ENHANCED MALARIA PARASITE TRANSMISSION FROM HELMINTH CO-INFECTED MICE

GREGORY S. NOLAND, THADDEUS K. GRACZYK, BERNARD FRIED, AND NIRBHAY KUMAR*
Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland; Department of Biology, Lafayette College, Easton, Pennsylvania

Abstract. Helminth infections are prevalent in malaria-endemic areas, yet the potential for helminths to alter malaria transmission has not been closely examined. We used the Echinostoma caproni–Plasmodium yoelii murine model of co-infection to assess the impact of helminth co-infection on malaria transmission. In four replicate experiments, Anopheles stephensi mosquitoes exposed to co-infected mice five days post-malaria infection had a higher rate of infectivity (80.1%, n = 241) than those exposed to malaria only–infected mice (72.0%, n = 232, P = 0.039). Intensity of malaria parasite transmission was also greater, with approximately two-fold more oocysts (geometric mean = 19.2 versus 10.5, P = 0.004) and an increase in sporozoite burden observed in mosquitoes exposed to co-infected mice. Malaria parasite prevalence and anemia were similar between co-infected and malaria only–infected mice, which suggested that enhanced malaria parasite transmission was due to helminth-induced modulation of host responses.

INTRODUCTION

Malaria, a disease that affects more than 300 million people annually, is transmitted between vertebrate hosts by female Anopheles mosquitoes. Infectivity of the transmissible form of the parasite, the gametocyte, can be influenced by various vertebrate host factors, including cytokines, antibodies to parasites, and non-specific alterations to host physiology. Th1 cytokines, specifically tumor necrosis factor-α and interferon-γ, have been shown to reduce transmission of Plasmodium vivax by neutralizing infectivity of gametocytes in conjunction with other serum factors produced during paroxysm. Anti-bodies directed against sexual stage parasites can likewise reduce transmission, however, paradoxically, some sexual stage-specific antibodies can also enhance malaria transmission. Furthermore, in rodent malaria infections, decreases in host pH and bicarbonate levels have been shown to inhibit transmission independently of other serum derived factors by preventing exflagellation of microgametocytes.

Approximately two billion people in malaria-endemic areas also have various helminth infections. Concurrent infection with malaria and helminths is common, and numerous studies have documented significant interactions, both synergistic and protective in human populations. In animal models, similarly mixed profiles have been described. Patent helminth infections tend to exacerbate normally non-lethal P. yoelii and P. chabaudi infections, and provide protection against some, but not all, lethal P. yoelii, P. chabaudi, and P. berghei infections. Although the mechanisms contributing to such outcomes are not clearly defined, significant modulation of Th1 cytokines and anti-malaria antibodies has been reported during co-infection. We therefore hypothesized that such alterations to host response during concomitant helminth infection may also modulate the intensity of malaria parasite transmission. Additionally, because helminth infection has been found to increase the duration of malaria parasitemia, we sought to determine whether chronic helminth infection extended the permissive window of P. yoelii transmission, which is normally limited to the first five days of infection.

One previous study has suggested that helminths may modulate malaria transmission potential because helminth-infected individuals with mild P. falciparum malaria were more likely to carry gametocytes than patients with malaria alone. However, the effect on actual transmission to mosquitoes has never been addressed. We used an established rodent model of co-infection, using an intestinal trematode, Echinostoma caproni, and a non lethal rodent malaria parasite, Plasmodium yoelii, to evaluate the impact of helminth infection on malaria transmission by comparing malaria parasite burdens in mosquitoes exposed to helminth co-infected or malaria only–infected mice.

MATERIALS AND METHODS

Mice and parasites. Four- to six-week old male BALB/c mice were obtained from the National Cancer Institute (Bethesda, MD) and maintained in a pathogen-free micro-isolation facility in accordance with the National Institutes of Health guidelines for the humane use of laboratory animals. Mice were infected with 10–15 E. caproni metacercarial cysts, as described previously, and E. caproni infections were monitored by microscopic examination of weekly fecal collections. Approximately four weeks after helminth infection, groups of 3–5 E. caproni-infected mice and age- and sex-matched worm-free controls were infected with 1 × 10⁵ P. yoelii parasites (17X non-lethal strain) by intraperitoneal injection of infected erythrocytes. Each of four independent replicate experiments was initiated with the same frozen parasite pool and passed through one donor mouse prior to experimental infections. Parasitemia and gametocytemia were determined by counting 1 × 10⁴ and 1 × 10³ erythrocytes, respectively, in Giemsa-stained thin films of mouse tail blood. Anemia was determined by measuring packed cell volume of blood drawn by tail bleed into micro-capillary tubes.

