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Elizabeth C. Smith · Andrew W. Taylor-Robinson

Parasite-specific immunoglobulin isotypes during lethal and non-lethal murine malaria infections

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Abstract Production of parasite-specific antibodies is an important component of immunity to blood stage malaria infection, as shown by several previous studies in rodent models. However, no study has addressed the induction of humoral immunity by different parasites in a genetically homogeneous host population. Here, levels of parasite-specific immunoglobulin isotypes were measured during primary infections of *Plasmodium chabaudi* and of *Plasmodium yoelii* in inbred NIH mice inoculated with cloned lines of either avirulent or virulent erythrocytic parasites. Non-lethal infections were characterized by early and late significant upregulation of IgG2a and IgG1, respectively. In contrast, for lethal infections, a slower, reduced IgG2a response correlated with a rapidly fatal outcome prior to any significant synthesis of IgG1. It is proposed that the sequential upregulated synthesis of parasite-specific IgG2a (cytophilic) and IgG1 (non-cytophilic) is associated with protective immunity to blood stage malaria infections in mice. This may provide an immunological framework for examining humoral immunity to malaria in humans.

Introduction

Malaria is caused by protozoan parasites of the genus *Plasmodium*. Each year, 2–3 million children die from *Plasmodium falciparum* malaria, and up to 500 million people suffer clinical disease (World Health Organisation 1995). Despite considerable effort, a commercially available vaccine remains a distant prospect. One reason for this is our incomplete knowledge of what immune responses to aim for and how these may be achieved. There is, therefore, a basic need for the underlying principles of immunity to each stage of the malaria life cycle to be clarified.

Passive transfer of immunoglobulin (Ig) G from semi-immune individuals can partially protect against *P. falciparum* infection (Cohen et al. 1961; Sabchareon et al. 1991). While such a strategy is not practicable on an industrial scale, it indicates the feasibility of inducing immunity to malaria through antibody (Ab) mediation. However, understanding in detail the effector mechanisms to blood-borne plasmodia in humans has proved intractable since often little is known of the parasitological history and immune status of the subject and collection of blood samples is infrequent. Thus, while the modes of action of protective Ab against *P. falciparum* have been postulated from in vitro cultures of parasitized red blood cells (pRBC), murine models have been used for analysing effector mechanisms ex vivo and in vivo (Mohan and Stevenson 1998). Through these, protective immunity to the pathogenic asexual blood forms of malaria has been shown to involve both Ab-independent and Ab-mediated mechanisms (Mohan and Stevenson 1998).

A major recent advance in immunology has been the establishment of a simple paradigm for separating CD4 T helper (Th) cells into two main subsets according to cytokine secretion patterns, which determine their function (Mosmann and Coffman 1989), and the equilibrium between which underlies the regulation of most host-parasite relationships (Abbas et al. 1996). Th1 cells produce IL-2, IFN-γ and TNF-β, and through these mediate macrophage activation and delayed-type hypersensitivity responses. Th2 cells produce IL-4, IL-5, IL-6, IL-10 and IL-13, and provide optimal help for the maturation of B cells to plasma cells and for switching to most Ab isotypes. This paradigm is undoubtedly more complex than initially appreciated. Of relevance to the work presented here, IFN-γ, the principal Th1 effector cytokine, also regulates the

E.C. Smith · A.W. Taylor-Robinson (✉)
School of Biology, University of Leeds,
Clarendon Way, Leeds LS2 9JT, UK
E-mail: bgyawtr@leeds.ac.uk
Tel.: +44-113-3432893
Fax: +44-113-3432882

production of the opsonizing or cytophilic and complement-fixing Ig isotype IgG2a found in mice (Snapper and Paul 1987). Th2 cells stimulate the production of high levels of the non-complement-fixing IgG isotypes, IgG1 and IgG4, in mice and humans, respectively (Stevens et al. 1988).

Previous work by ourselves and others has firmly established that protective immunity to blood stage *Plasmodium chabaudi* AS involves both Th1 and Th2 cells, with a temporal shift in predominance from Th1- to Th2-regulated immune reactivity (Langhorne et al. 1989; Stevenson and Tam 1993; Taylor-Robinson et al. 1993). Soon after primary challenge, Th1 cells activate various non-specific immune mechanisms, such as nitric oxide (NO) production, to control a rapidly escalating acute parasitemia (so reducing parasite density). Later on, as the proportion of Th2 cells increases, IgG1-mediated immunity begins to operate, so facilitating the resolution of patent infection (Taylor-Robinson et al. 1993). With increasing parasite exposure through subsequent infections, levels of parasitemia are progressively diminished, reflecting more rapid induction of acquired Ab, so lessening the requirement for Th1 mediation (Taylor-Robinson et al. 1996). This shifting Th1/Th2 balance closely parallels the alteration in immune bias from susceptible young child to semi-immune adult in malaria-endemic areas (Taylor-Robinson 2002). Although a biphasic response is most apparent for primary *P. chabaudi* AS infection, we have proposed that the Th1-to-Th2 switch is a feature of all malaria infections in both experimental models and humans, but which, depending on the parasitological and immunological factors in each infected individual, may be masked by a very skewed immune response in favor of predominant regulation by one or other $CD4^+$ T cell subset (Taylor-Robinson 1998).

