

Short communication

A role of IgE and CD23/NO immune pathway in age-related resistance of Lewis rats to *Plasmodium berghei* Anka?Innocent Safeukui^{a,b,*}, Rémi Vatan^a, Mariette Dethoua^a, Hervé Agbo^a, Gilbert Haumont^a, Daniel Moynet^a, Denis Malvy^a, Philippe Vincendeau^{a,c}, Djavad Mossalayi^a, Pascal Millet^{a,c}^a EA 3677, Bases thérapeutiques des inflammations et infections, Université Victor Segalen Bordeaux II, 146 rue Léo Saignat, 33076 Bordeaux Cedex, France^b Unité d'Immunologie Moléculaire des Parasites, URA CNRS 2581, Institut Pasteur, 28 rue du Dr Roux, 75075 Paris, France^c Laboratoire de Parasitologie, CHU de Bordeaux, 1 rue Jean Burguet, 33071 Bordeaux Cedex, France

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Abstract

In contrast to young rats, adult rats given i.p. *Plasmodium berghei* Anka (PbA) control the parasitaemia and repair their anaemia. Here, we investigated whether IgE and CD23/NO immune pathway could be implicated in this age-related resistance of adult rats to PbA. Eight-week-old rats displayed significantly higher levels of plasma total IgE ($p=0.01$) and soluble CD23 ($p=0.003$) during the peak of parasitaemia, compared to 4-week-old rats. IgE Fc-binding antibody or aminoguanidine administration to parasitized 8-week-old rats slightly delayed blood parasite clearance or exacerbated anaemia. These data suggest that IgE and CD23/NO could play an important role in the resistance of adult rats experiencing PbA primary intraerythrocytic development.

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1. Introduction

Serum levels of IgE and nitric oxide (NO) raised during *falciparum* malaria [1–4], and their production increase with the age of patients [4,5]. While the observation that circulating IgE levels might correlate with disease complications suggests a pathogenic role of IgE [3], the association of high anti-*Plasmodium falciparum* IgE plasma levels with a reduced risk of developing clinical malaria suggests the involvement of IgE in protection [4]. Similarly, some studies suggest that NO is a causative agent of coma during cerebral malaria [2], others suggest that it is protective against severe malaria [1]. Therefore, both IgE and NO could either protect against the most severe manifestations of malaria or induce host-damaging irreversible process, and their effect according to

age needs to be clarified within the context of their functions mediated in the blood circulatory compartments.

The IgE-mediated functions have been shown to depend on IgE high- (Fcε RI) and low- (Fcε RII or CD23) affinity receptors [6]. Fcε RI is abundant in mast cell and basophil membranes and is expressed at much lower levels in Langerhans' cell, monocyte, platelet and eosinophil membranes [6]. CD23 is widely expressed as membrane-bound molecule on leucocytes such as B cells, monocytes and sessile macrophages (in tissues such as liver, spleen and bone marrow) that could be loaded with plasma-derived IgE [6]. The red pulp of the spleen is the sole compartment where the parasite-hosting red blood cells are expected to interact with mast cells and macrophages. Most of the studies deciphering the complexity of the IgE-mediated processes during *Plasmodium* infection have focused mainly on mast cells and the Fcε RI. The cross-linking of CD23 on macrophages by potential ligands such as IgE-containing immune complexes will activate transcription of iNOS gene that induces NO production [7]. In the absence of binding, the membrane CD23 will undergo physiological cleavage into

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a soluble form, sCD23 [6]. Recently, field investigations suggest that low expression of CD23 on monocyte surface [8] or high level of plasma sCD23 [9] was associated with severe malaria. Here, a model of Lewis rat receiving *Plasmodium berghei* Anka-harboured syngeneic rat red blood cells (*Pb*-iRBCs) was used in order to shed some light on the age-related role of IgE and CD23/NO immune pathway in preventing the most severe manifestations of experimental malaria.

