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Fcγ receptor-mediated phagocytosis of *Plasmodium falciparum*-infected erythrocytes *in vitro*

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SUMMARY

Although convincing evidence exists for the role of immunoglobulin G (IgG) antibodies in immunity to malaria, antibody titres do not usually predict protection. In this study we have assessed the interaction between *Plasmodium falciparum*-infected erythrocytes (PE), opsonized with immune serum containing different amounts of IgG antibody isotypes, with either THP-1 cells, *ex-vivo* human monocytes or IIA1.6 transfectant cells expressing FcγRIIa-Arg/Arg131 or –His/His131 allotypes. Our results show that PMA-treated THP-1 cells were capable of phagocytosing serum-opsonized PE by FcγRI (CD64) and FcγRIIa (CD32), acting synergistically. The known FcγRIIa polymorphism motivated us to examine its influence on IgG isotype-mediated phagocytosis of opsonized PE with human monocytes and the IIA1.6 transfectant cells expressing either allelic forms. Regardless of the cell type, PE phagocytosis with FcγRIIa-His/His131 was highest following opsonization with a predominantly IgG3-containing immune serum pool. In contrast, PE phagocytosis with FcγRIIa-Arg/Arg131 tended to be higher with an IgG1-containing pool. These results suggest a genetically determined influence of effector cell phenotype on IgG antibody–pathogen interaction in *P. falciparum* malaria.

Keywords antibodies FcγR human phagocytes *Plasmodium falciparum*

INTRODUCTION

Antibody responses play a critical role in immune protection against asexual blood stages of *Plasmodium falciparum*. This has been demonstrated elegantly by passive transfer experiments using sera or purified immunoglobulins from adults resident in areas with hyperendemic malaria [1,2]. However, the mechanisms by which malaria-specific antibodies interfere with the development and/or multiplication of the asexual stages of human Plasmodia are still unclear. Druilhe and others have postulated that antibodies inhibit parasite growth in co-operation with either monocytes or neutrophils by antibody-dependent cellular inhibition (ADCI) [3,4] or immunophagocytosis [5] through cell-surface expressed Fc receptors after binding their parasite target.

The binding of the Fc portion of IgG to Fcγ receptors (FcγR) on the surfaces of monocytes, macrophages and neutrophils provokes biological functions such as antibody-dependent cellular cytoxicity (ADCC) (ADCI in malaria), phagocytosis, antigen presentation, release of infammatory mediators and enhancement of surface antigen expression, as well as generation of reactive oxygen species ([4], for a review see [6]). In humans, there are three classes of FcγR that bind cytophilic IgG (IgG1 and IgG3), namely: FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16). FcγRI is a high-affinity receptor capable of binding monomeric IgG1, IgG3 and IgG4. FcγRII and FcγRIII are low-affinity receptors, interacting with only IgG in complexed or aggregated form. Human FcγRII is a 40 kDa glycoprotein that is expressed on a variety of cells such as granulocytes, monocytes/macrophages, platelets, B cells, endothelial cells of the placenta and some T-cell subsets. The FcγRIIa subtype is expressed on neutrophils and monocytes/macrophages and initiates phagocytosis, ADCC and cellular activation. Recent studies provide evidence that a structural and functional polymorphism at position 131 of this receptor leads to a point mutation resulting in a switch from an arginine (R) to a histidine (H) residue in the proximal Ig-binding domain which greatly affects receptor affinity and specificity [7]. FcγRIIa-H131 exhibits an affinity for IgG2 not seen with the FcγRIIa-R131 [8] with consequent functional differences.

In vitro assays using immune sera seeking correlates for protection against malaria showed that FcγIIa receptors are involved [4]. Recent observations in two separate malaria studies showed that this polymorphism may have an influence on protection against this disease [9,10]. In all these studies, as well as in larger sero-epidemiological surveys, the quality of the antibody response, which is reflected in the distribution of the IgG isotype

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class(es), have been stressed [11–14]. Bouharoun-Tayoun and Druihle [11] observed differences in the distribution of Ig subclasses between clinically protected and non-protected individuals, with cytophilic isotypes (IgG1 and IgG3) being dominant in the protected individuals. In this context FcγRIIa-Arg/Arg, which binds IgG1 and IgG3 but not IgG2 [7], would be expected to be more efficient than the His/His allotype. FcγRIIa-His/His binds IgG1, IgG2 and IgG3, albeit with different affinities. Our own previous results show that ADCI *in vitro* is mediated predominantly by IgG3 [15], whereas Shi and colleagues [16], in a similar study, showed IgG1 to be more important.

