

Interactions Between Worm Infections and Malaria

Mathieu Nacher

Equipe d'Accueil EA 3593, Campus St. Denis, Faculté de Médecine, Antilles-Guyane, Cayenne, French Guiana; E-mail: m_nacher@lycos.com

Abstract

Helminths are the most prevalent parasitic infections and malaria is the deadliest parasitic disease. Helminths have been reported to be protective against the severe forms of malaria but they were also possibly linked to increased malaria-incidence and gametocyte carriage. Connecting the dots between observations suggests that statistical regularities throughout the evolution of worms and malaria parasites in the same hosts, may have led to the emergence of non-zero interactions as observed in iterated prisoners dilemma games. Thus by protecting the host, helminths protect themselves and their reproductive potential, but also favor the dissemination and reproduction of *Plasmodium falciparum*. The proximate causes of this evolutionarily stable strategy might be mediated by IgE and the CD23/NO pathway, the protective role of IL10 in helminth-infected patients, and possibly the hematological consequences of worms. The chronic activation of the CD23/NO pathway might be instrumental in down-regulating the expression of cytoadherence receptors thus reducing sequestration of parasitized red blood cells in the deep organs. Mild anemia in helminth-infected patients might favor gametocytogenesis and send attractive cues to the vector.

This framework leads to numerous testable hypotheses and could explain certain singularities regarding the double edged role of IgE and NO. Among these hypotheses, there are 2 practical ones: the impact of helminths on malaria vaccine candidates, and the theoretical risk of increasing the severity of malaria after anthelmintics. The capacity for increased IgE responses could thus have been vital in our ancestor's wormy and malarious past. Allergies may be what remains of it in the modern world.

Index Entries

Malaria; helminths; NO; IGE; CD23; IL-10.

With more than 400 million people affected and more than 1 million deaths across the world each year, malaria represents a major public health challenge. The deadliest parasite, *Plasmodium falciparum*, and the most prevalent parasites, worms (one fourth of humanity are infested today [1]) have been evolving in the same populations for thousands of years. These combined statistical regularities have exerted constant selective pressures on our ancestors. This article discusses interactions that have been observed between these parasites and attempts a few possible explanatory hypotheses for the modalities of this durable interaction.

Plasmodium falciparum

Among the four plasmodial species in humans, only *Plasmodium falciparum* is directly responsible for death (2). The infected female anopheles mosquito injects a few plasmodial sporozoites (usually 8–15) during its blood meal on the human host. The sporozoites will rapidly reach the liver where a first amplification takes place, releasing 10^5 – 10^6 merozoites in the blood circulation usually 5–6 d after the infective mosquito bite. Merozoites will then invade erythrocytes of all ages and multiply, thereby leading to the destruction of both parasitized and nonparasitized red blood cells. Each merozoite matures and generates around 20 new merozoites in 48 h; these are then capable of infecting other erythrocytes, thus amplifying the number of parasites about tenfold at each cycle. After a few erythrocytic cycles, usually 11 d after the mosquito bite, parasites will become detectable and malaria symptoms will appear.

The infected erythrocytes express numerous plasmodial proteins on their membrane. Among these, Pfemp1 (*Plasmodium falciparum* erythrocyte membrane protein 1) is clumped in knoblike protuberances that have the ability to adhere to endothelial cytoadherence receptors such as the constitutively expressed CD36 and the inducible ICAM1. This leads to the sequestration of the parasites in post-capillary

venules, thus allowing to evade the splenic filter. Parasite growth is logarithmic and will stop because of the immune response, or because of the reduction in suitable red blood cells. When the host is nonimmune and has no protective factors, multiplication of the parasite will take place with little limitation, and a significant proportion of the circulating parasite biomass will be sequestered in the deep tissues, leading to severe malaria. The combination of excessive parasite multiplication and sequestration will reduce oxygen transport and create vascular obstruction, which leads to tissular hypoxia, metabolic acidosis, and organ dysfunction. Schematically, when erythrocyte destruction predominates the clinical picture will be severe anemia, whereas when sequestration predominates the presentation will often be cerebral malaria. Although it is not in the interest of the parasite to kill the host, the low-oxygen microenvironment created by anemia or vascular obstruction seems to favor the conversion to plasmodial sexual stages, gametocytes (3). These sexual stages, when absorbed by a female mosquito, will form a zygote, which generates sporozoites capable of perpetuating the cycle in another human host.

