

High Levels of Immunoglobulin E Autoantibody to 14-3-3 ϵ Protein Correlate With Protection Against Severe *Plasmodium falciparum* Malaria

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Plasmodium falciparum infection generally induces elevated total plasma levels of immunoglobulins, some of which recognize self- or parasite-specific antigens. To our knowledge, we are the first to report high levels of functional immunoglobulin E (IgE) autoantibodies recognizing brain 14-3-3 protein ϵ in asymptomatic *P. falciparum* malaria. 14-3-3 ϵ protein belongs to a family of proteins that binds to CD81, a member of the tetraspanin superfamily elicited in hepatocyte invasion by sporozoites. Levels of expression of 14-3-3 ϵ protein were found to be increased in vivo and in vitro during *Plasmodium yoelii* and *P. falciparum* intra-hepatic development. Collectively, these results indicate that self-reactive IgE is produced during malaria. In addition, the negative correlation between levels of self-reactive IgE to 14-3-3 ϵ protein and parasitemia in asymptomatic malaria due to *P. falciparum* supports a role for these IgE molecules in defense mechanisms, probably by interfering with development of liver-stage parasites through the CD81 pathway.

Plasmodium falciparum infection induces hyperglobulinemia characterized by elevated levels of plasma immunoglobulin G (IgG) and immunoglobulin E (IgE) [1–5]. Several studies pointed out a role for these polyclonal IgE molecules, in association with proinflammatory cytokines, in both malaria pathogenesis and protection [3, 4–10]. A fraction of them are functional parasite-specific IgE that can cross-link IgE receptors and induce mast cell degranulation in vitro in the presence of *P. falciparum* antigens [3, 5–10]. In addition, deposition of IgE in microvessels of postmortem brain tissues [11] and elevated levels of IgE associated

with high tumor necrosis factor α and nitric oxide levels have been found in patients with cerebral malaria (CM) [5, 11, 12]. To date, no information exists concerning the repertoire of autoreactive IgE produced in *P. falciparum*-infected individuals, contrary to IgG that recognized plasmodial antigens as well as self-components from various tissues and organs.

We and others have shown that levels of autoantibody to brain antigens increased with *P. falciparum* malaria severity. In particular, IgG targeting voltage-gated calcium channels, α -2-spectrin, or β -tubulin-3 in the brain were described in CM patients [13–15]. Furthermore, α -2-spectrin and β -tubulin-3 IgG autoantibodies were positively correlated with increased plasma concentrations of cytokines previously associated with CM [13–15]. These findings clearly support the involvement of autoantibodies in malaria, either as pathological factors or as biomarkers of neuropathology. Insights into the production and clinical relevance of autoantibodies in CM are only beginning. In this report, we studied the repertoire of IgE autoantibody

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to brain antigens in groups of patients with asymptomatic malaria (AM), uncomplicated malaria (UM), severe noncerebral malaria (SM), and CM. We found that IgE autoantibody to brain 14-3-3 ϵ protein is produced in *P. falciparum*-infected individuals. We also observed that circulating levels of IgE that recognize 14-3-3 ϵ protein in the human brain and *P. falciparum* or *P. yoelii*-infected hepatocytes but not blood-stage *P. falciparum* parasites were elevated in asymptomatic malaria. Furthermore, the fact that IgE from AM patients was more efficient at inducing in vitro mastocyte degranulation suggests a functional role of IgE in protective mechanisms against severe disease during *P. falciparum* malaria.

14-3-3 proteins compose a large family of highly conserved acidic polypeptides that act as mediators in signal transduction events and cell cycle regulation [16]. In addition, 14-3-3 ϵ protein is also associated with CD81, a member of the tetraspanin family of integral membrane proteins. CD81 has been involved in infection of hepatocytes by either *P. falciparum* or *P. yoelii* sporozoites [17, 18]. Finally, the increased expression of 14-3-3 ϵ proteins in vitro during the development of *P. falciparum* and in vivo within liver cells of C57/BL6 mice infected with *P. yoelii* sporozoites suggests that CD81 signaling events could be mediated by 14-3-3 adapter proteins [17] and supports our hypothesis of a protective role of anti-14-3-3 self-reactive IgE in asymptomatic malaria.

