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Opsonic activity of human immune serum on *in vitro* phagocytosis of *Plasmodium falciparum* infected red blood cells by monocytes

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SUMMARY

In vitro human monocytes from normal blood donors ingest red blood cells infected with *Plasmodium falciparum* more efficiently than normal red blood cells (NRBC). The phagocytic activity of human monocytes for infected red blood cells (IRBC) is greatly enhanced by the addition of immune sera obtained from individuals living in areas with endemic malaria. In contrast, the addition of sera obtained from individuals recovering from a first infection, or pooled normal sera, does not result in increased phagocytosis of IRBC. The phagocytosis enhancing activity of immune sera is associated with the IgG fraction and IgG depleted sera do not stimulate phagocytosis. Enhanced immune serum mediated phagocytosis occurs as a result of opsonization of IRBC. This was demonstrated by experiments in which monocytes or IRBC were preincubated with immune serum prior to the phagocytic assay. The opsonic activity could be absorbed by IRBC but not by NRBC. The opsonization of IRBC and subsequent phagocytosis were also dependent on the stage of development of the intracellular parasite. IRBC containing schizonts and trophozoites were preferentially phagocytosed as compared with ring forms. The role of malaria induced surface alterations and/or malaria surface antigens in the opsonization of IRBC by immune sera is discussed. These experiments suggest that phagocytosis of *P. falciparum* IRBC by monocytes may play a role in the immune elimination of malaria infection in humans.

INTRODUCTION

Host defence mechanisms against malaria involve both humoral and cellular immunity but their respective roles have not been completely elucidated (Brown, 1976). There is ample evidence that anti-malarial antibodies can modify the course of malaria infections in primates. Passive transfer of immunoglobulins purified from the sera of adults living in endemic areas cause a dramatic fall in the asexual forms of *Plasmodium falciparum* and *Plasmodium malariae* in children (McGregor, Carrington & Cohen, 1963; Cohen, Butcher & Crandall, 1969). Similarly, human IgG purified from sera of patients from endemic areas in Nigeria provided partial protection to Aotus monkeys infected with *P. falciparum* (Diggs *et al.*, 1972). The mechanism(s) by which antibody mediated protection occurs is unclear. Two main hypotheses have been proposed; (1) anti-malarial antibody may bind to the surface of merozoites and block the invasion of RBC (McGregor *et al.*, 1963; Miller,

Abbreviations: NRBC = normal red blood cells; RBC = red blood cells; IRBC = infected red blood cells; PBS = phosphate-buffered saline; MEM = minimal essential medium; FCS = fetal calf serum.

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Powers & Shiroishi, 1977); (2) anti-malarial antibodies may opsonize infected red blood cells (IRBC) and/or merozoites which are subsequently phagocytosed by monocytes and macrophages (Shear, Nussenzweig & Bianco, 1979; Hunter, Winkelstein & Simpson, 1979). Consistent with the first hypothesis are the experiments which have demonstrated that sera from some individuals living in endemic areas can inhibit the growth of *P. falciparum* cultures *in vitro* (Wilson & Phillips, 1976). The second hypothesis is supported by evidence of involvement of the mononuclear phagocyte system in humans with acute malaria. Splenomegaly is observed in 70–80% of patients and hepatomegaly in 50% (Neva *et al.*, 1970). Histologically, enlargement of spleen and liver is primarily due to a tremendous hyperplasia of the reticuloendothelial system (Russel *et al.*, 1963). The phagocytic function of the mononuclear phagocytes is increased during malaria infection as shown by the increased rate of clearance of colloidal particles from the blood stream of these patients (Sheagren *et al.*, 1970). In addition, at the time of crisis in the infection, as the parasite count begins to decline, phagocytes have been observed to avidly engulf parasite pigments, parasitized red blood cells, and even NRBC (Russel *et al.*, 1963).

In this study we have investigated the capacity of human monocytes, either alone or in the presence of human serum from individuals with various degrees of immunity towards malaria, to phagocytose *P. falciparum* infected red blood cells.