The contribution of the host and the parasite to the balance of Ab-dependent and Ab-independent activation varies according to polymorphisms in both organisms (Stevenson et al. 1982; Sayles and Wassom 1988). It has been shown previously that inbred strains of mice of varying haplotype produce different IgG responses upon infection with a single strain of parasite, *Plasmodium yoelii* 17XNL (Taylor et al. 1988). By corollary, the aim of the present study was to address the induction of humoral immunity by different species of plasmodia in a single strain of inbred mouse. Inbred NIH mice were infected with four different malarias, representing two pairs of genetically closely matched parasites. Within each combination, infection with one strain is self-resolving and the other is lethal. Comparison of Ig isotypes thereby enabled analysis of the contribution of the humoral response to parasite-induced immunity and/or immunopathology. The finding of sequentially raised levels of IgG2a and IgG1 in non-lethal infections suggests this pattern of Ab production may provide a surrogate marker of immunity to malaria in mice, with parasite-specific IgG1 production associated with the resolution of parasitemia to subpatency.

Materials and methods

Mice and parasites

Female NIH ($H\text{-}2^q$) inbred strain mice were obtained, guaranteed specific-pathogen-free, from Harlan Olac (Bicester, UK) and used at 8–10 weeks of age. Routine screenings of mice for bacterial, viral and unintentional protozoal infections proved negative throughout the study period. Blood stage parasite isolates AS and 7/F1 of *P. chabaudi chabaudi*, and 17XNL and 17XL of *P. yoelii yoelii* (hereafter called *P. chabaudi* AS, *P. chabaudi* 7/F1, *P. yoelii* 17XNL and *P. yoelii* 17XL, respectively), were provided by the WHO Registry of Malaria Parasites, University of Edinburgh, UK. It should be noted that in the literature *P. chabaudi* 7/F1 and *P. yoelii* 17XNL are also known, respectively, as *P. chabaudi* clone 7F IP-PC1 (Hommel et al. 1982) and *P. yoelii* YM (Yoeli et al. 1975). Experimental mice were infected by i.v. inoculation of 1×10^5 pRBC in 0.2 ml RPMI 1640 medium (Gibco, Paisley, UK), following a single passage from liquid nitrogen storage. Parasitemias were determined daily by examining Giemsa's stained thin blood smears by oil immersion light microscopy (Taylor-Robinson and Phillips 1993). Depending on parasite density, either 500 RBC or 50 fields of view were counted to determine % parasitemia.

Collection of serum

On alternate days (or occasionally less frequently for *P. chabaudi* AS) throughout each course of infection, mice were killed and exsanguinated by cardiac puncture while under light carbon dioxide anesthesia. Between 1.0–1.2 ml blood per mouse was collected in glass capillary tubes and allowed to clot for 1 h each at 37°C, then at 4°C. Serum was removed from the clot, microcentrifuged at 300 *g* for 5 min and individual samples were stored at –20°C until assayed for Ig content (Taylor-Robinson and Phillips 1994). Normal serum was obtained from naive non-infected mice in an identical manner.

Measurement of parasite-specific antibodies

The two-site sandwich enzyme-linked immunosorbent assay (ELISA) used to determine levels of serum Ab was modified from that described previously (Taylor-Robinson and Phillips 1994). A lysate of pRBC was used as capture antigen (Taylor-Robinson and Phillips 1992; Taylor-Robinson and Phillips 1993). For each of the four different parasites used, a lysate was prepared by freeze-thawing peripheral blood collected from previously malaria-naive mice infected with the corresponding parasite when they had parasitemias $>25\%$. The protein concentration of the lysates was determined using an Exact, Sensitive, Low interference (ESL) kit (Boehringer Mannheim, Lewes, UK). Each lysate was diluted in phosphate-buffered saline (PBS) (pH 7.4) to give a coating concentration of 20 μg/ml and 100 μl/well applied to round-bottomed 96-well ELISA plates (Maxisorp; Nunc, Paisley, UK). After washing with 0.05% v/v Tween 20 in PBS, followed by blocking of excess binding sites with a solution of 2% v/v bovine serum albumin (BSA) in PBS for 1 h at room temperature, plates were incubated in quadruplicate with test sera, diluted to 1/50 in PBS, for 2 h at 37°C. Plates were washed extensively before detection of parasite-specific Ab using a rat anti-mouse horseradish peroxidase-conjugated monoclonal Ab (mAb) specific to IgG1 or IgG2a (Serotec, Oxford, UK). mAb to IgG1 (MCA 336P) or IgG2a (MCA 421P) were diluted in 0.5% BSA, 0.05% Tween 20 in PBS to 1.0 and 0.5 g/ml, respectively, and applied to plates for 1 h at 37°C before final washing. Plates were developed with 3,3′, 5,5′-tetramethylbenzidine at 1 mg/ml in glycine buffer (pH 10.4) as substrate, incubated in the dark at room temperature and the reaction stopped after 30 min using 50 μl/well 2 M H_2SO_4. Absorbance was determined at 450 nm by measurement of optical density (OD) using an Emax microplate reader (Molecular Devices, Crawley,