Thus, polymorphisms which may alter the relative antibody affinity of receptor(s) expressed on effector cells involved in antibody-mediated protection may ultimately influence disease outcome. Understanding the mechanism(s) of these interactions may help in the design of effective vaccines. To address this, we have designed experiments using a human monocytic cell line, THP-1, transfectant cell lines expressing the different allelic forms of FcγRIIa, as well as human monocytes in immunophagocytosis assays using well characterized sera from malaria exposed individuals.

MATERIALS AND METHODS

Serum donors

Serum samples were obtained from 23 semi-immune adults (18–54 years of age) from Lambaréné, a town in Gabon where *P. falciparum* malaria is hyperendemic [17]. As control, we used a pool of serum obtained from malaria non-exposed Europeans. Based on antibody quantification by ELISA described below we created different serum pools from malaria exposed and naive individuals: (i) P1: non-immune pool from non-exposed Europeans; (ii) P2: immune serum pool containing both IgG1 and IgG3; (iii) P3: immune serum pool containing predominantly IgG1; and (iv) P4: immune serum pool containing predominantly IgG3.

Monoclonal antibodies

The following monoclonal antibodies were obtained from Medarex (Annandale, NJ, USA): mouse anti-hFcRγI (CD64) MoAb 22 (mIgG1), mouse anti-hFcRγII (CD32) MoAb IV.3 (mIgG2b), mouse anti-hFcRγIII (CD16) MoAb 3G8 (purified Ig), FITC-labelled MoAb IV.3. Mouse anti-hFcRγII (CD32) MoAb AT10 was obtained from Dr Thomas Valerius (University of Erlangen-Nürnberg, Germany), and mouse anti-hFcγRI (CD64) clone 10·1 from Biozol, Germany. The mouse anti-hCD36 (mIgG1) clone CLB-IVC7 was obtained from Research Diagnostics INC, Flanders, NJ, USA. For inhibition studies, human monocytes and THP-1 cells were preincubated for 25 min at room temperature with anti-FcγR antibodies at the following concentrations: 10 μg/ml CD64 (anti-hFcγI clone 10·1); 5 μg/ml IV.3 (anti-hFcγRII); 0·5 μg/ml AT10 (hFcγRII); 10 μg/ml MoAb 3G8 (anti-hFcRγIII); 10 μg/ml isotype control MoAb (Sigma, Germany) were used as appropiate.

P. falciparum culture and antigen preparation

A *P. falciparum* isolate cys007, obtained from a child presenting with severe *P. falciparum* malaria at the Albert Schweitzer Hospital, Lambaréné, Gabon was adapted for *in vitro* culture according to the method of Trager and Jensen [18] using RPMI-1640 medium (Sigma, Germany) buffered with 25 mM Hepes, and supplemented with 25 mM sodium bicarbonate, 2 mM L-glutamine, 300 mM hypoxanthine and 10 μg gentamicin per ml (GIBCO, Paisley, UK). Parasites were grown in culture medium supplemented with 10% non-immune sera (prescreened) in an atmosphere of 5% CO_2, 5% O_2 and 90% N_2 and subcultured with O-positive erythrocytes depleted of lymphocytes (University Hospital, Tübingen, Germany).

To prepare crude schizont antigen for ELISA, the isolate was grown to a parasitemia of 3–5% with a majority of the parasites in the schizont stage. The cultures were enriched and synchronized by selective high-gradient magnetic sorting (MACS; Miltenyi Bio Tec, Bergisch Gladbach, Germany). Briefly, cultures were passed through a prewashed column (2% fetal calf serum (FCS) in phosphate-buffered saline (PBS)) in a magnetic field. Captured infected cells were eluted following removal of the column from the magnetic field. Synchronized and enriched parasites and uninfected erythrocytes used for culture were washed twice with PBS followed by controlled lysis with 0·1% saponin, 0·06 N NaCl, sonication in the presence of enzyme inhibitors and centrifugation at 10 000 ***g*** for 10 min at 4°C. The protein concentration of both the crude lysate and erythrocyte preparations were estimated by Bio-rad protein assay (Bio-rad laboratories GmbH, Munich, Germany).