METHODS

Study Population

All patients included in this study were children aged 0.1–6 years (mean age, 2.6 years) recruited between 1996 and 2000 at the Owendo Pediatric Hospital and the Libreville Hospital Center in Gabon, an area where malaria transmission is endemic. The study design was approved by the ethics committee of the local health office. Informed consent was obtained from parents or guardians. All experiments were done in accordance with ethical guidelines for research involving human subjects that were based on the Declaration of Helsinki and on the guidelines of the Institut Pasteur Paris. The patients were classified according to World Health Organization (WHO) guidelines [19] for the definition of malaria cases: Uncomplicated malaria (UM) and severe malaria (SM). Patients studied were composed of, 60 UM, 29 SM with severe anemia (hemoglobin level, <5 g/dL), or hypoglycemia (glucose level, <2.2 mmol/mL), and 17 CM with a Blantyre coma score ≤ 2 , or >1 convulsive episode during 24 hours before admission with post-critical comatose >15 minutes. In addition, 2 control groups from the same area were also studied: 17 were uninfected children (EC) who had a *P. falciparum*-negative thin blood smear, and 22 had AM without clinical manifestation of malaria but with a *P. falciparum*-positive thin blood smear. Venous blood was collected on ethylenediaminetetraacetic

acid in sterile vacutainers from each patient on the day of hospitalization (day 0), before any treatment, 7 days later (day 7), and 30 days later (day 30). Parasitemia was assessed by counting asexual forms of *P. falciparum* on thin blood smears after Giemsa staining.

P. falciparum 3D7 and *P. yoelii* 265BY Sporozoites

P. falciparum and *P. yoelii* 265BY sporozoites (strain NF54) were isolated after dissection of salivary glands of infected *Anopheles stephensi* obtained from the Department of Medical Microbiology, University Medical Center St. Radboud, Nijmegen, the Netherlands, and the Institut Pasteur Paris, France, respectively.

In Vitro *P. falciparum* and In Vivo *P. yoelii* Liver Stages

P. falciparum liver-stage parasites were obtained in vitro as follows. Hepatocytes were isolated from human liver biopsy specimens obtained from patients undergoing partial hepatectomy after oral informed consent at the Service de Chirurgie Digestive, Hépatobilio-Pancréatique et Transplantation Hépatique, Hôpital Pitié-Salpêtrière, Paris, France. The study was approved by the Institutional Review Board (Comité de Protection des Personnes) of the CHU Pitié-Salpêtrière, Assistance Publique-Hôpitaux de Paris, France, and was done in accordance with French national ethical guidelines, under Article L. 1121-1 of the Code de la Santé Publique. Hepatocytes were isolated from the biopsy specimens by use of 2-step enzymatic perfusion as previously described [18]. For Western blot analysis, purified hepatocytes were cultured in 6-well plates at 2.5×10^6 cells per well. The cultures were maintained at 37°C in 5% CO₂ for 24 hours before infection. Infection was done by adding 2.5×10^6 *P. falciparum* sporozoites per well. Infected and noninfected cultures were then stopped at 3 hours, 24 hours, and 4 days after infection, and proteins were extracted according to the protocol described below. For *P. yoelii* hepatic-stage protein extracts, livers from control and C57BL/6 mice infected intravenously with 40 000 *P. yoelii* sporozoites were isolated 42 hours after infection.

Culture of Blood-Stage *P. falciparum* Parasites

Erythrocytic stages of the *P. falciparum* malaria parasite line 3D7 were cultured using candle jar desiccators as previously described [20]. The culture medium was Roswell Park Memorial Institute 1640 medium (Gibco-BRL) supplemented with 0.5% AlbuMix (Gibco BRL). The cultures were maintained in 6-well tissue culture plates (NUNC). Parasitemia was 5% at the start of culture and reached 25% after 6 days. Culture medium and fresh red blood cells were added every other day.

Self-Reactive IgE Repertoire Analysis

To analyze the IgE autoantibody repertoire against human brain proteins, we used a modified Panama blot method [14]. In brief, brain extract proteins (200 μ g per gel, Medley, Ozyme, France) were separated by sodium dodecyl sulfate

polyacrylamide gel electrophoresis (SDS-PAGE), followed by quantitative immunoblotting with the different serum samples (diluted 1:5) incubated at 4°C for 3 days to increase resolution (Miniblot incubation units, Immunetics, Cambridge, MA). A pool of AM patient sera (diluted 1:10) was used as positive control and phosphate-buffered saline (PBS)–0.2% Tween as a negative control. IgE reactivities were revealed using anti-human IgE coupled to alkaline phosphatase (diluted 1:10 000) for 1 day at room temperature (mouse IgG2b monoclonal antibody against human IgE clone GE-1, Sigma, St. Louis, MO). The quantitative signals revealed by the NBT/BCIP reaction were normalized between membranes, using IGOR 3.16 software (Wavemetrics, Lake Oswego, OR) [21]. Profiles of the reactivity of standard and patient plasma samples with brain proteins were separated into 14 sections. Each section corresponded to the IgE reactivity recognizing a band with a defined molecular weight.