UK). No standard of known concentration for each Ig isotype was available so results were expressed directly as OD_{450} values and compared to an internal standard of normal NIH mouse serum obtained from 8–10 week old naive female mice. This provided a background value of non-specific responsiveness to each of the four pRBC lysates used.

Statistical analysis

Ab measurements are shown as mean ± SD. Differences in the production of Ig isotypes between experimental groups were analysed using an unpaired two-tailed Student's *t*-test, with $P < 0.05$ considered to be significant. For parasitemias, interquartile ranges were < 10% of median values but, for the purposes of clarity, these are not shown in the figures.

Results

Course and outcome of primary infection

Primary *P. chabaudi* AS infection was non-lethal and comprised an acute primary parasitemia that peaked at 59% on day 10 and which resolved to subpatency by 18 days post infection (dpi), followed by one or more recrudescent parasitemias (shown for the first 45 dpi in Fig. 1A). The kinetics of infection were in line with previous observations in NIH mice (McLean et al. 1982; Taylor-Robinson and Phillips 1998) and other inbred strains (Cox et al. 1987; Gilks et al. 1990). In contrast, *P. chabaudi* 7/F1 caused a virulent acute infection that peaked at 71% on day 9, and to which mice succumbed between 9–12 dpi (Fig. 2A), as noted previously in outbred OF-1 mice (Hommel et al. 1982).

Primary infection with *P. yoelii* 17XNL showed an acute parasitemia that rose steadily to reach a peak of 27% on day 15, but which then rapidly cleared to ≤ 0.001% by 19 dpi (Fig. 3A). *P. yoelii* 17XL infection, on the other hand, was rapidly uniformly lethal, with a parasitemia of 79% by day 5 and with no mice surviving beyond 7 dpi (Fig. 4A). The respectively non-lethal and lethal courses of *P. yoelii* 17XNL and 17XL infection in NIH mice were therefore essentially similar to those in other inbred strains of mice in which they have been described, notably BALB/c and C57/BL (Taylor et al.1988; Shear et al. 1989).

Parasite-specific Ig production during *P. chabaudi* AS infection (non-lethal)

Levels of IgG2a were significantly increased by 5 dpi (Fig. 1B) compared to control sera from uninfected mice (OD 450 nm = 0.342 ± 0.015; $P < 0.05$). Production was markedly elevated from days 13–17 ($P < 0.01$ compared to day 22; $P < 0.005$ compared to all other times of infection), coincidental with the descending primary parasitemia (Fig. 1A), but gradually diminished from the time of resolution of the acute infection. The initial production of IgG1 in response to infection was not significantly elevated compared to control normal serum (OD 450 nm = 0.255 ± 0.044; $P > 0.05$, 3–15 dpi) (Fig. 1B). From the time of clearance of parasitemia to subpatency (from day 17 onwards), the levels of IgG1 became significantly raised and remained at a high level throughout the remainder of the observation period. IgG1 production 35–45 dpi was markedly greater than at previous times of infection ($P < 0.001$, 3–15 dpi; $P < 0.005$, 17 dpi; $P < 0.01$, 22–28 dpi) and compared to control normal serum ($P < 0.001$).