For immunophagocytosis assays, 3–5% parasite cultures containing mainly mature trophozoite or schizonts were sychronized by plasmagel flotation (Plasmagel, Laboratoire Roger Belon, France). Enriched and synchronized parasite preparations in some cases were trypsinized by exposure to 0·1 mg/ml trypsin-EDTA solution (GIBCO, Paisley, UK) or by chymotryptic digestion with 0·1 mg/ml chymotrypsin solution (Sigma, Germany) at 37°C for 1 h. All preparations were washed twice in RPMI prior to assays.

ELISA

Detection of human IgG, IgG1, IgG2, IgG3 and IgG4 antibodies reacting with Cys007 *P. falciparum* schizont lysate was carried out as described by Aribot *et al*. [12], with some modifications. Briefly, 96-well plates (Corning Inc Costar®, Corning, NY, USA) were coated overnight at 4°C with 50 μl of *P. falciparum* antigen or erythrocyte extracts (5 μg/ml in carbonate buffer at pH 9·6). Following overnight incubation, plates were washed four times with PBS/0·5% Tween. 200 μl of 1% bovine serum albumin (BSA) in PBS was added to each well and incubated for 2 h at 37°C to block non-specific binding. The blocking step was followed by washing four times with PBS–Tween (0·5%). Serum samples diluted at 1 : 200 in PBS–Tween (0·5%) were applied (50 μl/well) in duplicate and incubated overnight at 4°C. In each plate, positive and negative control plasma pools were included, as well as four wells referred to as blank. In the blank wells only PBS–Tween (0·5%) was added. Following overnight incubation, plates were washed four times with PBS–Tween (0·5%). For total IgG determination, incubation after test samples was followed by application of 50 μl of horseradish peroxidase conjugated goat antihuman IgG (Fc-specific) (Sigma, St Louis, MO, USA) to individual wells and incubation in a waterbath at 25°C for 2 h.

For determination of IgG isotypes, 50 μl of antihuman IgG subclass-specific monoclonal antibodies (Caltag, San Francisco, CA, USA) diluted in PBS–Tween (0·5%) was dispensed into individual wells and incubated overnight at 4°C. The following antibodies were used: MoAb IgG1 (6069) 0·5 mg/ml (1/1000); IgG2 (6014) 0·5 mg/ml (1/2000); IgG3 (6047) 0·5 mg/ml (1/2000) and IgG4 (6023) 0·5 mg/ml (1/1000). Overnight incubation was

followed by four washes in PBS–Tween and addition of 50 μl of horseradish peroxidase conjugated goat antimouse IgG (Sigma, St Louis, MO, USA) diluted at 1 : 10 000 in PBS–0·5% Tween to individual wells.

Incubation with conjugate (2 h at room temperature) was followed by development with 100 μl per well of a chromogen-containing substrate mixture (TMB, Kirkegaard & Perry, Gaithersburg, MD, USA). The reaction was stopped after 15 min by addition of 50 μl of 1 M phosphoric acid per well. The antibody response measured as optical density (O.D.) was determined using an ASYS Hitech DigiScan microplate reader with a 450-nm filter and a reference filter of 550 nm. The background (mock-cultured uninfected erythrocyte extract) for each serum was subtracted from the O.D. reading of the response to the parasite lysate.

Cells and determination of FcγRIIa genotypes and expression
THP-1 cells obtained from DMSZ (German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany) were grown in RPMI-1640 supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 25 mM HEPES, 1 mM sodium pyruvate (Sigma). Cells were differentiated to become adherent, elongated and macrophage-like cells by the addition of PMA (phorbol 12-myristate 13-acetate) at 20 ηM/ml for 72 h. FcγRIIa transfectants IIAI.6 Arg/Arg or His/His were a kind gift from Dr van de Winkel (University of Utrecht, the Netherlands). Cells were cultured as described by Rodriguez *et al*. [19] in RPMI-1640 supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 25 mM HEPES, 1 mM sodium pyruvate and 0·8 mg/ml Geneticin (G418, Life Technologies, GIBCO BRL, Gaithersburg, MD). FcγRIIa expression was confirmed by flow cytometry using direct immunofluorescence with FITC-labelled MoAb IV.3. Transfectant FcγRIIa expression was observed to be stable during the course of experiments.

