 2013; Orlov *et al*. 2012; Elias *et al*. 2013; Walker *et al*. 2014). The basically unaltered expression of HLA-DR on circulating myeloid DCs as well as the slight reduction on plasmacytoid DCs (Woodberry *et al*. 2012) is puzzling in this context. A possible explanation could be that parasite activated DCs might preferentially migrate out of the circulation, as suggested previously based on field and *in vitro* data (Pichyangkul *et al*. 2004). Taken together, whether *Plasmodium* infection in humans induces or impairs DC activation and functionality and how this affects T-cell priming remains an important question for future investigations.

https://doi.org/10.1017/S0031182015000761 Published online by Cambridge University Press

Infection-induced lymphocyte sequestration

In parallel with emerging parasitaemia and immune activation, transient lymphopenia has been consistently observed in the early phases of a blood-stage infection (Fig. 2g) (Rzepczyk *et al*. 1996; Church *et al*. 1997; De Mast *et al*. 2008; Woodberry *et al*. 2012; Scholzen *et al*. 2014). A likely explanation is sequestration (Rzepczyk *et al*. 1996), since lymphocyte subset numbers recover shortly after treatment (Woodberry *et al*. 2012). Increased secretion of the chemokine CXCL9 (Ockenhouse *et al*. 2006; Bijker *et al*. 2013; Elias *et al*. 2014) and a selective reduction in circulating B-cell populations expressing the CXCL9 receptor CXCR3 during acute blood-stage (Elias *et al*. 2014) corroborate this explanation. Within the B-cell compartment, changes in proportions of different B-cell subsets could further be linked to their degree of expression of the receptor for BAFF (Fig. 2h) (Scholzen *et al*. 2014), a cytokine that has previously been shown to alter the responsiveness of B-cells for chemotactic stimuli (Badr *et al*. 2008). Whether in other lymphocyte populations, subsets with specific chemokine receptor signatures are equally transiently decreased has not yet been investigated.

CHMI-induced T-cell regulation

Induction of regulatory T-cells (Tregs) is a well-reported consequence of blood-stage infection in field settings and model systems (Scholzen *et al*. 2010) and also evident during CHMI. Ten days after sporozoite inoculation, when blood-stage parasites are usually detected by microscopy and volunteers treated, expression of the Treg transcription factor Foxp3 was increased, correlating with an increase in circulating bioactive TGFβ (Walther *et al*. 2005). CD4 + CD25hiCD69-Tregs were expanded (Fig. 2i) and mediated suppression of parasite-specific responses during infection (Fig. 2j) (Walther *et al*. 2005). That loss of lymphocyte proliferative capacity could be restored by addition of IL-2 (Rzepczyk *et al*. 1996) suggests that parasite-induced Tregs might function by competition with effector T-cells for IL-2 (Hofer *et al*. 2012). Further data indicate that this parasite-specific regulatory response can extend beyond acute infection; while 4 weeks after CHMI, *in vitro* CD4 and CD8 T-cell responses to unrelated antigens were unaffected as such, they were reduced when PfRBC were added to the assay (Todryk *et al*. 2009), indicating a suppressive function of parasite antigen re-called Tregs. The longevity of this regulatory response and life-cycle stage specificity of these Tregs, and therefore their potential impact on subsequent liver- and/or blood-stage infections, remain elusive.

Link between immune activation and parasitaemia

Immune activation and regulation during the early stage of blood-stage infection appear to be dependent directly on duration of exposure and parasite load: after CHMI, peak inflammatory cytokine production correlated with the length of time until a defined level of parasitaemia was reached (Walther *et al*. 2006), and expansion of CD4 + CD25hiCD69-Tregs was the more prominent the earlier parasites reached the threshold of detection (Walther *et al*. 2005). Moreover, peak parasitaemia correlated with subsequent maximum plasma levels of IFNγ and BAFF as well as proliferation of various B-cell subsets including plasma blast, classical and atypical memory B-cells (MBCs) (Fig. 2h) (Scholzen *et al*. 2014). While no causal relationships can be directly inferred from such correlations, parasite-driven induction and expansion of Tregs, IFNγ and BAFF production, and B-cell proliferation is consistent with findings from *in vitro* studies using PfRBC stimulated peripheral blood mononuclear cells (PBMCs) (Donati *et al*. 2004; Scholzen *et al*. 2009; Kumsiri *et al*. 2010).

Already at the time of first diagnosis, CHMI volunteers show similar pattern recognition receptor and pro-inflammatory cytokine gene transcription as naturally exposed individuals at more advanced stages of infection (Ockenhouse *et al*. 2006), while transcription of stress-related heat-shock proteins and other markers of inflammation as well as the induction of (counter)-regulatory pathways are much less pronounced (Ockenhouse *et al*. 2006). Markers and signs of inflammation and innate activation then substantially increase in the days after treatment. This includes the frequency of symptoms, fever (Church *et al*. 1997) and changes in haematological parameters (Church *et al*. 1997; de Mast *et al*. 2007, 2008, 2009*a*, *b*; Roestenberg *et al*. 2007) as well as production of the acute-phase protein CRP (Harpaz *et al*. 1992; Hermsen *et al*. 2003), complement activation (Roestenberg *et al*. 2007), cytokine secretion (Hermsen *et al*. 2003), B-cell, monocyte and DC activation (Scholzen *et al*. 2014). Since this is found across different treatment regimes, the most likely explanation is that the abrupt release of material from drug-killed and otherwise sequestered mature blood-stage parasites into the circulation leads to an initial exacerbation of immune activation, before all parasite material is finally removed.

INDUCTION AND MAINTENANCE OF ANTI-MALARIAL IMMUNE MEMORY

Priming of parasite-specific T-cell memory

Transcriptional analysis of PBMCs from CHMI volunteers provides circumstantial evidence for the initiation of adaptive immune responses already during the pre-patent period (Ockenhouse *et al*.

https://doi.org/10.1017/S0031182015000761 Published online by Cambridge University Press

2006). This includes (i) increased transcription of Fc receptors such as CD16, which can enhance antigen capture (Dobel *et al*. 2013), (ii) genes involved in antigen processing and presentation such as subunits of the immune proteasome, chaperones involved in MHC class I peptide loading and MHC class II molecules and (iii) glycolytic enzymes, which mark the metabolic switch from naive/resting to effector responses (Yang and Chi, 2012). Consistently, parasite-specific T-cell responses are induced after a single, primary CHMI (Fig. 2k) (Walther *et al*. 2005; Todryk *et al*. 2009; Teirlinck *et al*. 2011, 2013; Orlov *et al*. 2012; Elias *et al*. 2013; Walker *et al*. 2014). Parasite-specific responses are consistently detectable using intact and lysed blood-stage schizonts (Walther *et al*. 2005; Todryk *et al*. 2009; Teirlinck *et al*. 2011, 2013; Orlov *et al*. 2012; Elias *et al*. 2013; Walker *et al*. 2014) or sporozoites as a stimulus (Teirlinck *et al*. 2011). Responses to individual parasite antigens appear to be variable between CHMI studies: some detect peptide-specific responses to apical membrane protein (AMA)-1 and merozoite surface protein (MSP)-1 (Elias *et al*. 2013; Walker *et al*. 2014), while others fail to do for entire panels of sporozoite, liver- and blood-stage antigens (Todryk *et al*. 2009; Teirlinck *et al*. 2011). Relatively low frequency of T-cells specific for individual antigens may explain this discrepancy. Antigen-specific T-cell responses appear largely allele-specific for the challenge strain (Elias *et al*. 2013), but also show potential for cross-reactivity (Elias *et al*. 2013; Teirlinck *et al*. 2013).