MATERIALS AND METHODS

Monocytes. Fifty millilitres of heparinized blood (10 units/ml) from normal volunteers of the A+ blood group were obtained after informed consent. One volume of blood was diluted with 1 volume of 0·15 M phosphate-buffered saline (PBS). Aliquots of 30 ml were layered over 15 ml of Ficoll-Hypaque (1·077, sp. gr.; Ficoll 440, Pharmacia Fine Chemicals, Uppsala, Sweden, and Rompacon, Cilag-Chemie, Schaffhausen, Switzerland) and the samples were centrifuged at 400 ***g*** for 20 min at 4°C. Mononuclear cells were collected from the interface, washed three times with cold PBS and resuspended in Eagle's minimum essential medium (MEM) (Flow Laboratories, Irvine, Scotland). Differential leucocyte counts were performed on peroxidase-stained (Kaplow, 1965) cytocentrifuged smears. Cell suspensions consisted of mixtures of monocytes (22–40%) and lymphocytes. Granulocyte contamination was less than 2%. Viability, as assessed by the trypan blue dye exclusion test, was always more than 95%.

Culture of P. falciparum. The isolate SGE1 of *P. falciparum* was used. Parasites were grown in human RBC of A+ blood group by established methods (Trager & Jensen, 1976; Perrin *et al.*, 1980). NRBC were also cultured for 3–4 days to serve as control. The *P. falciparum* cultures were not synchronized and contained all erythrocytic stages (rings, trophozoites and schizonts). These cultures were enriched in mature parasites by differential centrifugation on the basis of their lower density. In brief, the IRBC were reconstituted as a 50% suspension of MEM and poured into glass tubes (10 cm long, containing 1 ml). The tubes were centrifuged for 15 min at 1,200 ***g***. Following centrifugation, the upper brown layer was collected. These preparations had parasitemia varying between 18 and 38% and were enriched in mature, erythrocytic stages of *P. falciparum* (ring forms: 10–18%, trophozoites: 25–50% and schizonts: 24–50%). This mild purification procedure did not affect the parasite viability.

In some experiments, preparations of mature forms (trophozoites and schizonts) and preparations of immature forms (ring forms) were tested separately. Both types of preparation were obtained from synchronized cultures (Lambros & Vanderberg, 1979). They were adjusted to a parasitemia of 9% and contained more than 98% of either ring forms or trophozoites and schizonts respectively.

Human sera. All sera were from blood group A+ individuals as follows: (a) sera from individual blood donors; (b) pooled sera from 20 blood donors; (c) sera from individuals living in areas endemic for malaria: six of these sera were from adult individuals living in Keneba, The Gambia, two were from adults living in the Ivory Coast; all these individuals had a history of repeated malarial infection, and (d) sera from European individuals recovering from a first acute *P. falciparum* infection, collected between 10 and 20 days after a curative treatment with chloroquine.

Immunofluorescent antibody (IFA) titre. Serum levels of antibodies to malaria were determined by the indirect fluorescent antibody technique with multispot antigen slides using rabbit antibody against human Ig labelled with fluorescein isothiocyanate as second reagent (O'Neill & Johnson, 1970).

Phagocytic assay. In each experiment, NRBC and IRBC were from the same blood donor. Fifty microlitres of either IRBC or NRBC containing 5×10^6 cells were mixed with 50 μl of the monocyte suspension ($2{\cdot}5 \times 10^5$ monocytes) and 20 μl of the serum to be tested in 3 ml sterile plastic tubes. In some experiments, no serum was added and in others, fetal calf serum (FCS) was used instead of human serum. The tubes were centrifuged at 200 ***g*** for 5 min at 4°C in order to increase contact between erythrocytes and monocytes. After centrifugation, tubes were incubated for 30 min at 37°C in a humidified 5% CO_2 and 95% air atmosphere. Pellets were then resuspended with a pasteur pipette and cytocentrifuged smears were performed. The number of phagocytosed RBC was determined morphologically on Giemsa-peroxidase stained smears. Results are expressed as the number of monocytes containing phagocytosed RBC and as the number of RBC endocytosed by 100 monocytes. In all cases at least 400 monocytes from duplicate smears were counted at × 1,000 magnification under oil immersion, by two independent investigators.