Human peripheral blood mononuclear cells (PBMC) were isolated from whole blood, from donors who gave informed consent, by standard Ficoll-Paque (Pharmacia, Uppsala, Sweden) gradient centrifugation of heparin-treated blood from healthy, malaria non-exposed European donors. PBMC at 2 × 10/ml of culture medium plus 10% FCS were plated onto 8-well glass chambered slides (Nalge-Nunc, Naperville, IL, USA) and left to adhere for 2 h, after which non-adherent cells were washed off.

The FcγRIIa-R-H131 genotype of THP-1 cells and human monocytes was determined by a nested sequence-specific primer-polymerase chain reaction (SSP-PCR) on genomic DNA as described by Carlsson *et al*. [20].

Phagocytosis assay
Immunophagocytosis assays were performed as described by McGilvray *et al*. [21], with some modifications. Differentiated THP-1 cells, monocytes or transfectant cells adhered to 8-well glass chambered slides (Nalge-Nunc) were employed to study phagocytosis. First, we determined the optimal serum concentration for opsonizing *P. falciparum*-infected erythrocytes (PE) by incubating the PEs with different concentrations of the heat-inactivated human serum pool, P2 (0, 5, 10, 20 and 50%) for 1 h at 37°C. In blocking assays, the FcγR I and II of THP-1 cells were blocked for 25 min with saturating amounts of anti-FcγR MoAb at room temperature. Following blocking of Fc receptors, cells were washed twice with RPMI-1640.

To study antibody-mediated phagocytosis mediated by Fcγ receptors, opsonized PE following treatment with 50% serum (heat-inactivated for 30 min at 55°) for 1 h at 37°C or mock-opsonized parasites (PE) were resuspended in 500 μl of RPMI-10% FCS-L-glutamine and added to different cells at a PE : cell ratio of 25 : 1 in duplicate. For each assay, non-infected erythrocytes (UE) were included and plates rotated gently for 4 h at 37°C, 5% CO_2. At the end of the 4-h incubation period, non-adherent PE or UE were washed thrice with RPMI-1640, and adherent but non-internalized PE or UE were lysed in ice-cold distilled water for 30 s. All cell preparations were fixed with methanol and stained with Giemsa. Phagocytosis was assessed by light microscopy by counting of 200–500 cells. Only erythrocytes within the phagocytic cell outline were considered in the estimation of phagocytosis. The average phagocytosis index was calculated as the percentage of effector cells with clear evidence of internalized PE measured in duplicate wells.

Statistical analyses
Differences between continuous variables were assessed using the nonparametric Mann–Whitney *U*-test, where a two-tailed $P < 0.05$ was considered significant.

RESULTS

We evaluated the role of FcγRI and FcγRIIa in IgG-mediated immunophagocytosis of *P. falciparum in vitro* using the human monocytic cell line, THP-1, genotyped human monocytic cells and a transfectant cell line IIAI.6, expressing different allelic forms of the FcγRIIa. In our initial experiments, we used the human monocytic cell line, THP-1, that has previously been shown to kill *P. falciparum* parasites *in vitro* [22]. First, we performed phagocytic assays to determine the phagocytic capacity and optimal serum concentration for opsonization. These cells proved able to internalize opsonized PE and the process was observed to be dependent on the serum concentration (Fig. 1). UEs were not ingested by cells (data not shown).

