Brief Definitive Report

Interleukin 6 Production in Experimental Cerebral Malaria: Modulation by Anticytokine Antibodies and Possible Role in Hypergammaglobulinemia

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Summary

The production of Interleukin 6 (IL-6) was studied during experimental cerebral malaria (ECM) induced by *Plasmodium berghei* ANKA (PbA) infection. IL-6 is present in the serum of mice with ECM, the highest concentrations being observed in mice with full-blown neurological syndrome. High IL-6 levels were also observed, however, in the absence of pathology in nonlethal malaria infection. These data suggest that IL-6 is produced in large amounts during malaria infection, but does not play a major role in the pathogenesis of ECM. A modulation of IL-6 production in ECM was achieved by in vivo treatment with other anticytokine antibodies: antibodies to interferon (IFN-γ) or to tumor necrosis factor (TNF) abolished the rise of IL-6, while anti-IL-3 and anti-granulocyte/macrophage colony-stimulating factor antibodies only partially prevented this rise, suggesting that the two cytokines IFN-γ and TNF are important intermediates in IL-6 production. Passive immunization against IL-6 did not prevent ECM, but significantly reduced serum IgG levels in malaria-infected mice. Thus, by its effects on B cells, IL-6 may be involved in hypergammaglobulinemia and immune-complex diseases, e.g., glomerulonephritis observed during malaria infection.

Experimental cerebral malaria (ECM) is a hyperacute lethal syndrome reproducing some features of human cerebral malaria (1), the most severe complication and cause of death of *Plasmodium falciparum* infection (2). We have shown that (*a*) ECM is strictly dependent upon the presence of T cells (3) and is accompanied by a marked expansion of monocyte/macrophage pool in lymphoid organs (4); (*b*) overproduction of TNF plays a central pathogenic role (5); and (*c*) a cascade of T cell–derived cytokines is required for this TNF overproduction to occur (4, 6).

IL-6 is a cytokine with pleiotropic effects that can be produced by a variety of cell types (7, 8), including monocytes, T cells, and endothelial cells (9), which are central to the lesion of CM (5). Also, it has been shown that the synthesis and release of IL-6 can be induced by TNF (10, 11). Therefore, we have analyzed the possible contribution of IL-6 to the pathogenesis of ECM.

Materials and Methods

Mice. Female CBA/Ca mice, originally obtained from Bomholtgard (Ry, Denmark) and bred in our laboratory, were 6–8 wk old at the time of infection.

Infection. *P. berghei* ANKA (PbA) infection was initiated as described (3). 17 XNL *Plasmodium yoelii* was from Dr. J.H.L. Playfair (Middlesex Hospital, London, UK).

IL-6 Assay. IL-6 activity was assayed by using the 7TD1 bioassay (7) revealed by MTT (12). The 7TD1 hybridoma cell line was kindly donated by Dr. J. Van Snick, Ludwig Institute, Brussels, Belgium.

Anticytokine Antibodies. Rabbit anti-mouse TNF (5), anti-mouse IL-3, and anti-mouse granulocyte/macrophage (GM)-CSF (4) antisera (produced in our laboratory), and rat anti-mouse IFN-γ (F3) IgG2a mAb (13) were injected in malaria-infected animals following protocols previously shown to be protective against ECM (4–6). 6B4, a rat IgG1 anti-murine IL-6 mAb (500 NU/ml on 1 U of murine rIL-6, one neutralizing unit (NU) being defined as the reciprocal of the dilution of the antibody leading to a 50% inhibition of IL-6–induced growth of 7TD1 cells), was kindly donated by Dr. J. Van Snick (14) and used as ascitic fluid.

Immunoglobulin Assays. Serum IgG and IgM levels were measured using ELISA, according to a procedure derived from that of Izui et al. (15).

Antimalarial Antibodies. Serum anti-*P. berghei* antibodies were measured by ELISA and by slide immunofluorescence as previously described (3).

Statistical Analysis. Means were compared using the nonparametric Mann-Whitney test.