Absorption studies. In order to determine whether the phagocytosis promoting factors were cytophilic or opsonic, absorption studies were performed. The effect of preincubation of NRBC or IRBC with immune serum (or normal serum pool as control) on phagocytosis was tested. Twenty microlitres of immune serum were added to 50 μl of IRBC or NRBC suspension (5×10^6 cells). The mixture was incubated for 30 min at 37°C. The cells were then washed twice in 3 ml of MEM and centrifuged at 350 ***g*** for 15 min at room temperature. The supernatant fluid was aspirated and the RBC were resuspended in 20 μl of normal serum pool and 50 μl of MEM. Fifty microlitres of monocyte suspension ($2{\cdot}5 \times 10^5$ cells) were added. Afterwards, the phagocytic assay was carried out. In a second set of experiments, 20 μl of immune serum or normal serum pool were added to $2{\cdot}5 \times 10^5$ monocytes in 50 μl, and the mixture was incubated for 30 min at 37°C. After two washes, 20 μl of normal serum pool and IRBC or NRBC were added to the monocytes. The phagocytic assay was carried out as described above. In a third set of experiments, the effect of absorption of immune and non-immune serum with IRBC or NRBC on phagocytosis by monocytes was studied. A pellet of 10^9 IRBC or NRBC was resuspended and incubated with immune serum or normal serum pool (400 μl) for 30 min at 37°C. The mixture was then centrifuged at 350 ***g*** for 10 min at room temperature. The sera were collected and 20 μl of each were added to either IRBC and monocytes or NRBC and monocytes for the phagocytic assay.

Preparation of IgG serum fraction and IgG depleted sera. Normal IgG and immune IgG were prepared from the serum of a normal pool and from the serum of an adult living in Keneba by DEAE-cellulose chromatography (Fahey & Terry, 1978). These preparations contained only IgG as shown by immunoelectrophoresis analysis. The same sera were depleted of IgG by passage of 1 ml of serum through a 5 ml protein A-sepharose column (Pharmacia) (Hjelm, Hjelm & Sjöquist, 1972). The sera were reconstituted to their original volume by negative pressure dialysis and dialysed against MEM overnight before use.

Statistical analysis. The Wilcoxon ranking test was used for analysis of paired values and the *t*-test for unpaired values (Snedecor & Cochran, 1967).

RESULTS

Basic experimental conditions

In every instance, the phagocytic assay was performed using monocytes, NRBC or IRBC and sera from donors or patients of blood group A+. In 10 experiments the percentage of monocytes phagocytosing NRBC varied between 3 and 6%. The dose response and kinetics of phagocytosis were determined; (a) a ratio of 20 RBC for 1 monocyte was optimal, (b) centrifugation of the monocyte-RBC mixtures permits shortening of the duration of the assay and enhances phagocytosis by monocytes of both NRBC and IRBC and (c) an incubation period of 30 min at 37°C of the monocytes-RBC mixture was chosen to permit visualization of phagocytosed RBC prior to their degradation within the monocytes.

Phagocytosis of infected and non-infected RBC in the presence of normal human serum

Monocytes from seven normal blood donors phagocytosed IRBC to a greater extent than NRBC in the presence of normal serum; 11·5±4% of monocytes phagocytosed IRBC versus 7·6±2·9% for NRBC ($P<0.02$). Preferential phagocytosis of IRBC was also observed in the absence of serum; 8·2±1·8% of the monocytes phagocytosed IRBC versus 3·2±0·8% for NRBC ($P<0.05$), or, in the presence of FCS; 10·2±0·8% of the monocytes phagocytosed IRBC versus 3·3±1·6% for NRBC ($P<0.05$) (results expressed as the mean±1 s.d. of three experiments using in each instance monocytes from three different blood donors).

