Clin. exp. Immunol. (1986) **63,** 343–353.

Characterization of the humoral immune response in *Plasmodium falciparum* malaria. III. Factors influencing the coexpression of antibody isotypes (IgM and IgG-1 to 4)

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(Accepted for publication 31 July 1985)

SUMMARY

Isotypes (IgM and IgG-1 to 4) of anti-*P. falciparum* antibodies were investigated in sera of malarial patients or immune donors by enzyme linked immunosorbent assay (ELISA) and two indirect immunofluorescence assays (IFAs), one staining intra-erythrocytic parasites of all stages and the other a restricted number of parasite antigens deposited in the membrane of infected erythrocytes by invading merozoites (Perlmann & Wahlgren, 1983; Perlmann *et al.*, 1984, Wahlgren *et al.*, 1985a). There was no correlation in overall antibody titres between the two IFAs. Antibodies of both IgM and all four IgG isotypes were detected in both assays. With the IFA for intracellular parasites a brilliant fluorescence was obtained with antibodies of all isotypes. However IgG-2 antibodies often gave staining restricted to the surface of schizonts. The incidence and reactivity in individual sera of antibodies of the different isotypes did not relate to the immune status of the donors (acute infection or clinically immune) but related well to the degree of malarial exposure as reflected by the overall antibody titres. This, in all three assays, high titred sera frequently contained antibodies of all isotypes while low titred sera usually only contained antibodies of IgM, IgG-1 and IgG-3 isotype. On average, the overall expression of antibodies of different isotypes in individual sera appears to reflect a sequential down-stream (5′ to 3′) activation of the corresponding Igh-C genes in *P. falciparum* specific B-cell clones.

Keywords *Plasmodium falciparum* malaria IgG subclasses immunofluorescence ELISA

INTRODUCTION

Immunoglobulins of the different IgG-isotypes vary in their chemical structures and biological functions (Natvig & Kunkel, 1973; Spiegelberg, 1974). Generally, their concentrations in sera and body fluids do not directly reflect the frequencies of peripheral B cells expressing the corresponding isotypes (Simmons *et al.*, 1981). In humans, IgG subclass-restricted antibody responses to different types of immunogens have been described (Yount *et al.*, 1968). The factors regulating isotype production by B cells are being studied intensely in a variety of model systems (Howard & Paul, 1983). However, those which regulate isotype expression in the course of natural infections such as

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malaria are largely unknown. As antibodies of different isotypes may differ considerably in their protective capacity better knowledge of the regulatory processes governing isotype expression in infection is urgently needed.

In a first attempt to investigate these problems we have studied the coexpression and reactivity of antibodies of IgM- and the four different IgG-isotypes to *P. falciparum* antigens in a large number of individual human sera. Antibodies to intracellular parasites were studied by a conventional immunofluorescence assay (IFA) and by enzyme linked immuosorbent assay (ELISA) (Lundgren *et al.*, 1983; Wahlgren *et al.*, 1983a). In parallel, antibodies to parasite antigens deposited in the surface of infected erythrocytes by the invading merozoites were investigated in a modified IFA (Perlmann & Wahlgren, 1983; Perlmann *et al.*, 1984; Wahlgren *et al.*, 1985a; Franzén & Wahlgren, 1985). Antibodies to these latter antigens may be important for protection as they have been shown to efficiently inhibit parasite reinvasion *in vitro* (Wåhlin *et al.*, 1984) and to rise in parallel with the acquisition of clinical immunity (Wahlgren *et al.*, 1985b).

MATERIALS AND METHODS

Parasites. The Tanzanian F32 strain of *Plasmodium falciparum*, isolated in 1978, cultivated according to Trager and Jensen (1976) in red blood cells (RBC), blood group O, at a haematocrite of 5%.

Sera. (1) *Sera investigated by immunofluorescence.* Sera from Swedish donors (S) were either from patients with *P. falciparum* infection for the first time or from patients with a history of previous infections. Colombian sera (C) were all from patients with acute *P. falciparum* malaria but with unknown previous exposure. Some of the Liberian sera (L) were presumably from immune adults living in a malaria holoendemic environment (Björkman *et al.*, 1985). Other sera were from Liberian children with different degrees of clinical immunity.

(2) *Sera investigated by ELISA.* These sera were from children or adolescents (2–15 years) all living in a holoendemic area of Liberia (Wahlgren *et al.*, 1985b).

Mouse monoclonal antibodies (MoAb). Mouse MoAb were purchased from Seward Laboratory, London, UK (now Unipath Ltd, Bedford, UK). The antibodies were derived from the following clones: anti-IgM, AF6 (1·8 mg/ml); anti-IgG-1, JL512 (4·0 mg/ml); anti-IgG-2, GOM II (2·6 mg/ml); anti-IgG-3, ZG4 (4·4 mg/ml); anti-IgG-4, RJ4 (2 mg/ml) (Lowe *et al.*, 1982).

