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Worms can worsen malaria: towards a new means to roll back malaria?

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Recent studies in Africa and Asia indicate that different helminthic infections adversely affect the clinical outcome of malaria infections. This suggests that helminths can influence the acquisition of immunity against *Plasmodium*. Worms could constitute a confounding factor in the assessment of efficacy of malaria-control intervention, including vaccine prototypes in clinical trials. These observations have fundamental and practical consequences; if the deleterious effect of worms on malaria is confirmed, treatment of helminths would offer an affordable, strongly effective and novel means to roll back malaria. With this article, we hope to induce others to conduct similar studies in different regions.

Mixed infections

In contrast to laboratory models, humans infected with malaria are exposed to a large number of concomitant acute or persistent infections due to viruses (e.g. hepatitis B, influenza and HIV), bacteria (e.g. *Shigella* and *Staphylococcus*) and a variety of parasites (e.g. other protozoa, intestinal worms, schistosomes and filariae) [1–3]. Each of these pathogens, particularly those causing chronic infection, can influence the host immune system. Because of the large number of pathogens interacting in a dynamic manner over time, the resulting multifactorial immunological network is beyond systematic analysis and might explain why these issues have seldom been addressed. In mixed infections induced in experimental models, the burden of one or both of the infectious agents might be increased, one or both might be suppressed or one might be increased and the other suppressed [2]. However, little is known about these interactions in humans and their relevance to clinical disease.

Insight into malarial immunity provides new hypotheses

Recent studies of the acquisition of immune protection against malaria that focused primarily on clinical investigations in humans [4,5] provided information about the potential interaction between helminthic infections and malaria.

Immunity to malaria erythrocytic stages builds up progressively in children from endemic areas. The immune can control parasite loads and tolerate parasitemia without clinical manifestations of the disease by the age of 15–25 years [6]. The extremely slow development of protective immunity, spanning more than ten years, is intriguing in immunological terms because it occurs even in areas where inhabitants are subjected to more than one infective mosquito bite everyday and who, during the first 8–12 years of their life, consistently harbor daily parasite loads of 1×10^8 to 1×10^{11} infected erythrocytes [7]. It is difficult to imagine how a protective antigen could be so poorly immunogenic that it required exposure to such large amounts of antigen for so long to induce an immune response [8].

Rigorous *in vitro* investigations of natural interactions between humans and *Plasmodium falciparum* have shed light on the immunological mechanisms responsible for this paradoxical situation. The central role of antibodies in acquired immunity was established by the classical work of Cohen *et al.* in The Gambia in 1961 [9]. Their observations were repeated in Thailand, where parallel *in vitro* investigations demonstrated that, to be effective at clearing parasites, the passively transferred immunoglobulin (Ig)G had to cooperate with blood monocytes in an antibody-dependant cellular cytotoxicity (ADCC) mechanism [5]. This ADCC mode of antibody action implies that only the two cytophilic antibody subclasses that can bind to receptors on monocytes – IgG1 and IgG3 – are involved in protection. This, in turn, prompted an analysis of the isotypic distribution of malarial antibodies in subjects from areas endemic for malaria [8]. The results obtained supplied the first clue towards solving the enigma because the slow acquisition of immunity was found to be related not to an absence of early immune responses but to an abnormal predominance of non-cytophilic antibodies in children exposed to *P. falciparum* for extended periods (i.e. during childhood and adolescence) and to a switch towards dominance of cytophilic classes when protection was reached [8]. This observation was subsequently confirmed by other studies [10–12]. It raised the question of why such an isotypic imbalance lasted for so long and, more pertinently, how and why it could change during adolescence towards adulthood (i.e. from the age of 12 to 25 years).

The hormonal changes that occur during adolescence have not been reported to have a particularly strong influence on the immune system [13]. However, it is known that children differ markedly from adults in their susceptibility to helminthic infections. Children usually

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suffer a higher prevalence of particularly large loads of helminths [3]. This difference might contribute to the slow acquisition of immunity to malaria because helminth infections are known to drive immune responses towards the production of the non-cytophilic subclasses (IgG2, IgG4 and IgM), whereas protection against malaria is associated with the presence of the IgG1 and IgG3 cytophilic subclasses [8,10–12].