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/90/11/1505/04 $2.00
Volume 172 November 1990 1505–1508

Results

Kinetics of IL-6 during Malaria Infection. Series of mice that are genetically susceptible to CM (CBA/Ca) were inoculated with PbA-infected erythrocytes, and bled daily from day 4 to day 7. Serum IL-6 remained in the range of noninfected mice (<15 U/ml) before the 7th day of malaria infection. As can be seen in Fig. 1, elevated serum IL-6 levels were already seen in mice with early signs of neurological syndrome (i.e., isolated palsies, mild ataxia, referred to as "early CM") (n = 4). The highest IL-6 serum levels were observed in mice with full-blown cerebral malaria, which occurs in 80–90% of PbA-infected mice (5) (hemi- or tetraplegia, convulsions, severe ataxia, tendency to roll over upon stimulation, and eventually coma, referred to as "CM") (n = 9). Serum IL-6 levels were also markedly elevated on day 7 of the nonlethal *Plasmodium yoelii* infection (n = 10), and gradually returned to normal by day 21, thus following a pattern very similar to the parasitemia in these animals (Fig. 2).

Modulation of IL-6 by Anticytokine Antibodies. As can be seen on Fig. 3, the rise in serum IL-6 concentrations was completely abolished in mice treated with anti-TNF (seven mice tested, $p < 0.001$) or anti-IFN-γ antibodies (eight mice tested, $p < 0.001$), whereas the combined treatment with anti-IL-3 and anti-GM-CSF antibodies, which totally prevents the rise of TNF (4), only partially (although significantly $p = 0.02$) reduced the IL-6 production in infected mice (nine mice tested).

Effects of Anti-IL-6 Antibody Treatment. 20 CBA/Ca mice were infected with PbA and 10 were injected intraperitoneally on days 0 and 4 with 0.2 ml of 6B4 mAb or medium as control. In anti-IL-6 mAb–treated infected mice, IL-6 was not detected in the serum at day 7 or 8 of infection (<15 U/ml, 9 mice tested). However, anti-IL-6 mAb failed to prevent ECM, which developed in 8 of 10 mice, as in 8 of 10 infected untreated mice. Anti-IL-6 mAb treatment significantly reduced the malaria-associated rise in serum IgG as detected on day 7 of infection (Table 1), but had no effect on IgM levels. Treatment of PbA-infected mice with another rat mAb (anti-IFN-γ) did not affect serum IgG levels (Table 1). In contrast to total IgG, serum-specific antimalarial antibody levels were not affected by anti-IL6 mAb treatment (221.4 ± 46.2 OD units by ELISA and 2,478.6 ± 1,505.8 titration units by immunofluorescence) compared to 165.3 ± 45.7 OD units and 2,041 ± 1,745.5 in control infected mice.

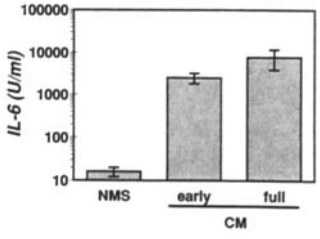

Figure 1. Serum IL-6 levels on day 7 after infection by PbA in CBA/Ca mice. (*NMS*) Normal mouse serum: age- and sex-matched CBA/Ca mice; early CM: starting neurological syndrome; full CM: full-blown neurological syndrome. See text for explanations. Results are expressed as means ± SEM.

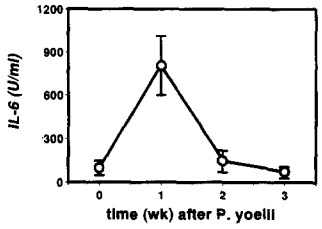

Figure 2. Kinetics of serum IL-6 during nonlethal malaria. CBA/Ca mice (n = 10) were infected with *P. yoelii*. Results are expressed as means ± SEM.

Discussion

In this paper we show that IL-6 is produced in considerable amounts during blood stage infection by malaria parasites, and that this cytokine may be involved in hypergammaglobulinemia rather than in the pathogenesis of the cerebral complications. Our results also indicate that malaria-induced IL-6 production depends upon other cytokines, since it can be modulated by anticytokine antibodies administered in vivo. At least one signal for IL-6 production is provided by TNF, as shown on brain macrophages or astrocytes in vitro (16), since anti-TNF antibody treatment prevents the rise of IL-6 in serum of plasmodium-infected mice, in agreement with experiments in *Escherichia coli* LPS-injected baboons (16). The rise in malarial-induced serum IL-6 was abolished by anti-IFN-γ antibody but only partially prevented by the dual treatment with anti-IL-3 and anti-GM-CSF antibodies. In other models, both IL-1 (9) and IFN-γ (11) also have a role in IL-6 production. Several infectious diseases have been already reported to induce IL-6 (17–20). In the context of malaria, IL-6 has been found in mice after sporozoite infection (21) and in man with severe falciparum infection (22). IL-6, at least in vitro, can be produced by brain cells (16, 23), in addition to the three cell types involved in ECM.