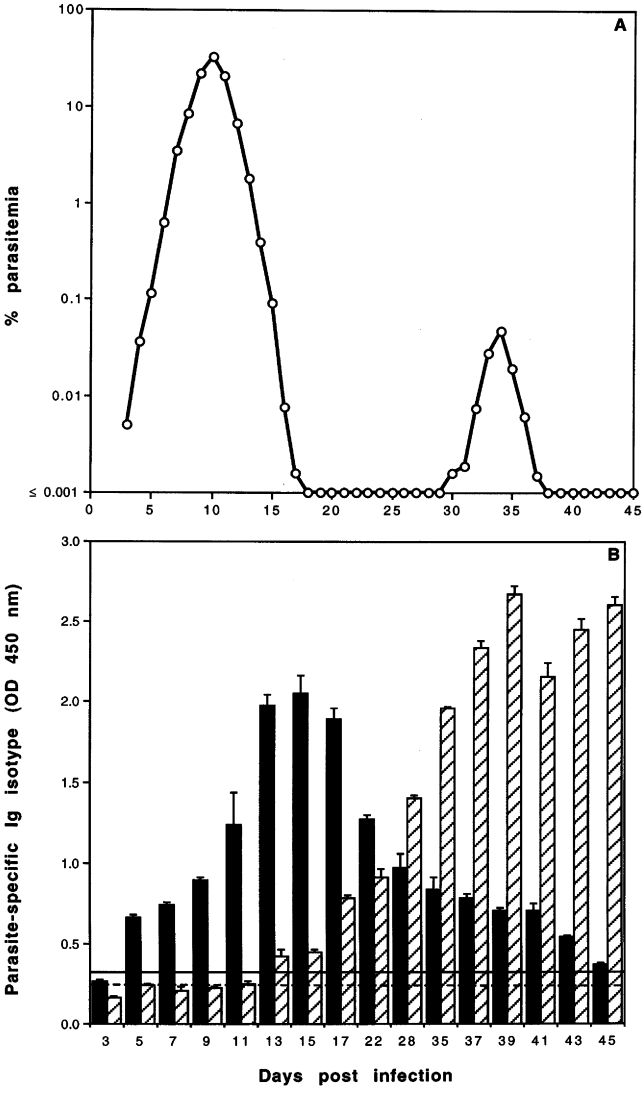

Fig. 1A, B Parasite-specific IgG isotype responses of NIH mice during the course of primary infection of *P. chabaudi* AS. Female mice were infected with 1×10^5 pRBC on day 0 and parasitemias, **A**, were determined daily by examination of Giemsa's stained thin blood smears. Values are medians of parasitemias of nine mice per group. Interquartile ranges (not shown) were < 10% of the median. Serum was collected on the days indicated and measured by isotype-specific ELISA for levels, **B**, of IgG2a (*filled bars*) or IgG1 (*striped bars*). Results are expressed as mean OD value ± SD ($n = 4$) for serum samples pooled from three mice sacrificed at each time point. Control absorbance values (IgG2a = *solid horizontal line*; IgG1 = *dashed horizontal line*) were provided by normal mouse serum obtained from age-matched, sex-matched NIH mice. Data are representative of three separate experiments

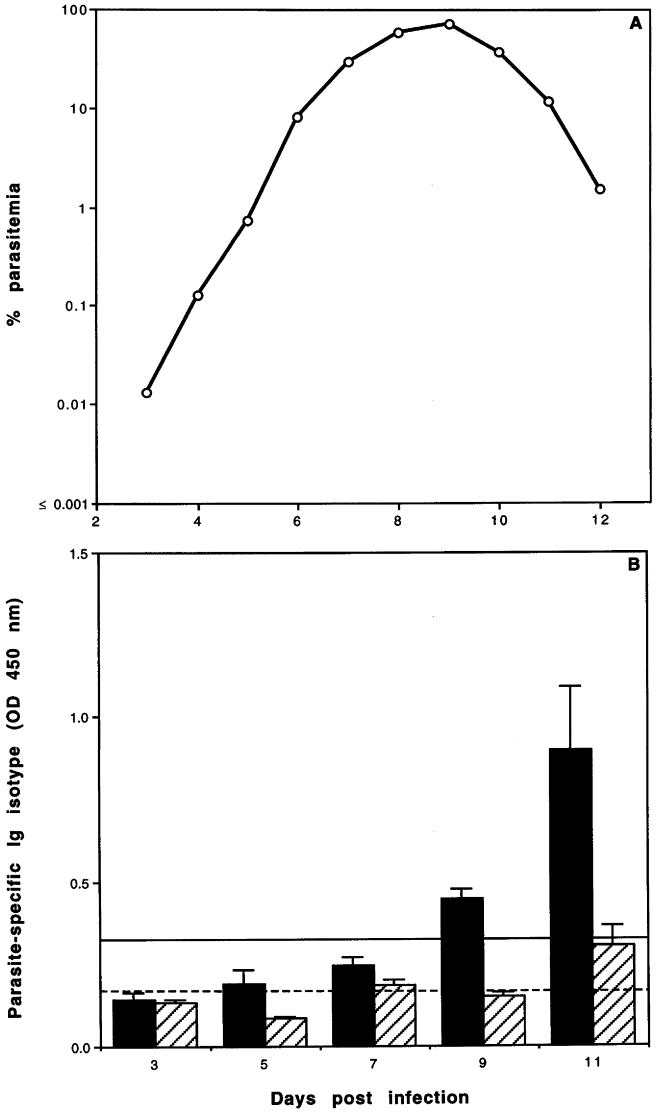

Fig. 2A, B Parasite-specific IgG isotype responses of NIH mice during the course of primary infection of *P. chabaudi* 7/F1. Female mice were infected with 1×10^5 pRBC on day 0 and parasitemias, **A**, were determined daily. Serum was collected on the days indicated and measured for levels, **B**, of IgG2a (*filled bars*) or IgG1 (*striped bars*). Control absorbance values: IgG2a = *solid horizontal line*; IgG1 = *dashed horizontal line*. Experimental details are as for legend to Fig. 1

