

Immunoglobulin E (IgE) containing complexes induce IL-4 production in human basophils: effect on Th1–Th2 balance in malaria

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Abstract

Human basophils are potent producers of IL-4 following cross-linking of the high affinity receptor for IgE (FcεR1). Elevated levels of both total- and malaria-specific IgE have been demonstrated in sera from people living in malaria-endemic regions. Whether or not these IgE antibodies are pathogenic is unclear. Serum containing high IgE levels obtained from malaria individuals was used to establish whether IgE-immune complexes could induce IL-4 production in human basophils. The basophils, obtained from healthy donors, were primed with 10 ng/ml of IL-3 before being transferred to wells containing goat anti-human IgE or human antimalarial IgE-immune complexes. IL-4 was induced upon stimulation of human basophils by plate bound IgE-containing immune complexes. Basophils treated similarly but with goat anti-IgG/human antimalarial- IgG-immune complexes did not secrete IL-4. Similarly mononuclear cells depleted of basophils in parallel culture did not secrete IL-4. Thus, human basophils may contribute to the polarization of T-helper type 2 in the (Th2) responses in malaria hosts via IgE-induced IL-4 production.

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1. Introduction

Parasite infections are often associated with increased levels of circulating basophils and eosi-

nophils, markedly increased serum levels of IgE and increased numbers of mast cells and/or basophils in the affected tissue. Elevated serum levels of specific and polyclonal IgE are usually a hallmark of atopy and helminthic infection (Jarrett and Miller, 1982). We and others have shown that people living in malaria-endemic areas have elevated serum levels of both total- and anti-

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malarial-specific IgE (Desowitz, 1989; Perlmann et al., 1994). When examining serum samples from children and adults living in malaria-endemic areas in different African and Asian countries, 85% of the donors had significantly elevated levels of IgE and more than 60% of these had detectable anti-malarial specific IgE antibodies (Perlmann et al., 1994). However, the role of these IgE antibodies in malaria is unknown.

IgE interacts with IgE-receptors expressed on a variety of different cell types. There are two major types of IgE receptors. One form is the low-affinity receptor FcεRII (CD23) constitutively expressed on B cells and induced by IL-4 on macrophages (Vercelli et al., 1988), some T cells, human eosinophils and platelets (Delespesse et al., 1992). Cross-linking of CD23 on macrophages or other CD23-bearing effector cells by IgE-containing immune complexes is thought to play a pathogenic role, via TNF-mediated pathways (Perlmann et al., 1994; Dugas et al., 1995; Perlmann et al., 1999, 1997). Although these data seem to favour a pathogenic role of malarial induced IgE, they do not exclude other roles for this immunoglobulin isotype.

IgE also binds to the high affinity IgE receptor (FcεRI) present primarily on mast cells and/or basophils in both mice and humans. Upon cross-linking of the high affinity IgE receptors or in response to IL-3, basophils rapidly release cytokines such as IL-4 and IL-13 (Plaut et al., 1989; Brunner et al., 1993). The cytokine milieu is an important factor in the polarization of helper (Th) cells. This is for example evident in the murine *P. chabaudi chabaudi* system where it has been shown that a population of IL-3 responsive IL-4 producing non-B non-T (NBNT) cells of basophil origin expand in the spleen and in the peripheral blood during acute infection (Poorafshar et al., 2000). This takes place at the same period when the Th cells switch from Th1 to Th2, needed for the complete resolution of the infection in this model (Helmby et al., 1998; Langhorne et al., 1990). However, a direct role of malaria-induced IgE in the activation of cells that express the high affinity receptors for IgE in humans is unknown. The aim of the present study was to determine whether IgE-

containing malaria sera have the capacity to induce IL-4 in NBNT cells of basophil origin.

2. Materials and methods

2.1. Materials

The following reagents and materials were obtained from the suppliers indicated: RPMI 1640, NaHCO₃, Hank's balanced salt solution (HBSS) without Ca²⁺ and Mg²⁺ (Gibco BRL, Paisley, UK); Ficoll and Percoll (Pharmacia, Uppsala, Sweden), recombinant human (rh) IL-3 and IL-4 (Sigma, Chemical Co., St. Louis, MO); basophil isolation kit (Miltenyi Biotech, Germany); IL-4 monoclonal antibody Elisa kit (Mabtech, Stockholm, Sweden). A pool of human sera with either high or low (undetectable) anti-malarial specific IgE but with similar levels of anti-malarial specific IgG was a kind gift from Anna Färnert (Karolinska Institute, Sweden).

