A Genetically Attenuated Parasite Vaccine Does Not Require Liver Stage Persistence to Elicit Sterile Protective Immunity against Sporozoite-Induced Malaria in Mice

To the Editor—We have read with interest the excellent recent report by Patrianti et al [1], in which infection treatment vaccination (ITV), with use of repeated vaccinations of mice with wild-type (WT) rodent malaria sporozoites followed by prophylactic primaquine (PQ) treatment, was used to induce sterile, lasting protective immunity against sporozoite challenge. PQ is a drug that kills parasite liver stages and, therefore, the authors concluded that persistence of liver stage parasites is not necessary to induce and maintain protective immunity. Furthermore, the authors noted that this differs fundamentally from vaccinations with irradiated sporozoites or genetically attenuated parasite (GAP) sporozoites, in which generation of an effective, lasting immune response depends on persistence of the parasite as liver stages. We respectfully disagree with the latter conclusion regarding GAPs.

Indeed, it has been shown that the degree of protection induced by vaccination with irradiated sporozoites can be diminished by treatment with PQ [2, 3]. It is difficult to directly evaluate the extent of this reduction, because different vaccination regimens, as well as different animal models, have been used over the years. In general, however, a reduction, rather than a total lack of protection, has been observed. For example, in one study [2], WT irradiated Plasmodium berghei sporozoite–vaccinated rats were treated with PQ. This resulted in a gradual loss of protection in the vaccinated rats: a 42% loss after 1 month and an 84% loss after 3 months. In another study, 3 weekly doses of irradiated P. berghei sporozoites were used to vaccinate mice [3]. Treatment of vaccinated mice with PQ had no effect on protection when mice were challenged 1 week after the last vaccination, but if challenged 5 months after the last vaccination, 50% of the mice lost protection [3]. Some information is available for GAP persistence as a prerequisite to induce and maintain protective immunity. The authors of one study found that PQ treatment of GAP-vaccinated mice led to a complete loss of protection when mice were challenged 1 month after the last vaccination [4]. However, persistence in the liver was not observed in a study using Plasmodium yoelii uis3− GAPs [5]. Furthermore, the P. yoelii p52/p36 GAP, which exhibits a severe defect in establishing a metabolically active liver stage and does not persist, also induces an effective immune response against WT challenge [6]. However, PQ was not used in these studies to assess its effect on protection.

To test whether liver stage persistence is necessary for GAP-induced protective immunity, we have designed an experiment in which BALB/c mice were vaccinated with P. yoelii uis3− [5] or WT sporozoites in a regimen comparable to that used in the study by Patrianti et al [1]. Initially, mice were vaccinated with WT sporozoites (10⁴) and treated with PQ 24 and 48 h after vaccination. In our hands, mice injected with WT sporozoites (n = 5) and treated with PQ (60 mg/kg) at 24 and 48 h after vaccination all developed blood stage parasitemia at day 4 after ITV (data not shown), indicating that PQ treatment 24 h after vaccination does not fully eradicate liver stages. Therefore, we decided to use 12 and 48 h after vaccination PQ treatment, which prevented the onset of blood stage infection in WT ITV. Mice were vaccinated with 10⁴ WT or uis3− sporozoites 3 times with a 2-week interval between vaccinations. Three months later, all mice, including the controls (uis3− vaccinated mice that were not treated with PQ and naive mice treated with PQ) were challenged with 10⁴ WT sporozoites. As expected, mice vaccinated with uis3− without PQ treatment or WT sporozoites with PQ treatment were completely protected (Figure 1). Importantly, however, vaccination with uis3− sporozoites followed by PQ treatment also resulted in complete protection against sporozoite challenge (Figure 1). As a control, mice treated with only PQ did become patent after sporozoite challenge (Figure 1). This demonstrates that GAPs do not need to persist as liver stages to induce complete, lasting protective immunity.

We conclude that the claim of a fundamental difference in the induction and maintenance of immune protection conferred by ITV versus GAP vaccination is untenable. It is likely that PQ treatment of WT parasite-inoculated mice creates a quasi-attenuated phenotype that is comparable to the phenotype described for GAPs (ie, early arrest in liver stage development and subsequent clearance of parasites from the liver) [5]. Does this imply that the expression of liver stage antigens does not significantly contribute to protection? We believe the answer is no, because even during the first 12 h of liver stage development, GAPs or WT parasites likely express many new antigens that can prime the immune response against liver stages. However, GAPs that arrest later in liver stage development, such as the recently described fatty acid biosynthesis mutants [7], likely express additional proteins and might therefore generate a more diversified immune response that targets a wider variety of antigens. GAPs that arrest late in liver stage development might,
Therefore, constitute a more potent vaccine when compared with early arresting GAPs or irradiated sporozoites. These are important topics for future investigations.

Reply to Mikolajczak et al

To the Editor—The correspondence from Mikolajczak et al [1] gives us an opportunity to refine the principles of 2 distinct whole-organism antimalaria vaccine concepts (Table 1). Akin to a classical vaccination scheme, live attenuated Plasmodium sporozoites can be administered in a prime-boost protocol. Irradiated [2] or genetically manipulated [3] sporozoites invade their target cells inside the liver, but life cycle progression to the pathogenic blood stage is blocked. In experimental murine and human vaccine trials, live attenuated sporozoites induce potent, long-lasting protection [3, 4] and are the current gold standard for antimalaria vaccine development. Intriguingly, vaccine-like immunity also develops upon exposure to natural sporozoites under drug cover, either by suppressive treatment of emerging blood-stage parasites [5, 6] or by causal prophylactic treatment [7].

As expected, Mikolajczak et al [1] were able to reproduce our data [7]. Vaccination with 3 doses of 10,000 Plasmodium yoelii sporozoites under primaquine cover induces 100% protection against reinfection 3 months later. We also showed substantial protection even when sporozoites were delivered by natural mosquito bite [7]. Interestingly, in their letter, Mikolajczak et al [1] combine the 2 approaches that lead to sterile protection in a single vaccination scheme. Although the interpretation of data remains inconclusive, it offers a path towards safe elimination of genetically attenuated parasites (GAPs) in vaccinees, a potentially important additional safety measure in experimental cohorts.

However, to test the roles of parasite persistence, a different experimental design is required. As established previously for the 2 approaches for live attenuated liver stages [8, 9], the vaccinations need to be done without drug treatment. Only then can acquisition of adaptive stage-specific immunity be attributed to the GAPs, rather than to the simultaneous primaquine-mediated growth arrest. To study

**Figure 1.** Prophylactic primaquine (PQ) treatment does not affect the level of protective immunity induced by Plasmodium yoelii uis3+ vaccination. BALB/c mice (8-week-old females from the Jackson Laboratories) were vaccinated with 3 intravenous doses of 10^5 P. yoelii 17 XNL wild-type (WT) or uis3+ sporozoites under prophylactic PQ treatment (60 mg/kg) 12 and 48 h after vaccination. Three months after the last vaccination, mice were challenged with 10^5 P. yoelii 17 XNL sporozoites. Mice were evaluated daily for the occurrence of blood stage parasites starting on day 3 after sporozoite challenge. Black line, mice vaccinated with WT sporozoites under prophylactic PQ treatment (n = 10); black triangles, mice vaccinated with uis3+ with prophylactic PQ treatment (n = 6); gray dots, mice vaccinated with uis3+ without prophylactic PQ treatment (n = 5); gray line, control group of naive mice treated with PQ without vaccination (n = 6).

### References


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