Phagocytosis promoting activity of human immune sera

Although normal monocytes preferentially phagocytose IRBC rather than NRBC, this effect is relatively modest. The next set of experiments was performed to evaluate the capacity of sera from individuals with varying degrees of immunity towards malaria infection to promote phagocytosis of IRBC. Sera from six individuals living in an endemic area in The Gambia were tested in the phagocytic assay. Each of these sera promoted phagocytosis of IRBC but not phagocytosis of NRBC (Table 1). These sera will be referred to as 'immune sera'. Sera from two individuals from the Ivory Coast, with respective antibody titres against *P. falciparum* of 1/400 and 1/800 also produced an enhancement of IRBC phagocytosis, respectively 28·4±3·2% and 26·9±4·8% IRBC ingested by 100 monocytes versus 7·2±1·3% and 6·5±0·6% NRBC ingested by 100 monocytes. Serial doubling dilutions in the normal serum pool of two immune sera (K8 and K14) were tested in the phagocytic assay. The phagocytosis promoting activity of these immune sera expressed as the percentage of monocytes containing IRBC was proportional to the concentration of immune serum in the assay and was effective for IRBC but not for NRBC; respectively 34·3 and 30% for 10% of serum K8 and K14, 25 and 20·5% for 2·5% and 16·4 and 15% for 1·25%. Phagocytosis of IRBC by monocytes was also tested in the presence of sera from seven europeans recovering from a first acute *P. falciparum*

Table 1. Effect of various immune sera on phagocytosis of IRBC and NRBC by monocytes

Patients' sera from The Gambia		% phagocytic monocytes	RBC ingested/ 100 monocytes	Anti-*P. falciparum* antibody titre
K2	IRBC	28·2±6·0*	34·2±5·8	1/3200
	NRBC	6·3±0·8	7·0±0·9	
K43	IRBC	32·5±8·7	39·7±10·20	1/6400
	NRBC	6·8±0·6	7·5±0·5	
K45	IRBC	32·0±2·7	38·0±3·5	1/12800
	NRBC	6·0±0·5	6·7±0·8	
K54	IRBC	30·5±2·3	36·2±1·0	1/6400
	NRBC	7·7±0·6	8·0±0·5	
K8	JRBC	30·8±5·6	39·5±7·9	1/6400
	NRBC	7·2±1·3	7·7±1·4	
K14	IRBC	31·7±6·8	41·3±9·8	1/12800
	NRBC	5·9±0·5	6·6±0·9	
Pooled serum	IRBC	9·5±1·0	10·3±1·0	negative
	NRBC	5·8±0·6	6·5±0·5	

* Each experiment was done with monocytes from three different individuals and results are expressed as the mean±1 s.d.

Using the *t*-test, a significant increase was found in phagocytosis of IRBC in the presence of individual immune serum as compared to that in the presence of pooled serum ($P<0.01$). Phagocytosis of NRBC in the presence of individual immune serum or normal pooled serum was not significantly changed ($P>0.05$).

infection. Despite the high levels of anti-*P. falciparum* antibodies detected in some of these sera (titres varying from 1/40 to 1/3200), they did not significantly increase phagocytosis of IRBC.

As IRBC preparations contained both IRBC and NRBC, the effect of the percentage of IRBC contained in the IRBC preparation used for the phagocytic assay was tested. IRBC were diluted with NRBC to keep constant the total number of RBC added in the assay (5×10^6). In the presence of normal monocytes and immune serum, the number of monocytes engulfing RBC decreased in parallel with the percentage of IRBC present in the IRBC preparation (Fig. 1). A similar effect was observed but at a lower level for the preferential phagocytosis of IRBC vesus NRBC in the presence of the normal serum pool.