Memory phenotype and cytokine profile of parasite-specific T-cells

Circulating memory T-cells can broadly be divided into central memory (CM) T-cells that can home to secondary lymphoid organs and largely lack immediate effector function, and effector memory (EM) T-cells with an altered chemokine receptor profile that preferentially home to non-lymphoid tissues (Sallusto *et al*. 2004). The more recently described tissue resident memory cells do not recirculate (Schenkel and Masopust, 2014) and can thus not easily be examined in human subjects. Parasite-specific T-cell responses measured in peripheral blood after a single CHMI are dominated by EM T-cells (Todryk *et al*. 2009; Teirlinck *et al*. 2011) and surprisingly stable for over at least a year (Teirlinck *et al*. 2011). These T-cell responses are largely of Th1 type origin, including secretion of IFNγ, TNFα, IL-2 and MIP-1α (Walther *et al*. 2005; Teirlinck *et al*. 2011, 2013; Orlov *et al*. 2012), while only few studies were able to detect parasite-specific IL-4 production (Todryk *et al*. 2009; Walker *et al*. 2014). In contrast to EM T-cells, CM responses are considerably weaker (Todryk *et al*. 2009; Teirlinck *et al*. 2011), do not correlate in magnitude with EM responses and appear to decline much faster than EM responses (Fig. 3b) (Todryk *et al*. 2009). This weak CM response might simply be due to preferential re-location of CM cells to lymphoid tissues (Brinkman *et al*. 2013). Alternatively, parasite-induced regulatory mechanisms may contribute since high parasite densities associated with lower CM, but not EM, responses at 4 and 12 weeks post-CHMI (Todryk *et al*. 2009). Moreover, the degree of Foxp3 expression during CHMI negatively correlated with IFNγ EM responses as late as 5 months post-CHMI (Todryk *et al*. 2009). Therefore, not just activation and priming, but also counter-regulation of effector T-cell responses is a consequence of a single *P. falciparum* infection.

The effect of repeated parasitaemia on T-cell memory is less well examined. Cohorts of Dutch and Tanzanian volunteers subjected to CHMI under very similar conditions (Obiero *et al*. 2015) showed a peculiar lack of parasite-specific IFNγ re-call responses in pre-exposed individuals, that failed to increase after CHMI (Obiero *et al*. 2015). Additionally, innate IFNγ responses in pre-exposed Tanzanians were also lower (Obiero *et al*. 2015). Since IFNγ production by innate lymphocytes such as NK-cells is at least partially dependent on T-cell derived cytokines such as IL-2 (Horowitz *et al*. 2010; McCall *et al*. 2010), the reduced responsiveness of adaptive T-cells may be one underlying reason of this lower innate response to the parasite. While no differences in regulatory T-cell levels were found that might also explain these findings, functionality and potential parasite antigen-specific enrichment of Tregs was not examined. Clearly, future studies are needed to investigate whether this difference in both innate and adaptive compartments may be due for instance to skewing to immune signatures other than Th1-type responses. Rather than being a sign of impairment, a more balanced, less Th1 driven cytokine profile may even be beneficial for the human host, since elevated anti-parasite IFNγ responses due to priming in earlier encounters associate with earlier clinical symptoms during blood-stage infection (Bijker *et al*. 2013). This would especially be true when in parallel with a less pronounced Th1 cytokine response – as in the above described Tanzanian CHMI cohort (Obiero *et al*. 2015) – other effector mechanisms, such as parasite-specific antibodies that might help to control parasitaemia, are also present.

Generation of antibody and MBC responses

A single CHMI clearly induces production of parasite-specific antibodies, directed against sporozoite and liver-stage antigens as well as the cross-stage antigen MSP-1 (Biswas *et al*. 2014; Elias *et al*. 2014; Nahrendorf *et al*. 2014; Walker *et al*.

https://doi.org/10.1017/S0031182015000761 Published online by Cambridge University Press

2014; Obiero *et al*. 2015). That a single infection is sufficient to induce parasite-specific antibody responses in a life-cycle exposure dependent manner is consistent with studies of travellers to malaria endemic countries: while recognition of blood-stage antigens or PfRBCs is usually limited to those travellers who experienced a symptomatic or asymptomatic malaria episode (Jelinek *et al*. 1995; Cobelens *et al*. 1998; Seed *et al*. 2006), seroconversion to circumsporozoite protein (CSP) can also be found in a fraction of those that were protected from blood-stage infection by chemoprophylaxis (Cobelens *et al*. 1998; Jelinek *et al*. 1998; Molle *et al*. 1999; Nothdurft *et al*. 1999; Knappik *et al*. 2002; Belderok *et al*. 2013). Also similar to a first naturally acquired infection in previously naive travellers (Elliott *et al*. 2007), CHMI generates antibody responses to multiple *P. falciparum* erythrocyte membrane protein-1 alleles (Turner *et al*. 2011), likely because multiple var genes can be transcribed simultaneously (Peters *et al*. 2002; Lavstsen *et al*. 2005; Wang *et al*. 2009). In addition, antibody maturation occurs: initially, both MSP-1_{19}-specific IgM and IgG titers increase, followed by a quick decline in IgM levels, while IgG titres are maintained with increasing avidity (Fig. 3c) (Walker *et al*. 2014). B-cell isotype switching is directed by follicular helper T-cells (McHeyzer-Williams *et al*. 2012), which although mainly confined to lymphoid tissue follicles, can also appear in the circulation (Locci *et al*. 2013; Boswell *et al*. 2014). The first attempt to associate CHMI-induced T- and B-cell responses showed an inverse correlation of MSP-1 IgG responses with parasite-specific T-cell re-call proliferation and IFNγ production, but the exact phenotype of these cells and typical follicular helper T-cell cytokine production was not investigated (Walker *et al*. 2014). Clearly, the induction and role of T-cell help for humoral responses in malaria requires further investigation. Next to antibodies, specific MBC responses to CSP and MSP-1 can be directly measured in PBMCs (Fig. 3c) (Elias *et al*. 2014; Nahrendorf *et al*. 2014). The magnitude of MSP-1_{19} specific antibody and MBC responses after a primary CHMI correlates with the degree of parasite exposure, determined by both duration and magnitude of blood-stage infection (Biswas *et al*. 2014; Elias *et al*. 2014; Walker *et al*. 2014). This exposure-dependency of humoral responses is also consistent with similar findings after repeated CHMI (Nahrendorf *et al*. 2014).