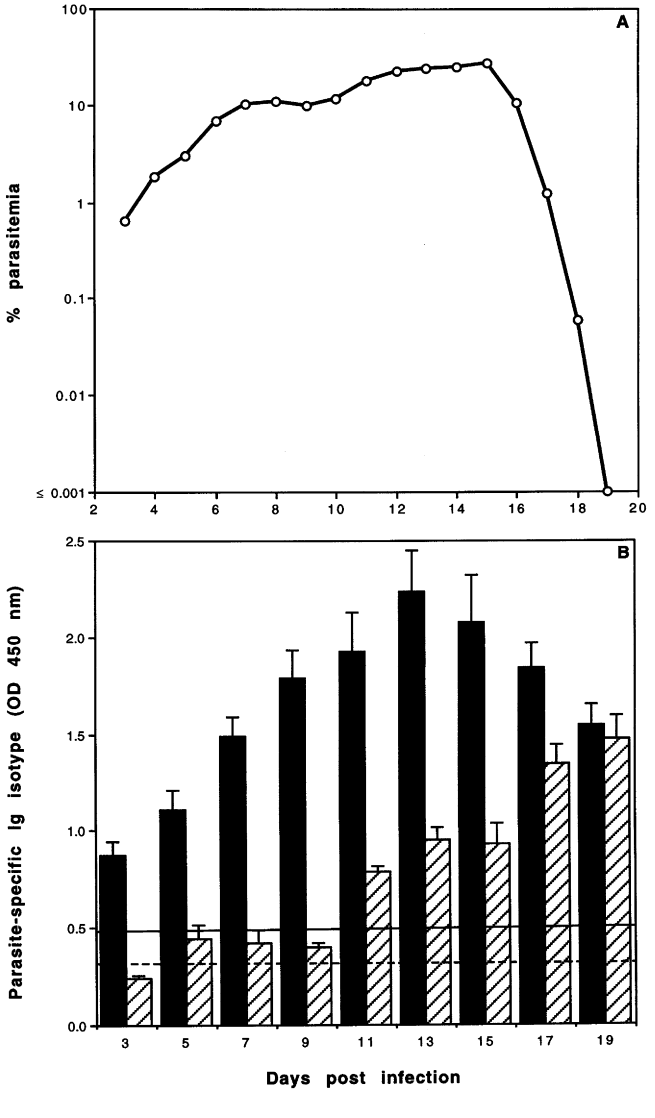

Fig. 3A, B Parasite-specific IgG isotype responses of NIH mice during the course of primary infection of *P. yoelii* 17XNL. Female mice were infected with 1×10^5 pRBC on day 0 and parasitemias, **A**, were determined daily. Serum was collected on the days indicated and measured for levels, **B**, of IgG2a (*filled bars*) or IgG1 (*striped bars*). Control absorbance values: IgG2a = *solid horizontal line*; IgG1 = *dashed horizontal line*. Experimental details are as for legend to Fig. 1

Parasite-specific Ig production during *P. chabaudi* 7/F1 infection (lethal)

Production of IgG2a was characterized by an elevated level on the last day of measurement (11 dpi, the last day of infection for 70% of mice) (Fig. 2B). This represented a significant increase compared both to previous times of infection ($P<0.01$, 3–7 dpi; $P<0.05$, 9 dpi) and to control normal serum (OD 450 nm $=0.322\pm0.041$; $P<0.01$). Prior to day 11, levels of IgG2a in infection sera were not significantly raised compared with control serum ($P>0.05$). Levels of IgG1 were uniformly low and were not significantly different to that for control normal serum (OD 450 nm $=0.172\pm0.024$) on any day of infection ($P>0.05$) (Fig. 2B).

During lethal *P. chabaudi* 7/F1 infection, therefore, the sequential upregulation of parasite-specific IgG2a and IgG1 observed in response to challenge with the non-lethal AS isolate (Fig. 1B) did not occur. Instead, there was no significant IgG1 response, while a marked later production of IgG2a appeared to correlate with severity of infection (Fig. 2B).

