Med Microbiol Immunol (2001) 189: 115–126

REVIEW

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Rodent malarias: the mouse as a model for understanding immune responses and pathology induced by the erythrocytic stages of the parasite

Received: 1 November 2000

Introduction

Rodent models of malaria have been used to investigate the consequences of the interactions between the immune system of the host and different developmental stages of the parasite. Infections can vary in virulence depending on the species and strain of *Plasmodium* and on the mouse strain (Table 1). In non-lethal infections (e.g. *P. chabaudi chabaudi*, *P. yoelii* 17XNL), resolution generally results in immunity to a second challenge infection with the same strain or species of parasite but not to a heterologous parasite. Some parasite strains/species are lethal only in particular strains of mice (*P. c. chabaudi*) and some are uniformly lethal (*P. berghei*, *P. yoelii* 17XL or YM). Most of the infections, whether lethal or non-lethal, display some of the features of severe malaria observed in human *P. falciparum* malaria (Table 1). These different patterns of infection and pathology allow the investigation of mechanisms involved in protective immunity and pathology.

The life cycle of the malaria parasite is complex with distinct phases which are either intracellular in hepatocytes or erythrocytes, or briefly extracellular. In addition, each stage is characterised by the expression of stage-specific proteins. Therefore, different immune effector mechanisms with different specificities are required for the elimination of these various forms. Although rodent models are not exact replicas of the human infection and disease, they provide much useful information about interaction of the host's immune system and the malaria parasite. In this review we will concentrate on the immune response generated against the erythrocytic stages, possible contribution of the immune response and cytokines to disease, and the relationship of these findings to human infection.

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Induction of the immune response to blood-stage parasites in mice

In the erythrocytic stages of the cycle, *Plasmodium* is either briefly extracellular or within erythrocytes, which neither contain the necessary antigen-processing machinery nor express major histocompatibility complex (MHC) class II or class I molecules to allow presentation to T cells. Therefore, the infected erythrocyte or the free merozoite can not be a direct target of specific effector $CD8^+$ or $CD4^+$ T cells, leaving the elimination of the parasite to antibody-dependent mechanisms or to non-specific mediators from macrophages or other cells.

The innate immune system including acute-phase proteins, alternate pathways of complement, phagocytes and natural killer (NK) cells may provide a first line of defence. In addition, components of the innate immune system play a crucial part in the initiation and subsequent direction of adaptive immune responses. Little is known about the role of many of the innate mechanisms in the early control of blood-stage parasites. Mice with high NK activity are generally resistant to parasites such as *P. c. chabaudi*, while strains such as A/J, DBA/2 and others with low NK activity and defects in phagocytic cells are susceptible to lethal infections [85].

In addition to their cytolytic activity, NK cells are an important source of interferon-γ (IFN-γ) [113]. Studies in *P. yoelii* infections have identified NK cells as one source of early IFN-γ production [22], which may be involved in activation of other cells such as macrophages and other antigen-presenting cells (APC), thus linking the innate and acquired response. Activation and proliferation of NK cells, however, requires IL-12 [59]. This cytokine is also a pre-requisite for the development of

Table 1 Plasmodium infections in different strains of mice. BALB/c mice can be susceptible or resistant to ECM caused by *P. berghei* ANKA depending on the source (*ECM* experimental cerebral malaria, *L* lethal, *NL* non-lethal)

Parasite	Strain	Mouse strain	Lethality	Experimental use
P. chabaudi chabaudi		CBA C57BL/6 BALB/c	NL	Immune mechanisms Malaria associated clinical signs/sequestration
	AS	129sv A/J DBA/2	L	Pathogenesis Chemotherapy Resistance and susceptibility
	CB	CBA C57BL/6		Immune mechanisms
P. chabaudi adami		BALB/c C57BL/6	NL	Immune mechanisms
P. berghei	ANKA	BALB/c C57BL/6 CBA	L	Pathogenesis ECM/sequestration
	K173	BALB/c CBA DBA C57BL/6	L	Pathogenesis Control for ECM
P. yoelii	17XL	CBA BALB/c C57BL/6 DBA	L	Immune mechanisms
		Swiss CBA	L	Pathogenesis ECM/ sequestration
	YM	BALB/c C57BL/6 DBA	L	Vaccine Pathogenesis Hypoglycaemia
	17XNL	CBA BALB/c C57BL/6 DBA	NL	Immune responses Vaccination Immune mechanisms
P. vinckei vinckei		BALB/c	L	Chemotherapy Pathogenesis Malaria associated clinical signs/sequestration
P. vinckei petteri	CR	C57BL/6 BALB/c	NL	Immune mechanisms