2. Materials and methods

2.1. Parasite and animals

P. berghei Anka (PbA) strain (uncloned line from Pr. I. Landau, Museum d'Histoire Naturelle, Paris, France; gift from Dr Jamal Khalife, Institut Pasteur, Lille, France) was used. This parasite strain, originally maintained in mice and adapted to Fischer rats by Pierrot and colleagues [10], was adapted to Lewis rats by at least two successive passages by intraperitoneal (i.p.) injection. All rats used in this study were female-specific pathogen-free Lewis rats purchased from Janvier (Le Genest Saint Isle, France). Once acclimated to our facility (1 week), 4-, 6- and 8-week-old rats received intraperitoneal (i.p.) injection of 10^7 *Pb*-iRBCs. Animal procedures were performed according to the European Economic Community guidelines “86/609”.

2.2. Rat plasma and peritoneal cell collection

Animals were anaesthetized with diethyl ether and blood from 4- or 8-week-old female rats inoculated or not with *Pb*-iRBCs was collected aseptically by retro-orbital venous sinus puncture. Plasma was separated by centrifugation. Peritoneal cells (PCs), aseptically collected by washing with 50 ml cold phosphate-buffered saline (PBS, pH 7.4, Life Technologies), were pelleted by centrifugation at $1200 \times g$ for 10 min at 4 °C, followed by washing twice with cold PBS. The viability of PCs determined by Trypan blue exclusion was always >90%. After counting, PCs were immediately used for further *in vitro* culture and exposure to DNP-binding IgE + DNP-BSA. Plasma was stored at –80 °C until used.

2.3. In vitro rat peritoneal cell handling

Peritoneal cells (10^6 /ml) suspended in culture medium (RPMI-1640, 100 U/ml penicillin, 100 µg/ml streptomycin and 10% heat-inactivated FCS, Eurobio, France) supplemented with 10 µg/ml rat IgE anti-DNP mAb plus 10 µg/ml DNP-BSA (both from Zymed Laboratories Inc., USA), in the presence or absence of 50 mg/ml of aminoguanidine hemisulfate (AG, Sigma), were incubated in duplicate at 37 °C in an air atmosphere containing 5% CO₂. PCs in culture medium alone or with AG were considered as control. For nitrite production measurement, culture supernatants were collected 3, 6, 10 and 24 h and immediately stored at –80 °C before further processing. Nitrite accumulation in the culture medium was assayed by a modified Griess reaction as previously described [11]. Supernatant nitrite concentration was used as

a marker for NO production. Culture medium, chemicals and FCS were endotoxin-free and were tested for the absence of direct activation effects on macrophages (the nitrite content in the supernatant being used as activation marker).

2.4. Quantification of plasma total IgE and soluble CD23

Total IgE antibodies and soluble CD23 (sCD23) were determined by ELISA. A total IgE antibody was determined using a rat IgE ELISA quantitation kit (No. E110-117, Bethyl Laboratories, USA) according to the furnisher's protocol. For plasmatic sCD23 measurement, microtiter plates (Nunc Maxisorp, VWR International) were coated with 2 µg/ml rabbit polyclonal antibodies anti-rat CD23 (gift from Dr Jamal Khalife, Institut Pasteur de Lille, France) in 0.05 M sodium carbonate/bicarbonate, pH 9.6, for 1 h at room temperature. Plates were washed three times with washing buffer (50 mM Tris, 0.14 M NaCl, and 0.05% Tween 20, pH 8) and unbound protein sites were blocked for 30 min at room temperature with 200 µl of blocking buffer (50 mM Tris, 0.14 M NaCl, and 1% BSA, pH 8). After washing three times, 100 µl sample (plasma from rat) diluted in sample buffer (50 mM Tris, 0.14 M NaCl, 1% BSA, and 0.05% Tween 20, pH 8) was then added in duplicate into the plates and incubated for 2 h at room temperature and then washed five times. 100 µl purified rat IgE diluted in sample buffer was added into the plates and incubated for 1 h at room temperature and then washed five times. To detect total sCD23, labelled anti-rat IgE (No. A110-117P, Bethyl Laboratories, USA) diluted 1 µg/ml in sample buffer was added and plates were incubated for 1 h at room temperature. The plates were washed five times and incubated for 20 min with the developing substrate TMB (FT-UP664780, Interchim). The reaction was stopped with sulfuric acid (2 N) and the optical density at 450 nm was immediately determined using a microplate reader (Fisher Scientific).