Parasite-specific Ig production during *P. yoelii* 17XNL infection (non-lethal)

IgG2a was elevated from very early during infection (Fig. 3B). On day 3, levels were significantly greater than

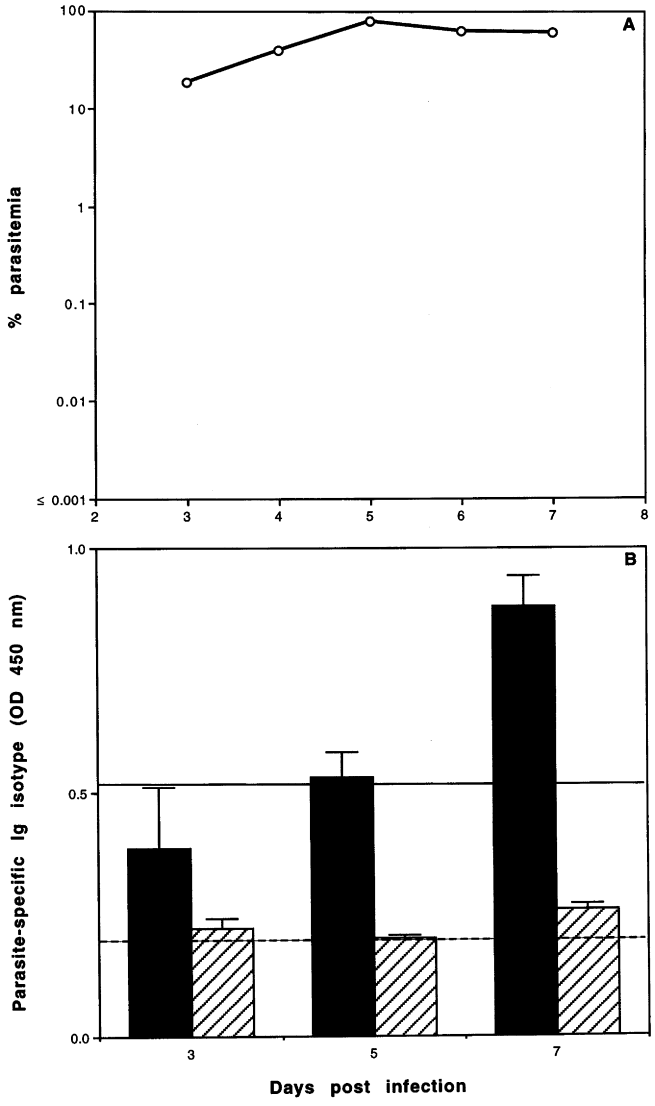

Fig. 4A, B Parasite-specific IgG isotype responses of NIH mice during the course of primary infection of *P. yoelii* 17XL. Female mice were infected with 1×10^5 pRBC on day 0 and parasitemias, **A**, were determined daily. Serum was collected on the days indicated and measured for levels, **B**, of IgG2a (*filled bars*) or IgG1 (*striped bars*). Control absorbance values: IgG2a = *solid horizontal line*; IgG1 = *dashed horizontal line*. Experimental details are as for legend to Fig. 1

those for control normal serum (OD 450 nm = 0.479 ± 0.062; $P < 0.05$) and remained markedly raised thereafter ($P < 0.01$, 5 dpi; $P < 0.005$, 7–19 dpi). By the time of parasite clearance on day 19 (Fig. 3A), however, production was significantly lower than peak levels 13 dpi ($P < 0.05$). Production of IgG1 increased throughout the course of infection (Fig. 3B). Initial levels were not significantly different from those detected in control normal serum (OD 450 nm = 0.320 ± 0.051; $P > 0.05$, 3–9 dpi), but from day 11 onwards were markedly elevated compared both to previous times of infection ($P < 0.01$, 11–15 dpi; $P < 0.005$, 17 dpi; $P < 0.001$, 19 dpi) and to control normal serum ($P < 0.01$, 11–15 dpi; $P < 0.001$, 17–19 dpi).

For non-lethal *P. yoelii* infection, therefore, production of significantly raised levels of parasite-specific IgG2a preceded that for IgG1 (Fig. 3B), as for *P. chabaudi* AS (Fig. 1). However, while this sequential Th1-to-Th2-regulated isotype switch was apparent for protective immunity to both parasites, it was more rapid for *P. yoelii* 17XNL, IgG2a peaking prior to the clearance of infection which coincided with the onset of IgG1 production.

Parasite-specific Ig production during *P. yoelii* 17XL infection (lethal)

IgG2a production was significantly raised on the last day of measurement (7 dpi, the last day of infection for 80% of mice) (Fig. 4B) compared both to previous times of infection ($P < 0.05$, 3–5 dpi) and to control normal serum (OD 450 nm = 0.513 ± 0.070; $P < 0.01$). Levels of IgG2a in infection sera from 3–5 dpi were not significantly raised compared with control serum ($P > 0.05$). Levels of IgG1 remained low throughout infection and were not significantly different to that for control normal serum (OD 450 nm = 0.194 ± 0.017; $P > 0.05$) (Fig. 4B).

