

IgE and tumor necrosis factor in malaria infection

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Abstract

IgE, the immunoglobulin instrumental in atopic diseases is also elevated in many infections. This paper reports on the occurrence and possible pathogenic role of IgE in human *Plasmodium falciparum* malaria, one of the most widely spread and severe infectious diseases world wide. Plasmodial infections induce IgE elevation in the blood of the majority of people living in malaria endemic areas and up to 5% of this IgE constitutes anti-malaria antibodies. Production of IgE is controlled by T cells and elevated IgE concentrations in the blood of malaria patients are the result of an increased ratio of T-helper 2 (Th2) over T-helper 1 (Th1) cells. The underlying Th1 to Th2 switch is controlled by a variety of environmental and genetic factors. The importance of the latter is demonstrated by the IgE levels occurring in monozygotic or dizygotic twins originating from malarious areas of Africa. While these levels were indistinguishable within monozygotic twin pairs, they were different within the dizygotic pairs. Comparison of the levels of total IgE or IgE anti-malaria antibodies in patients with uncomplicated malaria with those in patients with the severe form of the disease (cerebral malaria or severe malaria without cerebral involvement) indicate that these levels are significantly higher in the cases with severe disease. This is the reverse with IgG and suggests that IgE plays a role in malaria pathogenesis. An important pathogenic mediator causing malaria fever and tissue lesions is tumor necrosis factor (TNF), generally believed to be induced by toxins released from the parasite. However, sera from malaria patients can also cause TNF release from monocytes in a reaction dependent on the presence of IgE containing immune complexes or aggregates. This results in induction and cross-linking of Fc_ε receptor II (CD23) and by binding to and activating these cells, IgE will contribute to a local over-production of TNF in capillaries and post-capillary venules where *P. falciparum* parasites or their products accumulate in the severe forms of this disease. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Malaria; *Plasmodium falciparum*; Immunoglobulin; Pathogenic

1. Introduction

IgE is an immunoglobulin isotype which occurs only in mammals [1]. It is present in serum at very low concentrations, in humans normally ranging from 10 to 300 ng/ml. IgE is the immunoglobulin which is instrumental in IgE dependent allergies and asthma where it occurs at significantly elevated concentrations. It causes its effects by interacting both with antigen and with Fc_ε receptor bearing cells such as mast cells, basophils, eosinophils as well as monocyte/macrophages, platelets, and others. The major receptor responsible for the allergic responses is the ‘high affinity’ receptor, Fc_ε RI

[1,2]. A second major and widely distributed IgE receptor is the ‘low affinity’ receptor Fc_ε RII, also known as CD23 [3].

IgE is also elevated in many infections. Most well known are the conspicuous IgE elevations occurring in helminth infections [4]. As IgE mediated cellular reactions such as phagocytosis, antibody dependent cellular cytotoxicity or release of toxic mediators [5–7] can eliminate infecting parasites, it has been concluded that IgE antibodies have a protective role in these infections [8], a notion which is also supported by sero-epidemiological evidence [9,10]. However, these parasites are also attacked by other antibody dependent or independent immune reactions and the relative importance of IgE for protection in relation to other protective mechanisms remains to be clarified [11,12].

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2. IgE elevation in malaria

Elevations of the blood concentrations of IgE in malaria have first been reported by Desowitz et al. [13,14]. Our studies of IgE in *P. falciparum* malaria have shown that, on the average, 85% of the people living in malaria endemic parts of Africa or Thailand have such IgE elevations [15]. The IgE levels found roughly reflected malaria endemicity of the areas under investigation. Up to 5% of this IgE had antibody activity against a large number of *P. falciparum* antigens which also bound IgG antibodies; antigens giving rise to IgE antibodies exclusively have not been found thus far. High levels of IgE have recently also been seen in patients with *P. vivax* malaria (Haddad et al., in preparation). Production of IgE is under T cell control and primarily reflects the response of the immunoglobulin producing B cells to the cytokines IL4 or IL13, delivered by Th2 cells [16,17]. In line with this, the ratio of such CD4-Th2 cells over Th1 cells has been found to be increased in the blood of malaria patients [18]. As experimental malaria infections of mice also give rise to IgE elevation [19], it is likely that the elevated IgE concentrations seen here are induced by the malaria parasite itself rather than by concomitant infections, e.g. with helminths.

The switch from Th1 to Th2 responses, resulting in the shift of IgM/G- to IgE production by the B cells, is controlled by many factors including antigen dose, mode of immunisation, age (length of antigen exposure) (Fig. 1) [20], and others [21,22]. However, although environmental factors are important for inducing the switch, the propensity to develop IgE responses is also genetically regulated (for references and discussion see [23]). This has also been demonstrated by determining IgE levels in monozygotic and dizygotic twins [24]. An

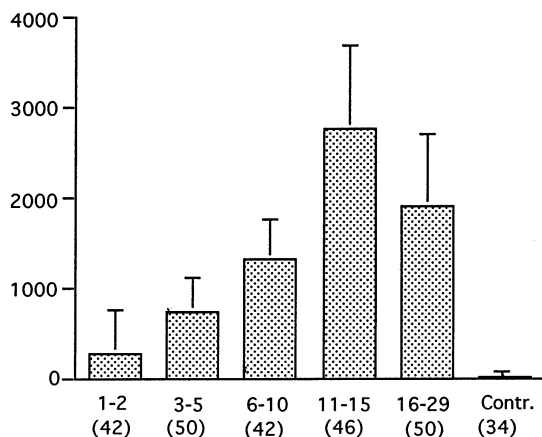


Fig. 1. IgE (ng/ml) $\pm$ S.E. in serum of donors of different age groups living in malaria endemic areas of Tanzania [20]. Number of donors in each age group given in parenthesis. Controls were 34 Swedish adults never exposed to malaria. IgE concentrations were determined by ELISA.