2.5. Statistical analysis

Statistical analysis of difference in means between infected young and adult rats or between infected and uninfected age-matched controls was performed using non-parametric Mann–Whitney or Wilcoxon rank tests. A *p* value less than 0.05 was considered significant in each experiment.

3. Results

3.1. Resistance of rats to PbA increases with age

The follow-up of parasitized rats showed that the onset of blood parasites was detected in all groups (4-, 6- and 8-week-old) at the same time point post-*Pb*-iRBCs inoculation (day 4), with parasitaemia values peaking between days 12 and 16 p.i., and being lower as rats are reaching the adult age (Fig. 1A). All 6- and 8-week-old rats cleared blood parasites and survived, while 8 out of 17 (47%) parasitized young rats (4-week-old) died by day 13 ± 3 p.i. and those survived cleared parasites (Fig. 1A). No sign of neurological symptoms was observed in any group.

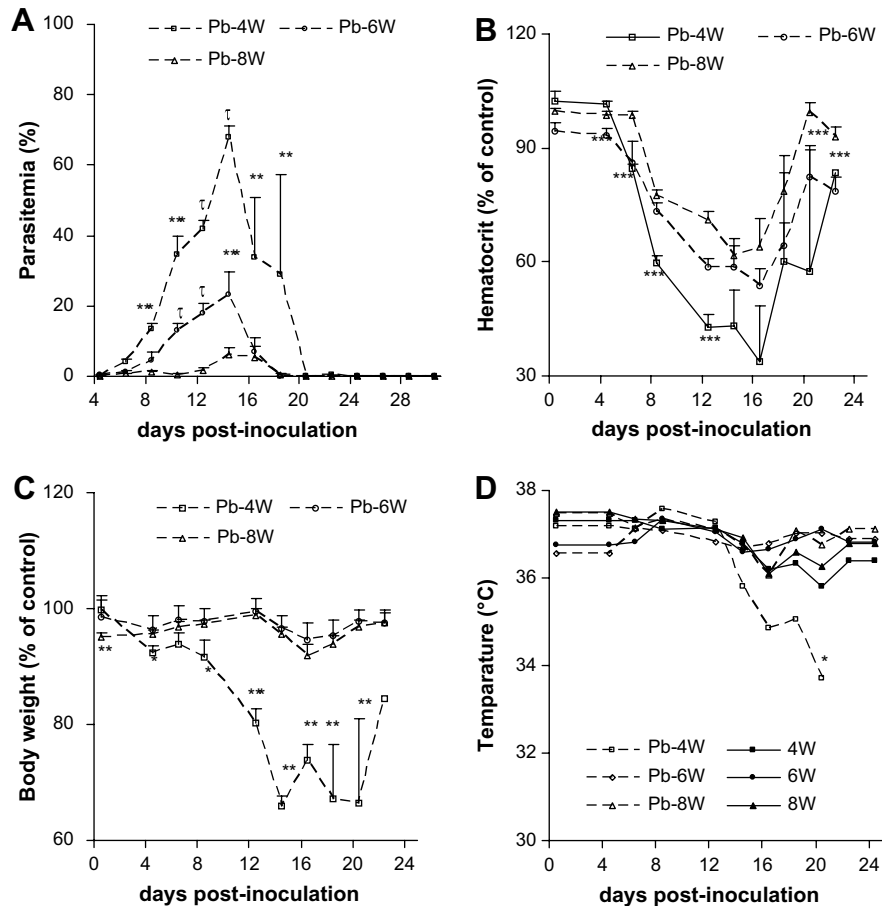


Fig. 1. Parasitaemia (A), hematocrit (B), body weight (C) and temperature (D) of 4- (4W), 6- (6W) and 8- (8W) week-old Lewis rats before (day 0) and/or following i.p. inoculation (Pb-) or not of 10^7 rat *Pb*-iRBCs. Data represent mean values $\pm$ SEM ($n = 5-9$ rats). Statistical significance: *, $p \leq 0.04$; **, $p \leq 0.02$; ***, $p \leq 0.009$; τ , $p < 0.0001$. p values represent comparison between *Pb*-4W or *Pb*-6W vs *Pb*-8W for parasitaemia, hematocrit and weight. For clarity of (D), the standard deviations are not shown.

