Short Report: Concurrent Infection with Heligmosomoides polygyrus Modulates Murine Host Response against Plasmodium berghei ANKA Infection

Kohhei Tetsutani,* Kenji Ishiwata, Motomi Torii, Shinjiro Hamano, Hajime Hisaeda, and Kunisuke Himeno
Department of Parasitology, Kyushu University Graduate School of Medicine, Fukuoka, Japan; Department of Tropical Medicine, The Jikei University School of Medicine, Tokyo, Japan; Department of Molecular Parasitology, Ehime University School of Medicine, To-on, Ehime, Japan

Abstract. We investigated whether concurrent infection with Heligmosomoides polygyrus, an intestinal nematode, modulated anti-malaria parasite immunity and development of experimental cerebral malaria (ECM) in mice. The C57BL/6 mice infected with Plasmodium berghei ANKA showed typical symptoms of ECM. Interestingly, preceding H. polygyrus infection did not alter ECM development, despite accelerated P. berghei growth in vivo. Our observation provides a new insight that ECM can be induced in a fashion independent of the immune responses affected by concurrent H. polygyrus. Differentiation between protective immunity and infection-associated host-damaging inflammatory response is urgently required for understanding the pathogenesis of cerebral malaria.

Malaria parasites cause the worst parasitic disease, with hundreds of millions of clinical cases annually worldwide. Severe malarial anemia and cerebral malaria are of particular importance clinically, both of which are responsible for millions of deaths. Cerebral malaria is considered to be a consequence of mechanical occlusion of the small blood vessels in the brain with parasitized red blood cells (RBCs), and/or of immunologic pathology attributed to local inflammation. Several lines of evidence supporting these hypotheses have been reported, yet the precise mechanisms underlying onset of cerebral malaria remain unclear.

A good rodent model for cerebral malaria is the infection of C57BL/6 mice with Plasmodium berghei ANKA, where the degree of immune activation reflects the extent of neurologic complications. However, intestinal helminths cause the largest number of parasitic infections among humans, but they usually cause nil to mild symptoms during their chronic infections. It has been reported that host immune responses are modulated with intestinal helminthes. Infections with these helminths increase susceptibility to viral, bacterial, or parasitic infections, and attenuate efficacy of several vaccines against infectious diseases; helminthic infections moderate allergic reactions or autoimmune conditions, which result from aberrant immune responses against foreign antigens or self-constituents.

In humans, the prevalence of malaria and infections with intestinal helminths overlap geographically, and the population in a given area suffers from both infections. It is supposed that infections with helminths affect the symptoms of malaria: Thai patients who have intestinal nematode infections suffer from malaria episodes more frequently. Conversely, symptoms of each episode are less severe and the frequency of cerebral malaria, pulmonary edema, or renal failure, all of which are associated with immunopathology, is lower when patients have dense helminthic infections.

It has been hypothesized that infections with intestinal helminths weaken protective immunity against co-existing malarial parasites, and simultaneously, the host-damaging inflammatory responses associated with malaria. To test this hypothesis, we examined the effects of concurrent infection with Heligmosomoides polygyrus on protective immunity and on experimental cerebral malaria (ECM) using C57BL/6 mice infected with P. berghei. All experiments using mice were conducted according to the guidelines for animal experimentation of Kyushu University.

Heligmosomoides polygyrus is a mouse intestinal nematode, which resides in the upper small intestine, and it is maintained through in vivo passage using male ICR mice. For infection, feces containing eggs were incubated on wet filter paper for a minimum of three days to allow eggs to develop into infective larvae. Male C57BL/6 mice at the age of eight to ten weeks were infected orally with 200 infective larvae by gastric intubation. Production of eggs began to be detected as early as 10 days after infection and continued for longer than eight weeks (data not shown). The mice did not show any central nervous system (CNS) symptoms during infection with H. polygyrus. After confirmation of H. polygyrus infection by feces examination, 2.5 × 10^4 P. berghei-infected RBCs were injected intraperitoneally (IP), at 14 days after H. polygyrus infection. Co-infection with P. berghei increased egg production by H. polygyrus slightly, without statistical significance (Figure 1).

Infection of C57BL/6 mice with P. berghei caused high lethality with CNS symptoms, which usually developed within days after infection. The C57BL/6 mice were orally infected with infective larvae of H. polygyrus. Fecal egg number was counted at the indicated days after infection with H. polygyrus. Data show means ± SE of four to six mice. Experiments were repeated three times with similar results.