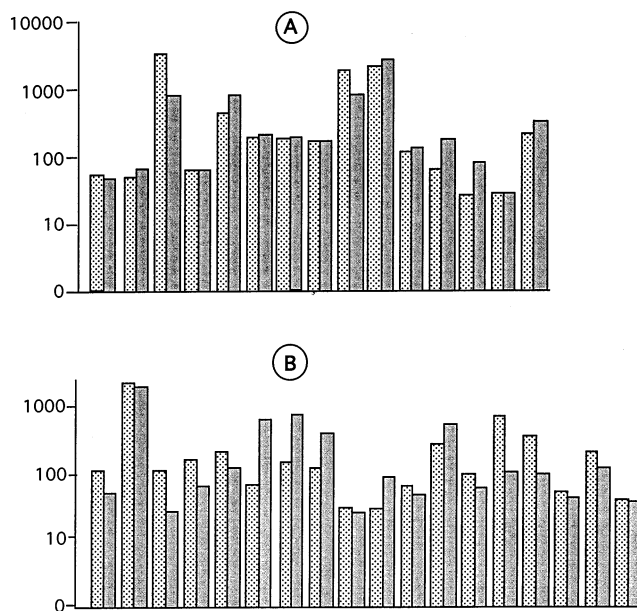


Fig. 2. IgE (ng/ml) in serum of 15 pairs of monozygotic (A) and 18 pairs of dizygotic twins (B). The differences within pairs in IgE levels were significantly higher ($P=0.035$, single factor ANOVA) for the dizygotic twins than for the monozygotic twins. For zygosity determinations, age, and methods see [25,27].

example of this from our studies is shown in Fig. 2 which presents levels of total IgE in twins from The Gambia and Madagascar. As may be seen, the differences in IgE concentrations within the twin pairs were less for the monozygotic than for the dizygotic twins and this was statistically significant. The genes regulating IgE levels are many, including loci located both outside and inside the MHC complex [23]. The twins included in our studies had previously been typed for HLA class II alleles or haplotypes (DRB, DQA, and DQB) when assessing their immune responses to *P. falciparum* [25]. No associations between the IgE levels shown in Fig. 2 and the donors' HLA class II haplotypes were seen, confirming that these levels are primarily controlled by other genes. This is similar to what has previously been noted in the analyses of humoral [25] or cellular [26] immune responses to *P. falciparum* antigens.

3. Role of IgE in malaria

Although it is likely that IgE mediated activation of various effector cells such as monocytes/macrophages contributes to the anti-plasmodial defence reactions, the possible role of IgE in protective immunity in malaria remains to be elucidated. When comparing the levels of IgE in patients with cerebral or severe malaria with those in uncomplicated *P. falciparum* disease, they were found to be significantly higher in complicated cases, of

which those with cerebral malaria consistently exhibited the highest levels [15,27]. (Fig. 3). A recent comparison of anti-plasmodial antibodies in two well characterized groups of 70 Thai patients with either severe (but not cerebral) or uncomplicated disease also indicated that IgE antibodies as well as total IgE were present at highest concentrations in the patients with severe malaria [28]. In contrast the concentrations of both IgG anti-plasmodial antibodies and total IgG were higher in the uncomplicated than in the severe cases. Thus, while IgG antibodies, known to be protective, may contribute to reduce disease severity, the IgE antibodies—regardless of whether or not they have a protective potential—may actually contribute to the pathogenesis of *P. falciparum* malaria. How they may do this will be discussed in the following.

4. The pathogenesis of *P. falciparum* malaria

Characteristic for *P. falciparum* malaria is the adherence of parasitized erythrocytes to the endothelial lining of small blood vessels [29,30]. This cytoadherence which is not shared by the other three plasmodial species afflicting humans is an essential feature of this infection and largely responsible for its extreme morbidity and mortality rates. Thus, in cerebral malaria the most severe form of *P. falciparum* disease, the sequestration of infected erythrocytes in capillaries and postcapillary venules may lead to microvascular obstruction of blood flow, resulting in hypoxia, ring hemorrhages, and tissue lesions [31]. Moreover, the binding of infected erythrocytes to vascular endothelium may also cause inflammatory reactions by recruiting monocytes and other leucocytes and by inducing endothelial activation and cytokine release [32]. Obviously, these mechanisms are not mutually exclusive [33].