Effect of blood-stage exposure on maintenance of humoral immune responses

There is a general notion that blood-stage parasites deregulate B-cell function, with negative effects on the maintenance of B-cell memory and antibody responses (Portugal *et al*. 2013; Scholzen and Sauerwein, 2013). However, CHMI data indicate that blood-stage parasite exposure has *per se* no negative impact on B-cell memory: despite transient loss of B-cells from the circulation during CHMI-induced acute blood-stage infection (Rzepczyk *et al*. 1996; Elias *et al*. 2014; Scholzen *et al*. 2014), previously vaccine- or whole sporozoite-induced antibody and MBC responses to liver- and cross-stage antigens were maintained rather than reduced after CHMI (Biswas *et al*. 2014; Elias *et al*. 2014; Nahrendorf *et al*. 2014). Blood-stage exposure during CHMI has further no negative impact on specific antibody avidity or functionality (Biswas *et al*. 2014), and can even boost previous experimentally or naturally induced responses by 2–10-fold (Biswas *et al*. 2014; Nahrendorf *et al*. 2014; Obiero *et al*. 2015). Of course, due to rapid treatment upon diagnosis, maximum densities of parasitaemia after CHMI range from only 1 to 100 parasites/μL^{-1} (Roestenberg *et al*. 2012), i.e. much lower than in natural infections. Nevertheless, even a history of naturally acquired infections does not impair B-cell memory re-called by CHMI; pre-existing antibody responses in Tanzanian volunteers were readily detectable in a large proportion of volunteers and, compared with malaria-naïve Dutch individuals, increased more strongly upon CHMI. This occurred even in sero-negative Tanzanians, providing evidence for a robust MBC response that was stably maintained even in those individuals in which plasma blast-produced antibody levels dropped below detection (Obiero *et al*. 2015). Similarly, antibody responses were also boosted in *P. vivax*-challenged Colombian individuals (Arevalo-Herrera *et al*. 2014). Together, these CHMI data support that the notion that slow development of clinically protective B-cell responses to malaria might not relate to induction or maintenance of memory itself but rather the polymorphic nature of malarial antigens in the wide variety of genetically distinct field strains (Struik and Riley, 2004).

Concluding remarks

Taken together, CHMI trials have revealed that the early phase of a primary *P. falciparum* infection is characterized by immune cell activation, re-distribution and inflammatory cytokine production, which coincide with blood-stage infection and are related to the degree of parasitaemia. Heterogeneity in the early inflammatory response appears to relate to differential presentation of clinical symptoms. Moreover, parasite-specific T- and B-cell memory responses are readily induced by a single infection and maintained for prolonged periods of time in the absence of re-exposure. B-cell responses are not perturbed by low dose blood-stage re-exposure, while parasitaemia does show some regulatory influence on Th1 type T-cell memory, which

https://doi.org/10.1017/S0031182015000761 Published online by Cambridge University Press

might contribute to limiting inflammation in ongoing and future encounters. Based on these findings, questions arise regarding the role of individual cell subsets in the early inflammatory/regulatory response to the parasite, the induction of adaptive responses and the quality of the immune response in future infections (Box 2). An exciting new development in CHMI is the controlled re-exposure of individuals with a history of natural pre-exposure. Comparative analysis of malaria-naive and naturally exposed individuals has already provided some insights into the effect of pre-exposure on parasite-specific re-call responses. It is further a promising approach to dissect differences in innate and adaptive immune cell activation during acute infection, and may provide novel insights into malaria-associated immune modulation leading to altered disease susceptibility.

Box 2. Outstanding questions

- **Which roles do individual NK, γδT-cell or monocyte subsets, and potentially innate lymphoid cells, play in the early inflammatory and regulatory response to the parasite?**
- **(How) does the inter-individual heterogeneity of these innate responses influence the induction of adaptive immune activation?**
- **Does expansion during malaria alter the composition of the γδT-cell compartment, which parasite life-cycle stage mediates this expansion and which consequences does this have in subsequent infections?**
- **How efficiently are parasite-specific follicular helper T-cell responses generated and how do they contribute to humoral immune responses?**
- **How long-lived are parasite-induced Treg responses and by which life-cycle stage are they re-called in future infections?**
- **Does apparently reduced functionality of DCs during CHMI reflect impairment or simply activation of these cells, and how does this relate to antigen-specific T-cell priming?**
- **Does cellular immune activation during a primary infection differ from that in naturally exposed individuals?**
- **(How) does repeated blood-stage exposure during natural (or experimental) infection skew innate and adaptive responses away from Th1 type cytokine secretion, and does this contribute to disease tolerance?**

FINANCIAL SUPPORT

A. S. and R. W. S. are supported by the Bill and Melinda Gates Foundation (grants OPP1080385 and OPP1091355). This work was further supported by the FP7-founded European Virtual Institute of Malaria Research (EVIMalaR, grant 242095).

REFERENCES

Arevalo-Herrera, M., Forero-Pena, D. A., Rubiano, K., Gomez-Hincapie, J., Martinez, N. L., Lopez-Perez, M., Castellanos, A., Cespedes, N., Palacios, R., Onate, J. M. and Herrera, S. (2014). *Plasmodium vivax* sporozoite challenge in malaria-naive and semi-immune Colombian volunteers. *PLoS ONE* **9**, e99754.

Artavanis-Tsakonas, K., Eleme, K., McQueen, K. L., Cheng, N. W., Parham, P., Davis, D. M. and Riley, E. M. (2003). Activation of a subset of human NK cells upon contact with *Plasmodium falciparum*-infected erythrocytes. *Journal of Immunology* **171**, 5396–5405.

Artis, D. and Spits, H. (2015). The biology of innate lymphoid cells. *Nature* **517**, 293–301.

Badr, G., Borhis, G., Lefevre, E. A., Chaoul, N., Deshayes, F., Dessirier, V., Lapree, G., Tsapis, A. and Richard, Y. (2008). BAFF enhances chemotaxis of primary human B cells: a particular synergy between BAFF and CXCL13 on memory B cells. *Blood* **111**, 2744–2754.

Belderok, S. M., van den Hoek, A., Roeffen, W., Sauerwein, R. and Sonder, G. J. (2013). Adherence to chemoprophylaxis and *Plasmodium falciparum* anti-circumsporozoite seroconversion in a prospective cohort study of Dutch short-term travelers. *PLoS ONE* **8**, e56863.