Effect of preincubation of RBC or monocytes with immune serum

Experiments were performed in order to determine whether the immune serum phagocytosis promoting factor(s) were opsonic or cytophilic. IRBC, NRBC and monocytes (preincubated with either immune serum or pooled normal serum) were added to the phagocytic assay which was performed in the presence of pooled normal serum. The results (Table 2) indicate that IRBC preincubated with immune serum but assayed in the presence of pooled normal serum were avidly phagocytosed. In contrast, preincubation of NRBC (results not reported on the table for NRBC; less than 5% phagocytic monocytes and less than 6% RBC ingested/100 monocytes) or monocytes with immune serum or normal serum pool did not result in a significant increase in phagocytosis of IRBC or NRBC. When RBC and monocytes were preincubated with immune serum and the phagocytic assay was also performed in immune serum, increased phagocytosis of IRBC was observed as expected. These results suggest that the phagocytosis promoting factor(s) binds to IRBC.

Effect of absorption of immune serum on phagocytosis of IRBC by monocytes

In a representative experiment, an immune serum (K2) or the normal serum pool were incubated with IRBC or NRBC respectively to determine whether IRBC or NRBC can deplete the phagocytosis promoting factor(s) present in the immune serum and decrease the preferential phagocytosis of IRBC observed in the absence of immune serum. The results reported in Table 3 demonstrate that incubation of the immune serum with IRBC, markedly reduced the capacity of the

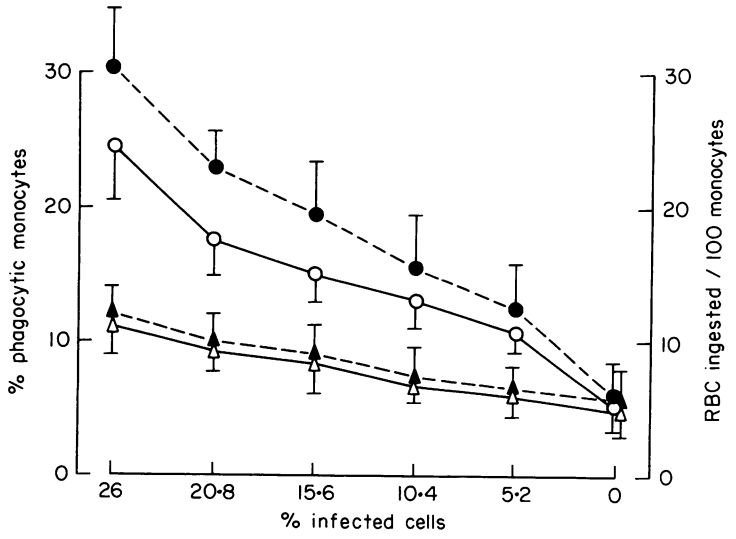

Fig. 1. Relationship of RBC phagoctyosis by monocytes to parasitaemia in presence of the same quantity of either immune serum (Patient K 14) (○ & ●) or normal serum pool (△ & ▲). Concentration of RBC was adjusted to 5×10^6 by addition of NRBC. Each point represents the mean ± 1 s.d. of monocytes taken from three normal donors. (○——△ = % phagocytic monocytes; ●- - - - -▲ = RBC ingested/100 monocytes).

Table 2. Effect of preincubation of IRBC or monocytes with immune or normal serum pool on phagocytosis by monocytes

Preincubation*	Cells and serum added† after wash	% phagocytic monocytes	RBC ingested/ 100 monocytes
IRBC + normal serum pool	Immune serum + monocytes	21·5 ± 6·1	26·7 ± 8·5
	Normal serum pool + monocytes	7·7 ± 3·2	8·0 ± 3·5
IRBC + immune serum	Immune serum + monocytes	21·3 ± 2·9	26·2 ± 4·0
	Normal serum pool + monocytes	18·5 ± 4·4	22·7 ± 6·7
Monocytes + normal serum pool	Immune serum + NRBC	4·5 ± 0·9	4·8 ± 0·8
	Normal serum pool + NRBC	5·3 ± 0·6	5·3 ± 0·6
	Immune serum + IRBC	22·3 ± 2·0	26·3 ± 2·3
	Normal serum pool + IRBC	8·7 ± 0·6	9·2 ± 0·8
Monocytes + immune serum	Immune serum + NRBC	4·7 ± 0·6	4·7 ± 0·6
	Normal serum pool + NRBC	4·2 ± 0·3	4L5 ± 0·5
	Immune serum + IRBC	22·3 ± 1·5	27·0 ± 1·0
	Normal serum pool + IRBC	8·3 ± 0·6	9·5 ± 0·5

