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Cutting Edge: Inhibition of IL-6 Trans-Signaling Protects from Malaria-Induced Lethality in Mice

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Circulating IL-6 levels correlate with the severity of blood-stage malaria in humans and mouse models, but the impact of IL-6 classic signaling through membrane IL-6Rα, as well as IL-6 trans-signaling through soluble IL-6Rα, on the outcome of malaria has remained unknown. In this study, we created IL-6Rα-deficient mice that exhibit a 50% survival of otherwise lethal blood-stage malaria of the genus Plasmodium chabaudi. Inducing IL-6 trans-signaling by injection of mouse recombinant soluble IL-6Rα in IL-6Rα-deficient mice restores the lethal outcome to malaria infection. In contrast, inhibition of IL-6 trans-signaling via injection of recombinant sGP130Fc protein in control mice results in a 40% survival rate. Our data demonstrate that IL-6 trans-signaling, rather than classic IL-6 signaling, contributes to malaria-induced lethality in mice, preceded by an increased inflammatory response. Therefore, inhibition of IL-6 trans-signaling may serve as a novel promising therapeutic basis to combat malaria. The Journal of Immunology, 2012, 188: 000–000.

Malaria remains a major health problem (1). All efforts to develop an antimalaria vaccine during the last 30 y have failed (2). This failure is particularly astonishing, because natural immunity against the bloodstages of the infectious agents, protozoan parasites of the genus Plasmodium, can be acquired, although only slowly, after repetitive infections. Formation and/or efficacy of protective mechanisms are apparently impaired by not-yet-understood parasite-induced host responses, which contribute to malaria morbidity and mortality (3). Presumably included among these host responses are those that are controlled by the pleiotropic cytokine IL-6, with its pro- and anti-inflammatory activities (4). Circulating IL-6 levels are increased in patients suffering from malaria caused by P. falciparum and P. vivax (5–8), which is often associated with polyclonal B cell activation (9). Conversely, decreasing IL-6 levels were described after antimalarial treatment (10) and are associated with decreasing hyperpyrexia (11) and decreasing parasitemia (12).

The mode of IL-6 action is complex (4, 13, 14). IL-6 signals through the specificity-defining membrane IL-6Rα, which requires the recruitment of two chains of the membrane receptor GP130 for signal transduction to activate the JAK/STAT pathway. This IL-6 classic signaling is restricted to those cells that express IL-6Rα on their surface (i.e., hepatocytes and immune cells). Nevertheless, IL-6 is also able to engage an alternative pathway through the naturally occurring soluble IL-6Rα (sIL-6Rα), which is derived by shedding of the ectodomain of membrane IL-6Rα and by alternative mRNA splicing. The IL-6/sIL-6Rα complex can communicate with all cells by binding to the ubiquitously expressed membrane GP130, thus initiating the so-called “IL-6 trans-signaling process” (13, 14). sIL-6Rα levels may also maintain the half-life of effective IL-6, as demonstrated previously (15). However, IL-6 trans-signaling can be inhibited when soluble GP130 (sGP130) binds and, thereby, inactivates the IL-6/sIL-6Rα complex (16). In this study, we show that IL-6 trans-signaling contributes to malaria-induced lethality.

Materials and Methods

Generation of IL-6Rα-deficient mice

Generation of IL-6Rαfl/fl mice was done, as described previously (17). Briefly, we flanked exons 2 and 3 of the IL-6Rα gene by loxP sites using standard gene-targeting techniques in C57BL/6-derived embryonic stem cells. To generate IL-6Rα-deficient (knockout; KO) mice (IL-6RαKO mice), IL-6Rαfl/fl mice were crossed with deleter-cre mice, and offspring were intercrossed. IL-6Rα deficiency in liver parenchymal cells (IL-6RαKO-L-KO mice) was generated, as described previously (17). Myeloid lineage-specific IL-6Rα-deficient mice (IL-6RαKO-MC mice) were created by crossing IL-6RαKO mice with LysM-cre mice and intercrossing offspring.

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Abbreviations used in this article: KO, knockout; p.i., postinfection; sGP130, soluble GP130; sIL-6Rα, soluble IL-6Rα.

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liver parenchymal cells (IL-6RαKO mice) (17) and in the myeloid lineage (IL-6RαMyel-KO mice). Macrophages and neutrophils in IL-6RαMyel-KO mice lack IL-6Rα (Supplemental Fig. 1F), which, upon IL-6 stimulation in vitro, were unable to activate STAT3 (Supplemental Fig. 1G).