Bertho, N., Blancheteau, V. M., Setterblad, N., Laupeze, B., Lord, J. M., Drenou, B., Amiot, L., Charron, D. J., Fauchet, R. and Mooney, N. (2002). MHC class II-mediated apoptosis of mature dendritic cells proceeds by activation of the protein kinase C-delta isoenzyme. *International Immunology* **14**, 935–942.

Bijker, E. M., Bastiaens, G. J., Teirlinck, A. C., van Gemert, G. J., Graumans, W., van de Vegte-Bolmer, M., Siebelink-Stoter, R., Arens, T., Teelen, K., Nahrendorf, W., Remarque, E. J., Roeffen, W., Jansens, A., Zimmerman, D., Vos, M., van Schaijk, B. C., Wiersma, J., van der Ven, A. J., de Mast, Q., van Lieshout, L., Verweij, J. J., Hermsen, C. C., Scholzen, A. and Sauerwein, R. W. (2013). Protection against malaria after immunization by chloroquine prophylaxis and sporozoites is mediated by preerythrocytic immunity. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 7862–7867.

Biswas, S., Choudhary, P., Elias, S. C., Miura, K., Milne, K. H., de Cassan, S. C., Collins, K. A., Halstead, F. D., Bliss, C. M., Ewer, K. J., Osier, F. H., Hodgson, S. H., Duncan, C. J., O'Hara, G. A., Long, C. A., Hill, A. V. and Draper, S. J. (2014). Assessment of humoral immune responses to blood-stage malaria antigens following ChAd63-MVA immunization, controlled human malaria infection and natural exposure. *PLoS ONE* **9**, e107903.

Boswell, K. L., Paris, R., Boritz, E., Ambrozak, D., Yamamoto, T., Darko, S., Wloka, K., Wheatley, A., Narpala, S., McDermott, A., Roederer, M., Haubrich, R., Connors, M., Ake, J., Douek, D. C., Kim, J., Petrovas, C. and Koup, R. A. (2014). Loss of circulating CD4 T cells with B cell helper function during chronic HIV infection. *PLoS Pathogens* **10**, e1003853.

Brinkman, C. C., Peske, J. D. and Engelhard, V. H. (2013). Peripheral tissue homing receptor control of naive, effector, and memory CD8 T cell localization in lymphoid and non-lymphoid tissues. *Frontiers in Immunology* **4**, 241.

Chulay, J. D., Schneider, I., Cosgriff, T. M., Hoffman, S. L., Ballou, W. R., Quakyi, I. A., Carter, R., Trosper, J. H. and Hockmeyer, W. T. (1986). Malaria transmitted to humans by mosquitoes infected from cultured *Plasmodium falciparum*. *American Journal of Tropical Medicine and Hygiene* **35**, 66–68.

Church, L. W., Le, T. P., Bryan, J. P., Gordon, D. M., Edelman, R., Fries, L., Davis, J. R., Herrington, D. A., Clyde, D. F., Shmuklarsky, M. J., Schneider, I., McGovern, T. W., Chulay, J. D., Ballou, W. R. and Hoffman, S. L. (1997). Clinical manifestations of *Plasmodium falciparum* malaria experimentally induced by mosquito challenge. *Journal of Infectious Diseases* **175**, 915–920.

Cobelens, F. G., Verhave, J. P., Leentvaar-Kuijpers, A. and Kager, P. A. (1998). Testing for anti-circumsporozoite and anti-blood-stage antibodies for epidemiologic assessment of *Plasmodium falciparum* infection in travelers. *American Journal of Tropical Medicine and Hygiene* **58**, 75–80.

https://doi.org/10.1017/S0031182015000761 Published online by Cambridge University Press

Collins, W. E. and Jeffery, G. M. (1999*a*). A retrospective examination of secondary sporozoite- and trophozoite-induced infections with *Plasmodium falciparum*: development of parasitologic and clinical immunity following secondary infection. *American Journal of Tropical Medicine and Hygiene* **61**, 20–35.

Collins, W. E. and Jeffery, G. M. (1999*b*). A retrospective examination of sporozoite- and trophozoite-induced infections with *Plasmodium falciparum* in patients previously infected with heterologous species of *Plasmodium*: effect on development of parasitologic and clinical immunity. *American Journal of Tropical Medicine and Hygiene* **61**, 36–43.

Collins, W. E. and Jeffery, G. M. (1999*c*). A retrospective examination of sporozoite- and trophozoite-induced infections with *Plasmodium falciparum*: development of parasitologic and clinical immunity during primary infection. *American Journal of Tropical Medicine and Hygiene* **61**, 4–19.

Collins, W. E., Jeffery, G. M. and Roberts, J. M. (2004). A retrospective examination of reinfection of humans with *Plasmodium vivax*. *American Journal of Tropical Medicine and Hygiene* **70**, 642–644.

Conroy, A., Serghides, L., Finney, C., Owino, S. O., Kumar, S., Gowda, D. C., Liles, W. C., Moore, J. M. and Kain, K. C. (2009). C5a enhances dysregulated inflammatory and angiogenic responses to malaria *in vitro*: potential implications for placental malaria. *PLoS ONE* **4**, e4953.

de Mast, Q., Groot, E., Lenting, P. J., de Groot, P. G., McCall, M., Sauerwein, R. W., Fijnheer, R. and van der Ven, A. (2007). Thrombocytopenia and release of activated von Willebrand factor during early *Plasmodium falciparum* malaria. *Journal of Infectious Diseases* **196**, 622–628.

De Mast, Q., Sweep, F. C., McCall, M., Geurts-Moespot, A., Hermsen, C., Calandra, T., Netea, M. G., Sauerwein, R. W. and van der Ven, A. J. (2008). A decrease of plasma macrophage migration inhibitory factor concentration is associated with lower numbers of circulating lymphocytes in experimental *Plasmodium falciparum* malaria. *Parasite Immunology* **30**, 133–138.

de Mast, Q., Nadjm, B., Reyburn, H., Kemna, E. H., Amos, B., Laarakkers, C. M., Silalye, S., Verhoef, H., Sauerwein, R. W., Swinkels, D. W. and van der Ven, A. J. (2009*a*). Assessment of urinary concentrations of hepcidin provides novel insight into disturbances in iron homeostasis during malarial infection. *Journal of Infectious Diseases* **199**, 253–262.

de Mast, Q., van Dongen-Lases, E. C., Swinkels, D. W., Nieman, A. E., Roestenberg, M., Druilhe, P., Arens, T. A., Luty, A. J., Hermsen, C. C., Sauerwein, R. W. and van der Ven, A. J. (2009*b*). Mild increases in serum hepcidin and interleukin-6 concentrations impair iron incorporation in haemoglobin during an experimental human malaria infection. *British Journal of Haematology* **145**, 657–664.

Dobel, T., Kunze, A., Babatz, J., Trankner, K., Ludwig, A., Schmitz, M., Enk, A. and Schakel, K. (2013). FcgammaRIII (CD16) equips immature 6-sulfo LacNAc-expressing dendritic cells (slanDCs) with a unique capacity to handle IgG-complexed antigens. *Blood* **121**, 3609–3618.