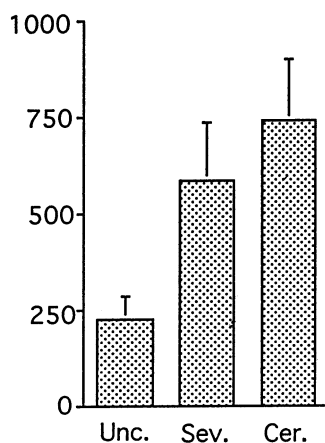


Fig. 3. IgE (ng/ml) $\pm$ S.E. in serum of Sudanese patients with cerebral (19), severe (6) or uncomplicated (12) *P. falciparum* malaria [27]. For methods and additional data from other areas see [15,27,28].

5. IgE and tumor necrosis factor

It has been known for several years that morbidity and mortality of *P. falciparum* malaria are related to the concentration of tumor necrosis factor (TNF) in the blood of the patients [34,35]. Although participating in protection (see below) this cytokine is considered to be the major pathogenic factor causing malaria fever and, in combination with cytoadherence, vessel lesions and hemorrhages [36]. Activated monocytes/macrophages are believed to be the primary source of TNF in malaria. TNF release is considered to be caused by parasite derived 'toxins', particularly GPI (glycosylphosphatidylinositol) containing polypeptides which have been shown to induce TNF in vitro [37,38]. However, monocytes are readily activated but activation does not necessarily have to involve parasite products. Thus it has been shown in other systems that IgE can also efficiently activate monocytes to kill parasites by inducing surface expression and cross-linking of CD23, the low affinity receptor for IgE [39]. This cross-linking leads to activation of iNOS (inducible nitric oxide synthase), accumulation of cGMP and cAMP, activation of the transcription factor NF- κ B and through this of the genes coding for TNF, and other cytokines [7]. Thus, IgE dependent monocyte activation gives rise to the release of both NO and TNF, two important effector molecules in malaria immunity [40–42].

Comparison of IgE and TNF concentrations in the blood of patients with uncomplicated or severe malaria indicated that these increased in parallel with the severity of the disease [27], suggesting that IgE may also be implicated in TNF induction and pathogenesis of this infection. To more directly establish this, human mononuclear blood cells were incubated in the presence of human serum in microplates coated with rabbit antibodies to human IgE. Such incubations led to TNF release significantly elevated above background when the sera were from malarious donors. Moreover, TNF release was well correlated with the IgE anti-malaria antibody concentrations in the sera and was abolished if they had first been depleted of such antibodies by running them over immunosorbent-columns binding human IgE [27]. Some typical results are also shown in Table 1. Similar results were obtained when the incubation plates had been coated with *P. falciparum* antigen originating from lysates of infected erythrocytes. In contrast, no TNF release above background was obtained when the plates had been coated with antibodies to human IgG. The most efficient effector cells were adherent mononuclear cells, i.e. monocytes.

Taken together, these results confirm that exposure of monocytes to IgE containing immune complexes or aggregates activates them to produce TNF and further suggest that such mechanisms may also be of importance in malaria pathogenesis [43]. Thus, in *P. falciparum*

Table 1

IgE in malaria sera contributes to the induction of TNF release from mononuclear cells^a

Malaria sera (donor)	Total IgE (ng/ml)		TNF (pg/ml)	
	Before	After	Before	After
1	3059	136	1550	130
2	849	95	1320	0
3	259	51	1590	630
4	145	23	860	120
5	52	4	1360	960

^a Mononuclear blood cells (2×10^5 /well) from normal human donors were incubated for 20 h at 37°C in micro titration plates coated with rabbit antibodies to human IgE. Incubation was in the presence of malaria sera before and after depletion of IgE (For further details and controls see [27]).

parum malaria, a local overproduction of TNF in small vessels, e.g. in the brain, may be induced by the interaction of monocytes with deposited immune complexes containing malaria antigen and IgE antibodies. The elevated concentrations of IgG anti IgE antibodies also present in the blood of these patients [27] may contribute to a local overproduction of TNF in a similar way.

In view of the fact that the concentration of IgG anti-malarial antibodies in the patients is much higher than that of the corresponding IgE antibodies [15,28] and that both IgG and IgE antibodies appear to be directed against the same epitopes [15], it must be asked how IgE antibodies can compete with IgG antibodies for malaria antigen to form the complexes initiating monocyte activation. The answer to this question is not known. However, it may be assumed that the IgE antibodies which appear relatively late in the course of an immune response have, on the average, an elevated affinity for antigen and therefore may compete successfully for binding. In any event and whatever the explanation, the finding of IgE deposits on infected erythrocytes and in the walls of small brain vessels from patients with cerebral malaria (Y. Maeno, P. Perlmann, H. Perlmann, Y. Kusakara, M. Aikawa, manuscript submitted) supports the conclusion that IgE induced TNF release is involved in the pathogenesis of severe malaria.

Acknowledgements

The work in the authors' laboratory was supported by grants from the Swedish Agency for Research Cooperation with Developing countries (SAREC), the Swedish Medical Research Council (MFR) and the European Union (EU). The skilful assistance of Margareta Hagstedt is gratefully acknowledged.

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