Various studies have been conducted to explore the possible influence of worms on the occurrence of clinical malaria. A preliminary investigation was carried out in Madagascar in two cohorts of children aged 2–15 years whose clinical malaria status was closely monitored for several years. Children in the first cohort ($n=36$) were treated with the anthelmintic drug levamisole, whereas those in the second cohort ($n=21$) served as controls. During the two-year follow up, a profound decrease (of 60%) in malaria incidence, parasite densities and spleen rates was observed among the treated group compared with the non-treated, age-matched control individuals*.

An effect as significant as that of sickle-cell anemia

Further studies were initiated under epidemiological conditions in which no preliminary anthelmintic treatment by levamisole, which has immunomodulatory effects, was necessary.

One was conducted in the village of Dielmo (Sénégal), where all 220 inhabitants were actively followed on a daily basis to record malaria incidence [7]. All intestinal helminthic infections had been cured at the initiation of the field study, although a stool examination revealed that 13 of the 80 children in the village had become reinfected. The relative risk (RR) of clinical malaria over a one-year period (for six months preceding and six months following the stool examination) was reduced in helminth-free children compared with helminth-positive children carrying *Ascaris*, *Ancylostoma* or *Trichuris* [14]. This reduction was similar in magnitude (RR=0.65 and $p=0.003$) to that conferred by the sickle-cell trait (RR=0.57 and $p=0.06$) [14].

The deleterious influence of helminths was confirmed by another important worm species, *Schistosoma*, in an epidemiological situation in which contamination by *Schistosoma mansoni* alone could be addressed [15]. Among 512 children living in Richard-Toll (Sénégal) aged 5–15 years, the incidence of malaria attacks was higher in subjects infected with *Schistosoma* than in non-infected subjects; the incidence was particularly pronounced in subjects with the highest helminthic loads {>1000 eggs per gram (epg) of feces [7]}, whereas the pattern was more complex for low (1–100 epg) and medium (101–400 epg) egg loads. However, the overall RR in the absence of schistosomiasis was the same as that observed in worm-free individuals in Dielmo (RR=0.59). It is noteworthy that, in these three studies, there was no indication of shared exposure to helminths and malaria, which have distinct means of transmission (although a more systematic analysis might be required to exclude fully the elements of shared exposure in particular individuals), and there were no cases of malnutrition that could constitute confounding factors.

There has been just one study in Asia that reported what might, at first, seem to be a conflicting observation [16]. It was found that *Ascaris lumbricoides* infections are less common in patients who present with cerebral malaria, thus prompting the suggestion that worms might protect against cerebral malaria. The Thai study [16] differed from the other studies in that it was carried out in adults and addressed resistance to cerebral malaria, which probably differs in its mechanisms from the anti-parasite immunity premunition. However, subsequent analyses of the same set of Thai patients' medical records suggested the opposite; contrary to the initial report, these analyses found an increased prevalence of mixed or successive infections by *Plasmodium vivax* and *P. falciparum* in subjects carrying *Ascaris* [17] or *Trichuris* [18] infections, an increased intensity of malarial anemia in the presence of helminths [19], and an increase in gametocytemia [20]. These findings indicate an increased susceptibility to *Plasmodium* in individuals infected with worms. A larger study in Western Thailand by the same research group [21] confirmed the deleterious effect of worms; among 731 villagers – 62% of them harboring intestinal helminthic infections – *P. falciparum* malaria attacks were less prevalent in worm-free individuals (RR=0.55 and $p=0.001$). In a study of 476 pregnant women from Malawi who were exposed to malaria and concomitant hookworm infections, it was observed that women infected with hookworms were at 1.80 times higher risk of having malaria than women not infected†. A positive association between infections with *A. lumbricoides* and the occurrence of *P. falciparum* parasitemia was described in a cohort of 1100 children and mothers in Zaire [22]. Finally, an African study of subjects with either cerebral malaria or high densities of *Plasmodium* [23] also suggests a lower risk (RR=0.45) in helminth-free individuals compared with *Ascaris* carriers. A similar trend was reported for *Schistosoma haematobium*, although the small number of individuals infected did not enable significance [23]. This report is in contrast to the initial observation from Asia [16] but is consistent with all other studies in non-cerebral malaria patients.