D'Ombrain, M. C., Hansen, D. S., Simpson, K. M. and Schofield, L. (2007). gammadelta-T cells expressing NK receptors predominate over NK cells and conventional T cells in the innate IFN-gamma response to *Plasmodium falciparum* malaria. *European Journal of Immunology* **37**, 1864–1873.

Donati, D., Zhang, L. P., Chene, A., Chen, Q., Flick, K., Nystrom, M., Wahlgren, M. and Bejarano, M. T. (2004). Identification of a polyclonal B-cell activator in *Plasmodium falciparum*. *Infection and Immunity* **72**, 5412–5418.

Druilhe, P., Hagan, P. and Rook, G. A. (2002). The importance of models of infection in the study of disease resistance. *Trends in Microbiology* **10**, S38–S46.

Duncan, C. J. and Draper, S. J. (2012). Controlled human blood stage malaria infection: current status and potential applications. *American Journal of Tropical Medicine and Hygiene* **86**, 561–565.

Elias, S. C., Collins, K. A., Halstead, F. D., Choudhary, P., Bliss, C. M., Ewer, K. J., Sheehy, S. H., Duncan, C. J., Biswas, S., Hill, A. V. and Draper, S. J. (2013). Assessment of immune interference, antagonism, and diversion following human immunization with biallelic blood-stage malaria viral-vectored vaccines and controlled malaria infection. *Journal of Immunology* **190**, 1135–1147.

Elias, S. C., Choudhary, P., de Cassan, S. C., Biswas, S., Collins, K. A., Halstead, F. D., Bliss, C. M., Ewer, K. J., Hodgson, S. H., Duncan, C. J., Hill, A. V. and Draper, S. J. (2014). Analysis of human B-cell responses following ChAd63-MVA MSP1 and AMA1 immunization and controlled malaria infection. *Immunology* **141**, 628–644.

Elliott, S. R., Payne, P. D., Duffy, M. F., Byrne, T. J., Tham, W. H., Rogerson, S. J., Brown, G. V. and Eisen, D. P. (2007). Antibody recognition of heterologous variant surface antigens after a single *Plasmodium falciparum* infection in previously naive adults. *American Journal of Tropical Medicine and Hygiene* **76**, 860–864.

Engwerda, C. R., Minigo, G., Amante, F. H. and McCarthy, J. S. (2012). Experimentally induced blood stage malaria infection as a tool for clinical research. *Trends in Parasitology* **28**, 515–521.

Epstein, J. E., Rao, S., Williams, F., Freilich, D., Luke, T., Sedegah, M., de la Vega, P., Sacci, J., Richie, T. L. and Hoffman, S. L. (2007). Safety and clinical outcome of experimental challenge of human volunteers with *Plasmodium falciparum*-infected mosquitoes: an update. *Journal of Infectious Diseases* **196**, 145–154.

Erdman, L. K., Finney, C. A., Liles, W. C. and Kain, K. C. (2008). Inflammatory pathways in malaria infection: TLRs share the stage with other components of innate immunity. *Molecular and Biochemical Parasitology* **162**, 105–111.

Feachem, R. G., Phillips, A. A., Hwang, J., Cotter, C., Wielgosz, B., Greenwood, B. M., Sabot, O., Rodriguez, M. H., Abeyasinghe, R. R., Ghebreyesus, T. A. and Snow, R. W. (2010). Shrinking the malaria map: progress and prospects. *Lancet* **376**, 1566–1578.

Frevert, U., Nacer, A., Cabrera, M., Movila, A. and Leberl, M. (2014). Imaging *Plasmodium* immunobiology in the liver, brain, and lung. *Parasitology International* **63**, 171–186.

Fuertes Marraco, S. A., Scott, C. L., Bouillet, P., Ives, A., Masina, S., Vremec, D., Jansen, E. S., O'Reilly, L. A., Schneider, P., Fasel, N., Shortman, K., Strasser, A. and Acha-Orbea, H. (2011). Type I interferon drives dendritic cell apoptosis via multiple BH3-only proteins following activation by PolyIC *in vivo*. *PLoS ONE* **6**, e20189.

Gun, S. Y., Claser, C., Tan, K. S. and Renia, L. (2014). Interferons and interferon regulatory factors in malaria. *Mediators of Inflammation* **2014**, 243713.

Harpaz, R., Edelman, R., Wasserman, S. S., Levine, M. M., Davis, J. R. and Sztein, M. B. (1992). Serum cytokine profiles in experimental human malaria. Relationship to protection and disease course after challenge. *Journal of Clinical Investigation* **90**, 515–523.

Hermsen, C. C., Konijnenberg, Y., Mulder, L., Loe, C., van Deuren, M., van der Meer, J. W., van Mierlo, G. J., Eling, W. M., Hack, C. E. and Sauerwein, R. W. (2003). Circulating concentrations of soluble granzyme A and B increase during natural and experimental *Plasmodium falciparum* infections. *Clinical and Experimental Immunology* **132**, 467–472.

Herrera, S., Fernandez, O., Manzano, M. R., Murrain, B., Vergara, J., Blanco, P., Palacios, R., Velez, J. D., Epstein, J. E., Chen-Mok, M., Reed, Z. H. and Arevalo-Herrera, M. (2009). Successful sporozoite challenge model in human volunteers with *Plasmodium vivax* strain derived from human donors. *American Journal of Tropical Medicine and Hygiene* **81**, 740–746.

Herrera, S., Solarte, Y., Jordan-Villegas, A., Echavarria, J. F., Rocha, L., Palacios, R., Ramirez, O., Velez, J. D., Epstein, J. E., Richie, T. L. and Arevalo-Herrera, M. (2011). Consistent safety and infectivity in sporozoite challenge model of *Plasmodium vivax* in malaria-naive human volunteers. *American Journal of Tropical Medicine and Hygiene* **84**, 4–11.

Herrington, D. A., Clyde, D. F., Murphy, J. R., Baqar, S., Levine, M. M., do Rosario, V. and Hollingdale, M. R. (1988). A model for *Plasmodium falciparum* sporozoite challenge and very early therapy of parasitaemia for efficacy studies of sporozoite vaccines. *Tropical and Geographical Medicine* **40**, 124–127.

Hofer, T., Krichevsky, O. and Altan-Bonnet, G. (2012). Competition for IL-2 between regulatory and effector T cells to chisel immune responses. *Frontiers in Immunology* **3**, 268.

Horowitz, A., Newman, K. C., Evans, J. H., Korbel, D. S., Davis, D. M. and Riley, E. M. (2010). Cross-talk between T cells and NK cells generates rapid effector responses to *Plasmodium falciparum*-infected erythrocytes. *Journal of Immunology* **184**, 6043–6052.