Although in recent years drug resistance has worsened the situation, humans have evolved with malaria for thousands of years despite the absence of treatment. A number of protective host factors have permitted survival from malaria: hemoglobinopathies, polymorphisms of adhesion molecules, or promoter genes involved in immunity illustrate malaria mortality's impact on our genome (2). The considerable plasmodial antigenic variation makes the acquisition of immunity a slow, transmission-dependent, and, as unsuccessful vaccine trials have shown, a somewhat sketchy process. Protective antimalarial immunity combines cellular and humoral immune effectors, in both their specific and nonspecific components. The absence of immunity increases the risk of severe malaria (most deaths from malaria are children under 5 yr), but it also seems

that the nature of the immune response can be part of its pathogenesis (i.e., by increasing cytoadherence receptors, or pro-inflammatory cytokines such as TNF- α) (4). In areas of high endemicity, after repeated infections, the host finally develops a state of premunition against malaria in which parasites are chronically present in the blood but their numbers are limited by nonspecific immune effectors (notably nitric oxide), and, most of the time, they do not generate any symptoms (5).

IgE, CD23, and Nitric Oxide

The oxidation of the terminal guanido nitrogen atoms of L-arginine generates nitric oxide (NO), a reactive short-lived radical that acts as a vasodilator, a neuromediator, and a regulator of other immune effectors, and is a cytostatic or cytotoxic agent against a variety of parasites (6,7). Its secretion during the immune response is regulated by the inducible nitric oxide synthase (iNOS). This enzyme has a complex induction pathway with both Th1 and Th2 cytokines as potential activators. Although the role of interferon (IFN)- γ is often put forward, the stimulation of the low-affinity receptor for IgE (Fc ϵ RII/CD23) is also a potent activator of iNOS and TNF- α (6). Following stimulation by IFN- γ or IL-4, the expression of the CD23 receptor is upregulated on a variety of cells, including macrophages, platelets, endothelial cells, epithelial cells, and B-lymphocytes (8). The CD23 receptor can be activated by ligands such as IgE complexes, membrane and soluble CD21, CD11b, and CD11c, and L-fucose. In the absence of ligands to stabilize it, the CD23 is cleaved into a soluble form that has pleiotropic and, notably, IgE-inducing properties (8). Although the cross-linking of the CD23 receptor stimulates iNOS and TNF- α , it also leads to the release of IL-10, which then inhibits both iNOS and TNF- α , and thus acts as a regulatory negative feedback on NO release (9–11).

NO, IgE, and Severe Malaria

Among the immunological studies of malaria conducted in the last decade, the study of the role of NO has generated much debate. It was first proposed that NO was a pathogenic factor causing coma during cerebral malaria (12,13). However, other studies suggested that, on the contrary, it was a protective factor against cerebral malaria (14,15). Similar discrepancies exist regarding the role of IgE. It has been observed that both total and *Plasmodium*-specific IgE were increased in severe malaria when compared to “mild” malaria (16), suggesting that IgE was a pathogenic factor during severe malaria (17,18). In this perspective, case-control studies were conducted in Thailand to determine whether intestinal helminths, which are major inducers of IgE secretion, had any relation to severe malaria. These studies showed that, after adjusting for confounders, helminths were associated with a dose-dependent protection against both cerebral malaria and malaria-associated renal failure (19–21). In addition, it was observed that helminth-infected patients had significantly increased concentrations of reactive nitrogen intermediates (RNIs), which are the end products of the short-lived NO. The quantity-dependent increase of RNI concentrations in helminth-infected patients was linked to high IgE concentrations in these patients. RNI concentrations were positively correlated with total IgE concentrations and negatively correlated with the soluble CD23 receptor, which results from the cleavage of the membrane CD23 when it is not stabilized by its ligands. Finally, RNI concentrations were negatively correlated with the schizont/trophozoite ratio, presumably a reflection of the sequestered parasite biomass. Overall, this suggested that IgE complexes in helminth-infected patients induced the release of NO through the CD23/NO pathway, thereby reducing sequestration and protecting against severe malaria (21). This, however, does not exclude other helminth-related immunological mechanisms such as nutritional changes in immunity or erythro-