$CD4^+$ Th1 cells upon interaction with APC. Therefore, the event initiating both activation of NK cells and acquired immune responses in a malaria infection is likely to be the induction of IL-12. This cytokine has been detected in the plasma of *P. c. chabaudi*-infected mice as early as 2 days after infection [87]. Injection of recombinant IL-12 into A/J mice, which are susceptible to a lethal *P. c. chabaudi* infection renders these mice able to resolve their infection [125], suggesting that IL-12-dependent processes may be important for eliciting a host response sufficient to eliminate or control the parasite. The most likely source of IL-12 in the infection is the APC. Bone-marrow-derived dendritic cells (DC), macrophages and B cells are all possible APC with capacity to present antigens from infected erythrocytes. However, only the DC is thought to be able to respond directly to pathogens without a second activating signal [4, 142]. We have shown that *P. c. chabaudi* parasites are capable of rapidly activating DC to produce cytokines including IL-12 and to up-regulate costimulatory molecules such as CD40 and CD86 independently of T cells, NK cells and IFN-γ (Seixas et al., submitted). This rapid activation could account for the early IL-12, NK and $CD4^+$ T cell IFN-γ response observed in *P. c. chabaudi* and *P. yoelii* infections.

Role of $CD4^+$ T cells and $\gamma\delta$ T cells in the immune response to the parasite

Experiments over many years have established the importance of T cells in the development of an effective immune response to malaria [12, 83]. $CD4^+$ T cells are critical for controlling erythrocytic stage parasites [1, 62, 83, 106]. However, in both humans and mice there is also an expansion of $\gamma\delta$ T cells during and following an acute malaria infection, indicating that they maybe contributing to parasite control or to the pathology of the infection [36, 116]. In humans, *P. falciparum*-activated $\gamma\delta$ T cells preferentially express a T cell receptor (TCR) with

Vγ9Vδ2 or Vγ9Vδ1 chains [6, 13], and in mice the responding splenic population is similarly restricted (Vγ2Vδ4 TCR chains [116]). These cells are able to secrete IFN-γ and tumour necrosis factor-α (TNF-α) [36], and in the case of humans, $\gamma\delta$ T cells can inhibit *P. falciparum* growth in vitro [28]. In mice, $\gamma\delta$ T cells, although not essential to the resolution of the infection, do play some role. In their absence acute parasitaemias are prolonged and elimination of parasites is delayed [70, 116]. Activation of $\gamma\delta$ T cells is dependent on IL-2 [57], and this dependency may explain findings showing that $CD4^+$ T cells are necessary for their expansion in mouse [137] and human infections [92]. The parasite molecules responsible for stimulating the $\gamma\delta$ T cells have not been fully characterised, but they could be host proteins induced by infection or released by damaged cells [7]. In *P. falciparum*, $\gamma\delta$ T cells appear to recognise phosphorylated nonpeptidic ligands [11, 21] that are present in host erythrocytes. In the mouse it is not known which components are involved in $\gamma\delta$ cell activation but these red cell components could be potential candidates.

$CD4^+$ T cells do not limit parasite growth directly, but achieve that effect by activating other effector cells to eliminate the parasite. The two major subsets of $CD4^+$ T cells are Th1 and Th2, although Th0 and Th3 have also been described [43, 93]. Th1 cells produce IL-2, IFN-γ and TNF-β and initiate cell-mediated immune responses via macrophage activation. IFN-γ also regulates the production of opsonizing or cytophilic antibodies such as IgG2a in the mouse [18] and is associated with inflammation that can cause tissue damage and pathology. Th2 cells produce IL-4, -5, -6, and -13 and regulate antibody-dependent responses. They also produce IL-10, which has anti-inflammatory properties and can antagonise Th1 responses inhibiting macrophage activation [90]. Th2 cells can mediate their effect by providing help to B lymphocytes for the production of antibodies [18].

The $CD4^+$ T cell response in rodent malarias has been most extensively studied in non-lethal *P. c. chabaudi* (AS) infections. In this model, it has been shown that both Th1 and Th2 cells are important for resolution of infection but the timing of their appearance is critical [68, 71, 124]. In the early stages of the infection in resistant mouse strains there is a rapid and large production of IFN-γ, which can be detected in the plasma 2–3 days before the peak of parasitaemia [82]. At this time IFN-γ is produced mainly by $CD4^+$ T cells, indicating a Th1–type response, which is maintained for at least the first 14 days of infection [71, 82]. Although $CD4^+$ T cells are less well characterised in other rodent infections, early Th1 and IFN-γ responses have been detected in both lethal and non-lethal *P. yoelii* infections and in a lethal *P. vinckei vinckei* infection [118].