2.2. Purification of basophils

Basophils were purified by two methods: one comprising Percoll density gradient centrifugation and the other cell depletion in a two-step procedure consisting of a Ficoll density gradient step and a negative selection step by MACS as described below. Briefly, 50 ml blood from healthy volunteers were drawn into EDTA vacutainer tubes and subsequently layered on either discontinuous Percoll gradients (densities 1.088, 1.079 and 1.070) or on Ficoll. Density gradient centrifugation was done at 400 × *g* for 25 min or 500 × *g* for 30 min at room temperature (rt) for Percoll or Ficoll gradients, respectively. After centrifugation the interphases were pooled and washed twice with ice-cold HBSS. The basophil-rich interphase was collected at 1.079 and 1.070 density. Mononuclear cells (MNC: lymphocytes, monocytes and less than 1% basophils), collected at the upper interphase of the Percoll gradient were used as controls. The Ficoll interphase was collected, washed twice with ice-cold phosphate buffered saline (PBS) supplemented with 2 mM EDTA and 0.5% FCS and the cells were ready for subsequent

negative selection by MACS. Total cell counts and viability were determined by staining with trypan blue and counting in a Neubauer chamber.

2.3. Magnetic cell separation (MACS)

Basophil enrichment was performed using MACS basophil isolation kit (Miltenyi Biotech) essentially according to the manufacturers' instructions. Briefly, the cell pellet obtained from Ficoll density gradient separation was resuspended in PBS/0.5% FCS/2 mM EDTA (herein referred to as buffer). The same buffer was subsequently used throughout the MACS selection steps. The cells were mixed with IgG-containing blocking reagent followed by addition of hapten-labeled antibody cocktail (anti: -CD3, -CD7, -CD14, -CD15, -CD16, -CD36, -CD45RA and -HLA-DR) and subsequent incubation. Thereafter cells were incubated with anti-hapten micro-beads at recommended concentrations, layered onto a BS+ MACS column and eluted with ice-cold buffer. Unbound and bound fractions were recovered in two separate tubes. Total cell counts and viability in each fraction were determined by trypan blue exclusion. Cells were then resuspended in RPMI 1640 supplemented with 10% FCS, 25 µg/ml of gentamycin and 2 mM L-glutamine (C-RPMI).

2.4. Flow cytometry

Approximately one hundred thousand cells in FACS buffer (PBS/2% FCS) were stained at +4 °C for 15 min with phycoerythrin (PE) conjugated mouse anti-CD123 and fluorescein-isothiocyanate (FITC) conjugated mouse anti-HLA-DR (Beckton and Dickinson, San Diego, CA). As isotype controls, PE conjugated mouse IgG1 and FITC conjugated mouse IgG2a (Dako, Hamburg, FRG) were used. The cells were washed and resuspended in FACS buffer for immediate analysis. The cells were analyzed in a FACS Calibur using the CELLQUEST software (Beckton and Dickinson).

2.5. Culture and stimulation

Basophil or MNC enriched preparations were suspended at $0.5\text{--}1 \times 10^6$ cells per ml in C-RPMI.

The cells were primed with 10 ng/ml IL-3 for 15 min at 37 °C. Thereafter $0.5\text{--}1 \times 10^5$ cells per well in a final volume of 100 µl were incubated in round-bottomed 96-well micro titer plates containing various stimulants. The plates were pre-coated overnight with 100 µl per well of either purified goat anti-human IgE or purified goat anti-human IgG (Vector Lab., Burlingame, USA) at a concentration of 10 ng/ml or with Percoll fractionated (crude) parasite antigen at 100 µg/ml. Then the plates were washed with PBS and subsequently incubated with 100 µl per well of pooled human sera diluted 1:10 in PBS from malaria-infected children containing high or low/undetectable IgE but similar IgG levels or with 100 pg/ml of IgE from a myeloma cell line. Following 1 h of incubation at 37 °C the plates were washed to remove unbound antibodies before addition of cells. The supernatants were collected after 18 h of incubation at 37 °C, humidified in 5% CO₂ chambers and stored at -20 °C until IL-4 was assayed by sandwich ELISA.