cyte characteristics (22), or the homeostatic properties of IL-10, which seems to protect against severe malaria (23,24), and is stimulated by both helminth infections (25) and the cross-linking of CD23 (11).

The possibility of a causal link between helminths and protection from severe malaria was suggested by (1) the dose-effect trend; (2) the strength of the association; (3) the repetition of the observation after controlling for confounders (26); (4) studies conducted in Anjouan suggesting that *Ascaris*-infected malnourished patients had a suppression of malaria (27); (5) the negative correlation between IgE deposition in the placental vasculature and parasitized red blood cell sequestration (28); (6) the presence of an animal model showing that rodents were protected from cerebral malaria by prior injection of filarial parasites (29); (7) the plausibility of the underlying immune mechanism being reinforced by the presence of an in vitro model showing that the stimulation of the CD23/NO pathway in human lung endothelial cells reduced ICAM1 expression and cytoadherence of parasitized red blood cells and killed parasites (Traore et al., in press).

The ability of CD21 to stimulate the CD23 receptor predicted that RNI concentrations should also correlate with sCD21, which has biological effects similar to IgE complexes (30). This was confirmed by another study in which cerebral malaria cases and hyperparasitemic controls both had biological profiles suggesting an activation of the CD23/NO pathway by IgE and CD21 (31). However, these profiles were different, suggesting that, in cerebral malaria, the induction and stimulation of CD23 and NO release were acute, whereas in controls, the CD23/NO pathway had been chronically stimulated. Thus, in helminth-free patients, recent *Plasmodium*-mediated CD23 expression might lead to a great availability of membrane receptors for ligands and to an intense unrestrained *post hoc* NO release that is conceivably pathogenic. In contrast, in helminth-infected

patients, because of the chronic IL-4-mediated induction of both the CD23 receptor and its ligand, a greater proportion of the membrane CD23 receptors were expressed and crosslinked at the earliest stages of plasmodial multiplication. IgE complexes might thus stimulate and block the remaining available CD23 receptors, as well as downregulate the acute IgE synthesis induced by malaria. An early moderate NO release could reduce ICAM1 expression and cytoadherence thus protecting the human host against sequestration-related complications (31). In addition to this "pharmacological" chronic versus acute ligand-receptor stimulation hypothesis, the release of IL-10 following chronic CD23 stimulation (11) in helminth-infected patients could have antagonized the proinflammatory effects of TNF- α (23,24) and downregulated the transcription of both iNOS and TNF- α genes (6). Thus an early stimulation of TNF- α and iNOS could subsequently be moderated by this negative feedback. This hypothetical framework could explain a number of singularities and reconcile the apparently conflicting views that IgE could be pathogenic (17,18) (*post hoc* secretion, no downregulation) and protective (19,32) (when chronic stimulation leads to an early control of malaria and downregulation of immune effectors). It also enables the reconciliation of conflicting views about the pathogenicity (12) vs protection (14) conferred by NO: it can be both protective and pathogenic depending on the dynamics of NO-induction (31).

