Enhancement of naturally acquired immunity against malaria by drug use

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Combination of chemoprophylaxis with chloroquine and so-called 'controlled human malaria infections' has been shown to induce sustained and fully protective immunity against malaria in experimental settings. This opens possibilities of translating this approach into an effective and applicable strategy for the field. We review the different ways in which antimalarial drugs have been used for prevention of malaria in endemic settings and discuss the possibilities and challenges of applying a strategy of drug use and naturally acquired infection in the field.

Introduction

Malaria remains one of the most important infectious diseases worldwide, causing almost 655,000 deaths per year, of which the majority are children under 5 years of age. Intense malaria control interventions during the past decade have successfully established a reduction of more than 50% in either confirmed cases of malaria or malaria admissions and deaths in 11 countries of the WHO African region (WHO, 2011a). However, increases in the number of malaria cases in 2009 in Rwanda, Sao Tome and Principe and Zambia, which previously reported reductions, illustrate the fragility of the current successes. This underscores the need for additional and innovative strategies.

Availability of an effective vaccine would greatly contribute to malaria control and elimination. It is well known that clinical immunity is acquired in endemic areas after a number of years and a sufficient number of naturally acquired infections (Snow & Marsh, 1995). The search for malaria vaccines against Plasmodium falciparum has been pursued for decades, with the main focus on the development of subunit-vaccines, but with limited success. Twenty candidate vaccines are currently under clinical investigation but only one product, RTS,S, has progressed into a phase 3 field trial having recently shown encouraging indications of protection in an interim evaluation (RTS,S Clinical Trials Partnership, 2011; WHO, 2011b).

One of the shortcomings of subunit-vaccines is the inability to appropriately address the significant antigenic diversity of target epitopes and the often-observed poor immunogenicity of the soluble parasite-derived proteins used. Against that background a whole-parasite approach may perform better. Indeed, immunization with sporozoite forms has consistently been shown to induce >90% protection in rodents and humans in experimental set-ups (Friesen & Matuschewski, 2011; Hoffman et al., 2002).

Transmission intensity varies greatly in sub-Saharan Africa where individuals can be subjected to up to 10 infectious bites per night at certain periods of the year. Here, we explore the idea that using medicines together with naturally acquired infection could result in the induction of protective immunity. We will review the different ways in which antimalarial drugs have been used for prevention of malaria in endemic areas and reflect on the possibilities and challenges of applying a strategy of drug use together with naturally acquired infection in the field (see Table 1 for an overview of the interventions discussed in this review).

Drug applications for prevention of malaria in endemic areas

Millions of travellers visit malaria-endemic areas for short periods of time while using chemoprophylaxis for prevention of malaria. Such practice has never been considered as a realistic strategy for endemic populations. Reasons include lack of sustainability, problems with acceptance by the community, risk of emerging drug resistance and concerns about impairment of the development of natural immunity (Greenwood, 2010).

Nevertheless, the potential effects of drug administration on development of clinical protection in natural settings have been explored in a number of studies. Different recipes of chemoprevention have been applied, including mass drug administration in the general population and application of drugs in specific risk groups including children, infants and pregnant women (Greenwood, 2010).
Mass drug administration (MDA) involves the prescription of antimalarial drugs to whole populations without screening for the presence of parasitaemia. This can be done either directly, when a curative dose of the antimalarial drug is given to an entire population, or indirectly, when the antimalarial is added to food, usually to salt.

Several studies have been conducted since the late 1950s, which show that MDA does substantially reduce the incidence of clinical malaria and parasite prevalence but that the impact of MDAs was transitory (von Seidlein & Greenwood, 2003). Evaluation and interpretation of the true effects are likely compromised by missing a substantial proportion of *P. falciparum* infections due to the limited sensitivity of microscopic parasite detection (Okell et al., 2009). Submicroscopic parasitaemia and frequent asymptomatic parasite carriage widely exist in endemic areas, particularly in those with very low endemicity. However, it is currently unknown what the effects on the immune response are and how these individuals manage to control malaria.

Chemoprophylaxis is the administration of a drug in such a way that its blood concentration is maintained above the minimum inhibitory level. Travellers who visit malaria-endemic areas use chemoprophylaxis to prevent malaria, and in endemic areas it has been applied for specific risk groups, such as children. In children, it can effectively reduce overall mortality and clinical malaria attacks; it also improves mean haemoglobin levels, reduces severe anaemia and improves school attendance (Geerligs et al., 2003).