Lethal *P. yoelii* infection was characterized, therefore, by a poor IgG response, observed as an insignificant difference compared with normal NIH mouse serum of production of parasite-specific IgG1 and raised production of IgG2a not before the last day of infection (Fig. 4B). Similar to *P. chabaudi* 7/F1 infection (Fig. 2), upregulation of parasite-specific IgG2a was insufficient to prevent a rapidly lethal outcome of infection, serving merely as a marker of severe infection.

Discussion

The present study examines changes in the production of IgG2a and IgG1 during primary blood stage infection of NIH mice with either lethal or non-lethal strains of *P. chabaudi* and *P. yoelii*. As regulation of these isotypes is influenced by the prevailing Th1/Th2 cytokine balance (Abbas et al. 1996), this complements a recent analysis of cytokine and inducible NO synthase (iNOS) mRNA expression during infections of the same host/parasite combination to determine whether absolute or temporal differences in the activation of individual cytokines are associated with resolution or exacerbation of disease (Taylor-Robinson and Smith 1999). That study revealed a lesion in production of IFN-γ, TNF-β and iNOS early in *P. chabaudi* 7/F1 and *P. yoelii* 17XL infections. Here, it is shown that fulminating parasitemia and rapid death occurs just as any appreciable parasite-specific IgG2a is detected, well before any presumptive switch to predominant IgG1 synthesis. Production of IgG2a is common to all infections but its rapid upregulation is a feature of self-resolving infections only, i.e. *P. chabaudi* AS and *P. yoelii*.

The four malarias used herein were specifically chosen to comprise two pairs of virulent and avirulent parasites, *P. chabaudi* 7/F1 (lethal) with *P. chabaudi* AS (non-lethal), and *P. yoelii* 17XL (lethal) with *P. yoelii* 17XNL (non-lethal). Within each pairing, parasites are close genetic matches (Landau and Boulard 1978; Cox 1988), and between which primary infections are thought to elicit either largely Ab-independent (*P. chabaudi*) or Ab-dependent (*P. yoelii*) immune responses (Long 1988). While the *yoelii* group parasites show a predilection for invasion of reticulocytes and the *chabaudi* group either prefers normocytes or no has preference (Landau and Boulard 1978; Cox 1988), as one of each pair of parasites is lethal and the other non-lethal the difference in the outcome of infection cannot be attributed per se to a variation in RBC preference. In addition, the varying rate of hematopoiesis among different mouse strains was controlled. This represents a compounding factor when interpreting infections across heterogeneic host backgrounds (Yap and Stevenson 1992). However, despite the aim that by matching parasites as closely as possible any differences in the kinetics and outcome of infection may be most likely attributed to the major changes in immune responsiveness that were observed, it was apparent that within each pair, the growth rate of the ascending parasitemia was greater for the lethal parasite than for its non-lethal counterpart (Figs. 1A and 2A; 3A and 4A). Thus, as has been noted before for *P. yoelii* in A/Tru mice (Fahey and Spitalny 1986), a rapid rate of replication probably contributes to the virulence of lethal parasites.

Non-lethal *P. yoelii* infection has for a number of years been regarded as a model of Ab-dependent immunity to blood stage malaria, requiring a $CD4^+$ T cell-regulated humoral immune response for resolution (Long 1988). The differential secretion of Ig isotypes shown by the present work is consistent with our previous findings (Taylor-Robinson and Smith 1999) which indirectly supported a role for the Th2-upregulated IgG1 in parasite elimination (Taylor-Robinson et al. 1993) by showing significant IL-4 and IL-10 levels at resolution of *P. yoelii* 17XNL infection, but further suggested a requirement for Th1 mediation for control of the preceding acute parasitemia. In this respect, suppression of acute infection with *P. yoelii* 17XNL is achieved though much the same mechanisms of immunity as control of *P. chabaudi* infection, immunity to which is conventionally considered Ab-independent (Cavacini et al. 1990). This finding is supported by the recent observation that *P. yoelii* 17XNL infection is more severe in IFN-γ-deficient mice (van der Hyde et al. 1997). It is noteworthy that the very early significant production of IgG2a by *P. yoelii* 17XNL-infected mice in the present study may be attributed to IFN-γ synthesis within 24 h of infection with these avirulent parasites (De Souza et al. 1997). From the present study, it appears that the previously reported distinction between Ab-dependent and -independent immunity to murine plasmodia may be based on a relative difference in the timing of IgG1 upregulation. For non-lethal *P. yoelii*, this is coincident with clearance, but for *P. chabaudi* AS occurs only after primary parasitemia is resolved. An updated definition would be that resolution of acute infection is and is not, respectively, via a Th2-driven IgG1-dependent mechanism.