Possible Evolutionary Benefits of a Helminth-Mediated Th2 Bias

Although total and specific IgE are increased in helminth infections, they usually do not eliminate worms. Moreover, evolution seems to have selected antigenic determinants with potent IgE-inducing capabilities. *Ascaris*, notably, is highly allergenic (2). If the function of IgE was only to get rid of worms, it would seem detrimental for *Ascaris* to induce it. Such a conspicuous Th2 bias must therefore have con-

ferred evolutionary advantages. One benefit could have been to limit superinfection and worm numbers, thereby avoiding competition for limited resources as well as preventing potentially fatal surgical complications. Given that malaria has been a major selective force in human history (33), it is also possible that this marked immunogenicity was selected because it allowed the hosts to survive and disseminate more *Ascaris* eggs. This would explain why studies found that *Ascaris* seemed to be more protective against cerebral malaria than other worms (19). Immunity was tuned in a manner that allows to limit worm infestation without totally eliminating established worms, thus leading to chronic IgE stimulation and IL-10 modulation (25). It was normal to have worms in human history; therefore, evolution must have selected the most favorable immunogenetic characteristics in populations with a Th2 bias (34). The CD23/NO pathway has the advantage of stimulating and then refraining NO and TNF- α release (6,9,11), without first stimulating Th1 cytokines which have the ability to upregulate cytoadherence.

From Immunology to Epidemiology

One of the major interests in the above hypothesis is that IgE, CD21, and CD23 can be upregulated by specific pathogens, thereby connecting immunology and epidemiology. Environmental Th2 and IgE inducers in the tropics are numerous: measles (35), malnutrition (36), filarial parasites (29), insect bites (37,38), and allergens are a few that could thus be suspected to influence the risk of severe malaria. HTLV1 upregulates CD21 expression (39) and CD23 is closely linked to EBV (EBV receptor), which also induces CD21 (40), thereby leading to the testable predictions that EBV and HTLV1 infections might also favorably alter the course of malaria. Ultraviolet radiation, which is omnipresent in the tropics and influences the evolution of parasitic diseases (41), also has the ability to modulate the Th1/Th2 equilibrium, notably IL-10 (42), thereby

potentially interfering with CD23/NO-mediated immunity.

Is There More to Worms Than Protection Against Severe Malaria?

Evolutionary biology has shown that there is a link between virulence and reproduction (43). Increased virulence at an individual level might increase the reproductive rate, but it may also kill the host, which probabilistically is not likely to be a very successful parasite strategy. This evolutionary link between virulence and reproduction might be illustrated by the observation that, in addition to protection from severe malaria, helminth-infected patients with mild *falciparum* malaria were more likely to have gametocytes than patients without helminths (44). This observation seemed to result from the fact that helminth-infected patients had lower hemoglobin concentrations than helminth-free patients (45), a condition that seems favorable for gametocytogenesis (3,46).

Surprisingly, although worms protected against severe malaria, it was also observed that the incidence of malaria increases in hookworm-infected patients (47). Similarly, in Africa, *Schistosoma haematobium* infection was associated with increased *falciparum* malaria incidence (48). Hypothetically, this could result from the host's increased mosquito attractiveness or decreased mosquito-avoidance behavior due to anemia in hookworm or *Schistosoma*-infected patients (49), or from a Th2 immunological bias suppressing IFN- γ -mediated immunity against liver stages (4).

Ultimately, by increasing incidence and avoiding severe complications of malaria, helminths might also allow to keep a nonsterilizing chronic stimulation of specific and nonspecific malaria immunity. Such low-level chronic parasitemia in helminth-infected subjects is suggested by observations that antihelminth treatment in high-transmission areas was followed by an increase in symptomatic malaria (50). On the contrary, however, helminth-

related immunomodulation might also lead to adverse consequences on the evolution of diseases caused by other pathogens, such as mycobacterial diseases (51) or HIV (52). This might now be of great evolutionary significance given the epidemiological transition that has recently put the burden of HIV and tuberculosis at the forefront.

Lessons From Animal Models

Similar intriguing complex interactions between pathogens have been observed in animal models. In many cases, the result of a combination of pathogens in the same host is not the addition of their respective pathologies. Sometimes, behind the noisy nuisance of a single pathogen lies a silent benefit in the ecosystem. Although a pathogen might cause fitness reduction, it might also prevent even greater fitness reduction due to another coinfecting pathogen, thereby leading to a net result that is beneficial in terms of fitness (53). Thus, looking at isolated elements does not presume the pathogen's overall function in the ecosystem. A variety of agonist and antagonist interactions have been observed in animal models between viruses and parasites (54), between bacteria and parasites (55–57), and between helminthic and protozoan parasites (58). However, these models are still reductions of the complexity of the real world (53) in which pathogens are nonrandomly associated by a network of positive and negative feedback loops that stabilize into durable interactions.