Parasite intraerythrocytic development resulted in hematocrit decrease, the onset of which was earlier in the 4- and 6-week-old rats (Fig. 1B). The rapid fall in hematocrit values observed over the first 8 days post-*Pb*-iRBCs inoculation (40, 27 and 22% in 4-, 6- and 8-week-old rats, respectively) was associated with only a modest rise in parasitaemia to 13.4, 4.5 and 1.4% for 4-, 6- and 8-week-old rats, respectively. The reduction of hematocrit levels was maximal between days 12 and 18 which correspond to the interval of the peak of parasitaemia, and was statistically significantly more important in young rats (4-week-old), compared to adult rats (6- and 8-week-old) (Fig. 1B). The investigation of clinical parameters showed that only 4-week-old rats exhibited a gradual loss in body weight to reach a maximum of 34% when compared to age-matched controls (Fig. 1C). Young rats also exhibited a gradual loss in body temperature during the entire course, and was significantly lower at day 20 p.i. when compared to age-matched controls ($p = 0.04$) (Fig. 1D).

3.2. Plasma total IgE and sCD23 levels increase with rat age

Total IgE and sCD23 levels, similar at baseline (day 0) between 4- and 8-week-old rats, raised during *Pb*A infection in

both rat groups (Fig. 2A and B). Susceptible 4-week-old rats displayed a significantly higher titer of total IgE and sCD23 at day 6 post-*Pb*-iRBCs inoculation, compared to adult rats ($p < 0.0001$ for both IgE and sCD23) (Fig. 2A and B). However, both IgE ($p = 0.01$) and sCD23 ($p = 0.003$) plasma content were higher in adult than in young rats at day 12 post-*Pb*-iRBC inoculation.

3.3. Naïve 8-week-old rat peritoneal cells exposed to IgE/DNP-BSA produce more nitrite than cells from 4-week-old rats

The nitrite content was 2.4-fold more important in the PC supernatants prepared from 8-week-old rats and co-incubated with IgE/DNP-BSA, compared to those from 4-week-old rats (Fig. 2C). This nitrite production was time-dependent (Fig. 2C) and was prevented by the specific NOS inhibitor AG (Fig. 2D).

3.4. Anti-IgE Ab or AG injection slightly decreases resistance of parasitized 8-week-old rats

Parasitaemia means were significantly higher at days 16 ($p < 0.04$) and 18 ($p < 0.009$) post-anti-IgE injection (*Pb*-8W/anti-IgE), compared to those receiving control Ig (*Pb*-8