Administration of recombinant IFN-γ protects mice against a lethal *P. yoelii* in a dose-dependent fashion but has little effect on the course of infection with the non-lethal variant [118]. *P. c. chabaudi*-infected mice depleted of IFN-γ by antibody treatment, or that are unable to respond to IFN-γ because of a defective IFN-γ receptor, have prolonged acute-phase parasitaemias, with some mortality, and a delay in parasite elimination [29, 82]. These data suggest that this cytokine is important in the initial phase of the infection. One of the major functions of IFN-γ is the induction of macrophage activation with the consequent production of TNF-α, IL-1 and IL-6 and soluble mediators such as nitric oxide (NO) and reactive oxygen species (ROS). Thus, IFN-γ can augment the initial cytokine response of DC initiated by direct interaction with the parasite.

Treatment with exogenous TNF-α at the beginning of the infection protects susceptible strains of mice against an otherwise lethal infection of *P. c. chabaudi*, and also delays patency and reduces peak parasitaemia in *P. c. adami* infections [123]. TNF-α is thought to mediate its anti-parasitic effect via the induction of soluble mediators which include NO and ROS. However, the role of NO and ROS in parasite killing is still controversial. Although the parasite can be damaged by these mediators in vitro [97], in vivo data are conflicting. *P. yoelii*- and *P. c. chabaudi*-specific Th1 T cell lines secreting IFN-γ can transfer protection to immunodeficient mice [1, 132]. However, treatment with L-N^{γ}-monomethylarginine to block NO did not alter the course of infection of a *P. yoelii* infection [1], but did abrogate the protective effect of the *P. c. chabaudi*-specific T cell clone [131]. *P. c. chabaudi* infections in inducible NO synthase-defective mice are not significantly different from infections in wild-type (WT) mice [30]. These observations support the idea that IFN-γ plays a critical role in activating effector cells such as macrophages to produce TNF-α, which exerts its effect indirectly by inducing anti-parasitic factors, but not necessarily NO.

Functionally heterogeneous subsets of human $CD4^+$ Th cells responding to malarial antigens have been described in humans [135]. Although attempts to correlate T cell responses and particular cytokine secretion with protective immunity against acute *P. falciparum* have been without much success, there are some indications that Th1-like responses and TNF-α production can play some role in immunity. In vitro, IFN-γ responses have been observed from peripheral blood mononuclear cells of patients recovering from acute *P. falciparum* infections, and plasma levels of IFN-γ and NO have been shown to correlate with mild malaria and development of immunity [102]. Recent studies in Gabon, comparing patients with mild and severe *P. falciparum* malaria, revealed that, although the level of TNF-α in serum correlated with severity of disease, a higher capacity of blood leukocytes to produce TNF-α in vitro was associated with faster reduction of fever, accelerated cure and good prognosis [91]. The in vitro and in vivo functions of many cytokines during the human infection are not fully understood, and the role of cytokines in the different phases of infection is still far from clear.

In *P. c. chabaudi*, as the primary infection progresses there is a shift in the pattern of the $CD4^+$ T cell response [68, 71]. After 3 weeks of infection the predominant

$CD4^+$ T cells are Th2. The importance of Th2 cells in parasite elimination is difficult to demonstrate directly. Mice lacking a key Th2 cytokine, IL-4, (IL-4 KO) are able to control and eliminate a primary *P. c. chabaudi* [140]. However, parasitaemias were elevated and the clearance delayed when compared with WT mice [140]. Despite the lack of IL-4, knockout mice made malaria-specific antibodies of all isotypes including IgG1, suggesting that some other cytokine could replace IL-4 for many of the Th2 functions [140]. The initial source of IL-4 that induces Th2 development and the mechanism of stable Th2 commitment is still not entirely clear. Recently the IL-1 receptor-related molecule T1/ST2 has been reported to distinguish a subset of $CD4^+$ T helper cells of the Th2 subtype, indicating that T1/ST2 expression plays a role in the development of Th2-like cytokine responses [134]. Another Th2-specific factor, GATA3, involved in Th2 development has been described [100]. It would be important to study these molecules in malaria to understand their role in the development of the Th2 response in the later phase of the *P. c. chabaudi* infection.

The timing of the switch to the Th2 response may be critical for the development of protective immunity. Recent studies comparing cytokine production during infection with lethal and non-lethal *P. yoelii*, showed that high levels of both IFN-γ and IL-10 were produced in the lethal infection (maximum at day 6), whereas IL-10 was not detectable in the non-lethal infection [58]. $CD4^+$ T cells were the major source of IL-10, indicating that the lethal strain activated both Th1 and Th2 cells [58].