Surprisingly, infection with blood-stage \textit{P. chabaudi} malaria took a lethal course in IL-6RαFL control mice (Fig. 1A), IL-6RαKO mice (Supplemental Fig. 1H), and IL-6RαLKO mice (Supplemental Fig. 1I), whereas 50% of IL-6RαKO mice were able to self-heal and, thus, survive the infection (Fig. 1B). The precrisis phase of infection was similar, reaching peak parasitemia on day 8 p.i. Mice succumbed to infection during the subsequent crisis phase characterized by dramatically decreasing parasitemia (Fig. 1B). \textit{P. chabaudi}-induced lethality is known to result from multiple organ failure that varies in individual mice (18). Control IL-6RαKO mice exhibited a continuous increase in circulating sIL-6Rα during the precrisis phase, from 16 to 190 ng/ml on day 8 p.i., whereas sIL-6Rα was absent in IL-6RαKO mice (Fig. 1C). The infection-induced increase in circulating IL-6 followed a biphasic pattern in both control and IL-6RαKO mice, with the first peak on day 1 p.i. and the second peak on day 8 p.i. (Fig. 1D), and the latter being higher in IL-6RαKO mice.

![FIGURE 1](http://www.jimmunol.org/)
Similarly, the proinflammatory cytokines IL-1β and TNF-α exhibited a biphasic increase in IL-6RαFL control mice during infection (Fig. 1E, 1F), whereas IL-6RαKO mice had lower IL-1β and TNF-α on day 8 p.i. In the liver, IL-1β and TNF-α are known to induce acute-phase and other innate responses (13). Indeed, the hepatic expression of two acute-phase proteins, serum amyloid A3 and C-reactive protein, was lower in IL-6RαKO mice than in IL-6RαFL control mice on day 8 p.i. (Fig. 1G, 1H). Importantly, the inflammatory response in IL-6RαMyel-KO mice and IL-6RαL-KO mice on day 8 p.i. (Supplemental Fig. 1J, 1K) was intermediate to that in IL-6RαFL mice and IL-6RαKO mice, indicating that only complete IL-6Rα deficiency effectively reduced inflammation.

Approximately 70% of circulating IL-6 was estimated to bind to sIL-6Rα (16), and the IL-6/sIL-6Rα complex is known to induce IL-6 trans-signaling in all cells via membrane-bound GP130. Therefore, it is conceivable that a lethal outcome of malaria is due to the P. chabaudi-induced increase in circulating endogenous sIL-6Rα in control IL-6RαFL mice causing increased IL-6 trans-signaling. To examine this hypothesis, we next aimed at restoring IL-6 trans-signaling by injecting mouse recombinant sIL-6Rα into IL-6RαKO mice. Injections of 1 μg sIL-6Rα on days 1, 4, and 7 p.i. caused an increase in circulating sIL-6Rα from undetectable levels to 70 ng/ml on day 8 p.i. (Fig. 2A) and a concomitant decrease in circulating IL-6 from 600 to 110 pg/ml (Fig. 2B). These data suggest the occurrence of IL-6 trans-signaling that was also evidenced by increased phosphorylation of hepatic STAT3, a downstream target of IL-6 signaling (Fig. 2C). Control IL-6RαFL mice and IL-6RαKO mice did not exhibit any hepatic STAT3 phosphorylation at the steady state. At peak parasitemia, the control mice displayed greater STAT3 phosphorylation than did IL-6RαKO mice (Fig. 2C). STAT3 phosphorylation was activated in response to IL-6 signaling induced by malaria, as well as by other factors (e.g., other GP130-acting cytokines or IFNs and IL-10) (4, 13). Remarkably, however, a much stronger STAT3 phosphorylation was also observed in IL-6RαKO mice injected with recombinant sIL-6Rα (Fig. 2C).