Jelinek, T., Nothdurft, H. D. and Loscher, T. (1995). Evaluation of circumsporozoite antibody testing as a sero-epidemiological tool for the detection of *Plasmodium falciparum* infection in non-immune travelers. *Tropical Medicine and Parasitology* **46**, 154–157.

Jelinek, T., Bluml, A., Loscher, T. and Nothdurft, H. D. (1998). Assessing the incidence of infection with *Plasmodium falciparum* among international travelers. *American Journal of Tropical Medicine and Hygiene* **59**, 35–37.

Knappik, M., Peyerl-Hoffmann, G. and Jelinek, T. (2002). *Plasmodium falciparum*: use of a NANP19 antibody-test for the detection of infection in non-immune travellers. *Tropical Medicine and International Health* **7**, 652–656.

Korbel, D. S., Newman, K. C., Almeida, C. R., Davis, D. M. and Riley, E. M. (2005). Heterogeneous human NK cell responses to

Plasmodium falciparum-infected erythrocytes. *Journal of Immunology* **175**, 7466–7473.

Kumsiri, R., Potup, P., Chotivanich, K., Petmitr, S., Kalambaheti, T. and Maneerat, Y. (2010). Blood stage *Plasmodium falciparum* antigens induce T cell independent immunoglobulin production via B cell activation factor of the TNF family (BAFF) pathway. *Acta Tropica* **116**, 217–226.

Kushwah, R. and Hu, J. (2010). Dendritic cell apoptosis: regulation of tolerance versus immunity. *Journal of Immunology* **185**, 795–802.

Langhorne, J., Ndungu, F. M., Sponaas, A. M. and Marsh, K. (2008). Immunity to malaria: more questions than answers. *Nature Immunology* **9**, 725–732.

Lavstsen, T., Magistrado, P., Hermsen, C. C., Salanti, A., Jensen, A. T., Sauerwein, R., Hviid, L., Theander, T. G. and Staalsoe, T. (2005). Expression of *Plasmodium falciparum* erythrocyte membrane protein 1 in experimentally infected humans. *Malaria Journal* **4**, 21.

Liehl, P., Zuzarte-Luis, V., Chan, J., Zillinger, T., Baptista, F., Carapau, D., Konert, M., Hanson, K. K., Carret, C., Lassnig, C., Muller, M., Kalinke, U., Saeed, M., Chora, A. F., Golenbock, D. T., Strobl, B., Prudencio, M., Coelho, L. P., Kappe, S. H., Superti-Furga, G., Pichlmair, A., Vigario, A. M., Rice, C. M., Fitzgerald, K. A., Barchet, W. and Mota, M. M. (2014). Host-cell sensors for *Plasmodium* activate innate immunity against liver-stage infection. *Nature Medicine* **20**, 47–53.

Liu, Z. and Roche, P. A. (2015). Macropinocytosis in phagocytes: regulation of MHC class-II-restricted antigen presentation in dendritic cells. *Frontiers in Physiology* **6**, 1.

Locci, M., Havenar-Daughton, C., Landais, E., Wu, J., Kroenke, M. A., Arlehamn, C. L., Su, L. F., Cubas, R., Davis, M. M., Sette, A., Haddad, E. K., International AIDS Vaccine Initiative Protocol C Principal Investigators, Poignard, P. and Crotty, S. (2013). Human circulating PD-1+CXCR3-CXCR5+ memory Tfh cells are highly functional and correlate with broadly neutralizing HIV antibody responses. Immunity 39, 758–769.

Lovegrove, F. E., Pena-Castillo, L., Mohammad, N., Liles, W. C., Hughes, T. R. and Kain, K. C. (2006). Simultaneous host and parasite expression profiling identifies tissue-specific transcriptional programs associated with susceptibility or resistance to experimental cerebral malaria. *BMC Genomics* **7**, 295.

Martini, F., Paglia, M. G., Montesano, C., Enders, P. J., Gentile, M., Pauza, C. D., Gioia, C., Colizzi, V., Narciso, P., Pucillo, L. P. and Poccia, F. (2003). V gamma 9 V delta 2T-cell anergy and complementarity-determining region 3-specific depletion during paroxysm of nonendemic malaria infection. *Infection and Immunity* **71**, 2945–2949.

McCall, M. B., Netea, M. G., Hermsen, C. C., Jansen, T., Jacobs, L., Golenbock, D., van der Ven, A. J. and Sauerwein, R. W. (2007). *Plasmodium falciparum* infection causes proinflammatory priming of human TLR responses. *Journal of Immunology* **179**, 162–171.

McCall, M. B., Roestenberg, M., Ploemen, I., Teirlinck, A., Hopman, J., de Mast, Q., Dolo, A., Doumbo, O. K., Luty, A., van der Ven, A. J., Hermsen, C. C. and Sauerwein, R. W. (2010). Memory-like IFN-gamma response by NK cells following malaria infection reveals the crucial role of T cells in NK cell activation by *P. falciparum*. *European Journal of Immunology* **40**, 3472–3477.

McCarthy, J. S., Sekuloski, S., Griffin, P. M., Elliott, S., Douglas, N., Peatey, C., Rockett, R., O'Rourke, P., Marquart, L., Hermsen, C., Duparc, S., Mohrle, J., Trenholme, K. R. and Humberstone, A. J. (2011). A pilot randomised trial of induced blood-stage *Plasmodium falciparum* infections in healthy volunteers for testing efficacy of new antimalarial drugs. *PLoS ONE* **6**, e21914.

McHeyzer-Williams, M., Okitsu, S., Wang, N. and McHeyzer-Williams, L. (2012). Molecular programming of B cell memory. *Nature Reviews Immunology* **12**, 24–34.

Mestas, J. and Hughes, C. C. (2004). Of mice and not men: differences between mouse and human immunology. *Journal of Immunology* **172**, 2731–2738.

Miller, J. L., Sack, B. K., Baldwin, M., Vaughan, A. M. and Kappe, S. H. (2014). Interferon-mediated innate immune responses against malaria parasite liver stages. *Cell Reports* **7**, 436–447.

Mitchell, A. J., Roediger, B. and Weninger, W. (2014). Monocyte homeostasis and the plasticity of inflammatory monocytes. *Cellular Immunology* **291**, 22–31.

Molineaux, L., Trauble, M., Collins, W. E., Jeffery, G. M. and Dietz, K. (2002). Malaria therapy reinoculation data suggest individual variation of an innate immune response and independent acquisition of antiparasitic and antitoxic immunities. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **96**, 205–209.

Molle, I., Petersen, E. and Buhl, M. R. (1999). Retrospective evaluation of exposure to *P. falciparum* using antibodies to circumsporozoite protein and to cultured *P. falciparum* antigens. *Scandinavian Journal of Infectious Diseases* **31**, 69–71.

