

IgE ELEVATION AND ANTI-*PLASMODIUM FALCIPARUM* IgE ANTIBODIES: ASSOCIATION OF HIGH LEVEL WITH MALARIA RESISTANCE

Srisin Khusmith¹, Jarinee Panitchakorn¹, Srivicha Krudsood², Polrat Wilairatana³
and Sornchai Looareesuwan³

¹Department of Microbiology and Immunology, ²Department of Tropical Hygiene,
³Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University,
Bangkok, Thailand

Abstract. The total IgE and anti-*Plasmodium falciparum* IgE antibodies were determined by enzyme linked immunosorbent assay (ELISA) in 480 children and adults living in malaria endemic area along Thai-Myanmar border, Kanchanaburi Province, western Thailand. Approximately 73.13% of tested individuals had elevated levels of total IgE with a range of 160-998 ng/ml. 20.5% of these IgE were specific to *P. falciparum* blood stage antigens, with a range of 78-353 µg/ml. However, the levels of total IgE were not significantly correlated with those of specific IgE ($r = 0.083$). The elevation of anti-*P. falciparum* IgE antibodies seems to be age dependent. The prolonged or repeated exposure to malaria parasites is necessary for the induction of specific IgE response as indicated by the finding of a significant correlation between the levels of *P. falciparum* specific IgE and the number of malaria attacks ($r = 0.551$, $p = 0.01$). Interestingly, among the specific IgE responders, 20 individuals naturally exposed to malaria but without clinical malaria reported had high levels of both total IgE and anti-*P. falciparum* IgE antibodies, with mean values of 418.67 mg/ml and 146.25 ng/ml, respectively. It is likely that the antibodies from such specific IgE responders could mediate phagocytosis *in vitro*.

INTRODUCTION

In human falciparum malaria, stimulation of CD4⁺ T cells from immune donors with malaria antigens *in vitro* frequently induces either IFN- γ or IL-4 secretion, indicating the occurrence of distinct Th1- or Th2 like cells in blood of these individuals (Troye-Blomberg *et al.* 1990). Generally, IL-4 is involved in switching immunoglobulin isotype production by B cells from IgM to IgE (Finkelman *et al.* 1990). The occurrence of an allergic response and subsequent detection of specific IgE antibody in a volunteer who had been vaccinated with *Plasmodium falciparum* sporozoite antigen produced by recombinant technology (Ballou

et al., 1987) suggests the possibility that *Plasmodium* specific reaginic antibody may develop in individuals exposed to endemic malaria. Approximately 85% of children and adults living in areas of high *P. falciparum* endemicity had elevated blood levels of both total IgE and anti-plasmodial IgE antibodies (Perlmann *et al.*, 1994; Desowitz, 1989; 1993), suggesting that plasmodial infection may directly contribute to IgE elevation. In general, elevated levels of IgE in serum reflect an underlying imbalance in the ratio of T helper cells, in favor of Th2 cells. The Th1 to Th2 switch is controlled by many factors including antigen, dose and structure, length of antigen exposure, mode of immunization and host genetics (Constant and Bottomly, 1997). The latter has been shown by the studies in African twins, in whom IgE levels were very similar in homozygous twin but varied widely in heterozygous pairs (Perlmann *et al.*, 1999). In infectious disease, IgE usually acts on parasites or parasitized cells through the intervention of effector cells.

Correspondence: Dr Srisin Khusmith, Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Bangkok 10400, Thailand.
Tel: +66 (0) 22460056, 22469000 ext 1594;
Fax: +66 (0) 22468340
E-mail: tmskm@mahidol.ac.th.

Interaction of IgE containing immune complexes with Fcε receptors on different types of effector cells is known to induce a variety of responses which can be protective and/or harmful for the host ((Perlmann *et al*, 1996). A possible role of IgE in pathogenesis of the disease was suggested by the finding of significantly higher IgE levels in patients with cerebral malaria than in those with uncomplicated malaria. However, the relative importance of anti-plasmodial IgE antibodies for protection against malaria has not yet been clarified.

MATERIALS AND METHODS

Sera

Serum samples were obtained from 480 consenting male and female individuals aged between 2->60 years, living in malaria endemic area along the Myanmar border at Bongty subdistrict, Kanchanaburi Province, western Thailand. The study included those with and without clinical malaria reported. Blood smears stained with Giemsa were examined for malaria parasites. The control sera were obtained from 30 healthy individuals residing in Bangkok where malaria is not endemic. They had no history of malaria exposure and denied traveling to any endemic area in the past two years, hence would be most unlikely to have been exposed to malaria during the time of study. The sera were stored at -70°C.

