

Research note

Increased production of acute-phase proteins corresponds to the peak parasitaemia of primary malaria infection

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Abstract

Recent studies have implicated non-specific mediators associated with CD4⁺ T cells of the T helper 1 subset in resistance to experimental malarias. As part of continuing studies into the multifactorial role of nitric oxide and other contributors to the innate immune response in control of acute-phase malaria infection, the production of the acute-phase proteins, caeruloplasmin and serum amyloid P, following infection of naive mice with blood stages of the rodent malaria parasite *Plasmodium chabaudi* was investigated. Levels of both acute-phase proteins in the serum of infected mice were significantly elevated on days 7–12 post-infection compared both to other times of infection, and to background levels detected in uninfected control mice. These times corresponded to the ascending and peak primary parasitaemia, when production of interferon- γ , tumour necrosis factor- α and nitric oxide is known to be raised. Although it is not apparent whether the production of caeruloplasmin and serum amyloid P has a causal effect in reducing parasitaemia or is simply a by-product of innate immunity, the detection of increased levels of circulating acute-phase proteins may act as a useful surrogate marker of high level parasitaemia, and therefore, of blood-borne malaria pathology. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. Text

The murine malaria *Plasmodium chabaudi* is a recognised model for examination of acquired immunity to the asexual erythrocytic stages of malaria parasites, including *P. falciparum* infection of humans [1]. NIH (H-2^q) mice infected with *P. chabaudi* develop a self-resolving primary infection, lasting up to 2 months, consisting of an acute primary parasitaemia that peaks on day 10 and lasts 15–18 days, followed by usually 1–2 minor patent recrudescences. Ourselves and others have reported previously the biphasic nature of the protective CD4⁺ T cell response during a primary *P. chabaudi* infection in mice [2–4]. This is characterised by an early T helper (Th)1 predominant response responsible for controlling acute infection proceeded by a Th2-regulated antibody-mediated resolution of low level parasitaemia [5–7]. A key element in the initial non-specific response to malaria infection is the rapid synthesis of proinflammatory mediators, such as interferon- γ , tumour necrosis factor- α and nitric oxide, to limit a rapidly escalating acute parasitaemia [7–10], thereby allowing the relatively slower development of acquired, specific effector mechanisms relevant to parasite clearance. With increasing parasite exposure through subsequent infections, levels of parasitaemia are progressively diminished, reflecting more rapid induction of acquired antibody, so lessening the requirement for Th1 mediation [10,11]. This shifting Th1/Th2 balance, from an initial cell-mediated immunity to its downregulation upon multiple infection, closely parallels alteration in immune bias from susceptible toddler to semi-immune adult in malaria-endemic areas [12].

This study evaluated the production of the major acute-phase proteins of mice, caeruloplasmin and serum amyloid P, secreted by IL-1-stimulated hepatocytes, during the early stages of *P. chabaudi* infection, at a time when non-specific immunity is considered responsible for reducing parasite density [1]. Although acute-phase proteins have been examined in the context of the inflammatory response to a variety of infectious diseases [13], little is known of their production during malaria. A previous study of human malaria infection un-

der laboratory conditions showed an increased serum level of the major acute-phase protein found in humans, C-reactive protein, in naive, but not in immunised subjects, following challenge with *P. falciparum* sporozoites [14]. In addition, increased levels of C-reactive protein have been associated with severe *P. falciparum* infection under conditions of natural exposure [15,16], but whether these had a beneficial or deleterious effect was not substantiated. It is known, however, that C-reactive protein can inhibit the penetration of erythrocytes by merozoites, by binding to the surface membrane of the parasite [17], suggesting a possible contribution by acute-phase proteins to the non-specific immune response to malaria.

Blood stage parasites of the AS strain of *P. chabaudi* were stored in liquid N₂ and maintained by blood passage, as described previously [4]. For experiments, female NIH mice (Harlan Olac, Bicester, UK) aged 8–10 weeks were infected intravenously with 1×10^5 parasitised erythrocytes and parasitaemias were determined daily by examination of Giemsa's stained thin blood smears by light microscopy [4]. Throughout infection, sera were collected daily by bleeding mice from the tail for 4 mice per group of 12 [7]. Individual mice were bled at every third collection of serum (3 days apart) and at no time was there any indication that bleeding of mice for serum modulated the course of infection.