The most compelling evidence that Th2-type cells may be important for protective immunity is that after the acute infection, parasites cannot be eliminated without B cells or antibody [139, 141]. Early studies demonstrated that passive transfer of hyperimmune sera protected mice against challenge infection in non-lethal *P. yoelii* 17XNL model [51, 145]. More recently, it has been shown that mice with no B cells as a result of targeted gene deletions cannot eliminate *P. yoelii*, *P.c. chabaudi* and *P. c. adami* parasites and in some cases succumb to a rapid lethal infection [139].

There may be several mechanisms by which antibody mediates its protective effect: agglutination; complement-dependent lysis; neutralisation; inhibition of invasion of red blood cells [24, 34, 42]; and antibody-dependent cellular cytotoxicity or inhibition (ADCC or ADCI) [8]. Experiments from our laboratory have indicated that *P. c. chabaudi* infections can be resolved in the absence of the classical and alternative pathway of complement (Taylor et al., in preparation).

Passive transfer of immunity to *P. falciparum* in humans with immunoglobulin is most effective when the titres of the cytophilic antibodies, IgG1 and IgG3 are high [9]. Similarly, in mice the equivalent cytophilic isotype, IgG2a, has been associated with protection in *P. yoelii* infections [145]. These data support the idea that antibody-dependent phagocytosis or ADCC/I operating via Fc receptor binding may be important means of parasite elimination. However, mice lacking Fc receptor common gamma chain are able to resolve *P. yoelii* or *P. c. chabaudi* infection, suggesting that this mechanism is not crucial [110]. Antibody isotype is also apparently not a critical factor in the induction of immunity by vaccination with merozoite surface protein-1 (MSP1) and the protective efficacy is not dependent on Fc receptors [143].

$CD4^+$ NK T cells may provide an additional source of T cell help in malaria infections. These cells respond to parasite-derived glycosylphosphatidylinositol (GPI) in a CD1d-dependent manner, produce IL-4 and provide help for an antibody response [52, 88, 115, 126]. This pathway is, however, not a crucial component of the B cell response to malarial proteins, since CD1d-deficient mice are able to make comparable antibodies to the GPI-linked circumsporozoite protein.

Regulation of $CD4^+$ T cell responses

The induction of a Th1 response versus a Th2 response appears to be tightly regulated and can be affected by different factors, including cytokine environment, the type and dose of antigen and the expression of costimulatory molecules on APC [19]. Th1 and Th2 cells themselves cross-regulate the differentiation and activity of each other via the cytokines produced [19].

Professional APC such as DC, which are activated by contact with blood stage parasites (Seixas et al., submitted), capture antigen and present it to Th0-like T cells. At the same time, they produce IL-12 and, therefore, would be able to induce development of Th1 cells [77]. The switch to Th2 cells seems to take place as parasite levels are decreasing and thus antigen dose is reduced. Therefore, one possibility is that in *P. c. chabaudi* infections antigen dose is the critical factor in the switch. B cell-deficient mice, which cannot clear their infections, are unable to switch to a Th2 response during the infection. However, the retention of a Th1 response is not due to high levels of antigen as reduction of parasite numbers by treatment with chloroquine does not allow the switch to take place [69]. By contrast, adoptive transfer of immune B cells results in switching. Therefore, the presence of B cells or the antibodies they produce influences the development of Th2 responses. The factors and mechanisms involved in this switch are not yet identified.

The immune responses driven by Th1 and Th2 T cells are also influenced by a third type of T cell whose main function is suppression of the immune responses mediated by Th1 and Th2 [43]. Such Th3 cells produce transforming growth factor-β (TGF-β) in high levels and have been demonstrated in experimental models of colitis or diabetes [45, 96]. TGF-β is a regulator of inflammation, which would be important for the balance between control and elimination of parasites and prevention of pathology. Treatment of infected mice with neutralising antibody to TGF-β, resulting in increased plasma levels of TNF-α and IFN-γ, exacerbated

P. berghei virulence and transformed a non-lethal *P. c. chabaudi* infection into a lethal one [98]. In *P. falciparum* low levels of TGF-β in plasma of acute patients have been reported [144]. Levels of TGF-β seem to be inversely correlated with severity of malaria, suggesting that its role may be down-regulating the production of pro-inflammatory cytokines.