It is remarkable that the available results indicate an increased malaria risk of similar magnitude (55–80% and, in some subgroups such as those with high schistosomiasis loads, up to 125%) in distinct malaria-endemic areas that is caused by distinct species of worms [14,15,21,23].

These observations also tend to suggest that immunity to malaria might be acquired faster in a worm-free population. However, this remains to be confirmed by further rigorous studies. Paradoxically, an indication of this was supplied – albeit indirectly – by a recent study that reported no influence of intestinal nematodes on malaria incidence [24]. The study was conducted in an area of low and unstable malaria transmission, where the risk of this disease spread across all age groups, indicating that there was extremely little, if any, acquired immunity

* R. Jambou *et al.*, Gordon Conference, Oxford, July 1998.

† M.C. Tigpen *et al.*, abstract 333, 53rd Annual Meeting of the American Society of Tropical Medicine and Hygiene, Miami Beach, October 2004.

to malaria. Hence, worms could not negatively influence an immune protection that was absent.

Implications for rolling back malaria

These observations, all but one of which deal only with *P. falciparum*, have important fundamental and practical consequences. From a fundamental point of view, the influence of worms raises several immunological questions that are important for vaccine development. Initial epidemiological studies of worm–malaria interactions were prompted by the hypothesis that worms might trigger, through a T helper (Th)2 response, the production of non-cytophilic, clinically non-effective antibodies [8]. Having demonstrated a clinical consequence, the initial hypothesis remains to be confirmed by actual data; the underlying immunological mechanisms merit further investigation in both models and immunoclinical studies in populations of endemic areas. To date, evidence has been obtained only in rodents about the influence of worms on the immune response to malaria. In relation to increased *Plasmodium chabaudi* parasitemia in mice infected with *S. mansoni*, a significant decrease in tumor necrosis factor α production was observed, whereas both cellular and humoral responses to *S. mansoni* were suppressed [25]. Conversely, the influence of helminthic infections on the Th1–Th2 balance, which itself affects isotype balance, has been documented [26]. Further investigations must be performed separately for acute uncomplicated malaria and for cerebral malaria in light of the single conflicting report [16] and the loose case-definition of the report in Ref. [23].

The practical implications are huge. African children carry both the major burden of malaria and the highest worm burden. Eight of the nine studies discussed imply that treating one burden (the worms) could markedly relieve the other burden (malaria). However, none of them (except the study by Jambou *et al.*[*]) was designed to assess the consequences of deworming. The possibility that administration of a single dose of a cheap and easily administered anthelmintic such as albendazole (at a cost of US$0.02 per dose [27,28]) could markedly affect the malaria disease burden in African children deserves further investigation.

Linking malaria-control programs with programs directed at other parasitic or bacterial diseases has recently been suggested [27]. Combining the logistics could bring additional benefits (e.g. improving anemia by treating hookworm and malaria simultaneously). However, the influence of worm carriage on malaria incidence described earlier suggests that a synergistic, rather than just additional, effect could be generated by linking programs. The geographic distribution of the main worm infections – be they intestinal, lymphatic or mesenteric – largely overlaps with areas endemic for malaria [27]. Such measures, should their benefit be confirmed, would be easy to implement because novel anthelmintic agents such as albendazole and ivermectin have a wide spectrum of activity [27].

In the most optimistic scenario, deworming could induce, in children primed first by the parasite, an early switch to the cytophilic type of antimalarial responses associated with protection (instead of the non-cytophilic response usually generated) [8,11,12]. In other words, avoiding the deleterious impact of worms might not only lead to a temporary improvement but also contribute to earlier acquisition of a protective type of response that could be long term.