2.6. IL-4 ELISA

Analysis of IL-4 was conducted using IL-4 ELISA kit (Mabtech) according to the manufacturers' instructions. Briefly, 96-flat bottomed micro-titer plates were coated overnight at 4 °C with 1 µg/ml mouse anti-human IL-4 mAb. Then the plates were blocked with 100 µl per well buffer containing 1% BSA for 1 h. Following incubation for 2 h with samples or serial dilutions of recombinant human IL-4 as standard, biotinylated mouse anti-human IL-4 mAb diluted 1:1000 was added for 1 h. After incubation and washing 100 µl per well streptavidine alkaline phosphatase (diluted 1:100) were added and incubated for 4 h followed by addition of the chromogen solution *p*-nitro-phenyl-phosphate (Sigma, Chemical Co.), 1 mg/ml in 10% diethanol amine buffer containing 0.5 mM MgCl₂, pH 9.8. Plates were washed 4 × with Tristween-20 between incubation steps. All incubation steps were conducted at rt and PBS-tween was used in all steps as a diluent. Absorption was read at 405 nm.

2.7. Statistical analysis

Student's *t*-test was used for assessing statistical significance of data, and values were considered significant at the probability level less than 0.05. All data were expressed as mean $\pm$ S.E.M.

3. Results

3.1. Effect of purity on IL-4 production

Basophils express the IL-3 receptor (CD123) but not HLA-DR. All other cell types expressing CD123 also express HLA-DR (Bodger and Newton 1987). Therefore, the basophils were analyzed as CD123 positive and HLA-DR negative cells. A typical example is shown in Fig. 1 where the purity, determined by flow cytometry was 87%. Basophil purity using the single step percoll gradient method ranged from 10 to 25% whereas that of basophils enriched by MACS depletion ranged from 34 to 87%. Cells were primed with 10 ng/ml of IL-3 for 15 min at 37 °C before being transferred to wells containing IgE-immune complexes (IC). In line with what has been shown by others there was a great individual variation in IL-4 production (Schroeder et al., 1994). However, as

Table 1

Effect of percent basophil purity on IL-4 production

Individuals	Basophil purity (%)	IL-4 secretion (pg/ml) ^a
Donor 1	35	34
	87	109
Donor 2	20	21
	34	64
Donor 3	21	28
	34	50

^a Secretion of IL-4 cytokine from basophils stimulated with IgE immune complexes. Each paired point represents the release from basophils prepared from the same donor with differences in percent purity.

shown in Table 1 the production of IL-4 in the individual donor was well correlated to the purity of the basophil preparation.

3.2. IgFcεR1-induced IL-4 is only seen in basophil-enriched cultures

Lymphocytes are a source of IL-4 and since they were the main contaminants in our basophil-enriched cell preparations it was of interest to investigate whether or not FcεR1-dependent IL-4 production could be induced in MNC. Thus, MNC and basophil-enriched cell preparations from the same donor were stimulated in parallel

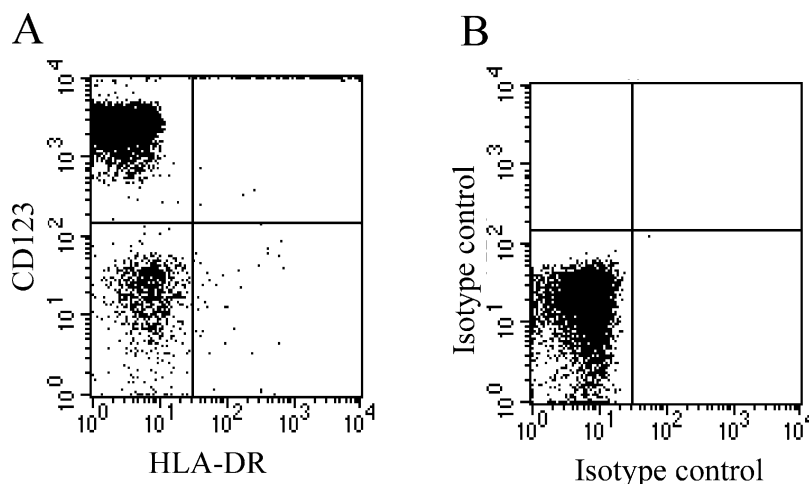


Fig. 1. Dot-plot illustration of flow cytometry of purified basophils. (A): the cells were stained for HLA-DR (FITC) and CD123 (PE), (B): isotype control (PE-conjugated mouse IgG1 and FITC-conjugated IgG2a) and subsequently analysed on FACSCalibur. Basophils express CD123 but not HLA-DR. This staining pattern was typical for the five samples analysed. The percent purity among donors varied between 34 and 87%.