To further increase adherence and sustainability and reduce the risk of inducing drug-resistance, a more targeted approach has been developed. Intermittent preventive treatment (IPT) is the administration of a full course antimalarial treatment at specified time points without parasite screening. This IPT restricts the use of antimalarials to specific risk groups at specified time points. The idea is that parasite exposure will be less to undesired subtherapeutic drug concentrations while concomitantly allowing a more effective generation of natural immunity in the intermittent periods between two doses of IPT (Schellenberg et al., 2005; Schultz et al., 1994). When administered in the existing health system during routine visits, for example during pregnancy or at infant-vaccinations, costs can be reduced and sustainability increased (Greenwood, 2010).

IPT was initially investigated in the context of pregnant women (IPTp), and subsequently extended to infants and children (IPTi, IPTc). IPTp with sulfadoxine–pyrimethamine (SP), administered two or three times during the second and third trimesters, effectively reduces disease burden and adverse outcomes of malaria in pregnancy by substantially reducing placental malaria [relative risk (RR) 0.48; 95% confidence interval (CI) 0.35–0.68, low birth weight (RR, 0.71; 95% CI, 0.55–0.92) and anaemia (ter Kuile et al., 2007). Moreover, IPTp is readily implementable and cost-effective (Brentlinger et al., 2007; Sicuri et al., 2010). The WHO therefore recommends IPTp with SP for areas with high or moderate transmission, and many countries have substantially scaled up delivery of IPTp, although coverage is still inadequate (van Eijk et al., 2011). IPTi has been shown to be safe in infants, with a protective efficacy of ~30% against clinical malaria and 21% against the risk of anaemia from the first dose until 12 months of age (Aponte et al., 2009). Unfortunately, levels of SP resistance are on the rise in many areas, requiring adaptation of SP regimes. This further underlines the need for new drugs or drug combinations and for innovative interventions.

### Immune responses in the context of reduced parasite exposure

More than 15 years ago, it was suggested that interventions that reduce malaria transmission and thus the level of exposure may interfere with the acquisition of natural immunity (Snow & Marsh, 1995; Trape & Rogier, 1996). This hypothesis, however, is difficult to address in the absence of an established immune correlate of clinical protection against malaria. More than two decades ago, a number of studies made attempts to investigate the effects of chemoprophylactic measures on humoral and/or cellular immune responses. The effect of insecticide-treated bed net use on antimalarial immunity has been investigated separately, showing inconclusive results that we will not explore further here (Diallo et al., 2007; Kariuki et al., 2003a, b).

**Table 1. Overview of interventions**

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Definition</th>
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<tr>
<td>Chemoprophylaxis</td>
<td>Administration of a drug in such a way that its blood concentration is</td>
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<td></td>
<td>maintained above the minimum inhibitory level</td>
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<tr>
<td>Mass drug administration (MDA)</td>
<td>Administration of a full therapeutic course of an antimalarial drug to a</td>
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<td></td>
<td>whole population at risk</td>
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<tr>
<td>Intermittent preventive treatment (IPT)</td>
<td>Administration of antimalarial treatment at specified time points,</td>
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<td></td>
<td>regardless of whether or not they are infected</td>
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<tr>
<td>Controlled human malaria infections (CHMI)</td>
<td>Exposure of healthy malaria-naïve volunteers to <em>Plasmodium</em>-infected</td>
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<td>under chemoprophylaxis</td>
<td>mosquito bites, whilst taking prophylactic drugs</td>
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<tr>
<td>Immunization by drug use and natural infection</td>
<td>Exposure to parasites under drug cover in malaria-endemic areas</td>
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Enhancement of immunity against malaria by drug use
While the majority of studies on humoral responses show a decrease in antibodies to malaria antigens after chemoprophylaxis (Bradley-Moore *et al.*, 1985; Cornille-Brögger *et al.,* 1978; Harland *et al.,* 1975; Hogh *et al.,* 1994; McGregor & Gilles, 1960; Otoo *et al.,* 1988; Voller & Wilson, 1964), this may simply represent less parasite exposure rather than an actual loss of protective immunity. Similar results were obtained in a number of IPT studies; IgG levels against crude *P. falciparum* lysate were significantly lower in Ghanian children after a single dose of SP compared to a placebo, without any evidence of rebound malaria (Schreiber *et al.,* 2007). In Senegal, a slightly decreased antibody response was measured in children receiving IPTc, when compared to non-treated controls (Boulanger *et al.,* 2010). In contrast, a study in Mozambique showed no significant difference in antibody responses between children receiving IPTi with SP and placebo-treated controls. Interestingly, one exception was observed here in that IgG and IgG1 responses to *P. falciparum* apical membrane antigen (AMA)-1 and/or merozoite surface protein (MSP)-19 at ages 5, 9 and 24 months were actually significantly increased in the SP group (Quelhas *et al.,* 2008). Therefore, these authors concluded that IPTi reduces the incidence of illness while allowing the development of naturally acquired antibody responses.