Vaccine trials risk flawed results

Currently, a large number of experimental antimalarial vaccines[‡] is either undergoing or about to enter clinical trials. Worms seem to be an important confounding factor in assessing the efficacy of these trials, and one that deserves to be investigated systematically in future vaccine trials. Indeed, the effects of deworming on clinical malaria incidence are expected to be markedly higher than the ~30% efficacy achieved by the best available vaccine [29] or the minimal efficacy target (also 30%) required to continue further vaccine development [30]. An imbalance in worm carriage between immunized and control groups in these trials could lead to the effectiveness of a potent experimental vaccine being missed (or, conversely, to an ineffective vaccine being considered promising). The presence of worms goes far beyond the problem of hyporesponsiveness mentioned previously [31]. It could lead to the induction of antibodies without protective effect, whereas the vaccine formulation might be able to induce immune responses of a protective type in a worm-free population. This concern applies not only to field trials in malaria-endemic areas but also to Phase I and II trials in naive Western volunteers, in whom *Enterobius* and *Trichuris* are not uncommon. This warrants detection and monitoring during the follow up.

Future perspectives

Many children are suffering and dying from malaria. If, as indicated by the studies discussed in this article, their status could be substantially improved by deworming and water sanitation, it would be important to document further this affordable means of control urgently. However, the single report that suggests an opposite effect [16], specifically that of protection against severe malaria, underscores the importance of conducting scientifically rigorous and carefully designed studies to explore this relationship fully before deworming can be considered as a novel means of antimalarial control.

In our opinion, the complexity of *Plasmodium* immune interactions in humans has been underestimated. The observations discussed should, at the very least, serve as a reminder that, in contrast to the laboratory animals and *in vitro* cultures that are the basis of much malaria research, individuals from malaria-endemic areas carry more than just a single pathogen. Helminth infections are a fact of life in areas endemic for malaria. The influence of these infections on the epidemiology and course of malaria infection represents a neglected area of research that deserves greater consideration, particularly in the context of evaluating the effectiveness of malaria-control interventions.

‡ Ninety-three trials have been inventoried by the Initiative for Vaccine Research at the World Health Organization (http://www.who.int/vaccine_research/documents/malaria_table.pdf).