Connecting the Dots

Although some of the above results need confirmation in other epidemiological settings, the general perspective that seems to arise is that helminths, humans, and *P. falciparum* have evolved toward mutual benefits: helminths protect their hosts so they can continue to lay their eggs; consequently, humans can survive to reach the reproductive age, and they might also be more likely to survive malaria in pregnancy. It is of interest that *Ascaris* and *Trichu-*

ris, which are transmitted through a fecal-oral cycle, were twice more prevalent in women than in men on the Thai-Burmese border (pers. comm.). Such a preferential transmission could have conferred protection against severe malaria to both pregnant women and their young children. Survival from malaria might also have been instrumental in selecting for the observed genetic predisposition to *Ascaris* (59).

The possible evolutionary benefits of a nonsterilizing immune response maintaining asymptomatic chronic malaria might have been related to advantages conferred by a perpetual state of readiness, with increased NO synthesis to control new plasmodial clones (14), and possibly resistance to other pathogens notably through endotoxine tolerance during Gram-negative infections (5). In addition, the acquisition of malaria tolerance in helminth-infected adults (50) of reproductive age could eventually have served as a fitness indicator influencing mate choice (53,60).

Finally, by infecting helminth-infected patients, *P. falciparum* might have more chances to reproduce and to generate genetic variability because of decreased host mortality, increased gametocyte carriage (44), and cross-fertilization (47,61). All worms do not have equivalent IgE-inducing potentials, and the blood loss due to hookworm, *Schistosoma*, and sometimes *Trichuris* might have hematological effects (i.e., reticulocytosis when the iron stores are intact, and later iron deficiency) with consequences for malaria. The combination of different worms might thus lead to complete the particular requirements of each parasitic partner to reach an optimal equilibrium. It is of interest to note that Americans of Filipino descent and African-Americans tend to have increased concentrations of IgE complexes and more allergies when compared to Caucasians (62–64). An explanation of this could be that their ancestors survived malaria and were able to transmit their genes because they had increased baseline IgE concentrations. What now represents a very unfortunate immunological hypersensitiveness in malaria-free areas might be

the remnant of a salutary immunogenetic trait in the wormy and malarious history of our ancestors. African-Americans and Asians also tend to have more autoimmune diseases (65–67). Again, this may have some relation to the selective pressures of malaria.

Conclusion: *Primum non nocere*

Populations living in malaria endemic areas are often multiparasitized. This can be somewhat puzzling from a medical perspective of disease causation that is often biased toward the noisy nuisance: parasites are bad and must be treated (34). However, in areas with scarce health resources, the natural equilibriums that have been selected by evolution might still be very effective. Unraveling the complex interactions between pathogens in order to obtain optimal health is therefore difficult in non-developed areas. Moreover, the fragmentation of research in noncommunicating specialties and chronic funding limitations make a comprehensive study of the interaction between different pathogens even more difficult. Given the apparent interactions between malaria and worms, factoring worms into the equation might increase our understanding of the ecology of malaria. Although the above observations may seem speculative and anecdotal, they lead to numerous testable hypotheses that are not devoid of practical consequences: a better understanding of the immune response, and of the part played by helminths might have very significant implications for successful malaria vaccines, as well as for the interpretation of vaccine trials (68). If vertical helminth eradication was performed without strengthening malaria treatment capabilities, it is theoretically foreseeable that a natural protection against severe malaria might be removed setting up conditions for cerebral malaria epidemics (19,22).

Acknowledgment

Mathieu Nacher is supported by the Howard Grant, Pasteur Institute Paris.