Relatively few studies have been conducted on cellular immune responses after chemoprophylaxis. These point towards higher lymphoproliferative responses and interferon (IFN)-γ production in the presence of a lower overall exposure to parasites (Hogh *et al.,* 1994; Otoo *et al.,* 1989).

**Clinical rebound after chemoprophylactic interventions**

One could argue that incidences of malaria morbidity and mortality are actually more relevant than immune responses after drug-based preventive measures. Prophylaxis with various drugs has been tested for children in many studies in Africa, Asia, Central America and the Pacific. Assessments showed that rebound malaria generally occurred from 3 months to 1 year after discontinuation of chemoprophylaxis, which was given for a period of 3 months to 5 years in infants aged less than 1 year up to children aged 5–10 years. Different end points were used to assess rebound malaria (Geerligs *et al.,* 2003).

Of 12 studies investigating rebound malaria after termination of chemoprophylaxis, nine did not show increased clinical malaria or parasitaemia (Geerligs *et al.,* 2003). For example, data from the famous Garki project in Nigeria, where pyrimethamine prophylaxis was combined with insecticide spraying, show large reductions in infant and child mortality rates during the intervention without increased morbidity or mortality afterwards (Molineaux & Gramiccia, 1980). Similar results were obtained in another Nigerian study that investigated the effects of chloroquine prophylaxis in children (Bradley-Moore *et al.,* 1985). Furthermore, when dapsone–pyrimethamine was administered to Gambian children for 2 years, between their 3rd and 5th birthdays, there was no increase in clinical malaria after cessation of the prophylaxis (Otoo *et al.,* 1988). The same lack of rebound malaria was shown in a study using pyrimethamine or chlorproguanil in 2–9-year-old children in Liberia (Björkman *et al.,* 1986). Finally, a non-immune adult population in Irian Jaya (West Papua), Indonesia, having used chloroquine for 1 year, initially showed an increased incidence of *P. falciparum* parasitaemia in the post-chloroquine group but did not show any significant difference in time to first parasitaemia and clinical malaria incidence in the complete 28 week follow-up period (Fryauff *et al.,* 1997).

Few studies have shown a significant increase in clinical malaria (Oyediran *et al.,* 1993; Saarinen *et al.,* 1988). Taken weekly, dapsone–pyrimethamine effectively reduced the risk of clinical malaria and severe anaemia when given to Tanzanian infants between 2 and 12 months of age. Once stopped, however, the group of treated children showed a significantly higher incidence rate of clinical malaria compared to the placebo group (Menendez *et al.,* 1997). Gambian children receiving dapsone–pyrimethamine between the ages of 6 months and 5 years during the transmission season developed an increased risk of clinical malaria in the year after stopping chemoprophylaxis. There was, however, a beneficial effect of a 15 % increase in survival rates during the overall surveillance period up till the age of 7 years (Greenwood *et al.,* 1995).

The perceived fear of a clinical rebound effect was one of the arguments for limiting the presumptive drug use to high risk groups and/or to specified time points. Drug levels that fall below inhibitory concentrations in-between IPT gifts would allow for limited parasite exposure and may, therefore, lead to building of immunity. Indeed, the first IPTi study actually showed a reduction in malaria incidence for a much longer period than could have been expected as a direct effect of the drug, suggesting that the desired enhanced acquisition of immunity did occur (Schellenberg *et al.,* 2005). Unfortunately, this effect has not been reproduced in later studies. In some occasions, even a small increase in malaria incidence was observed after cessation of the intervention (Konaté *et al.,* 2011; Mockenhaupt *et al.,* 2007) but, as was the case for chemoprophylaxis, most studies did not show a change in malaria incidence after the end of the intervention (Aponte *et al.,* 2009; Cairns *et al.,* 2008; Cissé *et al.,* 2006; Dicko *et al.,* 2011; Schreiber *et al.,* 2007).

There are large methodological differences between these studies, including age group, drug choice, dosage, frequency and duration of administration, malaria endemicity and clinical evaluation. Variation in these parameters prevents the possibility to draw unequivocal conclusions regarding the effects of drug use on the acquisition of natural immunity. The combined data, however, suggest that there is insufficient empirical evidence to support the
Protection by controlled human malaria infections under chemoprophylaxis

In contrast to data from a variety of field studies, there is strong experimental evidence that a combination of chemoprophylaxis and so-called ‘controlled human malaria infection’ (CHMI) (Sauerwein et al., 2011) can induce fully protective immune responses. Efficient induction of sterile protection against malaria can be achieved in rodents by inoculation of intact sporozoites under chemoprophylaxis (Belnoue et al., 2004; Borrmann & Matuschewski, 2011). In an analogous proof-of-concept CHMI study, malaria-naïve adult volunteers received 12–15 P. falciparum-infected mosquito-bites once a month for 3 months under chloroquine prophylaxis. In a subsequent challenge infection with five infected mosquitoes at 3 months post-immunization and discontinuation of chloroquine, immunized volunteers were completely protected. When re-challenged after >2 years, the majority of these volunteers was still fully protected against a CHMI. Long-lasting cellular immune responses, more specifically multifunctional effector memory T-cells that produce both IFN-γ and interleukin-2 upon ex vivo stimulation, are associated with protection (Roestenberg et al., 2009, 2011; Teirlink et al., 2011).