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References

1 Raso, G. *et al*. (2004) Multiple parasite infections and their relationship to self-reported morbidity in a community of rural Côte d'Ivoire. *Int. J. Epidemiol.* 33, 1092–1102
2 Cox, F.E.G. (2001) Concomitant infections, parasites and immune responses. *Parasitology* 122 (Suppl.), S23–S38
3 Manson-Bahr, P.E.C. and Apted, F.I.C. (2002) Helminthic infections. In *Manson's Tropical Diseases* (21st edn) (Cook, G.A.Z., ed.), pp. 1431–1597, Bailliere Tindall
4 Druilhe, P. *et al*. (1997) *In vivo* veritas – lessons from immunoglobulin-transfer experiments in malaria patients. *Ann. Trop. Med. Parasitol.* 91, 37–53
5 Bouharoun-Tayoun, H. *et al*. (1990) Antibodies that protect humans against *Plasmodium falciparum* blood stages do not on their own inhibit parasite growth and invasion *in vitro*, but act in cooperation with monocytes. *J. Exp. Med.* 172, 1633–1641
6 McGregor, I.A. (1972) Immunology of malarial infection and its possible consequences. *Br. Med. Bull.* 28, 22–27
7 Trape, J.F. *et al*. (1994) The Dielmo project. A longitudinal study of natural malaria infection in a community living in a holoendemic area of Senegal. *Am. J. Trop. Med. Hyg.* 51, 123–137
8 Bouharoun-Tayoun, H. and Druilhe, P. (1992) *Plasmodium falciparum* malaria: evidence for an isotype imbalance which may be responsible for delayed acquisition of protective immunity. *Infect. Immun.* 60, 1473–1481
9 Cohen, S. *et al*. (1961) γ-Globulin and acquired immunity to human malaria. *Nature* 192, 733–737
10 Polley, S.D. *et al*. (2003) Repeat sequences in block 2 of *Plasmodium falciparum* merozoite surface protein 1 are targets of antibodies associated with protection from malaria. *Infect. Immun.* 71, 1833–1842
11 Rzepczyk, C.M. *et al*. (1997) Humoral immune responses of Solomon islanders to the merozoite surface antigen 2 of *Plasmodium falciparum* show pronounced skewing towards antibodies of the immunoglobulin g3 subclass. *Infect. Immun.* 65, 1098–1100
12 Singh, S. *et al*. (2004) Identification of a conserved region of *Plasmodium falciparum* MSP3 targeted by biologically active antibodies to improve vaccine design. *J. Infect. Dis.* 190, 1010–1018
13 Hirokawa, K. *et al*. (1992) Aging and immunity. *Acta Pathol. Jpn* 42, 537–548
14 Spiegel, A. *et al*. (2003) Increased frequency of malaria attacks in subjects co-infected by intestinal worms and *Plasmodium falciparum* malaria. *Trans. R. Soc. Trop. Med. Hyg.* 97, 198–199
15 Sokhna, C. *et al*. (2004) Increase of malaria attacks among children presenting concomitant infection by *Schistosoma mansoni* in Senegal. *Malar. J.* 3, 43
16 Nacher, M. *et al*. (2000) *Ascaris lumbricoides* infection is associated with protection from cerebral malaria. *Parasite Immunol.* 22, 107–113
17 Nacher, M. *et al*. (2001) Contemporaneous and successive mixed *Plasmodium falciparum* and *Plasmodium vivax* infections are associated with *Ascaris lumbricoides*: an immunomodulating effect? *J. Parasitol.* 87, 912–915
18 Chaorattanakawee, S. *et al*. (2003) *Trichuris trichiura* infection is associated with the multiplicity of *Plasmodium falciparum* infections, in Thailand. *Ann. Trop. Med. Parasitol.* 97, 199–202
19 Nacher, M. *et al*. (2001) Association of helminth infection with decreased reticulocyte counts and hemoglobin concentration in Thai *falciparum* malaria. *Am. J. Trop. Med. Hyg.* 65, 335–337
20 Nacher, M. *et al*. (2001) Association of helminth infections with increased gametocyte carriage during mild *falciparum* malaria in Thailand. *Am. J. Trop. Med. Hyg.* 65, 644–647
21 Nacher, M. *et al*. (2002) Intestinal helminth infections are associated with increased incidence of *Plasmodium falciparum* malaria in Thailand. *J. Parasitol.* 88, 55–58
22 Tshikuka, J.G. *et al*. (1996) Multiple infection with *Plasmodium* and helminths in communities of low and relatively high socio-economic status. *Ann. Trop. Med. Parasitol.* 90, 277–293
23 Le Hesran, J. *et al*. (2004) Severe malaria attack is associated with high prevalence of *Ascaris lumbricoides* infection among children in rural Senegal. *Trans. R. Soc. Trop. Med. Hyg.* 98, 397–399
24 Shapiro, A.E. *et al*. (2005) Epidemiology of helminth infections and their relationship to clinical malaria in southwest Uganda. *Trans. R. Soc. Trop. Med. Hyg.* 99, 18–24
25 Helmby, H. and Troye-Blomberg, M. (1998) Altered immune responses in mice with concomitant *Schistosoma mansoni* and *Plasmodium chabaudi* infections. *Infect. Immun.* 66, 5167–5174
26 Pierce, E.J. *et al*. (1991) Downregulation of Th1 cytokine production accompanying the induction of Th2 responses by a parasitic helminth, *Schistosoma mansoni*. *J. Exp. Med.* 73, 159–166
27 Molyneux, D.H. and Nantulya, V.M. (2004) Linking disease control programmes in rural Africa: a pro-poor strategy to reach Abuja targets and millennium development goals. *BMJ* 328, 1129–1132
28 Anon (2004) Thinking beyond deworming. *Lancet* 364, 1993–1994
29 Alonso, P.L. *et al*. (2004) Efficacy of the RTS,S/AS02A vaccine against *Plasmodium falciparum* infection and disease in young African children: randomised controlled trial. *Lancet* 364, 1411–1420
30 Moorthy, V.S. *et al*. (2004) Malaria vaccine developments. *Lancet* 363, 150–156
31 Nacher, M. (2001) Malaria vaccine trials in a wormy world. *Trends Parasitol.* 17, 563–565