Preparation of malaria parasites and schizont burst antigens

The parasites *P. falciparum* SO strain were cultured in erythrocytes in RPMI-1640 medium supplemented with 10% normal human serum and 50 µg/ml hypoxanthine (Sigma, St Louis, USA) at 37°C in 5% CO₂ and 5% O₂ (Trager and Jensen, 1976). Stock cultures were synchronized by lysis of schizont-infected cells with 5% sorbitol (Lambros and Vanderburg, 1979). The parasite cultures were harvested when parasite density had reached 10% and concentrated by centrifugation over 60% dis-

continuous Percoll (Pharmacia, Uppsala, Sweden) gradient enriched for schizont-infected erythrocytes. For schizont burst antigen preparation, the schizont-infected cells were washed three times and resuspended in cultured medium at approximately 2 x 10⁷ cells/ml and incubated for 24 hours at 37°C. The supernatants were collected, pooled, extracted by freezing-thawing followed by sonication and stored at -70°C.

Total IgE determination by ELISA

Total serum IgE was determined by ELISA previously described (Perlmann *et al*, 1994) with some modification. The flat bottom 96-well micro ELISA plates (Costar, Cambridge, MA) were coated with rabbit anti-human IgE (DAKO A/S, Denmark) overnight at 4°C. The plates were incubated with phosphate buffered saline (PBS) containing 0.25% gelatin for 1 hour to saturate protein binding site. After washing of the plates, the test sera diluted to the appropriate concentrations in PBS containing 0.05% Tween (Sigma, St Louis, USA) were added to the wells and incubated for 1 hour at 37°C. The total IgE bound to the plates was then assayed with biotinylated ε-chain specific rabbit anti-human-IgE antibodies by incubating for 1 hour followed by alkaline phosphatase conjugated streptavidin (Sigma, St Louis, USA). The test was done in duplicate. Immunoglobulin concentrations were calculated from standard curves obtained by incubating the coated plates with five concentrations of purified myeloma protein IgE.

P. falciparum specific IgE antibody determination by ELISA

The anti-*Plasmodium* IgE antibodies were determined by enzyme-linked immunosorbent assay (ELISA). Briefly, the wells of microtiter plate (Costar, Cambridge, MA) were coated with 50 µl of parasite antigens (10 µg/ml). For analysis of IgE antibodies, the test sera were diluted 1:10 and incubated in the plates at room temperature overnight for optimal binding (Perlmann *et al*, 1994). Then the IgE antibody was assayed as described above for total IgE.

In vitro phagocytosis

Human blood monocytes from healthy individuals with no history of malaria were separated by Ficoll-hypaque (Histopaque-1077, Sigma, USA) density gradient centrifugation according to the method provide by the manufacturer and by attachment to serum coated plastic dish (Khusmith and Druilhe, 1983). This technique allowed the recovery of highly purified monocytes (95% on average). Cell viability based on dye exclusion test ranged from 90-95%. Approximately 10^6 washed monocytes suspended in RPMI 1640 supplement with 10% fetal bovine serum (Bio Whittaker, Belgium) were incubated for 1 hour with tested sera. The experiments were carried out in 20 sera from specific IgE responders without clinical malaria reported, 10 IgG-depleted sera with anti-IgG antibodies (DAKO A/S, Denmark) and 5 normal control sera. The test was done in duplicate. After washing, the cells were incubated at 37°C for 45 minutes with 10^7 merozoites and intact schizonts or in the presence of latex particles (latex 0.81; Difco Laboratories, USA). The latex particles were used as the control target for testing the functional activity of the monocytes.

Statistical analysis

The cut-off value for both total IgE and *P. falciparum* specific IgE antibodies was defined as mean antibody concentration + 2 standard deviation (SD) in 30 normal healthy controls. Differences between the responses were analysed by unpaired Student's *t*-test and correlations

between responses by linear regression analysis of Spearman rank test.

RESULTS**Total and anti- *P. falciparum* IgE antibodies elevation**

We were able to show that approximately 73.13% of tested individuals had elevated levels of total IgE in sera when compared to the mean value of normal control sera. Of these, 20.5% of this IgE was specific to *P. falciparum* blood stage antigens. The mean of total IgE of individuals in the area was 624 ng/ml with the range of 160-998 ng/ml, while *P. falciparum* specific IgE ranged from 78-353 ng/ml with the mean of 215 ng/ml. No such specific IgE response was found in normal healthy individuals (Table 1). However, there was no significant correlation between the levels of total IgE and those of *P. falciparum* specific IgE antibodies ($r = 0.082$).