References

- 1 Bundy, D., Sher, A., and Michael, E. (2000), *Parasitol Today* **16**, 273–274.
- 2 Manson, P., Cook, G.C., and Bell, D.R. (1996), *Manson's Tropical Diseases*, 20th ed., London, Philadelphia: W.B. Saunders.
- 3 Nacher, M., Singhasivanon, P., Silachamroon, U., Treeprasertsuk, S., Tosukhowong, T., Vannaphan, S., et al. (2002), *J Parasitol* **88**, 97–101.
- 4 Plebanski, M. and Hill, A.V. (2002), *Curr Opin Immunol* **12**, 437–441.
- 5 Clark, I.A., al-Yaman, F.M., Cowden, W.B., and Rockett, K.A. (1996), *Lancet* **348**, 1492–1494.
- 6 Dugas, B., Mossalayi, M.D., Damais, C., and Kolb, J.P. (1995), *Immunol Today* **16**, 574–580.
- 7 Vouldoukis, I., Riveros-Moreno, V., Dugas, B., Ouaz, F., Becherel, P., Debre, P., et al. (1995), *Proc Natl Acad Sci USA* **92**, 7804–7808.
- 8 Delespesse, G., Sarfati, M., Wu, C.Y., Fournier, S., and Letellier, M. *Immunol Rev* **125**, 77–97.
- 9 Ho, M., Sexton, M.M., Tongtawe, P., Looareesuwan, S., Suntharasamai, P., and Webster, H.K. (1995), *J Infect Dis* **172**, 838–844.
- 10 Vouldoukis, I., Becherel, P.A., Riveros-Moreno, V., Arock, M., da Silva, O., Debre, P., et al. *Eur J Immunol* **27**, 860–865.
- 11 Dugas, N., Palacios-Calender, M., Dugas, B., Riveros-Moreno, V., Delfraissy, J.F., Kolb, J.P., et al. (1998), *Cytokine* **10**, 680–689.
- 12 Al Yaman, F.M., Mokela, D., Genton, B., Rockett, K.A., Alpers, M.P., and Clark, I.A. (1996), *Trans R Soc Trop Med Hyg* **90**, 270–273.
- 13 Maneerat, Y., Viriyavejakul, P., Punpoowong, B., Jones, M., Wilairatana, P., Pongponratn, E., et al. (2000), *Histopathology* **37**, 269–277.
- 14 Anstey, N.M., Weinberg, J.B., Hassanali, M.Y., Mwaikambo, E.D., Manyenga, D., Misukonis, M.A., et al. (1996), *J Exp Med* **184**, 557–567.
- 15 Hobbs, M.R., Udhayakumar, V., Levesque, M.C., Booth, J., Roberts, J.M., Tkachuk, A.N., et al. (2002), *Lancet* **360**, 1468–1475.
- 16 Perlmann, H., Helmby, H., Hagstedt, M., Carlson, J., Larsson, P.H., Troye-Blomberg, M., et al. (1994), *Clin Exp Immunol* **97**, 284–292.
- 17 Perlmann, P., Perlmann, H., Flyg, B.W., Hagstedt, M., Elghazali, G., Worku, S., et al. (1997), *Infect Immun* **65**, 116–121.
- 18 Maeno, Y., Perlmann, P., Perlmann, H., Kusuvara, Y., Taniguchi, K., Nakabayashi, T., et al. (2000), *Am J Trop Med Hyg* **63**, 128–132.
- 19 Nacher, M., Gay, F., Singhasivanon, P., Krudsood, S., Treeprasertsuk, S., Mazier, D., et al. (2000), *Parasite Immunol* **22**, 107–113.
- 20 Nacher, M., Singhasivanon, P., Silachamroon, U., Treeprasertsuk, S., Vannaphan, S., Traore, B., et al. (2001), *Am J Trop Med Hyg* **65**, 834–836.
- 21 Nacher, M., Singhasivanon, P., Traore, B., Vannaphan, S., Gay, F., Chindanond, D., et al. (2002), *Am J Trop Med Hyg* **66**, 304–309.