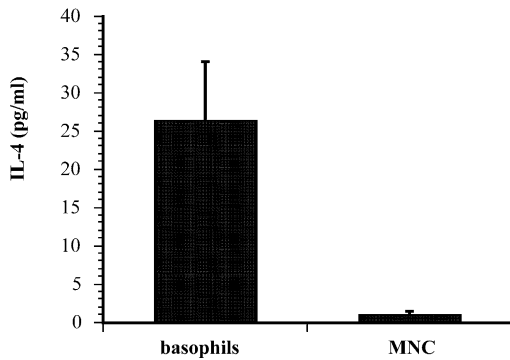


Fig. 2. Comparison between basophils and MNC in their capacity to produce IL-4. Basophils and MNC, isolated from the same blood specimen of different donors were stimulated with plate-bound IgE immune complexes for 18 h and IL-4 released into supernatants was measured by ELISA. Mean values $\pm$ S.E.M. of five experiments in duplicates are shown.

under similar experimental conditions. Fig. 2 shows the mean $\pm$ S.E.M. values of five different experiments. Basophil-enriched cells or MNC were incubated with IgE-IC formed by plate bound goat anti-human IgE in the presence of 100 pg/ml of human myeloma IgE. After this stimulation 26.3 ± 7.6 pg/ml IL-4 were detected in the basophil fraction, while only 1.1 ± 0.2 pg/ml was found in the MNC culture supernatants (t test; $P = 0.003$).

3.3. *FcεR1*-dependent IL-4 production induced by malaria immune sera containing elevated IgE levels

IL-4 production through cross-linking of high affinity IgE receptors is confined to basophils (Brunner et al., 1993; Mueller et al., 1994; Kon et al., 1998). In order to test whether or not malaria sera can induce IL-4 in basophils, a pool of malaria sera with high IgE content (diluted 1:10) was added to wells of 96-well tissue culture plates pre-coated overnight at 4 °C with 10 μg/ml of anti-human IgE. In parallel, a pool of sera containing similar levels of IgG but with undetectable IgE were used as controls. After washing off excess antibodies, basophil enriched cells or MNC were added and supernatants were assayed for IL-4. Stimulation of basophil-enriched cultures

with IgE-IC (plate-bound anti-IgE + IgE-rich sera) significantly induced IL-4 production (51 ± 8.5 pg/ml) compared with basophil-enriched cultures added to wells containing goat anti-human IgE alone (not shown) or IgG containing serum (33 ± 12 pg/ml), t -test: $P = 0.01$ (Fig. 3). In control wells with goat anti-human IgG followed by incubation with sera containing anti-malarial IgG antibodies, plate bound IC gave significantly lower IL-4 induction, 8 ± 4 pg/ml, $P = 0.005$ (Fig. 3). MNC treated similarly did not produce IL-4, (data not shown). Basophils cultured with crude plasmodium antigen did not produce IL-4 (data not shown). The levels of IL-4 obtained in these cultures were comparable to those produced in MNC control wells, suggesting that parasites do not induce IL-4 directly in an FcεR1-mediated pathway.

4. Discussion

Humans infected with *P. falciparum* malaria frequently exhibit elevated serum levels of total and *P. falciparum* specific IgE (Desowitz, 1989;