Protein Identification by Mass Spectrometry

Protein band was localized after 10% acrylamide gel SDS-PAGE separation of the brain protein extract. Asymptomatic individuals exhibiting the reactivity of interest were tested. Half of the gel was used to transfer protein onto a nitrocellulose membrane for Western blot analysis. The other half was stained with Coomassie Brilliant Blue R 250 (Bio-Rad, Marne la Coquette, France). Proteins were identified by peptide mass fingerprinting with a matrix-assisted laser desorption ionization time-of-flight mass spectrometer (4800 MALDI, Applied Biosystems, Framingham, MA). To search the National Center for Biotechnology Information nr database, monoisotopic masses were assigned using a local copy of Mascot GPS Explorer v3.6 (Matrix Science, London, United Kingdom).

Western Blot Analysis of 14-3-3 ϵ Protein Expression

Proteins from infected or uninfected samples of liver- or blood-stage parasites were extracted using RIPA lysis buffer (Interchim, Montlucon, France) complemented with a cocktail of protease inhibitors (Roche, Meylan, France). For Western blot analysis, briefly, parasite protein extracts (20 μ g of *P. falciparum* 3D7 blood-stage protein extract per lane and several concentrations for *P. falciparum* and *P. yoelii* liver stages) were separated by SDS-PAGE in a 10% polyacrylamide gel. Proteins were then transferred onto a nitrocellulose membrane (Whatman, Dassel, Germany) by semidry transfer. After blocking nonspecific sites with PBS–5% dry fat milk, membranes were then incubated with either asymptomatic patient plasma diluted 1:20 in PBS–0.1% Tween20 or polyclonal rabbit IgG anti-14-3-3 ϵ (Santa Cruz Biotechnology, Santa Cruz, CA). After washing and incubation with secondary goat anti-rabbit IgG coupled with horseradish peroxidase (Santa Cruz Biotechnology), revelation was done using the ECL kit from Pierce.

Quantification of IgE Anti-14-3-3 ϵ Protein by Enzyme-Linked Immunosorbent Assay (ELISA)

Levels of IgE and IgG specific for 14-3-3 ϵ protein were quantified by indirect ELISA, as follows. Briefly, 96-well flat-bottom plates were coated with 5 μ g/mL of 14-3-3 ϵ human protein (Sigma, France) for 2 hours at 37°C. Saturation was done with PBS–1% gelatin for 30 minutes at room temperature after 3 rounds of washing. Then, patients sera diluted at 1:10 in PBS–0.1% Tween (in duplicate) were incubated overnight at 4°C. Revelation of IgE or IgG specific to 14-3-3 ϵ protein was done after 5 rounds of washing by incubating the plates for 1 hour at 37°C with either mouse IgG2b anti-human IgE (ϵ chain) or anti-human IgG conjugated with alkaline phosphatase (Sigma-Aldrich, Saint-Quentin Fallavier, France) diluted at 1:5000 in PBS–0.1% Tween. Reactivity was assessed using the pNPP enzyme substrate ready-to-use solution (Interchim). The reaction was stopped after incubation for 30 minutes at 37°C with 1 M NaOH. The absorbance was measured at 405 nm using an Emax ELISA plate reader, and results were analyzed by the Sofmax software. All tests were done in duplicate.

IgE-Dependent Functional Assay

A rat mast cell line, RBL-2H3, transfected with a human Fc ϵ RI α -chain that triggers degranulation upon human IgE cross-linking was used [22]. Degranulation was monitored after brain antigen stimulation (1000, 100, and 10 ng/mL) by measuring the level of released β -hexosaminidase, as described elsewhere [22]. All results are expressed as the percentage of total β -hexosaminidase in the cells after correction for spontaneous release in unstimulated cultures.

Statistical Analysis

Nonparametric tests were performed, and median values were used to compare groups. The Mann–Whitney *U* test was used for comparisons between 2 groups, and the Kruskal–Wallis test was used to compare ≥ 3 groups. Spearman correlation was used to check for correlations between parameters. *P* values $< .05$ were considered statistically significant. For quantitative analysis of the multivariate self-reactive IgE data, principal component analysis (PCA) was used. PCA factor 1 is, by definition, the linear combination of single reactivity measurements that represent the maximum of information about a multivariate data set in terms of total variance. In this study, factor 1 represented 62% of total variance and had exclusively positive factor loads, so that it can be seen as a univariate approximation of IgE repertoire reactivity.

