Figure 2 The transition from *P. vivax* asymptomatic infection to clinical disease depends on the interaction of various known or potential parasite, host and external factors. Parasite factors include antimalarial drug sensitivity, multiplicative potential and relapsing patterns, erythrocyte invasion capabilities, induction of endothelial adhesion and the expression of variant genes important for host immune evasion. Host factors include age and gender, immunity, pregnancy, ethnicity and genetic polymorphisms, coexisting infections or chronic conditions. Other external factors include access to health care and effective antimalarial treatment, vectorial capacity in the area and other sociocultural factors.

the parasite causes severe or even lethal disease challenges our current comprehension of the pathophysiological mechanisms involved in these processes^{2,11}. Moreover, the differential impact of host, parasite and external factors needs to be further elucidated (Fig. 2).

P. falciparum causes clinical symptoms through two very different but possibly synergistic mechanisms¹²—toxin-induced activation of proinflammatory responses, through cytokine release, and blood flow obstruction of the microvasculature owing to cytoadherence and sequestration of parasitized red blood cells. Direct hemolysis and changes in red blood cell production may also lead to life-threatening severe anemia.

In *P. vivax* infections, the multiplication potential of the parasite is limited by its strict preference for reticulocytes¹¹, which significantly lowers the total parasite biomass compared to that in *P. falciparum* infections. But the

overall inflammatory response, of greater magnitude than that in response to *P. falciparum*¹³, may be crucial in the development of clinical symptomatology¹¹ and may also explain its comparatively lower pyrogenic threshold (parasitemia needed to cause fever). The enhanced inflammatory potential in combination with higher erythrocytic fragility resulting from *P. vivax*'s infection¹¹ may also account for its comparable potential to cause anemia.

Localized exacerbations of this acute inflammatory response that destroy parasitized erythrocytes in the lung microvasculature have also been proposed to cause acute respiratory distress syndrome (ARDS) in adults after commencing antimalarial therapy in hitherto uncomplicated *P. vivax* cases¹¹. In children with malaria ARDS is a rare entity, but the high reported^{6,7,14} incidence of respiratory distress or other severe presentations, including coma, often indistinguishable from *P. falciparum* disease, suggests common pathological mechanisms for both species.

Cytoadherence and erythrocyte sequestration, which are crucial in the pathophysiology of severe *P. falciparum* malaria and long believed not to occur in *P. vivax* infections, may also have an important role, either directly or as a means of localizing the inflammatory response to parasites adhering in the lungs, brain placenta or spleen. The recent proof of such phenomena in *P. vivax* infections *in vitro*¹⁵—albeit with a lower magnitude but with similar intensity than *P. falciparum*—anticipated by clinical observations¹⁶ has opened new avenues to explore how this parasite causes disease.

Future research at the bench will have to focus on furthering our understanding of the inflammatory response caused by *P. vivax* and identifying the specific cytokines involved in the response to this infection. Efforts are required to characterize potential surrogates of naturally

acquired immunity against this infection and to further define the molecular mechanisms of hypnozoite formation and reactivation—relevant for drug development—reticulocyte invasion, cytoadherence, sequestration and spleen clearance². Detailed histopathological descriptions of organ involvement, arising from autopsy reports of *P. vivax*-related fatalities, which have been rarely available in the literature, are now a priority. The study of host polymorphisms associated with protection against disease, such as Duffy trait, and the precise role in the pathophysiology of expanded *P. vivax* gene families linked to antigenic variation need to be better characterized². Overcoming current barriers for *P. vivax* research also requires the long-awaited development of a continuous *in vitro* culture system², which will be paramount to our understanding of this disease.

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■ BENCH TO BEDSIDE

Arming T cells to halt malaria

Michael F Good & Christian Engwerda

Like most organisms that have jumped from animals to humans, the parasite responsible for the most serious cases of malaria infec-

tion, *Plasmodium falciparum*, is a cunning evader and suppressor of host immunity, which makes vaccine development especially difficult. A thorough understanding of all potential antiparasitic immune mechanisms is therefore required to design an effective vaccine.

Until recently, the immune components able to kill malaria parasites resident within red blood cells were thought to be limited to

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antibodies, CD4⁺ T cells and $\gamma\delta$ T cells (found mainly in peripheral tissues)^{1–3}. In contrast, CD8⁺ T cells, a T cell subset known to kill tumor cells and virus-infected cells, were not thought to have a role, even though they were shown to kill the malaria parasite during its pre-erythrocytic stage within liver hepatocytes^{4,5}.

Over 20 years ago, in the *Plasmodium yoelii* mouse model of malaria, the role of CD8⁺ T cells during blood-stage infection was investigated inconclusively, with one study supporting cell-mediated protection⁶ and other studies arguing against it^{7,8}. In these studies, different methodologies were used, and the purity of cell populations was not always determined. One of the studies, in which the role of T cell subsets was studied in mice depleted of CD4⁺ or CD8⁺ T cells, showed that CD4⁺ but not CD8⁺ T cells could transfer protection⁸, suggesting that CD8⁺ T cells are not responsible for blood-stage immunity.

Nevertheless, a study by Imai *et al.*⁹ using a new method of immunization—a live challenge with nonlethal *P. yoelii* followed by subsequent challenges with a lethal strain of the same parasite species, instead of immunization by exposure to a single infection—suggests that we may need to re-evaluate the role of CD8⁺ T cells in immunity to the blood stages of malaria. If correct, this will alter the way we think about developing a malaria vaccine.