**FIGURE 2.** IL-6 trans-signaling causes lethal outcome to P. chabaudi malaria. Infected mice were injected i.p. with 1 μg recombinant sIL-6Rα on days 1, 4, and 7 p.i. Levels of sIL-6Rα (A) and IL-6 (B) in serum were determined on day 8 p.i. (n = 5–6). (C) Western blot of liver lysates using pSTAT3 Ab of uninfected and P. chabaudi-infected IL-6RαFL and IL-6RαKO mice on day 8 p.i. IL-6RαKO mice were injected with 1 μg recombinant sIL-6Rα and IL-6RαFL mice were injected with 16/8/8 μg sGP130Fc on days 1, 4, and 7 p.i., respectively. Calnexin: loading control. (D) P. chabaudi malaria in IL-6RαKO mice (n = 14) injected with 1 μg recombinant sIL-6Rα on days 1, 4 and 7 p.i. Levels of IL-1β (E) and TNF-α (F) in IL-6RαFL and IL-6RαKO mice on days 0 and 8 p.i. Significant differences (p < 0.01) were determined as in Fig. 1. "IL-6RαKO" mice, day 0 versus day 8 p.i., "IL-6RαFL" versus IL-6RαKO on day 8 p.i., "IL-6RαKO" versus IL-6RαKO injected with recombinant sIL-6Rα, "IL-6Rα" versus IL-6RαKO injected with recombinant sGP130Fc. Serum levels of sIL-6Rα (G) and IL-6 (H) in P. chabaudi-infected IL-6RαFL mice on day 8 p.i. (n = 6), with or without injections of sGP130Fc during the infection. *p < 0.01, Student t test. (I) Outcome of P. chabaudi malaria in IL-6RαFL mice (n = 14) injected with recombinant sGP130Fc on days 1 (16 μg), 4 (8 μg), and 7 (8 μg) p.i.
phosphorylation occurred upon injection of sIL-6R into IL-6RαKO mice (Fig. 2C), thus demonstrating the induction of IL-6 trans-signaling by recombinant sIL-6Rα. Importantly, all IL-6RαKO mice injected with sIL-6Rα succumbed to malaria during the crisis phase (Fig. 2D). This was associated with an increased inflammatory response to P. chabaudi malaria, as indicated by increasing levels of IL-1β and TNF-α in sIL-6Rα-injected IL-6RαKO mice that were similar to those found in control IL-6RαFL mice on day 8 p.i. (Fig. 2E, 2F). Notably, myeloid lineage cells, rather than nonimmune cells, were affected by IL-6 trans-signaling that induced inflammatory cytokine expression in ex vivo-isolated macrophages but not in primary hepatocytes (Supplemental Fig. 1L, 1M).

IL-6 trans-signaling can be inhibited by sGP130, which is derived by shedding of the ectodomain from membrane GP130 and alternative splicing (13, 16). This sGP130 is able to bind the IL-6/sIL-6Rα complex, thus preventing IL-6 trans-signaling (16). To further substantiate a critical role for IL-6 trans-signaling in the outcome of malaria, we also attempted to reduce IL-6 trans-signaling in the IL-6Rα-expressing control mice. When control IL-6RαFL mice were injected with mouse recombinant sGP130Fc protein (16, 8, and 8 µg) during infection on days 1, 4, and 7 p.i., respectively, endogenous sIL-6Rα decreased from 200 to 35 ng/ml (Fig. 2G), and concentrations of IL-6 decreased frominduced in mice injected with mouse recombinant sGP130Fc protein (16, 8, and 8 µg) during infection on days 1, 4, and 7 p.i., respectively, endogenous sIL-6Rα decreased from 200 to 35 ng/ml (Fig. 2G), and concentrations of IL-6 decreased from 350 to 100 pg/ml on day 8 p.i. (Fig. 2H). IL-6 trans-signaling was indeed inhibited, as evidenced by dramatically lowered STAT3 signaling was not affected (Supplemental Fig. 2A, 2B). Moreover, the infection-induced inflammatory response was decreased, as evidenced by lower levels of IL-1β and TNF-α (Fig. 2E, 2F). Importantly, under these inhibitory conditions of IL-6 trans-signaling, ~40% of the P. chabaudi-challenged control mice survived the infection (Fig. 2I). Remarkably, survival was not increased further when sGP130Fc doses were increased to 32/16/16 µg, whereas doses <16/8/8 µg on days 1, 4, and 7 p.i. did not prevent P. chabaudi-induced lethality (Supplemental Fig. 2C–G), revealing that only a dosing regimen that efficiently reduced sIL-6Rα in serum conferred protection from malaria-induced lethality (Supplemental Fig. 2C–G).

Collectively, this report provides unequivocal evidence that IL-6 trans-signaling, rather than IL-6 classic signaling, contributes to a lethal outcome of P. chabaudi malaria. Although the infection-induced increase in sIL-6Rα may be considered a beneficial response of the host that inactivates overshooting of IL-6 and its effects through membrane IL-6Rα, this beneficial response is increasingly superposed by the concomitant increase in IL-6 trans-signaling, which promotes harmful responses, ultimately impairing the development and efficacy of protective immunity. The cell types mediating such harmful responses remain to be identified. Remarkably, some data indicate that sIL-6Rα levels also increase with the severity of human malaria caused by P. falciparum and P. vivax (12, 23). Therefore, inhibition of IL-6 trans-signaling represents a novel promising therapeutic approach to combat human malaria; it is currently under investigation to treat human rheumatoid arthritis (24), human chronic liver disease (25), and human cachexia (26).

Disclosures

The authors have no financial conflicts of interest.

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