Nahrendorf, W., Scholzen, A., Bijker, E. M., Teirlinck, A. C., Bastiaens, G. J., Schats, R., Hermsen, C. C., Visser, L. G., Langhorne, J. and Sauerwein, R. W. (2014). Memory B-cell and antibody responses induced by *Plasmodium falciparum* sporozoite immunization. *Journal of Infectious Diseases* **210**, 1981–1990.

Nduati, E. W., Ng, D. H., Ndungu, F. M., Gardner, P., Urban, B. C. and Langhorne, J. (2010). Distinct kinetics of memory B-cell and plasma-cell responses in peripheral blood following a blood-stage *Plasmodium chabaudi* infection in mice. *PLoS ONE* **5**, e15007.

Nganou-Makamdop, K., van Gemert, G. J., Arens, T., Hermsen, C. C. and Sauerwein, R. W. (2012). Long term protection after immunization with *P. berghei* sporozoites correlates with sustained IFNgamma responses of hepatic CD8+ memory T cells. *PLoS ONE* **7**, e36508.

Nothdurft, H. D., Jelinek, T., Bluml, A., von Sonnenburg, F. and Loscher, T. (1999). Seroconversion to circumsporozoite antigen of *Plasmodium falciparum* demonstrates a high risk of malaria transmission in travelers to East Africa. *Clinical Infectious Diseases* **28**, 641–642.

Obiero, J. M., Shekalaghe, S., Hermsen, C. C., Mpina, M., Bijker, E. M., Roestenberg, M., Teelen, K., Billingsley, P. F., Sim, B. K., James, E. R., Daubenberger, C. A., Hoffman, S. L., Abdulla, S., Sauerwein, R. W. and Scholzen, A. (2015). Impact of malaria pre-exposure on anti-parasite cellular and humoral immune responses after controlled human malaria infection. *Infection and Immunity* **83**, 2185–2196.

Ockenhouse, C. F., Hu, W. C., Kester, K. E., Cummings, J. F., Stewart, A., Heppner, D. G., Jedlicka, A. E., Scott, A. L., Wolfe, N. D., Vahey, M. and Burke, D. S. (2006). Common and divergent immune response signaling pathways discovered in peripheral blood mononuclear cell gene expression patterns in presymptomatic and clinically apparent malaria. *Infection and Immunity* **74**, 5561–5573.

Orlov, M., Vaida, F., Finney, O. C., Smith, D. M., Talley, A. K., Wang, R., Kappe, S. H., Deng, Q., Schooley, R. T. and Duffy, P. E. (2012). *P. falciparum* enhances HIV replication in an experimental malaria challenge system. *PLoS ONE* **7**, e39000.

Peters, J., Fowler, E., Gatton, M., Chen, N., Saul, A. and Cheng, Q. (2002). High diversity and rapid changeover of expressed var genes during the acute phase of *Plasmodium falciparum* infections in human volunteers. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 10689–10694.

Pichyangkul, S., Yongvanitchit, K., Kum-arb, U., Hemmi, H., Akira, S., Krieg, A. M., Heppner, D. G., Stewart, V. A., Hasegawa, H., Looareesuwan, S., Shanks, G. D. and Miller, R. S. (2004). Malaria blood stage parasites activate human plasmacytoid dendritic cells and murine dendritic cells through a Toll-like receptor 9-dependent pathway. *Journal of Immunology* **172**, 4926–4933.

Portugal, S., Pierce, S. K. and Crompton, P. D. (2013). Young lives lost as B cells falter: what we are learning about antibody responses in malaria. *Journal of Immunology* **190**, 3039–3046.

Riley, E. M. and Stewart, V. A. (2013). Immune mechanisms in malaria: new insights in vaccine development. *Nature Medicine* **19**, 168–178.

Roestenberg, M., McCall, M., Mollnes, T. E., van Deuren, M., Sprong, T., Klasen, I., Hermsen, C. C., Sauerwein, R. W. and van der Ven, A. (2007). Complement activation in experimental human malaria infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **101**, 643–649.

Roestenberg, M., O'Hara, G. A., Duncan, C. J., Epstein, J. E., Edwards, N. J., Scholzen, A., van der Ven, A. J., Hermsen, C. C., Hill, A. V. and Sauerwein, R. W. (2012). Comparison of clinical and parasitological data from controlled human malaria infection trials. *PLoS ONE* **7**, e38434.

Roestenberg, M., Bijker, E. M., Sim, B. K., Billingsley, P. F., James, E. R., Bastiaens, G. J., Teirlinck, A. C., Scholzen, A., Teelen, K., Arens, T., van der Ven, A. J., Gunasekera, A., Chakravarty, S., Velmurugan, S., Hermsen, C. C., Sauerwein, R. W. and Hoffman, S. L. (2013). Controlled human malaria infections by intradermal injection of cryopreserved *Plasmodium falciparum* sporozoites. *American Journal of Tropical Medicine and Hygiene* **88**, 5–13.

Rzepczyk, C. M., Stamatiou, S., Anderson, K., Stowers, A., Cheng, Q., Saul, A., Allworth, A., McCormack, J., Whitby, M., Olive, C. and Lawrence, G. (1996). Experimental human *Plasmodium falciparum* infections: longitudinal analysis of lymphocyte responses with particular reference to gamma delta T cells. *Scandinavian Journal of Immunology* **43**, 219–227.

Sallusto, F., Geginat, J. and Lanzavecchia, A. (2004). Central memory and effector memory T cell subsets: function, generation, and maintenance. *Annual Review of Immunology* **22**, 745–763.

https://doi.org/10.1017/S0031182015000761 Published online by Cambridge University Press

Sauerwein, R. W., Roestenberg, M. and Moorthy, V. S. (2011). Experimental human challenge infections can accelerate clinical malaria vaccine development. *Nature Reviews Immunology* **11**, 57–64.

Schenkel, J. M. and Masopust, D. (2014). Tissue-resident memory T cells. *Immunity* **41**, 886–897.

Scholzen, A. and Sauerwein, R. W. (2013). How malaria modulates memory: activation and dysregulation of B cells in *Plasmodium* infection. *Trends in Parasitology* **29**, 252–262.

Scholzen, A., Mittag, D., Rogerson, S. J., Cooke, B. M. and Plebanski, M. (2009). *Plasmodium falciparum*-mediated induction of human CD25Foxp3 CD4T cells is independent of direct TCR stimulation and requires IL-2, IL-10 and TGFbeta. *PLoS Pathogens* **5**, e1000543.

Scholzen, A., Minigo, G. and Plebanski, M. (2010). Heroes or villains? T regulatory cells in malaria infection. *Trends in Parasitology* **26**, 16–25.

Scholzen, A., Teirlinck, A. C., Bijker, E. M., Roestenberg, M., Hermsen, C. C., Hoffman, S. L. and Sauerwein, R. W. (2014). BAFF and BAFF receptor levels correlate with B cell subset activation and redistribution in controlled human malaria infection. *Journal of Immunology* **192**, 3719–3729.