PE parasites expressing the variant surface antigen, PfEMP1 (*P. falciparum* erythrocyte membrane protein 1) are susceptible to CD36-mediated nonopsonic phagocytosis [21]. We examined

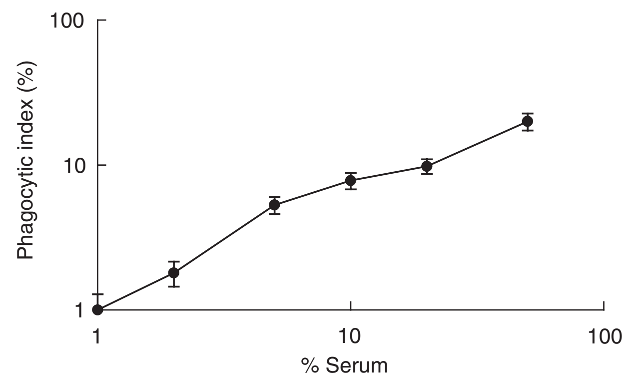

Fig. 1. Effect of the concentration of opsonizing immune serum on phagocytosis of *P. falciparum*-infected erythrocytes (PE). PMA-treated THP-1 cells were exposed to PE opsonized with 0, 5, 10, 20, 40 and 50% heat-inactivated immune serum pool, P2, for 4 h at 37°C. Values on the *x*- and *y*-axes are log-transformed.

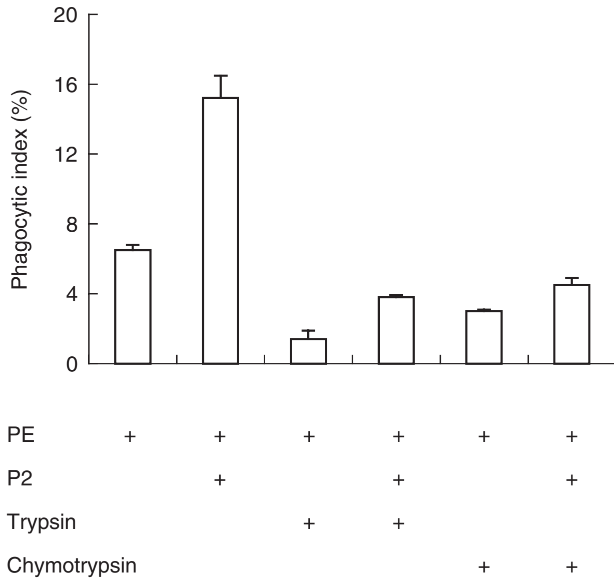

Fig. 2. Comparison of *P. falciparum* antibody-mediated phagocytosis following different enzymatic treatment. *P. falciparum*-infected erythrocytes (PE) were subjected to treatment with trypsin or chymotrypsin or untreated for 1 h at 37°C. Following enzymatic treatment, PE were either opsonized with P2 or mock-opsonized and incubated with monocytes for 4 h at 37°C.

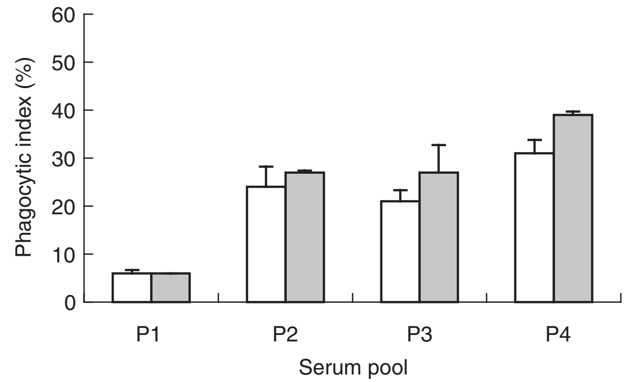

Fig. 3. Influence of interferon-gamma (IFN-γ) treatment on PMA-treated THP-1 cells on antibody-mediated phagocytosis of *P. falciparum*-infected erythrocytes (PE). PMA-treated THP-1 cells were stimulated with IFN-γ at 100 U/ml for 24 h and phagocytosis performed using PE opsonized with different IgG serum pools as described in Materials and methods. □, Unstimulated; ▩ IFN-γ-stimulated.

Table 1. Anti-schizont specific IgG isotype antibodies in sera from non-exposed and malaria-exposed individuals

Serum pool*	IgG	IgG1	IgG2	IgG3	IgG4
P1	0·1	0	0·1	0·1	0·1
P2	0·6	0·2	0·1	0·3	0
P3	0·7	0·6	0·2	0·1	0·1
P4	0·7	0·2	0·3	1·3	0

*Pools were created as described in Materials and methods. Values shown are mean optical densities (O.D.) of individual sera included in pools.

the role of *P. falciparum* parasite surface antigens in antibody-mediated phagocytosis. Treatment of PEs with trypsin or chymotrypsin greatly reduced phagocytosis of PEs (Fig. 2). In the presence of immune serum this trypsin-mediated reduction was less marked, suggesting that trypsin-insensitive surface expressed parasite antigens may persist.