* Preincubation for 30 min at 37°C with either a normal serum pool or serum from a patient from The Gambia (K 14) (serum concentration = 20%).

† After incubation, cells were washed twice with 3 ml MEM. Second incubation for 30 min at 37°C.

Table 3. Effect of absorption of immune and non-immune sera with IRBC or NRBC on phagocytosis by monocytes

Sera used for* absorption	RBC used for absorption	RBC used for† phagocytic assay	% phagocytic monocytes	RBC ingested/ 100 monocytes
Immune serum	IRBC	IRBC	17·2 ± 0·8‡	21·3 ± 1·8
		NRBC	7·8 ± 0·8	8·7 ± 1·3
	NRBC	IRBC	32·5 ± 4·4	40·5 ± 8·0
		NRBC	6·8 ± 0·8	7·2 ± 0·6
Normal serum pool	IRBC	IRBC	12·5 ± 1·3	15·2 ± 2·8
		NRBC	6·8 ± 0·8	7·7 ± 0·3
	NRBC	IRBC	13·8 ± 0·3	16·2 ± 0·6
		NRBC	7·8 ± 1·0	8·2 ± 1·3

* Normal serum pool and immune serum (K 2) were absorbed with NRBC or IRBC at 37°C for 30 min ($2·5 \times 10^8$ RBC/100 μl serum).

† For the phagocytic assay, monocytes ($2·5 \times 10^5$) were incubated with either NRBC or IRBC (5×10^6 cells) and 20 μl of the various absorbed sera.

‡ Each experiment was done with monocytes from three different individuals. Results are expressed as the mean ± 1 s.d.

immune serum to enhance the phagocytosis of IRBC by monocytes. In contrast, incubation of the immune serum with NRBC did not reduce the enhancement of the phagocytic activity of monocytes towards IRBC. In addition, the enhanced phagocytosis of IRBC as opposed to NRBC in the presence of the normal serum pool was not influenced by prior absorption of the normal serum pool with IRBC.

Evaluation of the opsonic effect of IgG preparations

In experiments performed using IgG purified from immune serum and IgG-depleted immune serum (Fig. 2), phagocytosis of IRBC was markedly enhanced upon addition of immune IgG. Inversely, IgG-depleted immune serum lost its activity. Immune IgG or IgG-depleted immune serum had no effect on phagocytosis of NRBC. IgG from a normal serum pool and normal serum pool passed through sepharose column displayed the same activity as the normal serum pool. These results show that opsonic antibodies of the IgG type are present in immune sera.

Opsonic activity of immune serum on IRBC containing ring forms, or trophozoites and schizonts

Immune serum enhanced the phagocytosis of IRBC containing mature forms (trophozoites and schizonts) (Fig. 3), whereas phagocytosis of IRBC containing rings was not enhanced by the addition of immune serum in the assay.

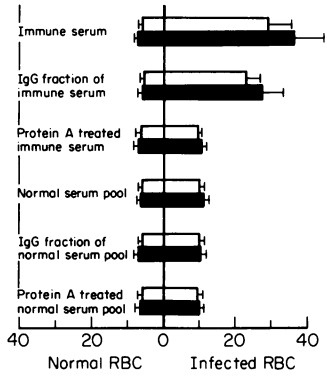

Fig. 2. Effect of Protein A treated serum and of IgG purified from immune serum or normal serum pool on the phagocytosis by monocytes of IRBC (Right) and NRBC (Left). Each bar represents the mean ± 1 s.d. of monocytes taken from three normal donors. (□ = % phagocytic monocytes; ■ = RBC ingested/100 monocytes).