Mosquito infections. At days five, eight, and thirteen post-malaria infection, mice were anesthetized with an intraperitoneal injection of ketamine (Ketaset, 100 mg/kg;Phoenix Pharmaceuticals, St. Joseph, MO) and acepromazine (5 mg/kg; Henry Schein, Melville, NY) mixed in saline. Cages of 3–5-day-old, starved, female Anopheles stephensi mosquitoes were then allowed to feed on individual mice for 30 minutes.
Unfed mosquitoes were removed immediately after exposure, and remaining mosquitoes maintained at 24°C and a relative humidity of 80%. Eight to twelve days post-feeding, mosquito midguts were removed, stained with 0.1% mercurochrome (Sigma, St. Louis, MO), and malaria oocysts were enumerated. Sporozoite burden was determined on day 14 in 2 experiments by homogenizing pooled mosquito thoraxes, at least 50 per group, in approximately 1 mL of Hanks’ balanced salt solution supplemented with 1% normal mouse serum. The homogenate was then filtered through nylon mesh, resuspended to a volume of 4 mL, and aliquots examined for sporozoites by hemocytometer. The resulting pooled sporozoite burden was expressed as the number of sporozoites per mosquito.

**Statistical analysis.** Normally distributed variables were compared using Student’s unpaired *t*-test. Oocyst counts were compared using Student’s unpaired *t*-test after log_{10} transformation. Comparison of proportions was performed using the chi-square test. Bivariate analysis was performed by calculating Pearson’s correlation coefficient between the geometric mean number of oocysts per mosquito for each cage of mosquitoes fed on individual mice and the following terms: anemia, *P. yoelii* asexual parasitemia, and *P. yoelii* gametocytemia at the time of feeding. Analysis was performed using Microsoft (Redmond, WA) Excel® and STATATA version 8.1 (STATA Corporation, College Station, TX).

**RESULTS**

**Increased infectivity and parasite burden in mosquitoes fed on co-infected mice.** To determine whether chronic helminth co-infection modulated the intensity of the *P. yoelii* transmission, *An. stephensi* mosquitoes were fed on *E. caproni–* *P. yoelii* co-infected or *P. yoelii* only–infected mice at day five post-malaria infection. Over four replicate experiments, mosquitoes exposed to co-infected mice had a greater proportion of malaria infection. Over four replicate experiments, mosquitoes fed on individual mice and the following terms: anemia, *P. yoelii* asexual parasitemia, and *P. yoelii* gametocytemia at the time of feeding. Analysis was performed using Microsoft (Redmond, WA) Excel® and STATATA version 8.1 (STATA Corporation, College Station, TX).

**Table 1**

Mosquito infectivity in four experiments in *Anopheles stephensi* mosquitoes fed on either *Echinostoma caproni–Plasmodium yoelii* co-infected (E + M) or *P. yoelii* only–infected (M) mice five days post-malaria infection

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Oocyst-positive mosquitoes/Total no (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E + M</td>
<td>M</td>
</tr>
<tr>
<td>1</td>
<td>71/78 (91.0)</td>
</tr>
<tr>
<td>2</td>
<td>56/69 (81.2)</td>
</tr>
<tr>
<td>3</td>
<td>34/43 (79.1)</td>
</tr>
<tr>
<td>4</td>
<td>32/51 (62.8)</td>
</tr>
<tr>
<td>Total</td>
<td>193/241 (80.1)</td>
</tr>
</tbody>
</table>

* *P* = 0.039, by χ² test.