RESULTS AND DISCUSSION

IgE autoantibody to brain antigens was studied for the first time in a parasitic disease. The IgE autoantibody repertoire

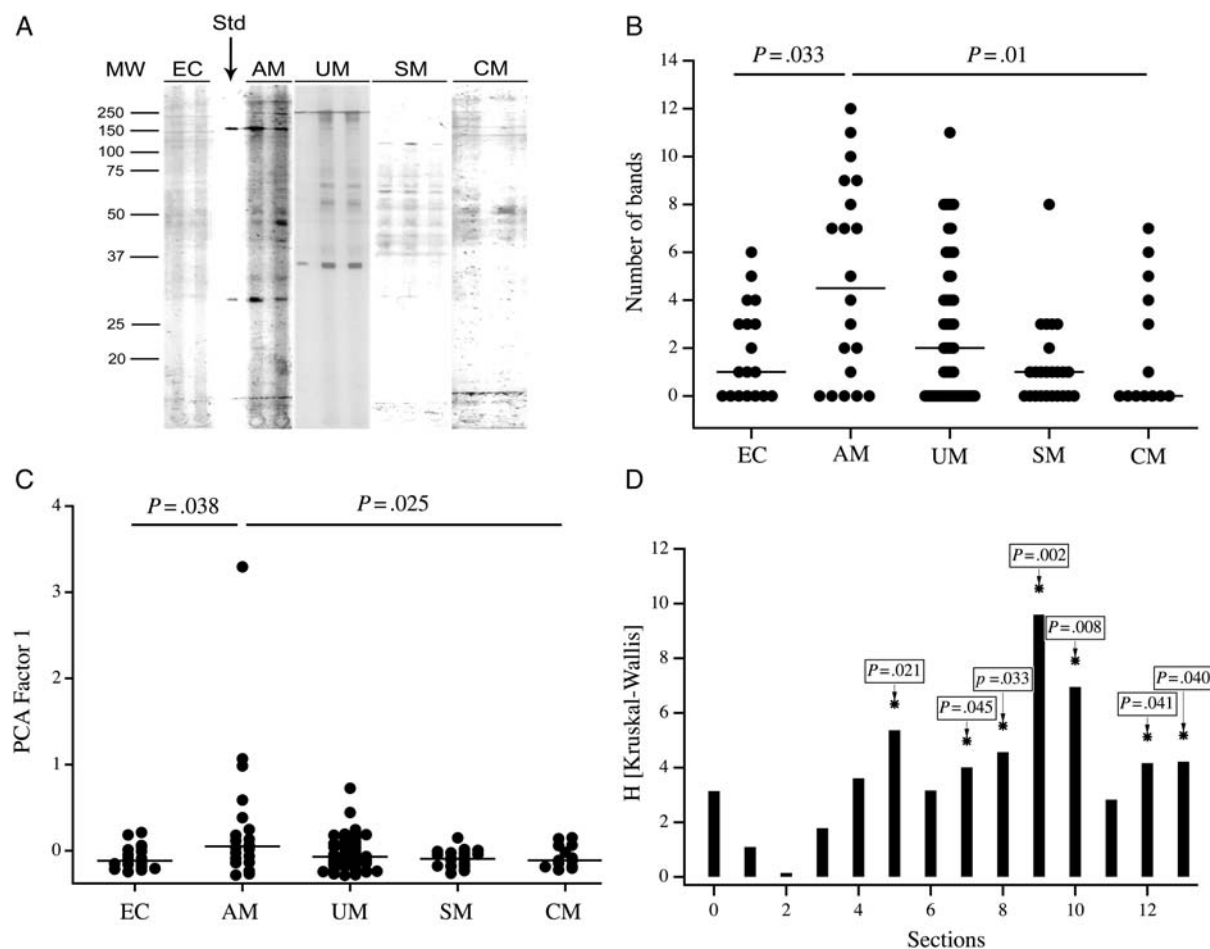


Figure 1. Analysis of self-reactivity profiles in *Plasmodium falciparum* malaria. *A*, Immunoblots of selected individuals showing immunoglobulin E (IgE) autoreactivity against brain extract. Each lane represents a different patient serum belonging to the groups mentioned on top. A pool of asymptomatic patients is used as standard (Std) to normalize between membranes. A broader range of bands is observed in patients with asymptomatic malaria (AM), compared with patients without malaria who lived in the region of endemicity (EC), patients with severe malaria (SM), and patients with cerebral malaria (CM). *B*, Median number of bands recognized by each group of patients. The diversity of autoantibodies repertoire was significantly higher in the AM group, compared with the EC group ($P=.033$) and the uncomplicated malaria (UM), SM, and CM groups ($P=.01$). *C*, Each membrane was divided into 14 sections that characterize each band of reactivity, and principal components scores were calculated. The AM group had significantly higher principal component analysis (PCA) factor 1 scores than the EC group ($P=.038$) and diseased groups (UM, SM, and CM) ($P=.025$). *D*, A Kruskal–Wallis test was performed to evaluate the sections highly recognized by the AM group as compared to the other groups. Sections 5, 7, 8, 9, 10, 12, and 13 were significantly recognized by AM patients ($P=.021$, .045, .033, .002, .008, .041, and .040, respectively).