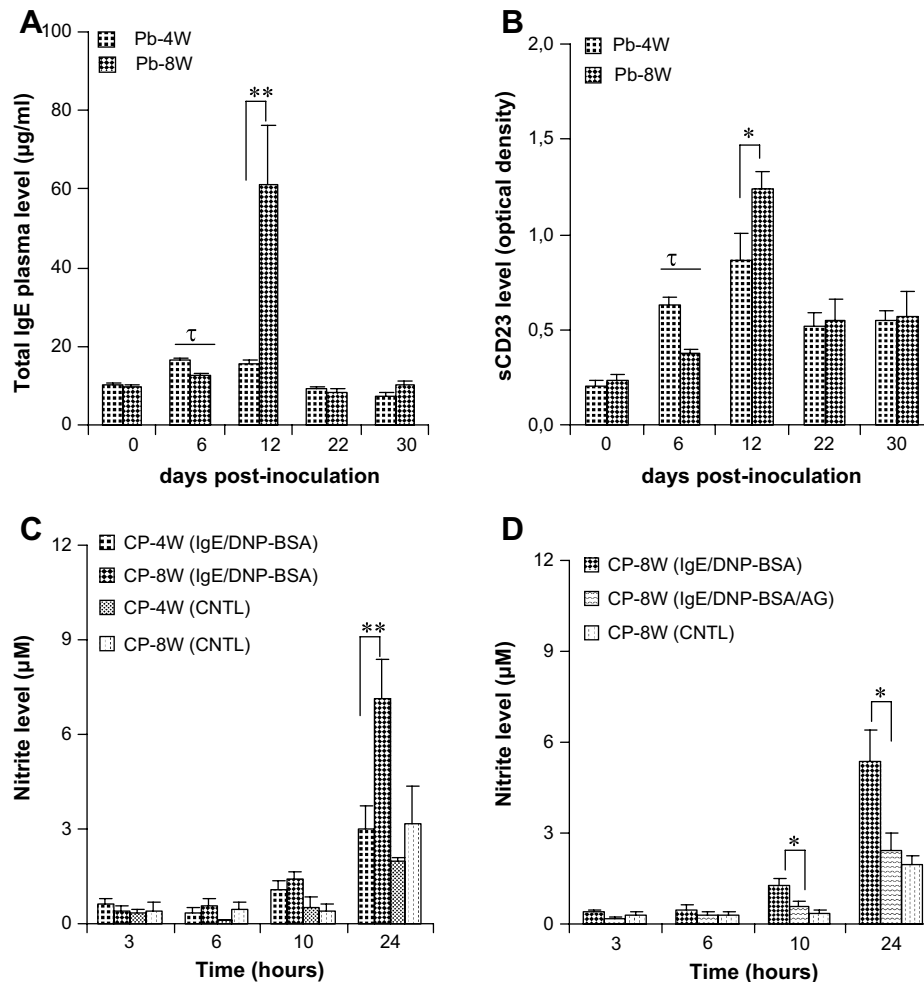


Fig. 2. Plasma levels of total IgE (A) and soluble CD23 (B) of 4- (*Pb*-4W) and 8- (*Pb*-8W) week-old Lewis rats before (day 0) and following i.p. injection of 10^7 rat *Pb*-iRBCs (results of three independent experiments with at least three rats/group in each experiment). Production of nitrite by peritoneal cells obtained from healthy rats (C and D). Peritoneal cells of 4- (CP-4W) or 8- (CP-8W) week-old were incubated with culture medium supplemented with (C) IgE/DNP-BSA (10 µg/ml both IgE and DNP-BSA), or (D) IgE/DNP-BSA plus aminoguanidine (AG, 50 mg/ml). Peritoneal cells incubated with culture medium alone were considered as control (CNTL) (results of at least three independent experiments). Supernatant nitrite concentration was used as a marker for NO formation. Each point represents mean values and SEM. Statistical significance: *, $p \leq 0.04$; **, $p = 0.001$; τ , $p < 0.0001$. p Values represent comparison between *Pb*-4W vs *Pb*-8W for plasma total IgE and soluble CD23, or CP-4W (IgE/DNP-BSA) vs CP-8W (IgE/DNP-BSA) for (C), or CP-8W (IgE/DNP-BSA) vs CP-8W (IgE/DNP-BSA/AG) for (D).

W/CNTL) (Fig. 3A1). Hematocrit level (Fig. 3B1), and means of weight and temperature (data not shown) were similar between both rat groups. Parasitaemia during the follow-up was similar between AG- and PBS-injected rat groups (Fig. 3A2). Compared to parasitized adult rats injected with PBS, those receiving AG during the same period displayed a significantly decreased hematocrit level between days 2 and 6 post-AG injection (which corresponds to days 10–14 post-*Pb*-iRBC inoculation) (Fig. 3B2). This hematocrit level decrease was followed by a significant slower recovery after complete clearance of blood parasites (Fig. 3B2). Means of weight and temperature were similar between AG and PBS rat groups (data not shown).