* Address correspondence to Kohhei Tetsutani, Department of Parasitology, Kyushu University Graduate School of Medicine, 3-1-1, Maidashi, Higashi-ku, Fukuoka, 812-0054, Japan. E-mail: tetztani@parasite.med.kyushu-u.ac.jp

FIGURE 1. Egg production of Heligmosomoides polygyrus. The C57BL/6 mice were orally infected with infective larvae of H. polygyrus. Fecal egg number was counted at the indicated days after infection with H. polygyrus. Data show means ± SE of four to six mice. Experiments were repeated three times with similar results.
FIGURE 2. Course of infection with *Plasmodium berghei* in mice co-infected with *Heligmosomoides polygyrus*. The C57BL/6 mice were infected with *P. berghei* 14 days after *H. polygyrus* infection and were analyzed for percentage parasitemia monitored by microscopic evaluation of thin blood films stained with Giemsa solution (A), and for experimental cerebral malaria (ECM) incidence (B). Five to six animals were used in each group. Experiments were repeated three times with similar results and two of them are shown. (A) Each line represents data from an individual mouse, and the numbers indicate day of mouse death. An asterisk indicates statistical significance between mice infected with *H. polygyrus*/*P. berghei* and those only with *P. berghei* using the Student’s *t* test. (B) *P. berghei*-infected animals were considered to have ECM when neurologic signs, described in the text, appeared. Statistical significance was not found between mice infected with *H. polygyrus*/*P. berghei* and those only with *P. berghei*. 
cells, interleukin-10, and indoleamine-2,3-dioxygenase (our also to the pathology of ECM. 13 Our preliminary results immunity. The classic Th1/2 balance, immunosuppressive mechanisms in-formed by immune responses in ECM development are also attenuated. 13 Although we have not addressed how H. polygyrus suppresses immunity, it has been postulated that protective Th1 responses are attenuated in the Th2-biased environment induced by concurrently infected H. polygyrus. 5 In addition to the classic Th1/2 balance, immunosuppressive mechanisms induced by H. polygyrus can explain the reduced protective immunity. Heligmosomoides polygyrus is now known to induce alternatively activated macrophages, regulatory T cells, interleukin-10, and indoleamine-2,3-dioxygenase (our unpublished observations). No matter what the mechanisms are, anti-malaria immunity is thought to be suppressed by H. polygyrus infection, which suggests that pathogenic processes formed by immune responses in ECM development are also attenuated. Therefore, there are some responses reported to participate in both protection and immunopathology: Interferon (IFN)-γ contributes not only to elimination of malaria parasites, but also to the pathology of ECM. 13 Our preliminary results showed that H. polygyrus suppressed IFN-γ production from antigen–specific splenic T cells in mice after immunization with the corresponding antigen (unpublished observations). However unexpectedly, concurrent infection with H. polygyrus did not alter ECM development. This might be explained by the fact that immune suppression/modulation induced by concurrent H. polygyrus infection is not probably spread into CNS: H. polygyrus infection is reported to suppress experimental airway hypersensitivity, 14 although there have been no studies showing that nematodes whose life-cycle in their host are limited in alimentary tract to prevent experimental autoimmune/allergic encephalomyelitis (EAE) in CNS, although Schistosoma mansoni 15 and Trichinella spiralis, 16 which are thought to have easier access to the host blood stream, are reported to modulate EAE pathology. An alternative explanation is that protective immunity against malaria parasites is simply different from the pathogenic responses. For instance, although CD8+ T cells are responsible for ECM development, 17 these cells are not supposed to contribute to protective immunity against blood-stage malaria parasites, mainly because of absence of MHC class I molecules on the surface of RBCs. Finally, we cannot strictly exclude the probability that ECM pathology develops so quickly that modulations by concurrent H. polygyrus infection, if any, might not be obvious in our experiments. More sensitive methods of ECM diagnosis and definition are required for further research.

In conclusion, our results showed that infection with an intestinal nematode increased malaria parasite growth in vivo but did not alter immunopathology in ECM development. Differentiation between pathogen-killing immunity and self-damaging inflammatory responses is essential for a complete understanding of the pathology of cerebral malaria, and for designing effective vaccine strategies.

Received April 19, 2008. Accepted for publication August 20, 2008.

Financial support: This work was supported by the Ministry of Education, Science, Sport and Culture of Japan (Grants 20390121, 19041056), and by the Uehara Memorial Foundation.

Authors’ addresses: Kohhei Tetsutani, Shinjiro Hamano, Hajime Hisaeda, and Kunisuke Himeno, Department of Parasitology, Kyushu University Graduate School of Medicine, 3-1-1, Maidashi, Higashiku, Fukuoka, 812-0054, Japan, Tel: +81-92-642-6117, Fax: +81-92-642-6118, E-mail: tetsutani@parasite.med.kyushu-u.ac.jp. Kenji Ishiwata, Department of Tropical Medicine, The Jikei University School of Medicine, 3-25-8, Nishi-shinbashi, Minato-ku, Tokyo, Japan, Motomi Torii, Department of Molecular Parasitology, Ehime University School of Medicine, Shitsukawa, Toon, Ehime 791-0295, Japan.

REFERENCES:

11. Finney CAM, Taylor MD, Wilson MS, Maiels RM, 2007. Expansion and activation of CD4+CD25+ regulatory T cells in...