- 22 Murray, M.J., Murray, A.B., Murray, N.J., and Murray, M.B. (1978), *Am J Clin Nutr* **31**, 57–61.
- 23 Ho, M., Schollaardt, T., Snape, S., Looareesuwan, S., Suntharasamai, P., and White, N.J. (1998), *J Infect Dis* **178**, 520–525.
- 24 May, J., Lell, B., Luty, A.J., Meyer, C.G., and Kreamsner, P.G. (2000), *J Infect Dis* **182**, 1570–1573.
- 25 Yazdanbakhsh, M., van den Biggelaar, A., and Maizels, R.M. (2001), *Trends Immunol* **22**, 372–377.
- 26 Nacher, M., Singhasivanon, P., Treeprasertsuk, S., Vannaphan, S., Traore, B., Looareesuwan, S., et al. (2002), *Ann Trop Med Parasitol* **96**, 5–13.
- 27 Murray, M.J., Murray, A.B., Murray, M.B., and Murray, C.J. (1977), *Am J Clin Nutr* **30**, 2117–2121.
- 28 Maeno, Y., Steketee, R.W., Nagatake, T., Tegoshi, T., Desowitz, R.S., Wirima, J.J., et al. (1993), *Am J Trop Med Hyg* **49**, 574–580.
- 29 Yan, Y., Inuo, G., Akao, N., Tsukidate, S., and Fujita, K. (1997), *Parasitology* **114**, 333–338.
- 30 Fremaux-Bacchi, V., Kolb, J.P., Rakotobe, S., Kazatchkine, M.D., and Fischer, E.M. (1999), *Immunopharmacology* **42**, 31–37.
- 31 Nacher, M., Singhasivanon, P., Silachamroon, U., Treeprasertsuk, S., Tosukhowong, T., Vannaphan, S., et al. (2002), *Parasite Immunol* **24**, 395–400.
- 32 Khusmith, S., Panitchakorn, J., Krudsood, S., Wilairatana, P., and Looareesuwan, S. (2001), *Southeast Asian J Trop Med Public Health* **32**, 696–701.
- 33 Hill, A.V., Allsopp, C.E., Kwiatkowski, D., Anstey, N.M., Twumasi, P., Rowe, P.A., et al. (1991), *Nature* **352**, 595–600.
- 34 Nacher, M. (2002), *Parasite Immunol* **24**, 391–395.
- 35 Bach, J.F. (2002), *N Engl J Med* **347**, 911–920.
- 36 Hagel, I., Lynch, N.R., Di Prisco, M.C., Sanchez, J., and Perez, M. (1995), *Trans R Soc Trop Med Hyg* **89**, 562–565.
- 37 Peng, Z., Arthur, G., Rector, E.S., Kierek-Jaszczuk, D., Simons, F.E., and Becker, A.B. (1997), *J Allergy Clin Immunol* **100**, 87–95.
- 38 Kamhawi, S., Belkaid, Y., Modi, G., Rowton, E., and Sacks, D. (2000), *Science* **290**, 1351–1354.
- 39 McNearney, T.A., Ebenbichler, C.F., Totsch, M., and Dierich, M.P. (1993), *Eur J Immunol* **23**, 1266–1270.
- 40 Larcher, C., Kempkes, B., Kremmer, E., Prodinger, W.M., Pawlita, M., Bornkamm, G.W., et al. (1995), *Eur J Immunol* **25**, 1713–1719.
- 41 Nacher, M., Carme, B., Sainte Marie, D., Couppie, P., Clyti, E., Guibert, P., et al. (2001), *J Parasitol* **87**, 1495–1498.
- 42 Garssen, J., Vandebruiel, R.J., De Gruijl, F.R., Wolvers, D.A., Van Dijk, M., Fluitman, A., et al. (1999), *Immunology* **97**, 506–514.
- 43 Gupta, S., Hill, A.V., Kwiatkowski, D., Greenwood, A.M., Greenwood, B.M., and Day, K.P. (1994), *Proc Natl Acad Sci USA* **91**, 3715–3719.