We next examined the potential role of IFN-γ in antibody-mediated phagocytosis by first exposing PMA-treated THP-1 cells for 24 h to recombinant human IFN-γ. For opsonization, we used pools of sera from non-exposed and malaria semi-immune individuals (see Materials and methods and Table 1). Our results show a non-significant trend for enhanced phagocytic activity in stimulated cells with the different pools (Fig. 3), suggesting that IFN-γmay be important for the induction of antibody-dependent phagocytosis of *P. falciparum*-infected erythrocytes. Several studies [23–25] have shown that IFN-γ stimulation enhances FcγRI expression but had no effect on FcγRII expression. The enhanced phagocytosis observed following stimulation could be the result of FcγI up-regulation which we also observed by flow cytometry (data not shown). The highest phagocytic indices were observed with pool P4, but the differences with P2 or P3 did not reach statistical significance.

We next assessed the relative contribution of the different FcγR (I or II) classes to the IgG-mediated phagocytosis of PE. Blocking experiments were performed with or without saturating amounts of different anti-FcγR MoAbs (Fig. 4). Phagocytosis of serum-opsonized PE was reduced by blocking of THP-1 cells with whole CD32 or whole CD64-blocking antibodies, whereas internalization of non-opsonized PE was unaffected. In the presence of both blocking antibodies, phagocytosis was reduced to background levels. Thus, IgG-induced phagocytosis proved dependent upon both monocyte FcγRI and FcRγIIa. This result suggests a role for both FcγRI and IIa in antibody-mediated phagocytosis of opsonized PE. FcγRI has no polymorphic forms and is expressed constitutively in monocytes/macrophages only. FcγRIIa, on the other hand, is expressed on a range of different phagocytic cells and there is evidence that a point mutation in position 131 is implicated in receptor function. Hence, FcγRIIa, the more abundant of the two receptors, might be expected to play a bigger role in antibody-mediated protection *in vivo*. We examined the influence of this polymorphism on immunophagocytosis of PE *in vitro* by assessing the phagocytic capacities of a transfectant cell line, IIAI.6, expressing either the RR or the HH polymorphic forms as well as of monocytes from individuals expressing homozygous FcγRIIa-R131 or IIa-H131 with no previous malaria exposure. We used well-characterized sera in an attempt to show the influence of cytophilic IgG antibody isotypes in the receptor–parasite interaction (Table 1). Transfectants expressing FcγRIIa-H/H131 as well as monocytes from FcγRIIa-H/H131 donors were significantly more efficient in phagocytosis of P4 opsonized parasites than were FcγIIa-R/R131 transfectants or FcγIIa-R/R131 human donors' monocytes (Fig. 5a,b). The serum pool P4 contains predominantly IgG3 antibodies with specificity for crude schizont extract of the parasite used in immunophagocytosis. Phagocytosis of PE opsonized with the P3 immune serum pool, containing predominantly anti-schizont IgG1, was higher in the presence of cells expressing FcγRIIa-R/R131 rather than FcγRIIa-H/H131, but significantly so only in the case of transfectants (Fig. 5a,b).