Age, malaria exposure and anti- *P. falciparum* IgE antibodies

The elevation of anti-*P. falciparum* IgE antibodies seems to be related to age by the majority of *P. falciparum* specific IgE responders are in the age group of 20-40 years. However, no significant correlation was found when the levels of specific IgE were tested with all ages of such individuals ($r = 0.082$). In contrast, the anti-*P. falciparum* IgE antibodies were significantly correlated with the numbers of

Table 1
Total IgE and *P. falciparum* specific IgE in sera of individuals living in malaria endemic area.

Donors	No.	Total IgE (ng/ml) Mean (range)	% IgE positive	Specific IgE (ng/ml)	%Specific IgE positive
Individuals in the area	480	624 (160-998)	73.13 (351/480) ^a	215 (78-353)	20.5 (120/480) ^b
Normal control	30	58 (20.5-88)		0	

^anumber of total IgE positive subjects/total tested subjects.

^bnumber of *P. falciparum* specific IgE positive subjects/total tested subjects.

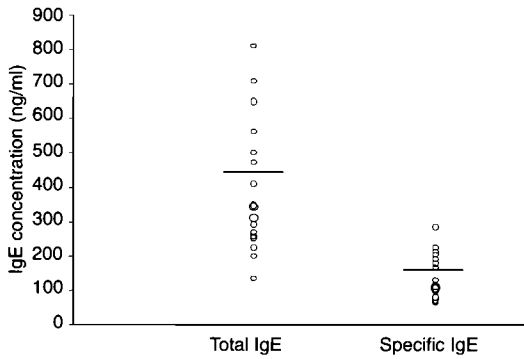


Fig 1—Total IgE and *P. falciparum* specific IgE antibodies in sera of individuals naturally exposure to malaria without clinical malaria reported.

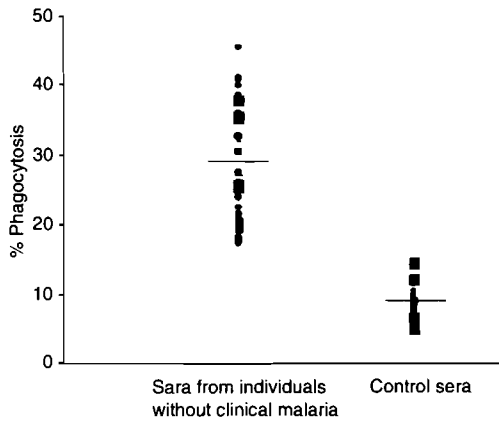


Fig 2—Enhanced phagocytosis by antibodies in sera of *P. falciparum* specific IgE responders who had no clinical malaria reported.

malaria attacks ($r = 0.551$, $p = 0.01$). However, among the specific IgE responders with current infection, there was a significant correlation between the level of anti-*P. falciparum* IgE antibodies and parasitemia ($r = 0.301$, $p = 0.01$).

Elevation of anti-*P. falciparum* IgE antibodies in endemic individuals without clinical malaria reported

Among these specific IgE responders, 20 individuals naturally exposed to malaria without clinical malaria reported had high level of IgE with the mean value of 418.66 ng/ml and 146.25 ng/ml for total IgE and anti- *P. falciparum* IgE antibodies, respectively (Fig 1).

Table 2
Enhanced phagocytosis of *P. falciparum* malaria parasites by antibody in sera of specific IgE responders who had no clinical malaria reported.

Sera ^a	% Phagocytosis	
	Before IgG depletion	After IgG depletion
IWCM 2	45.5	20.5
IWCM 4	25.5	13.5
IWCM 5	35.5	16.5
IWCM 7	27.5	15.5
IWCM 8	36.0	18.5
IWCM 9	21.5	11.5
IWCM 11	30.5	16.5
IWCM 13	26.0	14.0
IWCM 15	22.5	13.0
IWCM 17	18.0	9.5
10 Normal	5.0-9.5	2.5-4.5

^aDue to the limited amount of sera, only 10 out of 20 sera from individuals without clinical malaria reported (IWCM) could be tested.

Antibody-dependent phagocytosis

The monocytes specifically armed by anti-*P. falciparum* antibodies in sera of 20 *P. falciparum* specific IgE responders without clinical malaria reported could mediated phagocytosis of blood stage parasites *in vitro*, resulting in increased ingestion mostly merozoites, but few infected erythrocytes, varied from 18-45.5% (Fig 2). However, lower phagocytic activity was found after depletion of IgG in these 10 sera (9.5-20.5 % phagocytosis) compared to those of normal sera (2.5-4.5%) (Table 2).