In the last three decades, CD4⁺ T cells have been shown to be crucial in malaria immunity, not just as ‘helper’ cells for B cells secreting antibody, but also as ‘effectors’, killing malaria parasites indirectly by secreting inflammatory cytokines and activating other cell types, such as macrophages. Several groups have also shown that CD4⁺ T cell clones specific for the parasite were able to transfer malaria immunity to naive recipient mice¹⁰. Parasite-specific memory CD4⁺ T cells were found in the blood of human volunteers deliberately exposed to very low doses of malaria parasites with very few or no parasite-specific antibodies^{11,12}, supporting the case for a malaria vaccine based on the generation of parasite-specific CD4⁺ T cells¹³. Yet the potential for parasite-specific memory CD8⁺ T cells to be part of such a blood-stage malaria vaccine was not considered, despite these cells being readily detected after exposure¹¹.

Imai *et al.*⁹ have shown that CD8⁺ T cells can transfer immunity in a mouse model of malaria caused by the rodent parasite *P. yoelii*. Interestingly, the mice had to be repeatedly exposed to different strains of *P. yoelii* to develop protective CD8⁺ T cells. After infec-

Figure 1 Immunization protocol and the possible mechanisms by which CD8⁺ T cells kill blood stage malaria parasites. Two challenges with virulent *P. yoelii* L after infection with the avirulent strain induces a cellular immune response mediated by CD8⁺ T cells. After the second challenge, CD8⁺ T cells are activated and able to inhibit the blood stage of the parasite by inducing phagocytosis of infected erythrocytes by macrophages through release of IFN- γ . CD8⁺ T cells may also control disease progression by promoting perforin-mediated cytolytic functions. Adoptive transfer of memory CD8⁺ T cells protects naive mice from infection by blood-stage malaria parasites. This may open new avenues for developing a blood-stage vaccine based on cell-mediated immunity.

tion with avirulent *P. yoelii* NL, mice were protected against infection with virulent *P. yoelii* L; however, CD8⁺ T cells from these mice were not capable of transferring protection. In contrast, infection with the avirulent strain and two subsequent infections with the virulent strain yielded CD8⁺ T cells capable of adoptively transferring protection. These CD8⁺ T cells were isolated to 95% purity, causing concern that contaminating CD4⁺ T cells might be responsible for mediating protection, but depletion of CD4⁺ T cells from this enriched CD8⁺ T cell population did not affect the potency of the transferred cells, whereas depletion of CD8⁺ T cells destroyed their potency, confirming that the CD8⁺ T cells were responsible for the protection. These CD8⁺ T cells were also shown to be antiparasitic when transferred into RAG2-deficient mice that lack T cells, confirming that any endogenous, naive CD4⁺ T cells were not responsible for protection.

Once generated, how would CD8⁺ T cells and CD4⁺ T cells destroy parasites that reside within red blood cells, which do not express the major histocompatibility complex class I and II presenting molecules essential for T cell recognition? Many studies have shown that T cells can recognize parasite antigens presented by dendritic cells and other antigen-presenting cells. Adoptive transfer experiments with CD8⁺ T cells also showed the antiparasitic activity of these cells depended on interferon- γ (IFN- γ) production, the need for functional macrophages, and that efficacy was partly dependent on the production of the cytolytic molecule perforin⁹ (Fig. 1).

Although it is easy to reconcile the potential role of CD8⁺ T cell-derived IFN- γ for enhancing the phagocytic and microbicidal activation of macrophages, the perforin-mediated killing of parasite-laden macrophages by cytolytic CD8⁺ T cells proposed by the authors remains speculative and without any obvious benefit to the host. Further investigation is required to better understand the antiparasitic activity of perforin.

An important question yet to be answered is why transferred CD8⁺ T cells were ineffective after a single infection with the avirulent *P. yoelii* NL but became effective after two subsequent infections with the virulent strain. The authors suggested that this might be a ‘prime-boost’ strategy, but such regimens normally use two very different forms of immunogens, such as DNA followed by protein⁹.

An alternative explanation might be the mice develop an antibody response after the first infection with *P. yoelii* NL, which was also shown by the authors, keeping the second and third infections at microscopically undetectable levels. Studies in humans and in mice have shown that subpatent infections allow the expansion of parasite-specific CD8⁺ T cells^{11,14}. Irrespective of the mechanism, it seems that the authors’ immunization protocol established suitable immunological conditions, yet to be fully characterized, that allow the expansion of protective CD8⁺ T cells.

If other laboratories validate the findings of this study, we will need to think differently about how we design a blood-stage malaria vaccine. Vaccine strategies to stimulate CD8⁺

T cells differ from those used to induce CD4⁺ T cell responses—viral vectors, for example, are very effective inducers of CD8⁺ T cells, and whole-parasite vaccines could also be considered, as they may induce protective CD4⁺ and CD8⁺ T cell responses.

As the development of antibody-dependent malaria blood-stage subunit vaccines continues to be challenging, with no success beyond phase 1 trials, this new information on CD8⁺ T cells is very timely. The major challenge for subunit vaccines is that the vaccine candidates, based on parasite surface proteins, are poorly immunogenic and highly polymorphic.

Currently there are few strategies being

considered to overcome these obstacles. In contrast, there is evidence to suggest that the target antigens of T cells are highly conserved. Vaccines to induce T cell responses will have their own unique challenges, but they are worth pursuing. This recent study now gives more reasons to further consider these approaches.

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