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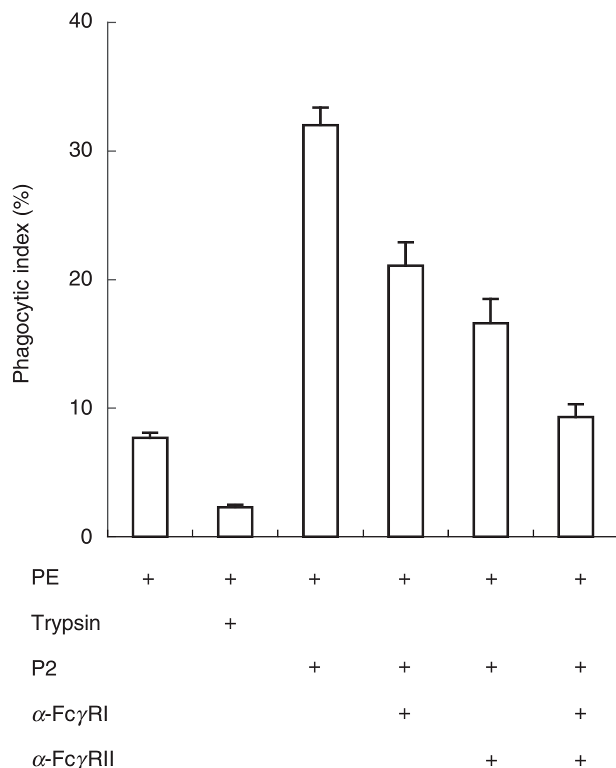

Fig. 4. Effect of FcγR-blocking monoclonal antibodies (MoAbs) on antibody-mediated phagocytosis of *P. falciparum*-infected erythrocytes (PE) by THP-1 monocytic cells. PMA-treated THP-1 cells were incubated with either mock-opsonized PE, trypsinized PE or P2 opsonized PE or cells were blocked with saturating amounts of either FcγRI and/or FcγRll blocking MoAbs and exposed to P2 opsonized PE. Data are mean ± s.d. of a representative of three different experiments.

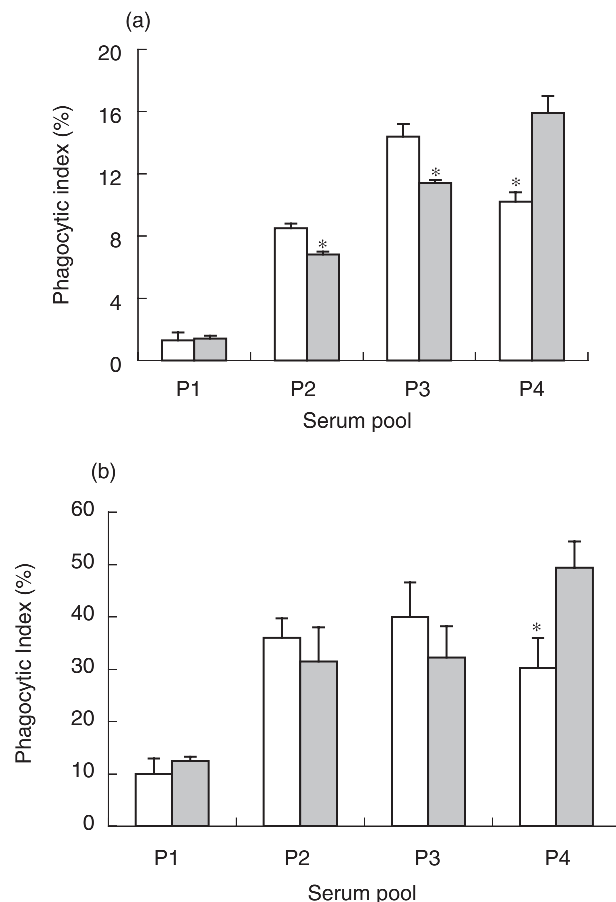

Fig. 5. Phagocytosis of *P. falciparum* by IIAI.6 transfectants and *ex vivo* monocytes. (a) IIAI.6 cells expressing FcγRlla-R131 or IIa-H131 were used as effector cells with different pools of sera-opsonized *P. falciparum*-infected erythrocytes (PE). Data are mean ± s.d. of two different experiments carried out in duplicate. (b) *Ex vivo* monocytes from FcγRlla-R131 or IIa-H131 human subjects simulating experiments in (a). Data in (b) are mean ± s.d. of three individuals in each group carried out in duplicate. **P* < 0·05 for comparison of phagocytic indices of cells expressing Arg-Arg *versus* His-His. □, Arg-Arg; ▒, His-His.