4. Discussion

Our study indicates that the resistance of rats experiencing asexual intraerythrocytic development of *Pb*A is age-

dependent with, IgE at least contributing to this increased resistance, even though it is not established that this high production of total IgE was due to increase of parasite-reactive IgE. This high production of IgE might protect adult rats against *Pb*A through the activation of monocyte/macrophage populations involving CD23 receptors, and thus stimulate the production of pro-inflammatory cytokine TNF- α [3] and NO [7] which might contribute to the inhibition of asexual development of *Pb*A [12], through direct anti-parasitic action or induction of phagocytosis by macrophages. This hypothesis is supported by our data showing a slightly delayed parasite clearance in parasitized adult rats receiving antibody targeting IgE Fc. Other possible mechanisms involved might be linked to antibody enhancement by IgE and T cell responses via CD23-positive B cells [13], or to CD23 playing a role by either enhancing antigen (Ag) processing and presentation to T cells through its binding of IgE/Ag complexes [14], or mediating phagocytosis of IgE-opsonized particles by

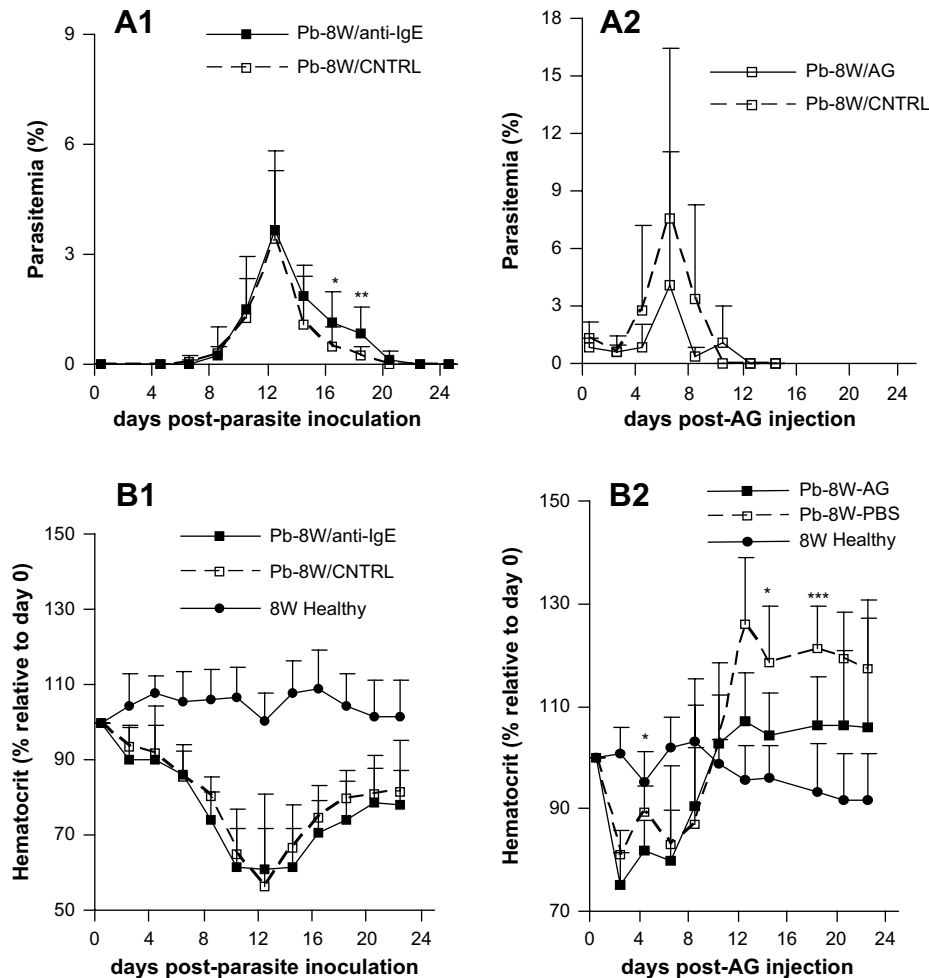


Fig. 3. Parasitaemia (A) and hematocrit level (B) of adult Lewis rats following parasite inoculation (*Pb*-8W), or not (8W healthy) and administration of either antibodies (A1 and B1) or AG (A2 and B2). For antibody administration: groups of 10 adult rats (8-week-old) were inoculated i.p. with 10^7 *Pb*-iRBCs. Two groups of five rats each randomly selected were inoculated i.v. with either 100 μ g of goat IgG polyclonal antibodies targeting specifically the rat IgE Fc (GARA/IgE(Fc)/7S, Nordic Immunology Laboratories, The Netherlands) (*Pb*-8W/anti-IgE) or IgG isotype-matched control antibody (*Pb*-8W/CNTRL) before (d0), and at days 1, 2, 10 and 11 post-*Pb*-iRBCs inoculation. For AG administration: groups of 10 adult rats (8-week-old) were inoculated i.p. with 10^7 *Pb*-iRBCs. Starting from day 8 post-*Pb*-iRBCs inoculation, five rats were randomly selected to receive 10 daily doses of AG i.p. injection (400 mg/kg/day) (*Pb*-8W/AG). The five others (parasitized control group) received during the same period only PBS (*Pb*-8W/CNTRL), which was also used as AG solvent. For all experiments, a group of five parasite-free rats was considered as healthy control and was not given any solution injection (8W Healthy). Parasitaemia and hematocrit level were monitored before (at day 0 only for antibody administered groups), and/or every 2 days post-antibody or AG/PBS inoculation. All experiments were performed in duplicate. Data represent mean values $\pm$ standard deviation of 5–10 rats. Statistical significance: *, $p < 0.04$; **, $p < 0.02$; ***, $p \leq 0.003$. p Values represent comparison between *Pb*-8W/anti-IgE or *Pb*-8W/AG vs *Pb*-8W/CNTRL.