was analyzed using a global approach based on an adapted quantitative 1-dimensional immunoblotting method associated with multivariate statistics [21]. The number of bands recognized between all patient groups differed significantly ($P=.049$), with the highest number observed in the AM group ($P=.033$ compared with the EC group and $P=.010$ compared with the other malaria groups; Figure 1*A* and 1*B*). Therefore, AM showed the most diverse repertoire of self-reactivities (>5 bands). Interestingly, up to 45% of AM patients recognized at least 5 different bands, but no EC patients or those with disease did so. PCA was used to better estimate the contribution of both the diversity and the specificity of reactivity

profiles in the different groups of patients (Figure 1*C*). The AM group had the highest scores of the first principal component, which also represent the highest IgE reactivity. Given this overall analysis, we identified 7 sections (5, 7, 8, 9, 10, 12, and 13) that discriminated AM from the other malaria groups on the basis of the migration scale (Figure 1*D*). Mass spectrometry, however, allowed protein identification for section 13 only. Considering an empirical threshold below which all uninfected individuals are distributed, we found that circulating IgE recognized section 13 in 40% of AM patients and 27% of UM patients. The peptide mass fingerprinting analysis of this section revealed different isoforms of the 14-3-3 protein

Table 1. Identification of Protein 14-3-3 by Mass Spectrometry

Protein Name	Accession No.	Protein MW (kDa)	Protein Score (%)
14-3-3 ϵ (human complexed)	gi67464424	26.9	99.99
14-3-3 ϵ isoform transcript variant	gi62131678	26.7	99.99

Peptide fingerprinting allowed the identification of the protein 14-3-3 in section 13. This protein had a 99.99% protein score. The human complexed 14-3-3 ϵ protein had a molecular weight of 26.9 kDa, while the isoform transcript variant had a molecular weight of 26.7 kDa.

Abbreviation: MW, molecular weight.

family (Table 1), among which the ϵ isoform had a protein score of 99.9%. Together, these data show for the first time the production of IgE self-reactive antibodies during *P. falciparum* malaria. In contrast to our previous observations in the same cohorts of *P. falciparum*-infected patients, which showed a preferential association of self-reactive IgG to brain proteins in

CM patients, the largest and the most diverse repertoire of IgE reactivity was observed in the asymptomatic group [14]. Note also that the IgE autoantibody response did not correlate with total IgE serum levels, parasite load, or age.

14-3-3 proteins are a family of highly conserved 30-kDa acidic protein isoforms that form a dimer acting as a molecular adaptor [23–25]. The ϵ isoform is abundantly expressed in neurons of the central nervous system and is mainly localized in the synapses and neuronal cytoplasm [26]. The binding motifs, potential functions, and pathological relevance of 14-3-3 proteins vary widely [16, 27–29]. They interact with key intracellular-signaling components and regulate signal cascades for differentiation, development, growth, apoptosis [30–32], and survival. As shown in Figure 2A, by use of immunoblotting, purified protein, and specific IgG to 14-3-3 protein as a positive control (lane 2), we confirmed that the ϵ isoform is present in the human brain protein extract used in the study (lane 3). In addition, circulating IgE from the AM group recognized a band at the same kilodalton level (Figure 2A, lanes 4 and 5). Although band intensity progressively decreased in the AM patients with time, the IgE autoractive response to 14.3.3 protein detected at day 0 persisted up to day 7 and day 30 upon treatment (Figure 2B). These results validate the presence of 14-3-3 ϵ protein in brain protein extract and the presence of self-reactive IgE in sera from asymptomatic *P. falciparum*-infected patients.

One possible explanation for the association of IgE anti-14-3-3 ϵ with asymptomatic malaria could be linked with a neuroprotective effect exerted by the antibody in blocking the expression and the release of the protein in neuronal synapses within the central nervous system [26]. Thus, this may result in protection against CM by improving the cross-talk between immune and nervous systems, which becomes ineffective during brain injury [24–26]. In line with this hypothesis, 14-3-3 protein has been found in spinal fluid from patients with different neurodegenerative disorders (eg, viral encephalitis, recent stroke, and Creutzfeldt-Jakob diseases) [33, 34]. Therefore, the association of high anti-14-3-3 IgE reactivity to brain protein and the elevated circulating IgE levels associated with a reduced risk of developing severe disease may support a role for IgE in protection against severe and/or fatal disease without suppressing parasitemia, as previously observed in other studies in humans [1, 33] and rodents [1, 35]. However, our findings do not exclude a role for self-reactive IgE in the inflammatory processes implicated in the pathogenesis of CM through cross-linking on Fc ϵ RI expressed by neutrophils migrating in the brain of CM mice infected with *P. berghei* ANKA, as recently demonstrated by Porcherie et al [36].