Evasion of the immune response

Several mechanisms might contribute to parasite evasion of the host defences: growth in a protected location; polyclonal activation of lymphocytes; antigenic variation; sequestration; preferential induction of antibodies with no protective function; down regulation of antigen-presentation; and induction of immune tolerance. Direct evidence for most of these mechanisms in rodent models is lacking.

Antigenic variation in erythrocytic stages of plasmodia has long been recognised in *P. knowlesi* and other primate, human and rodent species but the molecular basis for this was only recently elucidated. The variant antigens are families of polymorphic membrane proteins. One set of molecules with these characteristics in *P. falciparum* is *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1) encoded by a multigene family (VAR genes) [3, 119]. PfEMP-1 is expressed at the surface of malaria-infected erythrocytes and plays a role in allowing these cells to adhere to the vascular endothelium via molecules such as CD36 and ICAM-1 [2, 3, 35]. Other multigenic families besides VAR, such as Stevors and Rifins, have been identified in *P. falciparum* [15, 67]. Clonal antigenic variation thus provides a mean for the infected red blood cells to evade removal by the immune system. Sequestration of *P. c. chabaudi* parasites has been reported in liver and also brain [95] but the molecules involved in parasite adherence are not yet identified. Similarly, antigenic variation has been reported at the serological level in *P. c. chabaudi* [104] and multigenic families involved in host red cell selection have been identified in *P. yoelii* [108]. However, the equivalent of the VAR genes in rodent malarias has not yet been identified.

Interaction of the parasite with APC may not always result in activation. Certain strains of *P. falciparum* have been shown to inhibit the maturation of DC and their antigen-presentation capability [136]. This may be an explanation for the immunosuppression described in malaria infections. Presentation to T cells in the absence of the correct costimulation may induce anergy, apoptosis and clonal deletion. In *P. berghei* infection parasite-specific T cells are eliminated in vivo and this deletion involves apoptosis [47].

Pathogenesis of malaria

It is widely accepted that the causes of malarial disease are multifactorial. Common clinical complications include fever or chills, anaemia, hypoglycaemia, renal failure, malnutrition and most severe, cerebral malaria (CM), which is only caused by *P. falciparum* infections (for review see [81]). The complications of a *Plasmodial* infection are thought to be due to some extent to inappropriate inflammatory or immune-related responses by the host. It has been clearly demonstrated in rodent models that inflammatory cytokines produced during the acute infection, particularly TNF-α and IFN-γ, play a major role in pathogenesis [16, 37, 38, 72]. High levels of these cytokines are also found in humans with *P. falciparum* and *P. vivax* infections [65, 66] and a high level of plasma TNF-α is correlated with poor prognosis in *P. falciparum* infection [41, 56, 64]. However, whether there is any causal relationship between IFN-γ and TNF-α and disease severity in human will need to be clarified further.

There are several mouse models that can be used to study malarial pathogenesis (see Table 1). Several of the clinical complications found in humans can occur in the different rodent infections. However, although the severe complications may be similar in mouse and human, the pathogenic mechanism may be different. Therefore, extrapolation from mouse to human studies should be made with care. Until recently, *P. berghei* (ANKA) infection has been the major model for the study of pathogenesis of CM, and *P. yoelii* and *P. c. chabaudi* infections have been used to study the pathogenic processes involved in hypoglycaemia, anaemia and other clinical signs of the malarial infection [16, 20, 26, 72, 150].

Experimental cerebral malaria model

Infection of certain mouse strains (for example, C57BL/6 and BALB/c mice) with *P. berghei* (ANKA) results in adherence of monocytes to the endothelial cells of microvascular vessels in the brain, causing neurological complications with features similar to human forms of CM [109]. Mice die within 10 days due to these complications. Hence, this model has been used extensively as an experimental model (ECM) for the investigation of human CM. One criticism of this model is that mouse ECM is characterised by monocyte adherence to blood vessel endothelia [109], whereas CM caused by *P. falciparum* is associated with massive parasite sequestration in the brain [78]. However, recent studies of post-mortem samples obtained from African children who died from CM also has shown adherence of mononuclear cells in the brain [107]. In the future, *P. berghei* (ANKA) infections in F1 (BALB/c $\times$ C57BL/6 F1) mice may prove to be a more appropriate model than C57BL/6 since both infected erythrocytes and monocytes were found to adhere to endothelial cells in the brain, more closely resembling the human disease [46].