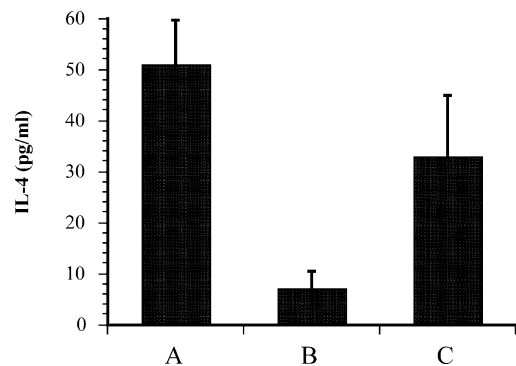


Fig. 3. The effect of IgE-containing immune complexes on IL-4 induction on basophil-enriched cells. Mean values $\pm$ S.E.M. of five experiments in duplicates are shown. (A) Sera containing elevated levels of IgE bound to plate-bound goat anti-h-IgE, (B) sera containing elevated levels of IgG bound to plate-bound goat anti-h-IgE, (C) sera containing elevated levels of IgG but undetectable IgE bound to plate-bound goat anti-h-IgE. Sera were obtained from malaria infected individuals.

Perlmann et al., 1994). In the present study we show that IgE in the form of immune complexes is a potent inducer of IL-4 in human basophils, supporting a regulatory role of IgE in the switch from Th1 to Th2 cells. This is further supported by the findings that elevated serum levels of IgE are well correlated with the ratio of IL-4/IFN- γ -producing cells in the blood (ElGhazali et al., 1997).

In the murine *P. chabaudi* system we and others have recently shown that IL-4 producing NBNT cells play an important role in providing the Th2 milieu needed for the final clearance of the parasite (Langhorne et al., 1990; Stevenson and Tam, 1993; Taylor-Robinson et al., 1993; Taylor-Robinson and Phillips, 1993). IL-4 producing NBNT cells are believed to be of mast cell or basophil origin (Seder et al., 1991). That basophils are involved in the IgE–Fc ϵ R-mediated IL-4 production is further demonstrated by the identification of the mouse mast cell serine protease (MMCP-8), which is thought to be the first basophil-specific marker in mouse (Poorafshar et al., 2000). In line with these findings we here show that human basophils are potent producers of IL-4 upon cross-linking of the IgE-receptors or in response to IL-3 which also has been reported by others (Brunner et al., 1993; Poorafshar et al., 2000).

The cells used in the present study were highly purified basophils albeit, other cell types expressing IgE-receptors could be involved in the IL-4 production. However, since control cultures containing mononuclear cells or any other contaminating cells produced significantly less IL-4 when stimulated in a similar manner as the basophils this is unlikely. One other contaminating cell type could be the eosinophil (MacGlashan et al., 1997). However, eosinophils have much lower cell densities and are not enriched by percoll-gradient centrifugation (Raghuprasad, 1982). Furthermore, eosinophils express HLA-DR and would have been seen in the FACS analysis. Thus, we find it unlikely that eosinophils are the IL-4 producers in our system.

No IL-4 was induced by the anti-human IgG reagents suggesting the involvement of IgE receptor in the triggering. Since basophils are devoid of the low affinity IgE- receptor (CD23) this strongly

argues for the involvement of the high affinity IgE receptors (Fc ϵ RI). The basophil-enriched preparation could be induced to produce IL-4 after stimulation with anti-human IgE alone. This may be due to in vivo bound IgE which is being cross-linked by the anti-human IgE (MacGlashan et al., 1997).

Our results support the conclusion that IgE, in addition to its role in inducing inflammatory mediators, plays an immunoregulatory role by inducing IL-4 production in Fc ϵ RI⁺ cells. Thus, cells such as basophils (Saito et al., 1988; Miller et al., 1994; Kon et al., 1998; Bogers et al., 2000) may present the ‘natural immunity analogue’ important for the polarization of Th2 cells (Haisch et al., 2001). Our finding also shows that plasmodium parasites, unlike schistosome eggs, are unlikely to directly cross-link these receptors prior to a specific immune response that subsequently induces production of parasite specific IgE antibodies. Elevated levels of serum IgE are a reflection of increased numbers of IL-4/or IL-13 producing cells of the Th2 type (Aebischer and Stadler, 1996; Finkelman et al., 1997). The reasons why malaria exposed individuals exhibit high levels of both total and anti-malarial IgE are not known. In any event our study is indicative of a complex interplay by which Th2 polarization and IgE elevation may be regulating each other via involvement of Fc ϵ RI-mediated-IL-4 production in basophils. Further elucidation of the molecular mechanisms underlying the switch towards Th2 differentiation in malaria infection will be of considerable interest for both vaccine development and pharmacological immunomodulation.

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