DISCUSSION

In this study we have investigated the role of Fcγ receptors (FcγR) in antibody-mediated phagocytosis using a human monocytic cell line, THP-1, human monocytes and IIAI.6 transfectant cells expressing the different FcγRIIa-Arg/His131 allelic forms. First, we confirmed that opsonization of *P. falciparum*-infected erythrocytes (PE) with immune serum enhances phagocytosis by PMA-treated THP-1 cells and that this process depends principally on trypsin-sensitive antigen(s) expressed on the PE surface [21,22]. We speculate that the residual amount of phagocytosis of PE we observed following trypsinization and opsonization in these experiments may reflect the involvement of trypsin-insensitive parasite-derived antigens, such as the rifins [26]. Turrini *et al.* [27] showed previously that monocyte-mediated phagocytosis of *P. falciparum*-infected human red blood cells involves both immune and nonimmune determinants as well as parasite factors. In addition, and as our results confirm, non-opsononized PE expressing PfEMP1 can be internalized by monocytes and macrophages [21], The enhancement of THP-1-mediated PE phagocytosis by IFN-γpretreatment confirms earlier findings by Kumaratilake *et al.* [22] and implies the involvement of FcγRI, expression of which is selectively increased following monocyte stimulation [23–25]. On the other hand, the highest levels of phagocytosis were seen consistently after opsonization of PE with a serum pool containing predominantly parasite-specific IgG3, the isotype favoured by FcγRII. It should also be noted that our own genotypic analysis showed that the THP-1 cell line we used expresses the His/His allele of FcγRIIa, which could influence the level of phagocytosis (see below). Receptor blockade experiments, nevertheless, indicated that the phagocytosis we observed here depended on both FcγRI and FcγRII.

Because, as noted above, THP-1 cells express FcγRIIa-His/His131, we undertook more detailed investigations of FcγR–IgG isotype interactions by using a transfectant cell line expressing either FcγRIIa-His/His131 or FcγRIIa-Arg/Arg131 as well as human monocytes expressing the two alleles in their homozygous form. Regardless of the cell type, PE phagocytosis with FcγRIIa-His/His131 was significantly higher following opsonization with the IgG3-containing immune serum pool, while there was a trend for higher phagocytic indices for the combination of FcγRIIa-Arg/Arg131-expressing cells with the IgG1 pool. For both receptor types, the hierarchy of the affinity of Fc binding places IgG3 first, followed by IgG1, the major difference between the two concerning IgG2 which is bound by the His/His type with an affinity equal to that of IgG1, but much less so by the Arg/Arg type [28].

Our results could therefore be an indication of a difference in the relative affinities for IgG1 and IgG3 of the two FcγRIIa types. A possible influence of the differing amounts of parasite-specific IgG2 present in the different pools (see Table 1) cannot be excluded, however, as this would be predicted to influence the profile seen with the His/His131 type. In any case, the profile of PE phagocytosis observed with the transfected cells was not altered by the presence of FcγRI on the monocytes used. Other studies have shown that inhibition of the growth of *P. falciparum* blood-stage parasites *in vitro* is mediated by co-operation between monocytes and malaria-specific IgG1 and IgG3, but not IgG2, via the FcγRIIa [4]. Controversies surround the role of IgG2 in protection against malaria. IgG2 can bind FcγRIIa-H131 but has always been regarded as a non-cytophilic antibody [29] and has been reported to be associated with a delay in acquisition of protective responses against malaria [11]. Contrastingly, studies by Aucan and colleagues [9] as well as Deloron and colleagues [30] have shown that parasite-specific IgG2 is associated with protection from malaria. In addition, among the antibodies directed to the PE surface during acute *P. falciparum* infections, IgG2 predominates (AE Tebo, unpublished observations), which may be of particular relevance to our findings here.

In conclusion, our study confirms the potential utility of THP-1 cells for the study of both FcγRI- and FcγRIIa-mediated immune phagocytosis of PE *in vitro*. Their importance in studying FcγRIIa-dependent phagocytosis may be limited because they express FcγRIIa-H131, which binds IgG3 and both IgG2 and IgG1. As an alternative, as we have demonstrated, transfected cell lines expressing FcγR provide a reliable and useful substitute for assessing parasite antigen-specific antibody functions. Our data provide evidence that the level of phagocytic activity directed against plasmodium-infected human erythrocytes may depend not only on the IgG isotype profile of the opsonizing serum but also on effector cell receptor polymorphisms.

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