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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: 4141–4144.

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 (sIL6Rα), 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-6Rαflxed 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-6Rαflxed mice were crossed with deleter-cre mice, and offspring were intercrossed. IL-6Rα deficiency in liver parenchymal cells (IL-6RαL-KO mice) was generated, as described previously (17). Myeloid lineage-specific IL-6Rα-deficient mice (IL-6RαM−/−KO mice) were created by crossing IL-6Rαflxed mice with LysoM−cre mice and intercrossing offspring.
malaria infections

Mice were kept under specific pathogen-free conditions and received water and food ad libitum. The experiments were approved by the state authorities. Only female mice aged 10–14 wk were used in the experiments. Challenge was i.p. with 10^7 erythrocytes parasitized with virulent P. chabaudi a, or 10 ng LPS for 4 h. mRNA expression for IL-1β, TNF-α, was determined in sera using commercially available ELISA kits (R&D Systems), according to the manufacturer’s manual.

Northern blot

Total RNA (20 μg) was glyoxylated, separated in agarose gels, transferred to positively charged Biodyne7PLUS nylon membrane (Pall, Pensacola, FL), and subjected to Northern blot analysis, as detailed previously (18). Ex vivo experiments

Macrophages were isolated from IL-6Rα FL, IL-6Rα KO, and IL-6Rα Myel-KO mice, as described previously (20), and stimulated in vitro with 25 ng/ml IL-6. Hepatocytes were isolated from IL-6Rα FL- and IL-6Rα KO- mice, as detailed previously (21). A total of 10^6 ex vivo macrophages and hepatocytes was stimulated in vitro with 25 ng/ml IL-6, 25 ng/ml IL-6 plus 200 ng/ml sIL-6Rα, or 10 ng LPS for 4 h. mRNA expression for IL-1β, TNF-α, and IL-6 was quantified by quantitative PCR analysis (20).

Results and Discussion

To address the role of IL-6 signaling in malaria, we decided to investigate experimental P. chabaudi malaria in the mouse, which shares several characteristics with P. falciaparum, which causes the most dangerous form of human malaria (22). First, we created an IL-6Rα-deficient mouse strain on the C57BL/6 background (Supplemental Fig. 1A–D). These IL-6Rα KO mice did not exhibit any apparent phenotype. In particular, the immune system did not appear to be grossly affected, as indicated by similar numbers of CD19+ B cells and TCR-β+ T cells in major immune organs (Supplemental Fig. 1E). Moreover, we also created IL-6Rα deficiency specifically in liver parenchymal cells (IL-6RαL-KO mice) (17) and in the myeloid lineage (IL-6Rα Myel-KO mice). Macrophages and neutrophils in IL-6Rα Myel-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 P. chabaudi malaria took a lethal course in IL-6Rα FL control mice (Fig. 1A), IL-6Rα Myel-KO mice (Supplemental Fig. 1H), and IL-6RαL-KO mice (Supplemental Fig. 1I), whereas 50% of IL-6Rα KO 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). P. chabaudi-induced lethality is known to result from multiple organ failure that varies in individual mice (18). Control IL-6Rα FL 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-6Rα KO mice (Fig. 1C). The infection-induced increase in circulating IL-6 followed a biphasic pattern in both control and IL-6Rα KO 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-6Rα KO mice.
Similarly, the proinflammatory cytokines IL-1β and TNF-α exhibited a biphasic increase in IL-6Rα^FL^ control mice during infection (Fig. 1E, 1F), whereas mouse recombinant sIL-6Rα into IL-6Rα^KO^ 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-6Rα^FL^ mice and IL-6Rα^KO^ 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-6Rα^KO^ 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

**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-6Rα^FL^ and IL-6Rα^KO^ mice on day 8 p.i. IL-6Rα^KO^ mice were injected with 1 μg recombinant sIL-6Rα and IL-6Rα^FL^ 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-6Rα^KO^ 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-6Rα^FL^ and IL-6Rα^KO^ mice on days 0 and 8 p.i. Significant differences (p < 0.01) were determined as in Fig. 1. IL-6Rα^FL^ mice, day 0 versus day 8 p.i., *IL-6Rα^FL^ versus IL-6Rα^KO^ on day 8 p.i., **IL-6Rα^FL^ versus IL-6Rα^KO^ injected with recombinant sIL-6Rα, †IL-6Rα^FL^ versus IL-6Rα^KO^ injected with recombinant sGP130Fc. Serum levels of sIL-6Rα (G) and IL-6 (H) in *P. chabaudi*-infected IL-6Rα^FL^ 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-6Rα^FL^ mice (n = 14) injected with recombinant sGP130Fc on days 1 (16 μg), 4 (8 μg), and 7 (8 μg) p.i.
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-6R 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 cells. 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 from 350 to 100 pg/ml on day 8 p.i. (Fig. 2H). IL-6 trans-signaling was indeed inhibited, as evidenced by a dramatically lowered STAT3 phosphorylation (Fig. 2C), whereas the MAPK/ERK- and tumor necrosis factor alpha, and IL-12(p70) in Malian children with severe malaria. This was associated with an IL-6 receptor activation, which promotes harmful responses, contributing to a lethal outcome of Plasmodium chabaudi malaria. The cell types mediating such harmful responses ultimately impairing the development and efficacy of protective immunity. The function of the soluble IL-6 receptor in vivo: sensation of human soluble IL-6 receptor transgenic mice towards IL-6 and prolongation of the plasma half-life of IL-6. IL-6. IL-8, IL-10, tumor necrosis factor alpha, and IL-12(p70) in Malian children with severe malaria. Immunol. Cell Biol. 77: 3033–3043.

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Disclosures
The authors have no financial conflicts of interest.