Seder, R. A., Chang, L. J., Enama, M. E., Zephir, K. L., Sarwar, U. N., Gordon, I. J., Holman, L. A., James, E. R., Billingsley, P. F., Gunasekera, A., Richman, A., Chakravarty, S., Manoj, A., Velmurugan, S., Li, M., Ruben, A. J., Li, T., Eappen, A. G., Stafford, R. E., Plummer, S. H., Hendel, C. S., Novik, L., Costner, P. J., Mendoza, F. H., Saunders, J. G., Nason, M. C., Richardson, J. H., Murphy, J., Davidson, S. A., Richie, T. L., *et al.* (2013). Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. *Science* **341**, 1359–1365.

Seed, C. R., Hamzah, J. and Davis, T. M. (2006). Evidence for undetected malaria infection in non-immune Australian travellers not taking chemoprophylaxis. *Acta Tropica* **99**, 62–66.

Sheehy, S. H., Spencer, A. J., Douglas, A. D., Sim, B. K., Longley, R. J., Edwards, N. J., Poulton, I. D., Kimani, D., Williams, A. R., Anagnostou, N. A., Roberts, R., Kerridge, S., Voysey, M., James, E. R., Billingsley, P. F., Gunasekera, A., Lawrie, A. M., Hoffman, S. L. and Hill, A. V. (2013). Optimising controlled human malaria infection studies using cryopreserved parasites administered by needle and syringe. *PLoS ONE* **8**, e65960.

Snounou, G. and Perignon, J. L. (2013). Malariotherapy – insanity at the service of malariology. *Advances in Parasitology* **81**, 223–255.

Stephens, R., Culleton, R. L. and Lamb, T. J. (2012). The contribution of *Plasmodium chabaudi* to our understanding of malaria. *Trends in Parasitology* **28**, 73–82.

Struik, S. S. and Riley, E. M. (2004). Does malaria suffer from lack of memory? *Immunological Reviews* **201**, 268–290.

Talley, A. K., Healy, S. A., Finney, O. C., Murphy, S. C., Kublin, J., Salas, C. J., Lundebjerg, S., Gilbert, P., Van Voorhis, W. C., Whisler, J., Wang, R., Ockenhouse, C. F., Heppner, D. G., Kappe, S. H. and Duffy, P. E. (2014). Safety and comparability of controlled human *Plasmodium falciparum* infection by mosquito bite in malaria-naive subjects at a new facility for sporozoite challenge. *PLoS ONE* **9**, e109654.

Teirlinck, A. C., McCall, M. B., Roestenberg, M., Scholzen, A., Woestenenk, R., de Mast, Q., van der Ven, A. J., Hermsen, C. C., Luty, A. J. and Sauerwein, R. W. (2011). Longevity and composition of cellular immune responses following experimental *Plasmodium falciparum* malaria infection in humans. *PLoS Pathogens* **7**, e1002389.

Teirlinck, A. C., Roestenberg, M., van de Vegte-Bolmer, M., Scholzen, A., Heinrichs, M. J., Siebelink-Stoter, R., Graumans, W., van Gemert, G. J., Teelen, K., Vos, M. W., Nganou-Makamdop, K., Borrmann, S., Rozier, Y. P., Erkens, M. A., Luty, A. J., Hermsen, C. C., Sim, B. K., van Lieshout, L., Hoffman, S. L., Visser, L. G. and Sauerwein, R. W. (2013). NF135.C10: a new *Plasmodium falciparum* clone for controlled human malaria infections. *Journal of Infectious Diseases* **207**, 656–660.

Todryk, S. M., Walther, M., Bejon, P., Hutchings, C., Thompson, F. M., Urban, B. C., Porter, D. W. and Hill, A. V. (2009). Multiple functions of human T cells generated by experimental malaria challenge. *European Journal of Immunology* **39**, 3042–3051.

Turner, L., Wang, C. W., Lavstsen, T., Mwakalinga, S. B., Sauerwein, R. W., Hermsen, C. C. and Theander, T. G. (2011). Antibodies against PfEMP1, RIFIN, MSP3 and GLURP are acquired during controlled *Plasmodium falciparum* malaria infections in naive volunteers. *PLoS ONE* **6**, e29025.

Verhage, D. F., Telgt, D. S., Bousema, J. T., Hermsen, C. C., van Gemert, G. J., van der Meer, J. W. and Sauerwein, R. W. (2005). Clinical outcome of experimental human malaria induced by *Plasmodium falciparum*-infected mosquitoes. *Netherlands Journal of Medicine* **63**, 52–58.

Walker, K. M., Okitsu, S., Porter, D. W., Duncan, C., Amacker, M., Pluschke, G., Cavanagh, D. R., Hill, A. V. and Todryk, S. M. (2014). Antibody and T cell responses associated with experimental human malaria infection or vaccination show limited relationships. *Immunology* **145**, 71–81.

Walther, M., Tongren, J. E., Andrews, L., Korbel, D., King, E., Fletcher, H., Andersen, R. F., Bejon, P., Thompson, F., Dunachie, S. J., Edele, F., de Souza, J. B., Sinden, R. E., Gilbert, S. C., Riley, E. M. and Hill, A. V. (2005). Upregulation of TGF-beta, FOXP3, and CD4+CD25+ regulatory T cells correlates with more rapid parasite growth in human malaria infection. *Immunity* **23**, 287–296.

Walther, M., Woodruff, J., Edele, F., Jeffries, D., Tongren, J. E., King, E., Andrews, L., Bejon, P., Gilbert, S. C., De Souza, J. B., Sinden, R., Hill, A. V. and Riley, E. M. (2006). Innate immune responses to human malaria: heterogeneous cytokine responses to blood-stage *Plasmodium falciparum* correlate with parasitological and clinical outcomes. *Journal of Immunology* **177**, 5736–5745.

Wang, C. W., Hermsen, C. C., Sauerwein, R. W., Arnot, D. E., Theander, T. G. and Lavstsen, T. (2009). The *Plasmodium falciparum* var gene transcription strategy at the onset of blood stage infection in a human volunteer. *Parasitology International* **58**, 478–480.

WHO (2013). World Malaria Report 2013. World Health Organization, WHO Press.

Woodberry, T., Minigo, G., Piera, K. A., Amante, F. H., Pinzon-Charry, A., Good, M. F., Lopez, J. A., Engwerda, C. R., McCarthy, J. S. and Anstey, N. M. (2012). Low-level *Plasmodium falciparum* blood-stage infection causes dendritic cell apoptosis and dysfunction in healthy volunteers. *Journal of Infectious Diseases* **206**, 333–340.

Wykes, M. N. and Good, M. F. (2008). What really happens to dendritic cells during malaria? *Nature Reviews Microbiology* **6**, 864–870.

Yang, K. and Chi, H. (2012). mTOR and metabolic pathways in T cell quiescence and functional activation. *Seminars in Immunology* **24**, 421–428.