**Figure 1.** Box plots of number of *Plasmodium yoelii* oocysts per mosquito in oocyst-positive *Anopheles stephensi* fed on *Echinostoma caproni–* *P. yoelii* co-infected mice (E + M) (n = 193 mosquitoes) or *P. yoelii* only–infected mice (M) (n = 167 mosquitoes) five days post-malaria infection (aggregate of four experiments). Boxes, with medians indicated, contain the middle 50% of observations (the interquartile range [IQR]), whiskers indicate the smallest and largest observations within the lower and upper fence (±1.5 × IQR), and outliers are observations outside the upper fence. Geometric mean number of oocysts were 19.2 (E + M) and 10.5 (M) (*P* = 0.004, by Student’s *t*-test of log-transformed values.)

In two of the four experiments, we also compared the resulting sporozoite burden between groups. In one experiment, mosquitoes fed on co-infected mice had approximately 10-fold more sporozoites per mosquito than those fed on malaria only–infected controls (7,150 versus 750 sporozoites). In the other experiment, an approximately two-fold increase (2,120 versus 1,260 sporozoites; n = 50 mosquitoes per group).

Gametocyte and total parasite levels in mice were not significantly different between co-infected and malaria only–infected mice on the day of mosquito feeding day five post-malaria infection (*P* = 0.60 and 0.08, respectively; Table 2). We also found no correlation between gametocytemia of individual mice and the numbers of oocysts detected in mosquitoes fed on either co-infected mice or malaria only–infected mice (r = −0.44 and −0.38, respectively). Likewise, there was no correlation between total parasitemia and oocyst burden for either group (r = −0.07 and = 0.57, respectively). Additionally, packed cell volumes were similar between groups at day five of malaria infection (*P* = 0.11), and also did not correlate with oocyst burdens for either co-infected mice or malaria only–infected mice (r = 0.53 and 0.75, respectively).

**Temporal restriction of *P. yoelii* infectivity.** Successful transmission of *P. yoelii* rapidly decreases at day six after an initial peak of maximal infectivity between days two and five.28 To determine whether chronic helminth co-infection mitigated this temporal restriction of transmission, mosquitoes were exposed to *E. caproni–* malaria co-infected or malaria only–infected mice on days 8 and 13 of malaria infection in each of 2 separate experiments. Although gametocytes and asexual malaria parasites were present in both groups of mice at similar levels on days 8 or 13 of malaria infection (Table 2),
**no oocysts were detected in mosquitoes fed on either group of mice (n ≥ 100 mosquitoes per group, combined). Helminth infection therefore seemed to have no effect on abrogating the temporal restriction of *P. yoelii* transmission.**

**DISCUSSION**

Co-infection with various helminth species occurs commonly in malaria-endemic areas, and the potential for helminths to influence malaria transmission has not been closely examined. In the present study, using a mouse model of *E. caproni* and *P. yoelii* co-infection, we observed that concurrent intestinal helminth infection in mice resulted in a significantly increased proportion of malaria-infected mosquitoes and a significantly increased parasite burden in infected mosquitoes at both oocyst and salivary gland sporozoite levels. We also found that the restriction of *P. yoelii* transmission to the first five days of infection was not extended by helminth co-infection.

Co-infecting parasites have previously been shown to influence transmission of mosquito-borne pathogens. Filarial larvae and malaria sporozoites were found to facilitate increased arbovirus dissemination in mosquitoes by disrupting the mosquito midgut and salivary glands, respectively.

In another study, filarial co-infection had no effect on malaria parasite transmission from humans to naturally fed mosquitoes, although low intensity of malaria infection may have precluded detection of an effect. Such mechanical disruptions are not possible in the present *E. caproni*–*P. yoelii* system because echinostome parasites exclusively inhabit the gut and do not produce blood stage forms capable of being ingested with malaria parasites during mosquito feeding.