The caeruloplasmin and serum amyloid P content of individual serum samples were measured by modification [16] of a two-site sandwich ELISA methodology for detection of serum proteins [18]. Briefly, wells of microtitre plates (Nunc maxisorp, Nunc, Paisley, UK) were coated with rabbit antibodies (10 μ g/ml; 100 μ l) to murine caeruloplasmin (IgG1; DAKO, High Wycombe, UK) or serum amyloid P (polyclonal IgG; Calbiochem, Nottingham, UK) diluted in carbonate–bicarbonate buffer, pH 9.6, and incubated overnight at 4°C. After washing with 0.05% v/v Tween 20 in phosphate-buffered saline (PBS) (pH 7.4; wash buffer), excess binding sites were blocked with a solution of 2% v/v bovine serum albumin (BSA) in PBS for 1 h at room temperature. Sera, diluted 1:100–1:1000 in wash buffer, were added (100 μ l)

in triplicate and incubated for 2 h at room temperature. Caeruloplasmin and serum amyloid P standards (DAKO and Calbiochem, respectively) prepared in wash buffer to cover the concentration range 0.05–50 mg/ml were included on each plate, as was a negative control of wash buffer alone. Following extensive washing, peroxidase-conjugated rabbit polyclonal IgG anti-mouse caeruloplasmin (DAKO) or biotinylated rabbit polyclonal IgG anti-mouse serum amyloid P (Calbiochem), diluted 1:4000 in 0.5% BSA, 0.05% Tween 20 in PBS, were added for a further 1 h incubation at room temperature. For the serum amyloid P ELISA, after washing, 100 μ l of peroxidase-conjugated streptavidin (Serotec, Oxford, UK) diluted 1:5000 in wash buffer was added and

plates were incubated for 1 h at room temperature. After a final wash, reactions were developed with *p*-nitrophenyl phosphate (Sigma, Poole, UK) at 1 mg/ml in glycine buffer (pH 10.4) as substrate and coloration was stopped after 15 min by adding 2 M sulphuric acid. Absorbance was determined at 490 nm by measurement of optical density using an EmaxTM microplate reader (Molecular Devices, Crawley, UK). Unknown concentrations of acute-phase proteins in test samples were calculated against standard curves calibrated with purified caeruloplasmin and serum amyloid P. Concentrations of each acute-phase protein were compared by Student's *t*-test, with $P < 0.05$ considered significant.