The sequential predominance of Th1- and Th2-type cytokines (Taylor-Robinson and Smith 1999) and their regulated Ig isotypes (Fig. 3) in response to non-lethal *P. yoelii* infection corroborates a prior investigation of *P. chabaudi* AS in mice (Mohan and Stevenson 1998) (Fig. 1) and bears out our proposal that for effective protection against blood stage malaria a Th1-to-Th2 switch is necessary for prompt reduction of parasite density and elimination of chronic parasitemia by non-specific and parasite-specific mechanisms, respectively (Taylor-Robinson 1998; Taylor-Robinson and Smith 1999). Certainly, in mice reinfected with *P. chabaudi* AS, Th2 activation is far more rapid than during primary infection, resulting in an immune response weighted heavily towards IgG1 mediation (Taylor-Robinson and Phillips 1992). Isotype switching in favor of IgG1 following reinfection is not confined to infection with avirulent parasites as it is also seen in otherwise susceptible BALB/c mice that survive primary *P. chabaudi* 7/F1 infection following chloroquine treatment (D'Império Lima et al. 1996).

We contend that the controlled production of non-specific acute phase mediators, such as IFN-γ, TNF-α and NO, through crossregulatory cytokine regulation involving IL-10, IL-12 and IL-18, amongst others (Moore et al. 1993; Stevenson et al. 1995), leads to limitation of parasitemia but without overt immunopathology (Taylor-Robinson 1998; Taylor-Robinson and Smith 1999). Why more severe infections fail to trigger an adequate Th1-type response, and how this is suppressed, is not fully understood and is the subject of our current investigations. Given that in infections for which the outcome is lethal a late increased synthesis of parasite-specific IgG2a does occur (Figs. 2B and 4B), it is interesting to note that inoculation of the IgG2a fraction of hyperimmune serum can modulate parasitemias of BALB/c mice infected with *P. yoelii* 17XL (White et al. 1991). This indicates that the failure to control acute infection is due not so much to an intrinsic lack of protective capacity of this isotype but to a lack of acceleration of its upregulation, and that of other Th1-type responses (Taylor-Robinson and Smith 1999) commensurate with the increase in primary parasitemia, so failing to reduce or delay the escalation of infection (Fahey and Spitalny 1986).

In the context of human malaria, although several studies have attempted to associate the distribution of Ab isotypes with protective immunity, an unequivocal pattern has not emerged (Wahlgren et al. 1983; Chizzolini et al. 1991; Bouharoun-Tayoun and Druilhe 1992). In general, sera with high Ab titers to pRBC antigens tend to contain IgM and IgG1 to IgG4 subclasses, while low titer sera comprise IgM, IgG1 and IgG3 Ab (Mohan

and Stevenson 1998). Observations made in the geographically distant sites of West Africa and Thailand indicate that the cytophilic subclasses IgG1 and IgG3 predominate in semi-immune adults while unprotected children and non-immune immigrants either possess non-cytophilic IgM or IgG2 classes or have an overall lower level of malaria-specific Ab (Thelu et al. 1991; Bouharoun-Tayoun and Druilhe 1992; Sarthou et al. 1997). Equally, persons infected with *P. falciparum* demonstrated significantly increased IgG3 titers specific for merozoite surface antigen 2 (MSA-2) (Taylor et al. 1995; Rzepczyk et al. 1997), a subclass that has been associated with responsiveness to the onset of acute bouts of infection (Aribot et al. 1996). Herein, since murine IgG2a is a cytophilic Ab, its elevated production in non-lethal malaria infections in mice correlates with human studies in which opsonizing Ab (IgG1 fraction) were thought to confer protection (Bouharoun-Tayoun and Druilhe 1992). Other clinical studies have highlighted the reduced risks of *P. falciparum* prevalence associated with IgG1 upregulation and the increase in transmittance of IgG1 across the placenta, so possibly contributing to congenital inheritance of immunity (Beck et al. 1995; Deloron et al. 1997).

On the basis of the results described here, we propose that in mice sequentially raised levels of IgG2a and IgG1 may be hallmark of self-resolving blood stage malaria infection. It is tempting to speculate that similar induction of Th1, then Th2, cells is needed to first reduce to, then maintain at, a subclinical threshold malaria in humans, and what limited data there are from the examination of Ig isotypes (Thelu et al. 1991; Bouharoun-Tayoun and Druilhe 1992; Beck et al. 1995; Taylor et al. 1995; Aribot et al. 1996; Rzepczyk et al. 1997; Sarthou et al. 1997) do collectively support this notion.

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