Increased production of TNF-α and $CD4^+$ T cell-derived IFN-γ is associated with the appearance of neurological signs of CM in *P. berghei* (ANKA) infections [37, 40, 112], and depletion of $CD4^+$ T cells,

TNF-α or IFN-γ prevents the neurological complications [39, 61, 111]. In support of an inflammatory involvement in ECM, administration of two cytokines, IL-10 and TGF-β, known to down-regulate TNF-α and IFN-γ production, can suppress or prevent the development of CM [60]. In addition, mice deficient for either IFN-γ receptor or both TNF-α receptors did not develop any clinical signs of CM [76, 112]. Surprisingly, it is the TNFR2 (p75) receptor which is associated with ECM and not the p55 receptor, through which the classical functions of TNF-α are thought to be transduced [75].

Up-regulation of TNF-α is followed by the increased expression of adhesion molecules, notably ICAM-1 and CD36, on endothelial cells [48]. These adhesion molecules are thought to mediate the sequestration of monocytes and parasites in different organs including the brain [2, 76]. Defective expression of ICAM-1 can prevent the development of CM in *P. berghei* (ANKA)-sensitive mice [31]. In addition to monocytes, there is an accumulation of platelets on the brain endothelia [105]. The consequence of this is controversial. One hypothesis is that platelets are prematurely lysed near endothelial cells and release intracellular materials that cause breakdown of blood-brain barrier and hence oedema in brain (a manifestation of CM in both human and mice) [74, 80]. Recently, platelets were also reported to induce ICAM-1 expression on the surface of endothelial cells in response to TNF-α stimulation [105].

Although less thoroughly investigated, other rodent models also reproduce some of the clinical signs of human CM and may be useful. Cytoadherence of erythrocytes infected with *P. yoelii* 17XL to microvascular vessel of brain has been demonstrated in Swiss mice [55]. These mice also showed symptoms of CM, and the pathogenesis has been shown to depend on the expression of ICAM-1 [54, 117].

Similarly, *P. c. chabaudi*-infected erythrocytes have also been found to adhere to brain vessels in mice using electron microscopy scanning [95]. Until now, no obvious signs of neurological involvement have been described for this infection. However, since the immunology of *P. c. chabaudi* infections is very well characterised and since antigenic variation has been described at the serological level in this model [104], it may be particularly useful for investigating the relationship between antigenic variation, sequestration and pathology.

A direct causal relationship between systemic TNF-α and CM in humans has been less easy to establish. Since *P. vivax* is accompanied by high plasma TNF-α levels without any neurological complications [53], it is thought that sequestration of *P. falciparum* and local responses in brain endothelium rather than systemic production of TNF-α are important for CM [10]. A single mutation at position 306 of the TNF promoter (TNF_{-306}), which results in higher production of TNF-α has been associated with a greater risk of CM in humans [148]. However, in vivo treatment of children with anti-TNF-α antibodies had little effect on the progression of CM, although fever was reduced [66, 138].

Restriction fragment length polymorphism analysis has shown that mouse strains can be divided into two groups according to the restriction pattern of intron 1 and 3′UTR of the TNF-α promoter (regions that are important for post-transcriptional regulation) [49]. Several mouse strains, e.g. BALB/c, DBA/2 and A/J (susceptible to *P. c. chabaudi* infections), share a similar pattern in intron 1 and 3′ UTR, whereas C57BL/6 and C57BL/10 (resistant to *P. c. chabaudi* infections) share another pattern [49]. Whether such host genetic factors are significant for the development of CM in mice will need to be further investigated.

Is there any involvement of TNF-α in hypoglycaemia?

Hypoglycaemia is another major complication in human malaria. In mouse models, blood glucose drops in most non-lethal infections as the acute parasitaemia increases and returns to normal as parasitaemia is controlled [20, 72]. However, the magnitude of hypoglycaemia is not always directly correlated with the level of parasitaemia. Hypoglycaemia can occur via several pathways: changes in glucose metabolism; impaired gluconeogenesis; and increased consumption of glucose by both host and parasites. TNF-α has been shown to increase activity of key enzymes involved in glucose transportation (glucose transportase I, GLUT1) [120] and glycolysis (phosphofrutokinase via increased concentration of fructose-2, 6-bisphosphate) [128]. Therefore, inflammatory cytokines could potentially be involved in the hypoglycaemia accompanying malaria infections.

The relationship between TNF-α and hypoglycaemia in vivo in malaria infections is not clear. In support of the view that TNF-α may play a role in hypoglycaemia is the observation that a single dose of recombinant TNF-α given to normal mice can reproduce the hypoglycaemia displayed by mice with a terminal infection of *P. vinckei petteri* [17]. In *P. c. chabaudi* infection, high levels of IFN-γ and TNF-α coincide with hypoglycaemia, and the drop in blood glucose is more pronounced in infected IL-10 knockout (IL-10 KO) mice where TNF-α and IFN-γ levels are elevated [20, 72]. However, experiments that have directly addressed the involvement of TNF-α do not support a role in hypoglycaemia. Administration of anti-TNF-α antibodies or drugs to inhibit TNF-α do not prevent the development of hypoglycaemia in *P. c. chabaudi* and *P. yoelii* YM infections [26, 27]. Furthermore, transgenic mice constantly expressing human TNF-α and mice given recombinant TNF-α both develop hyperglycaemia instead of hypoglycaemia [27, 127].