- 44 Nacher, M., Singhasivanon, P., Silachamroon, U., Treeprasertsuk, S., Krudsood, S., Gay, F., et al. (2001), *Am J Trop Med Hyg* **65**, 644–647.
- 45 Nacher, M., Singhasivanon, P., Gay, F., Phumratanaprapin, W., Silachamroon, U., and Looareesuwan, S. (2001), *Am J Trop Med Hyg* **65**, 335–337.
- 46 Price, R., Nosten, F., Simpson, J.A., Luxemburger, C., Phaipun, L., ter Kuile, F., et al. (1999), *Am J Trop Med Hyg* **60**, 1019–1023.
- 47 Nacher, M., Singhasivanon, P., Yimsamran, S., Manibunyong, W., Thanyavanich, N., Wuthisen, R., et al. (2002), *J Parasitol* **88**, 55–58.
- 48 Hammady, M., Dabo, A., Sangare, L., Sogoba, M., Thera, A., Dicko, A., and Doumbo, O. (2002), *Am J Trop Med Hyg* **67**, S128.
- 49 Nacher, M. (2002), *Med Hypotheses* **59**, 363–365.
- 50 Murray, J., Murray, A., Murray, M., and Murray, C. (1978), *Am J Clin Nutr* **31**, 1363–1366.
- 51 Diniz, L.M., Zandonade, E., Dietze, R., Pereira, F.E., and Ribeiro-Rodrigues, R. (2001), *Am J Trop Med Hyg* **65**, 852–854.
- 52 Bentwich, Z., Kalinkovich, A., Weisman, Z., Borkow, G., Beyers, N., and Beyers, A.D. (1999), *Immunol Today* **20**, 485–487.
- 53 Thomas, F., Poulin, R., Guegan, J.F., Michalakakis, Y., and Renaud, F. (2000), *Parasitol Today* **16**, 533–536.
- 54 Eckwalanga, M., Marussig, M., Tavares, M.D., Bouanga, J.C., Hulier, E., Pavlovitch, J.H., et al. (1994), *Proc Natl Acad Sci USA* **91**, 8097–8101.
- 55 Clark, I.A. (1979), *Infect Immun* **24**, 319–325.
- 56 Clark, I.A. (1979), *Parasite Immunol* **1**, 179–196.
- 57 Clark, I.A., Allison, A.C., and Cox, F.E. (1976), *Nature* **259**, 309–311.
- 58 Christensen, N.O., Nansen, P., Fagbemi, B.O., and Monrad, J. (1987), *Parasitol Res* **73**, 387–410.
- 59 Williams-Blangero, S., VandeBerg, J.L., Subedi, J., Aivaliotis, M.J., Rai, D.R., Upadhyay, R.P., et al. (2002), *Proc Natl Acad Sci USA* **99**, 5533–5538.
- 60 Kavaliers, M., Colwell, D.D., and Choleris, E. (2000), *Parasitol Today* **16**, 464–468.
- 61 Nacher, M., Singhasivanon, P., Gay, F., Silachamroon, U., Phumratanaprapin, W., and Looareesuwan, S. (2001), *J Parasitol* **87**, 912–915.
- 62 Orgel, H.A., Lenoir, M.A., and Bazaral, M. (1974), *J Allergy Clin Immunol* **53**, 213–222.
- 63 Roberts, E.M. (2002), *J Natl Med Assoc* **94**, 215–223.
- 64 Joseph, C.L., Ownby, D.R., Peterson, E.L., and Johnson, C.C. (2002), *Ann Allergy Asthma Immunol* **88**, 507–512.
- 65 McCarty, D.J., Manzi, S., Medsger, T.A., Jr., Ramsey-Goldman, R., LaPorte, R.E., and Kwok, C.K. (1995), *Arthritis Rheum* **38**, 1260–1270.
- 66 Serdula, M.K. and Rhoads, G.G. (1979), *Arthritis Rheum* **22**, 328–333.
- 67 Sullivan, K.E., Wooten, C., Schmeckpeper, B.J., Goldman, D., and Petri, M.A. (1997), *Arthritis Rheum* **40**, 2207–2211.
- 68 Nacher, M. (2001), *Trends Parasitol* **17**, 563–565.