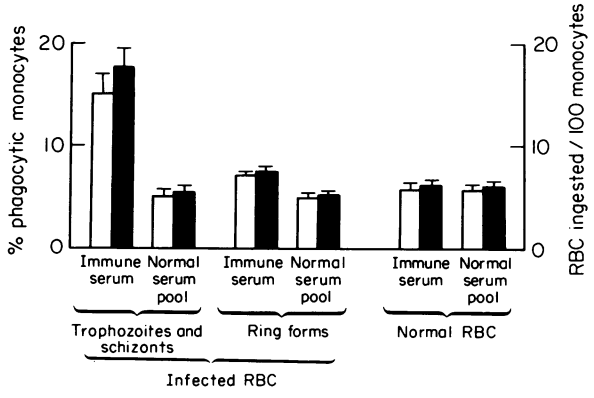

Fig. 3. Effect of immune serum or normal serum pool on phagocytosis by monocytes of IRBC containing parasites at various developmental stages. Open bars represent the % phagocytic monocytes and hatched bars the number of RBC ingested/100 monocytes. Each bar represents the mean ± 1 s.d. of monocytes taken from three normal donors. (□ = % phagocytic monocytes; ■ = RBC ingested/100 monocytes).

DISCUSSION

The present study demonstrates that human monocytes are able to phagocytose *P. falciparum* IRBC *in vitro*. This phagocytic activity is greatly enhanced by the presence of serum from individuals living in areas endemic for malaria. The phagocytosis promoting factor present in human immune serum is associated with opsonic IgG. Finally, only mature intra-erythrocytic developmental stages of *P. falciparum* are preferentially phagocytosed following addition of human immune serum in the phagocytic assay.

The *P. falciparum* IRBC preparations used in the phagocytic assay were prepared from *in vitro* cultures of *P. falciparum*. The suspensions were rich in trophozoites and schizonts which selectively accumulate on top of non-infected RBC on the basis of their lower density. This mild and quick purification procedure was selected because it avoids prolonged incubation and the numerous washes of IRBC preparations which are a drawback of other purification procedures (Reese, Langreth & Trager, 1979).

The monocyte preparations were obtained by the standard Ficoll-Hypaque cell separation procedure yielding a band of low density cells containing lymphocytes and monocytes. No attempt was made to further purify the monocyte population to avoid metabolic or surface changes that might alter monocyte functions. Macrophage activation occurs during malaria infection and the degree of this activation has been shown to modulate the extent of phagocytosis of *P. berghei* IRBC (Shear *et al.*, 1979). This parameter was not tested in our study since monocytes were from normal blood donors.

In our experimental conditions, IRBC were phagocytosed preferentially to NRBC by normal monocytes in presence of normal serum. Previous studies using a rodent model suggested that this phenomenon may be a consequence of non-specific binding of Ig to IRBC (Shear *et al.*, 1979). This effect is unlikely to occur in our system since increased phagocytosis of *P. falciparum* IRBC was also observed in serum-free medium. The increased phagocytosis of IRBC seems rather to be related to morphological changes of the IRBC surface (Kilejian, 1980).