The difference in the efficiency of inducing full protection is striking when compared to the situation in endemic countries where it may take years to acquire clinical protection. So, what is the critical difference between CHMI and chemoprophylactic interventions in the field? Several differences may contribute to, or account for, these results. (i), In the experimental studies, challenge infection was performed by a homologous strain, while the genetic diversity of Plasmodium in the field is immense. Therefore, observed protection may be strain-specific; however, we do not favour this explanation, since protection from a heterologous challenge has been shown after immunization with irradiated sporozoites by mosquito bites (Hoffman et al., 2002). (ii), Asexual parasites have been shown to suppress immune responses in rodent studies (Ocaña-Morgner et al., 2003); therefore, the presence of (submicroscopic) parasitaemia might be accountable for a compromised induction of protective immune responses in field studies. (iii), When hepatitis B virus (HBV) vaccine responders are boosted with hepatitis B envelope protein vaccine with or without a single dose of chloroquine, a substantial increase in HBV-specific CD8+ T-cells is observed in the individuals receiving chloroquine (Accapezzato et al., 2005). Some have argued that exposure to Plasmodium-infected mosquito bites induces malaria-specific regulatory T-cells in the skin and, therefore, parasite-specific immunotolerance, which blocks vaccine efficacy. Chloroquine may inhibit this induction of regulatory T-cells and, therefore, enhance the acquisition of immunity (Guilbride et al., 2010). As such, the known immune-modulating effects of the drug chloroquine may be, at least partially, responsible for the efficient induction of immunity in the CHMI model (Sauerwein et al., 2010). A clinical trial where immunization with CHMI under chloroquine is compared to another antimalarial drug that does not have these immune-modulating effects, for example mefloquine, will provide clarification on this hypothesis (Sauerwein et al., 2010).

Innovative application of drug use in the field

Now that the potential for induction of complete and sterile protection has been demonstrated under conditions of CHMI, one may consider the translation of this to a practical application under field conditions. Evidence for a proof-of-concept may be obtained from a study in an area with a short but intensive transmission season that approaches similar inoculation rates as CHMI (Borrmann & Matuschewski, 2011). This will allow for high exposure to pre-erythrocytic antigens while blood-stage infections are controlled. If transmission is low or virtually absent between the malaria seasons, one can evaluate the possibility of induced protective efficacy in the next season.

For safety reasons, alternatives to chloroquine monotherapy have to be used because of the widespread chloroquine-resistance. Recently, immunization with sporozoites of the rodent malaria Plasmodium berghei, in combination with azithromycin, pyrimethamine or primaquine, resulted in a high protective efficacy. Interestingly, when animals received azithromycin prophylaxis during sporozoite exposure, lower liver loads and superior protection were observed (Friesen & Matuschewski, 2011). The combination of azithromycin and chloroquine has demonstrated synergistic effect against parasite growth in vitro (Nakornchai & Konthiang, 2006) and has shown substantially improved clinical and parasitological outcomes compared to azithromycin or chloroquine monotherapy in vivo (Dunne et al., 2005). In treatment trials with azithromycin–chloroquine in Africa, this drug combination showed non-inferiority compared to mefloquine (van Eijk & Terlouw, 2011). Both drugs have been safely administered in all trimesters of pregnancy. Since the antimalarial activity of chloroquine is pleiotropic, drug resistance may be due to different mechanisms, each amenable to reversal by drug combination (Ginsburg, 2005). This opens up exciting possibilities of combining azithromycin and chloroquine as an effective drug combination for immunization by drug use and naturally acquired infections. In this way, even in areas with chloroquine resistance, the antimalarial effects of both drugs and the possibly beneficial immune-modulating effects of chloroquine could be combined. Another interesting
candidate might be dihydroartemisinin–piperazine, which is an effective first-line treatment for *P. falciparum* malaria in both adults and children (Four Artemisinin-Based Combinations (4ABC) Study Group, 2011; Sinclair *et al.*, 2009).

In conclusion, this concept of drug use and naturally acquired infections may be used in the road map towards innovative use of antimalarial drugs for the control or even elimination of malaria. Once field trials are as effective as the promising data from CHMI studies, the concept can hopefully be translated to an effective and applicable strategy for endemic populations.

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**References**


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