DISCUSSION

In the present study, we confirm and extend previous reports demonstrating the elevation of both total and *P. falciparum* specific IgE antibodies in sera of individuals living in endemic area in Thailand. As would be expected, the total IgE levels in 73.13% of tested individuals were elevated, ranging from 160-998 ng/ml when compared to those of normal

healthy controls which ranged from 20.5-88 ng/ml. These levels were lower than those previously reported in holoendemic areas. (Desowitz *et al*, 1989; Perlmann *et al*, 1994). Among these IgE responders, only 20.5% had elevated *P. falciparum* specific IgE antibodies. In any event, this observation was less than those previously reported in that 60% of Liberian adults (Perlmann *et al*, 1994) and 15-33% of adults in Papua New Guinea (Desowitz *et al*, 1989; 1993) had *P. falciparum* specific IgE antibodies. IgE binding was also shown in sera of African and Asian assayed by ELISA with synthetic peptides expressing antibody binding sites from two major merozoite polypeptides, Pf155/RESA and Pf322. Moreover, these donors had IgE antibodies to some malaria parasite antigens when tested in immunoblotting (Perlmann *et al*, 1994).

An important question posed by the results is what cause IgE elevation in malaria. High blood concentrations of both polyclonal and specific IgE are a hallmark of helminthic infection (Hagan *et al*, 1993; Dunne *et al*, 1992). However, the involvement of activated Th2 cells and ensuing IL-4 secretion in such infections has also been documented (King *et al*, 1993; Elghazali *et al*, 1997). As many of the individuals included in our study were from areas where helminthic infections are common such as opisthorchiasis, trichinellosis, hook worm etc, involvement of the helminthic infection in the elevation of IgE is obviously possible. In any event, IgE antibodies against *P. falciparum* must have been induced by malaria parasites, probably via Th2-involved pathway. However, the results do not exclude that this was possibly due to an effect on CD4⁺ T cell differentiation of concomitant infection, resulting in Th2 responses to "third party antigens" here by malaria parasites as shown in previous findings (Zwingenberger *et al*, 1991; Kullberg *et al*, 1992; Perlmann *et al*, 1994). However, as it has been shown in mouse model that infection of mice of certain haplotypes with *P. chabaudi chabaudi* also induce IgE elevation (Helmy *et al*, 1996; von der Weid and Langhorne, 1993), it is likely that IgE elevation in human *P. falciparum* infection is usually, although per-

haps not always caused by this parasite without any "help" from other pathogens.

Among the *P. falciparum* specific IgE responders, the elevation of anti-*P. falciparum* IgE antibodies seems to be related to age by the majority of individuals who had specific anti *P. falciparum* IgE antibodies are in the age group of 20-40 years. However, no significant correlation was found when the levels of specific IgE were tested with all ages of these specific IgE responders. In contrast, our results indicated that prolonged or repeated exposure to the parasite is necessary for the induction of specific IgE response by the data showing that the levels of anti-*P. falciparum* IgE antibodies among these responders were significantly correlated with the numbers of malaria attacks.

Obviously, among these specific IgE responders, 20 individuals naturally exposed to malaria, never been reported to have clinical malaria, had high level of total IgE and anti-*P. falciparum* IgE antibodies with the mean value of 418.66 ng/ml and of 146.25 ng/ml, respectively. In addition, these levels of specific IgE antibodies in such individuals were corresponding to the levels of IgG specific to the vaccine candidate epitopes on circumsporozoite protein (CSP) and merozoite protein antigens (MSP) (S Khusmith *et al*, manuscript in preparation).

For malaria, no conclusive evidence regarding IgE's possible protective or pathogenic functions is presently available. In infectious disease, in general, IgE usually acts on parasites or parasitized cells through effector cells. One of the receptor so called Fcε RII or CD23, occurred primarily on monocytes/macrophages and other hematopoietic cells (Delespesse *et al*, 1991), which is low affinity receptor, is induced to elevated expression by IgE dependent phagocytosis, cellular cytotoxicity and adhesion. Our observation is likely to be that the anti-*P. falciparum* antibodies in 20 specific IgE responders without any clinical malaria reported could armed specifically the monocytes and enhanced merozoite phagocytosis. However, we could not conclude that the antibody dependent phagocytosis is mediated

only by IgE since these sera also contain *P. falciparum* specific IgG antibodies. The significance of such findings is now under investigation.

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