macrophages expressing CD23 [15]. Recent study suggests that mast cells and mast cell-derived TNF- α mediate protection against murine malaria through cross-linking of IgE to Fc ϵ RI mast cells [16].

In our study, susceptible young rats displayed early (at day 6 post-infection) higher production of total IgE and sCD23 levels compared to adult rats. Total IgE levels were constant between days 6 and 12 p.i. in young rats, while they increased in adult rats during the same period. This stability of IgE levels in young rats was associated with a significant increase of plasma sCD23 levels. CD23 both positively and negatively regulates IgE synthesis [6]. Based on these data, we can hypothesize that a limited production or abnormal regulation of IgE might occur in susceptible rats. The fact that sCD23 also retains some capacity to bind IgE [17] may reduce the level of IgE necessary

to build an effective CD23/NO immune pathway. Furthermore, the early Th2-like immune response elicited in young rats could also be responsible for the lack of efficacy to control and coupled anaemia, as it has been suggested that cell-mediated mechanism was crucial to protect young rats from a primary infection [18]. This hypothesis is supported by previous study of Adam and colleagues [18] who found an early (at day 6 post-infection) production of IL-10 and detected spleen CD4+/CD25+ T cells during infection in young Fisher rats, and high level of spleen TCD8+/CD25-, macrophages, B cells, NK and NKT cells in adult rats. These authors also found a constant production of CD4+/CD25+ T cells during infection in young rats [18]. By inhibiting the development of parasite-specific Th1-like immune responses [19], TCD4+/CD25+ T cells (known as natural T regulatory cells) might contribute to

malaria pathogenesis, allowing malaria parasites to escape from the host immune responses [19].

In summary, our data provide experimental evidence for the activation of IgE and CD23 during primary infection of rats with PbA and for their implication in the protection of adult animals. Our data suggest that a particular attention should be drawn to IgE and CD23/NO that could play an important role in the age-related resistance of adults during *P. falciparum* primo-infection. Additionally, as vaccination of newborns represents a most effective strategy to prevent infectious diseases and as the early protection conferred by most existing vaccines is primarily based on antibody-dependent mechanisms, our results suggest that this strategy could be limited by immaturity of the immune system. Helminths (geographically spread in malaria endemic areas) are widely known to induce a strong IgE response that might confer protection against the development of severe malaria [20]. Such information may prove useful for current global control programs, which could thus cover both infections simultaneously, resulting in better efficiency.

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