The intracellular adaptor 14-3-3 ϵ protein can also associate with CD81, a member of the tetraspanin superfamily connected to a number of biological functions that are required for hepatocyte infection by sporozoites of *P. falciparum* in

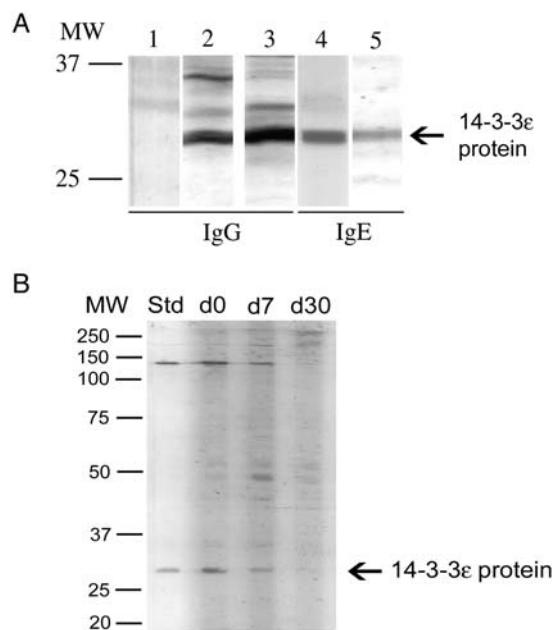


Figure 2. Identification of protein 14-3-3 ϵ as the target of the immunoglobulin E (IgE) self-reactive antibody. *A*, 14-3-3 ϵ protein was detected in healthy human brain extract (lane 3) but not in negative control (lane 1), using rabbit polyclonal immunoglobulin G (IgG) specific to 14-3-3 ϵ revealed with a goat polyclonal anti-rabbit IgG coupled to alkaline phosphatase. Positive control was done using purified 14-3-3 ϵ protein (lane 2). Representative immunoblot showing that the same band was revealed in serum of asymptomatic patients, using an anti-human IgE coupled to alkaline phosphatase (lane 4 and 5). *B*, IgE to 14-3-3 ϵ protein (indicated by the bottom arrow) was present in the serum at day 0 before treatment and persisted until day 7 and day 30 during treatment. Abbreviations: MW, molecular weight; Std, standard.