One explanation for the development of hypoglycaemia accompanying a rodent malaria infection is a pronounced hyperinsulinaemia, which has been reported in mice infected with *P. yoelii* and *P. c. chabaudi* [130].

Lipid products of the parasites may directly stimulate insulin production, thus causing the drop in blood glucose [129, 130]. Drugs that inhibit the production of insulin prevent the development of hypoglycaemia in mice infected with *P. yoelii* YM or *P. c. chabaudi* parasites [25]. These paradoxical conclusions suggest that it may be a combination of the cytokine responses and the parasites themselves that cause hypoglycaemia and that there could be a differential threshold for hyper- and hypoglycaemia induced via TNF-α.

Hypoglycaemia in mice may not have the same pathogenesis as in humans since, although high levels of TNF-α in human *P. falciparum* infection correlate with a drop in blood glucose, no hyperinsulinaemia is observed [41, 56, 64].

Anaemia

Anaemia is a feature of all rodent malaria infections and is thought to be the result of several processes, some of which could clearly involve inflammatory cytokines and immune responses. These include premature removal of erythrocytes from circulation [44, 89], auto-antibodies against erythrocytes [79, 133] and cytokine regulation of haematopoiesis [16, 84], all of which may contribute to malarial anaemia.

A common finding in all these studies is that TNF-α or an imbalance in favour of pro-inflammatory cytokines could be a significant factor [16, 84, 150] in the development of anaemia. TNF-α has been shown to suppress erythropoiesis both in mouse and human bone marrow cultures [16, 23, 103, 121], and enhance erythrophagocytosis [16]. Recombinant TNF-α given during or prior to a *P. vinckei* and *P. c. chabaudi* infection increases the degree of anaemia [16, 17]. Although the magnitude of anaemia at the peak of a *P. c. chabaudi* infection is not affected by elevated levels of TNF-α, administration of anti-TNF-α antibodies does result in a more transient anaemia (our unpublished observations). In agreement with these findings in mice, high TNF-α levels in serum, the TNF_{-238} allele and the lower plasma IL-10 to TNF-α ratio are associated with severe anaemia in Gambian children [63, 99].

Resistance and susceptibility

Some rodent malaria infections are lethal only in certain strains of mice. This allows us to investigate the mechanisms leading to susceptibility or resistance to infection. *P. c. chabaudi* infection is non-lethal in C57BL/6, B10D2, B10A and 129svj mice (see review [122]). However, in a few strains, such as A/J and DBA/2 mice, such infection leads rapidly to death (see review [122]). In these genetically susceptible mice, death within 10 days is associated with high parasitaemias rather than high levels of inflammatory cytokines (Fig. 1).

Susceptibility to a lethal infection of *P. c. chabaudi* [122] in A/J and DBA/2 mice and to lethal *P. yoelii* parasite strains in C57BL/6 mice [22] is associated with an early Th2-like response and lower TNF-α and IFN-γ responses in the spleen [50]. Treatment of susceptible A/J