In the phagocytic assay, phagocytosis of IRBC by human monocytes was markedly enhanced after addition of immune serum from individuals living in endemic areas. The humoral factor(s) promoting phagocytosis was associated with IgG and disappeared after removal of IgG from the immune sera. Preincubation of either IRBC or monocytes with immune serum only revealed the presence of opsonic antibodies. These results agree with *in vivo* experiments showing that macrophages and humoral factors behave synergistically to enhance destruction of IRBC in rodents infected with *P. berghei* (Criswell *et al.*, 1971; Green & Kreier, 1978). *In vitro* experiments in murine and primate systems have also shown that immune sera increase phagocytosis of IRBC by macrophages to a greater extent than non-immune sera (Brown & Hills, 1971; Chow & Kreier, 1972; Hunter *et al.*, 1979) and that this serum promoting factor is associated with opsonic IgG (Hunter *et al.*, 1979). In primary infections, other investigators have shown that the spleen, but not humoral factors, plays a major role in the clearance of IRBC (Quinn & Wyler, 1979a,b). The quality of the immune sera or the magnitude of the antibody response of the host could explain the discrepancies observed under different experimental conditions. In rodents, hyper-immune serum from rats challenged several times with *P. berghei* can reliably transfer protection to virgin rats whereas immune serum from rats rendered resistant to challenge by only one prior injection is less effective (Golenser, Spira & Zuckerman, 1975). In our experiments, the source of immune sera used in the phagocytic assay also plays a crucial role, since sera from individuals recovering from a first malaria infection did not promote phagocytosis of *P. falciparum* IRBC by monocytes.

Immune serum from individuals living in endemic areas enhances the phagocytosis of IRBC containing trophozoites and schizonts but not ring forms. The membranes of IRBC containing trophozoites and schizonts but not the membranes of IRBC containing ring forms have morphological alterations described as 'knob-like protrusions' (Kilejian, 1980). These knobs develop in parallel with the growth of parasites in RBC and they have been shown to be responsible for the adhesion of IRBC to venous endothelial cells (Luse & Miller, 1971). It is possible that adhesion of trophozoites and schizonts to monocytes in the absence of immune serum is related to the same phenomenon, thus explaining the non-immune phagocytosis of IRBC. It has also been

demonstrated that at least one malarial antigen is present in the knobs (Kilejian, 1980). Immunoelectronmicroscopic studies have shown that antibodies from monkeys immunized with *P. falciparum* react with the knobs (Langreth & Reese, 1979). Our absorption experiments support these findings, since the serum factor promoting phagocytosis is absorbed by intact IRBC but not by NRBC. Moreover the immune serum specifically enhances phagocytosis of IRBC containing trophozoites and schizonts but not IRBC containing rings. These experiments, however, do not exclude the possibility that opsonic antibodies may react with hidden determinants of the RBC membrane that are expressed as a consequence of malaria infection.

Some sera from individuals recovering from a first acute *P. falciparum* infection have a high level of anti-malarial antibodies as detected by indirect immunofluorescence; however, these sera do not enhance the phagocytosis of IRBC by monocytes. Indirect immunofluorescence is a global test for the detection of antibodies and does not reflect the heterogeneity of the antibody response. This point has been previously documented by testing the capacity of sera from individuals with various degrees of immunity towards malaria to react with ^{35}S-methionine-labelled *P. falciparum* antigens in immunoprecipitation experiments. It was found that some malarial antigens were only recognized by sera from individuals living in endemic areas but not by sera from individuals recovering from a first acute *P. falciparum* infection (Perrin *et al.*, 1981). It is possible that some malarial antigens, especially antigen(s) expressed in the knobs, are poorly immunogenic and that multiple exposures of the host to malaria infection is needed before significant amounts of antibodies are raised against these particular antigens. This may explain the differences observed in the capacity of various immune sera to enhance phagocytosis of IRBC.

The poor efficiency of sera from individuals recovering from a first infection versus the high efficiency of sera from individuals living in malaria endemic areas for the enhancement of phagocytosis of IRBC can also be related to experimental and epidemiological studies (McGregor & Williams, 1978). In humans, a single exposure to malaria infection has little or no protective effect for the host upon reinfection with *P. falciparum*. In contrast, adult individuals living in endemic areas have less frequent episodes of parasitemia than young children living in the same areas (McGregor & Williams, 1978). In addition, adults can control and rapidly clear their parasitemia upon reinfection (Wilson, Garnham & Swellingrebel, 1950; McGregor, 1960; McGregor & Williams, 1978). Our experimental data suggest that enhancement by opsonic antibodies of phagocytosis of IRBC by monocytes and macrophages may play a role in the clearance of malaria infection in these individuals.

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