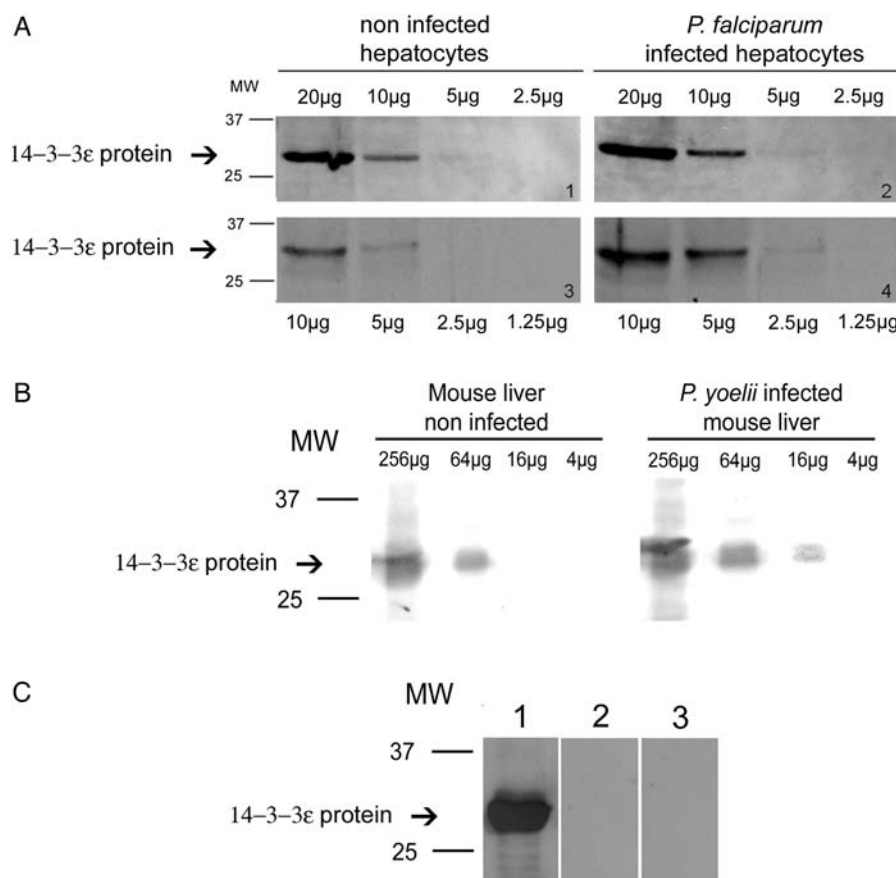


Figure 3. Expression of 14-3-3 ε protein during *Plasmodium falciparum* and *Plasmodium yoelii* liver stages but not in intraerythrocytic parasites. *A*, 14-3-3 ε protein was detected in different quantity of protein extract from noninfected and *P. falciparum*-infected hepatocyte cultures at 3 hours (*A* and *B*, respectively) and 24 hours (*C* and *D*, respectively) after sporozoite inoculation, using a rabbit polyclonal immunoglobulin G (IgG) specific to 14-3-3 ε revealed with a goat polyclonal anti-rabbit IgG coupled to alkaline phosphatase. *B*, Expression of 14-3-3 ε protein was detected in vivo in different amounts of liver extract from noninfected and *P. yoelii*-infected C57BL/6 mice, using a rabbit polyclonal IgG anti-14-3-3 ε protein revealed with a goat polyclonal anti-rabbit IgG coupled to alkaline phosphatase. *C*, 14-3-3 ε protein was not detected in extract of *P. falciparum* 3D7 blood stages with rabbit polyclonal IgG anti-14-3-3 ε protein (lane 2) nor with serum from asymptomatic patients (lane 3). Reactivity of purified 14-3-3 ε protein with the rabbit polyclonal IgG anti-14-3-3 ε protein revealed with a goat polyclonal anti-rabbit IgG coupled to alkaline phosphatase revealed by enhanced chemiluminescence was used as positive control (lane 1). Abbreviation: MW, molecular weight.

humans and *P. yoelii* in rodents [17, 18]. To test whether 14-3-3 ε protein plays a role in the CD81-dependent pathway associated with hepatocytes invasion by sporozoites and whether IgE specific to 14-3-3 ε protein can target the liver stage, we studied the intracellular expression of 14-3-3 ε protein in primary human hepatocyte cultures infected with sporozoites of the *P. falciparum* NF54 strain, using Western blotting and 14-3-3 ε protein-specific antibodies. Our data show that levels of expression of 14-3-3 ε protein within infected hepatocytes were increased at 3 hours and persisted at 24 hours after sporozoite penetration (Figure 3*A*). These results have been further confirmed in vivo in C57BL/6 mice infected with *P. yoelii* 265 BY sporozoites. We found a higher level of 14-3-3 ε protein in the liver of *P. yoelii*-infected mice 42 hours after sporozoite injection, compared with naive animals (Figure 3*B*).

It is noteworthy that 14-3-3 ε protein favors the activation of protein kinases in signal transduction pathways and represents a crucial integration of point of survival–death decisions in mammalian cells by interacting with BAD [30]. Thus, this suggests that during the process of infection by sporozoites, intrahepatic 14-3-3 ε protein can trap and hold phosphorylated BAD and liberate the antiapoptotic factor Bcl-2 to ensure cell survival and parasite multiplication [31, 32]. In addition, an isoform of the 14-3-3 ε protein was shown to be expressed in young trophozoites of *P. falciparum* parasites in the asexual blood stage [37]. Therefore, to exclude any reactivity with the parasite protein, the expression of 14-3-3 ε protein by intrahepatic forms of *P. falciparum* has been assessed in vitro by immunostaining using fluorescent anti-14-3-3 ε antibodies. A very weak fluorescence signal was detected