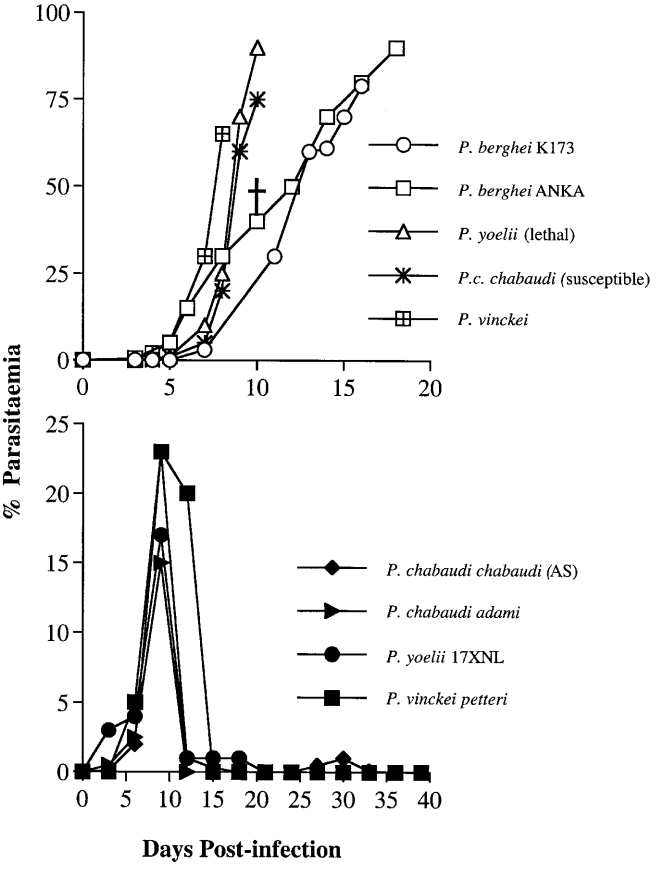

Fig. 1 Diagrammatic representation of the courses of primary erythrocytic infections with different lethal (**A**) and non-lethal strains of mouse malaria (**B**) (■ time of death due to neurological complications during *Plasmodium berghei* ANKA infections in susceptible mice)

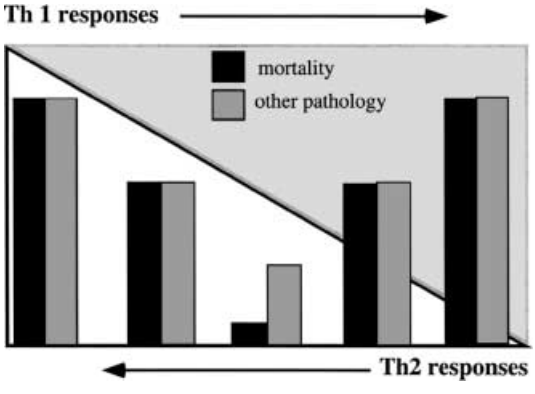

Fig. 2 The influence of pro-inflammatory cytokines on the development of immunity and pathology in experimental mouse malaria infections

mice with either recombinant (r) TNF-α or rIL-12 can rescue them from an otherwise lethal infection of *P. c. chabaudi* [86, 123].

Several loci, which are genetically linked to resistance to either *P. c. chabaudi* or *P. c. adami* (in A/J, C3He/J and SJL/J mice), have been mapped to chromosome 8 and 9 [32, 33]. The proteins encoded by genes in these chromosomes are still unknown but are linked to genes coding for several erythrocyte membrane proteins that may be involved in parasite invasion of red cells.

Resistance and susceptibility of mice to *P. c. chabaudi* is also influenced by gender. Male A/J mice suffer higher parasitaemias and greater mortality than female mice [32, 33, 122]. This increased mortality may be related to the male hormone, testosterone. When treated with testosterone, female mice became more susceptible to an otherwise non-lethal infection [94, 114] and castrated male mice are more resistant to *P. c. chabaudi* infection [149]. Since testosterone had been shown to suppress inflammatory responses by down regulation of IFN-γ production by $CD4^+$ T cells [101] and TNF-α and IL-12 production by macrophages [5, 14, 146, 147], it is possible that male mice have less pronounced pro-inflammatory response early in infection than female mice. Thus, they are less able to initiate an effective early response that can control the parasite. This gender difference in strains of mice such as A/J already less able to mount a pro-inflammatory response renders male mice unable to control a primary blood stage infection.

By contrast, when inflammatory cytokines are up-regulated, as for example in IL-10 KO mice, the susceptibility pattern is reversed [72, 73]. In this case female mice are more susceptible to a lethal infection and suffer a greater hypoglycaemia, hypothermia and loss of body weight [72]. Death in this case seems to be related to the effects of cytokines such as IFN-γ and TNF-α and not to an inability to control early parasitaemia [72, 73]. Male mice on this resistant background even with the defective IL-10 gene do not succumb to a lethal infection. Although their pro-inflammatory cytokines are up-regulated compared with WT mice, they are insufficient to cause death.

The pathology associated with *Plasmodium* infections in mice seems therefore not only to be caused by high parasitaemias but also by the cytokine responses of the host. A low initial inflammatory response is associated with high parasitaemia and mortality. On the other hand, enhanced inflammatory responses lead to a more severe disease and increased mortality without an increase in parasitaemia. Survival and immunity to infection require a balance in the cytokine response sufficient to activate the appropriate immune response but not enough to cause TNF-α-related pathology (Fig. 2).

Acknowledgements Work from the authors' laboratory was funded by the Medical Research Council (UK), The Wellcome Trust (UK) and the Biotechnology Programme of European Community. We would like to thank Meike Hensmann for the critical review of the manuscript.

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