Fig. 1 shows the production of caeruloplasmin

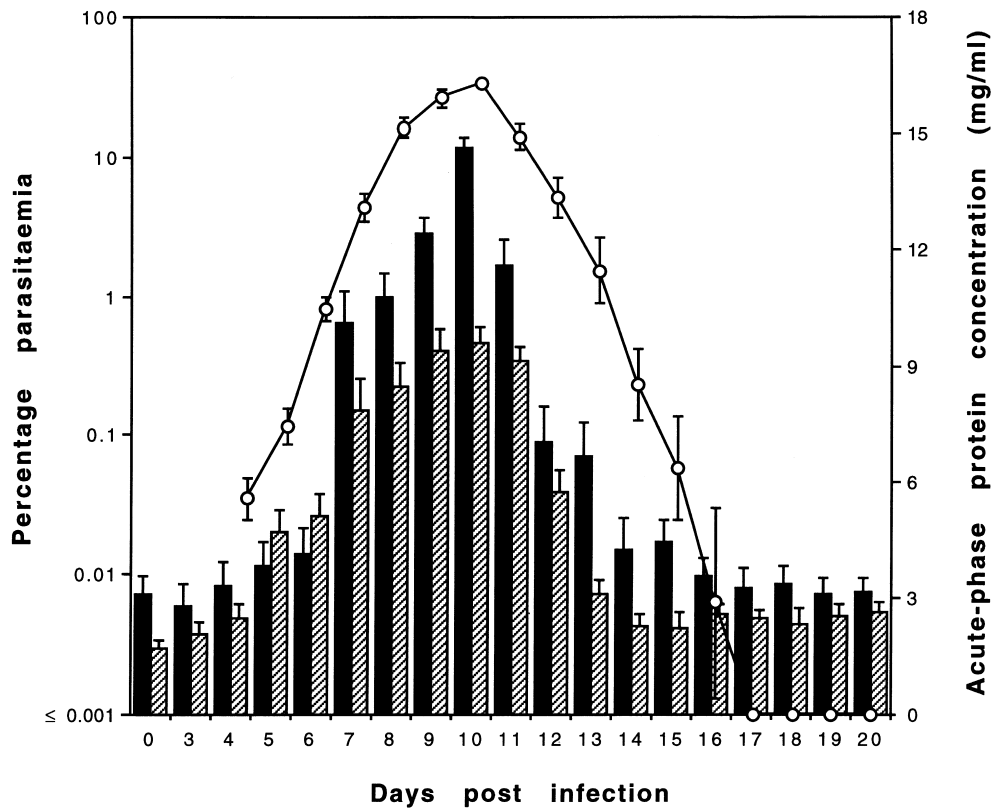


Fig. 1. Serum levels of caeruloplasmin (■) and serum amyloid P (▨) during the course of primary *P. chabaudi* AS parasitaemia (○) in NIH mice. Concentrations shown represent the mean \pm 1 S.D. of pooled mean values from three separate experiments. For each experiment, individual values were measured from four mice within each group on each day. Levels of both acute-phase proteins later in infection, days 21–75, and following reinfection, were not significantly elevated with respect to sera from uninfected control mice ($P > 0.05$).

and serum amyloid P in mice during acute infection with *P. chabaudi*. Caeruloplasmin levels in sera from infected mice on days 0–6 and 14–20 were not significantly different from those in control sera from uninfected mice (2.92 ± 0.45 mg/ml; $P > 0.05$). Similarly, for serum amyloid P, levels on days 0–4 and 13–20 post-infection were not significantly elevated compared to the control value obtained from uninfected mouse serum (2.47 ± 0.36 mg/ml; $P > 0.05$). In contrast, over the period corresponding to the ascending primary parasitaemia, peak parasitaemia and initial resolution of infection, spanning days 7–13 and 5–12 post-infection for measurement of caeruloplasmin and serum amyloid P, respectively, levels of these acute-phase proteins were significantly increased compared both to baseline control values and to concentrations at other times of infection ($P < 0.001$ – $P < 0.05$). Maximal production of both acute-phase proteins corresponded to the time of peak parasitaemia, 10 days post-infection.

An elevated production of caeruloplasmin and serum amyloid P appears to correlate directly with the rapid inflammatory response to initial infection and the onset of increased parasitaemia. As concentrations of both serum proteins were diminished later during primary infection and following further challenge, it is likely that acute-phase protein production may serve as a sensitive index of symptomatic malaria. This is in accord with the observations that concentrations of C-reactive protein in the serum of children with asymptomatic *P. falciparum* infections are lower than in those showing clinical symptoms [16] and that abrupt increase in C-reactive protein occurs during acute illness in individuals not previously exposed to malaria [14]. We are currently performing in vitro assays to determine whether caeruloplasmin, serum amyloid P and other acute-phase proteins have antimalarial properties in vitro, either in isolation or in the presence of other immune effector molecules. Initial findings suggest that acute-phase serum (day 10 post-infection) suppresses growth of *P. falciparum*-parasitised erythrocytes in vitro, but that this activity is ablated by treating the *P. chabaudi*-infected mice from which the serum was derived with antibody to IL-6 (unpublished data). Since it

is known that IL-6 stimulates the rapid synthesis of acute-phase proteins in vivo [19,20], it is tempting to speculate that acute-phase proteins may perform an important immunomodulatory function, in much the same way as nitric oxide has been shown to do [5,10,21,22], during the acute parasitaemia of blood stage malaria infections.

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