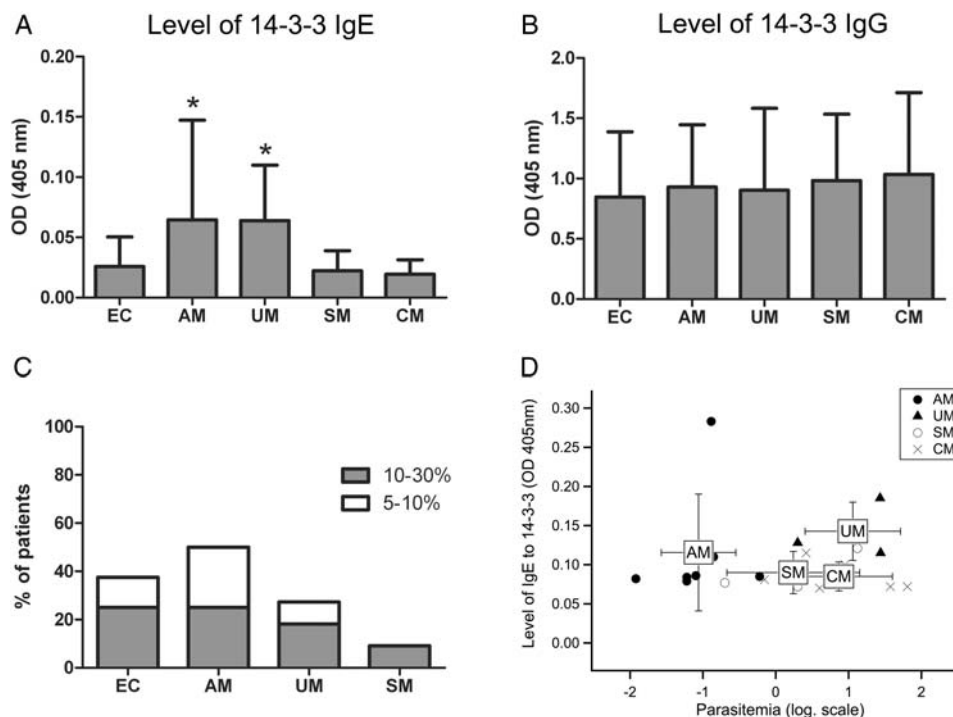


Figure 4. Levels of specific immunoglobulin E (IgE) and immunoglobulin G (IgG) to 14-3-3 ϵ protein and functional IgE in *Plasmodium falciparum*-infected patients. *A*, Levels of plasmatic IgE recognizing 14-3-3 ϵ protein in controls and different groups of *P. falciparum* malaria patients assessed by indirect enzyme-linked immunosorbent assay, using mouse IgG2b anti-human IgE (ϵ chain-specific) coupled with alkaline phosphatase. The level of IgE was significantly higher in asymptomatic malaria (AM) and uncomplicated malaria (UM) groups, compared with severe malaria (SM) and cerebral malaria (CM) groups (* P = .029 and P = .012, respectively). *B*, The levels of plasmatic IgG recognizing 14-3-3 ϵ protein were not significantly different in controls and groups of patients with *P. falciparum* malaria. *C*, Functional IgE in asymptomatic patients with *P. falciparum* infection. Distribution of patients with positive anti-brain functional IgE exhibiting different intensities of enzyme release per clinical group (low enzyme release, from 5% to 10%; moderate enzyme release, from 10% to 30%; and high enzyme release, >30%). *D*, Levels of IgE anti-14-3-3 ϵ protein and parasitemia discriminate the AM group from the other malaria groups.

in the primary hepatocytes 3, 24, and 48 hours after sporozoite penetration. We further checked whether IgE from infected patients recognized the 14-3-3 protein of erythrocytic-stage *P. falciparum* parasites and found no reactivity with the parasite extract (Figure 3C).

Because immunization with 14-3-3 ϵ supports a role for this protein in defense mechanisms against schistosomiasis [38–40], we therefore assessed by ELISA the prevalence of IgE to 14-3-3 ϵ protein in the sera of asymptomatic individuals, compared with the prevalence in other malaria groups. We found more 14-3-3 ϵ protein-specific IgE in AM and UM groups than in EC, SM, and CM groups (P = .029 and P = .012, respectively) (Figure 4A). Interestingly, AM patients had the highest ratio of IgE to 14-3-3 ϵ protein, whereas no difference was observed between groups for other immunoglobulin isotypes, such as IgG (Figure 4B). These results strongly suggest a role for IgE in protection against severe malaria [1, 35]. Note that the same group of patients presented the highest levels of functional *P. falciparum*-specific IgE [3]. Nevertheless, we cannot exclude the possibility that the low

levels of circulating IgE in SM and CM were due to an increase in IgE–14-3-3 ϵ protein complexes and/or IgE sequestration within tissues. To better estimate the efficiency of the monomeric IgE pool in AM sera, we performed a functional test based on the ability of the circulating IgE to induce degranulation of a rat mast cell line transfected with the human α -chain of Fc ϵ RI in the presence of brain antigens [22]. Sera from individuals randomly chosen and able to induce >5% β -hexosaminidase release, a value corresponding to spontaneous release, were considered to contain functional IgE. Among the *P. falciparum*-infected patients tested, those belonging to the AM group had the highest capacity to induce degranulation of the human Fc ϵ RI-transfected mast cell in the presence of brain protein extract (Figure 4C). The percentage of individuals with AM with positive functional IgE recognizing human brain proteins was 50%. The degranulation intensity in the AM group was between 5% and 30% of enzyme release. Interestingly, the functional efficacy of the degranulation test was not different from previous observations for *P. falciparum*-specific IgE in the same individuals [3]. In addition, we found

a negative correlation between levels of IgE anti-14-3-3 ϵ protein and parasitemia in AM patients. As shown in Figure 4D, AM patients are discriminated by these 2 factors, compared with the other malaria groups. These data suggest that self-reactive IgE to brain proteins, including IgE to 14-3-3 ϵ protein, could be implied in protection against disease in asymptomatic infection through antibody-mediated mechanisms triggering degranulation of mast cells, basophils, eosinophils, and/or monocytes/macrophages.

In summary, to our knowledge, these studies are the first to identify the production of IgE autoantibodies that interact with brain antigens during malaria. Collectively, these findings also provide additional support for protective mechanism(s) mediated by IgE that bind self-antigens during infectious diseases. They also reinforce the hypothesis that IgE may constitute an important factor in protective mechanisms against severe malaria.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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