The central findings of these studies were the consistent increase in the proportion of *Plasmodium*-infected mosquitoes and increased intensity of parasite burden in mosquitoes fed on co-infected mice over those fed on malaria only-infected controls. This consistency is remarkable given the inherent biologic variability in these types of experiments. So that these trends may be fully appreciated, we have presented the primary data by experiment in addition to pooled results. Increased mosquito infectivity and parasite burden could not be attributed to differences in gametocyte or asexual malaria parasite density or anemia between groups of mice. The similarity of gametocytemia contrasts with a previous report of increased *P. falciparum* gametocyte prevalence in helminth co-infected humans, although this increase was nullified after adjustment for anemia. Furthermore, we also did not observe a correlation between gametocytemia and oocyst production in either co-infected mice or malaria-only infected controls. A lack of correlation between gametocytemia and oocyst burden is routinely noted and is likely due to the predilection for rodent malaria gametocytes to sequester in capillaries close to the skin. Consequently, even if differences in gametocyte prevalence were detected between groups, it would be difficult to prove increased parasite transmission based solely on peripheral gametocyte density. Gametocyte density in the present study varied greatly between mice, and development of a *P. yoelii* gametocyte-specific quantitative detection assay, similar to the one recently designed for *P. chabaudi,* would prove useful in further investigating the relationship between gametocyte density and malaria transmission to mosquitoes.

Anemia is a prominent feature of many human helminth infections and represents a significant risk factor for malaria gametocyte carriage. In the present study, which used *E. caproni,* a non-hematophagous intestinal parasite, no significant difference was detected in packed cell volume between co-infected mice and those infected only with malaria (Table 2). Although prediuresis, the process by which mosquitoes concentrate erythrocytes during blood feeding, may compensate for low levels of anemia, the non-significant difference in anemia detected in the present study is unlikely to account for the observed increase in oocyst burden. One would predict that mosquitoes fed on anemic co-infected mice would have ingested fewer gametocyte-infected erythrocytes than those fed on malaria only-infected controls. We did not compare packed cell volume between groups beyond day five because we had already determined that transmission did not occur at later time points.

**Echinostoma caproni** co-infection increases the intensity and duration of late stage *P. yoelii* infection yet in the present study, we found that it did not alter the temporal restriction of *P. yoelii* transmission. On the basis of studies in *P. berghei,* decreases in host pH and bicarbonate levels induced by increasing parasitemia seem to prevent exflagellation of microgametes independently of other serum-derived transmission modulating factors. Because *P. berghei* and *P. yoelii* share similar patterns of transmission restriction, it is likely that host physiologic changes also influence infectivity of *P. yoelii* parasites. We conclude therefore that although *E. caproni* infection alters the magnitude of transmission during early infection, it does not abrogate the ensuing physiologic alterations that limit *P. yoelii* transmission temporally. This restriction, however, is unique to rodent malaria parasites, and the possibility remains for helminths to alter transmission throughout the course of patent, and even sub-patent, human malaria infections.

In summary, we have shown that infection in the vertebrate host with an intestinal helminth can enhance transmission of malaria parasites to the mosquito vector. We hypothesize that such interactions are likely due to helminth-induced changes to the host immune system. Because similar enhancement of malaria transmission may also occur during widespread hel-
mimuth co-infection in humans, current global deworming strategies may realize benefits beyond the alleviation of helminth infections. However, in light of studies that found an increase in malaria infections after anthelmintic treatment, further studies are required to properly evaluate such predicted outcomes and the mechanisms mediating enhanced transmission.

Received November 16, 2006. Accepted for publication February 13, 2007.

Acknowledgments: We thank Erik J. Fitzgerald for statistical advice.

Financial support: Gregory S. Noland is supported by a predoctoral fellowship from the Johns Hopkins Malaria Research Institute. Research in the laboratory of Nirbhay Kumar is supported by grants from the National Institutes of Health.

Authors’ addresses: Gregory S. Noland and Nirbhay Kumar, Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, 615 North Wolfe Street, Baltimore, MD 21205, Telephone: 410-955-7177, Fax: 410-955-0105, E-mail: gnoland@jhsph.edu and nkumar@jhsph.edu. Thaddeus K. Gracey, Departments of Environmental Health Sciences and Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, 615 North Wolfe Street, Baltimore, MD 21205 Telephone: 410-614-0898. Fax: 410-955-0105, E-mail: tgraczyk@jhsph.edu. Bernard Fried, Department of Biology, Lafayette College, Kunkel Hall 204, Easton, PA 18042, Telephone: 610-330-5463, Fax: 610-330-5705, E-mail: friedb@lafayette.edu.

REFERENCES