While IL-6 is induced by TNF (10, 11), two arguments do not support major role of IL-6 in the pathogenesis of ECM.

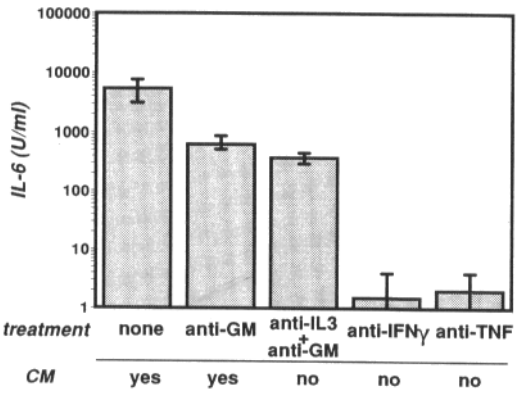

Figure 3. Modulation of serum IL-6 levels by in vivo treatment with anticytokine antibodies. While anti-TNF and anti-IFN-γ antibodies totally prevent the rise of serum IL-6, the combined treatment by anti-IL3 and anti-GM-CSF antibodies only partially reduces IL-6 levels. The relation with the protection is shown on the bottom line. See Materials and Methods for doses and schedule of injection of the antibodies. Results are expressed as means ± SEM.

Table 1. *Effect of Anti-IL-6 mAb on Serum IgG and IgM Levels in PbA-Infected Mice.*

Time after PbA	Treatment	IgG	p	IgM	p^*
		μg/ml		*μg/ml*	
Day 0	–	7,975 ± 1,520		180 ± 40	
Day 4	control	15,420 ± 3,490	–	202 ± 50	–
	anti-IL-6	10,780 ± 800..	NS	275 ± 19	NS
Day 7	control	21,333 ± 3,280	–	532 ± 75	–
	anti-IL-6 mAb	10,280 ± 2,700	0.017	481 ± 86	NS
	anti-IFN-γ mAb	25,562 ± 4,405	NS	ND	ND

Results are given as mean ± SEM.
* The levels of Ig in treated and control animals were compared with nonparametric Mann-Whitney test (ND, not determined; NS, not significant).

First, the dissociation between high levels of IL-6 and occurrence of ECM (in CBA/Ca mice treated by anti-IL-3 and anti-GM-CSF or in mice infected with the non lethal *P. yoelii* parasite). Second, the absence of protective effect of in vivo treatment with anti-IL-6 mAb on the development of ECM. In addition, unlike TNF (24, 25), IL-6 cannot directly cause tissue necrosis (Fiers, W., personal communication) and might even have a "protective" effect on some IL-1- or TNF-induced pathological reactions. For instance, IL-6 decreases the IL-1-induced PGE2 release (Dayer, J.M., personal communication).

IL-6 has been found to lead to maturation of activated B cells into Ig-secreting plasma cells (26). Since anti-IL-6 mAb prevents hypergammaglobulinemia without reducing the specific antimalarial humoral response, one has to hypothesize that cytokines other than IL-6 (such as IL-2 and IL-4) are more instrumental in specific antibody responses. IL-6 is thus possibly involved in malarial complications associated with hypergammaglobulinemia. In addition, overexpression of IL-6 has been suggested in the development of B cell lymphomas (27). IL-6 production might contribute to the higher frequency of Burkitt's lymphomas associated with plasmodium infections in epidemiological studies (28). The significance of IL-6 production in malaria may thus go beyond the cerebral syndrome.

We thank Dr. J. Van Snick for providing 7TD1 and 6B4 cells, and Dr. B. Allet and John Delamarter (Glaxo IMB, Carouge, Switzerland) for providing recombinant murine TNF, IL-3, and GM-CSF. The technical assistance of D. Gretener, C. Gysler, and C. Schwerdel is gratefully acknowledged.

This work is supported by grant 870107 (to G.E. Grau) from the United Nations Development Program/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases, grants 3.803.086 (to P.-H. Lambert), 3.650.0.87 (to P. Vassalli), and 3.930.0.87 (A. Fontana) from the Swiss National Science Foundation and the Sandoz Research Foundation. G.E. Grau is supported by the Cloetta Foundation, Zürich, Switzerland.

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Received for publication 7 March 1990 and in revised form 30 July 1990.

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