P. falciparum IFA. Indirect immunofluorescence of intracellular *P. falciparum* antigens was performed on monolayers of air dried infected erythrocytes (Lundgren *et al.*, 1983). For analysis of parasite antigens in the membrane of infected erythrocytes the monolayers were fixed with glutaraldehyde (GA) and air dried (Perlmann *et al.*, 1984). The slides were stained with a FITC conjugated sheep anti-human immunoglobulin, diluted 1/20 or with a biotinylated polyvalent anti-human immunoglobulin and FITC conjugated avidin. For analysis of the isotypes of antiparasite antibodies the antigen coated slides were incubated with test serum (diluted 1/25 1/40), mouse MoAb (1/20) and affinity purified and FITC-conjugated rabbit anti-mouse immunoglobulin antibodies (P/F ratio 3.25, diluted 1/10). Before conjugation the antibodies were extensively absorbed with human immunoglobulin. Indirect immunofluorescence of free parasites after saponin lysis of infected erythrocytes was performed as described by David *et al.* (1984).

P. falciparum ELISA. The assay was performed as before (Wahlgren *et al.*, 1983a,b) with an antigen from a Percoll-enriched trophozoite-schizont fraction of infected erythrocytes. Sera were extensively absorbed with human RBC ghosts and diluted: 1/1000; 1/5000, 1/25000 when tested for IgM and total IgG 1/200, 1/800, 1/3200 when tested for IgG isotypes (Wahlgren *et al.*, 1983a, b).

RESULTS

To assess possible differences in the sensitivity of the assays for the different IgG subclasses, the reagents used in this study were tested in ELISA in which the wells were coated with 10 μg of myeloma proteins of IgG-1 to -4 isotypes (Wahlgren *et al.*, 1983b). The order of sensitivity of the

assays was IgG-4 > IgG-3 > IgG-1 > IgG-2, with the test for IgG-4 being approximately four times as sensitive than that for IgG-2.

Immunofluorescence on intracellular parasites

In general, the serum titres for donors more extensively exposed to the infection were not higher than those for donors with less exposure (Table 1). Antibodies of IgM isotype and of all four IgG subclasses were found in 10/31 sera. However, while the presence of IgM, IgG-1 and IgG-3 antibodies was not obviously related to the overall titres, low titred sera only rarely contained IgG-4 antibodies and were frequently also deficient in IgG-2 antibodies. The extent to which antibodies of the different IgG subclasses were coexpressed in individual sera is shown graphically in Fig. 1a. In

Table 1. Immunofluorescence of intraerythrocytic *P. falciparum*

Patient	Immune-status	Titres	IgM	IgG-3	IgG-1	IgG-2	IgG-4
A.M. -C	A U	15,625	+	+	+	+s	−
12K303-L	− U	3,125	−	+	+	+s	+
Y1 -L	− U	3,125	−	+	+	+s	+
J-O.R. -C	A U	3,125	−	+s	+s	+s	−
H.M. -C	A U	3,125	−	+	+	+	+
X.R. -C	A U	3,125	+	+	+	+	−
G.T. -S	A U	3,125	+	+	+	+	+
B.L. -S	A F	3,125	+	+	+s	−	+s
B.J. -S	A F	3,125	+	+	+	+s	+
X.G. -L	− U	3,125	−	+	+	+s	−
12K304 -L	− U	3,125	+	+	+	+	−
ISL1 -L	− I	1,280	+	+	+	+	+
ISL2 -L	− I	1,280	+	+	+	+s	+
ISL3 -L	− I	1,280	+	+	+	+	+
ISL4 -L	− I	1,280	+	+	+	+	+
X12 -L	− U	625	+	+	+	+	+
Y11 -L	− U	625	+	+	+	+	+s
K.P. -S	A F	625	−	+	+	+s	−
A.C. -S	A U	625	+	+	+	+s	+
F.G. -C	A U	625	−	+	+	−	+
A-R.F -C	A U	625	−	+	+	+s	+
S.L. -S	A F	625	+	+	+	+s	−
B.T-W. -S	A U	625	+	+	+	+	+
B.P. -S	A U	625	−	+	+	+s	−
X29 -L	− U	625	+	+	+	+s	−
J-E.C. -C	A U	625	−	+	+	−	−
G.G. -L	− U	125	−	+	+	+s	−
X51 -L	− U	125	+	−	+	−	−
Y2 -L	− U	125	−	+	+	+s	−
Y.C. -S	A F	125	+	+	+	−	−
M.V. -C	A U	25	−	+	+	+s	−

Indirect immunofluorescence of intraerythrocytic parasites obtained with human sera (diluted 1/40) and monoclonal antibodies for IgM or the four human IgG subclasses and FITC-conjugated rabbit anti-mouse immunoglobulin. Overall anti-parasite titres are the reciprocal of the highest dilution giving positive staining. − = all parasites negative; + = all parasites positive; s = surface staining only of late stage parasites. C = sera from Colombian patients; S = Swedish, L = Liberian; A = patient with acute *P. falciparum* malaria; F = first infection; I = clinically immune; U = unknown exposure.

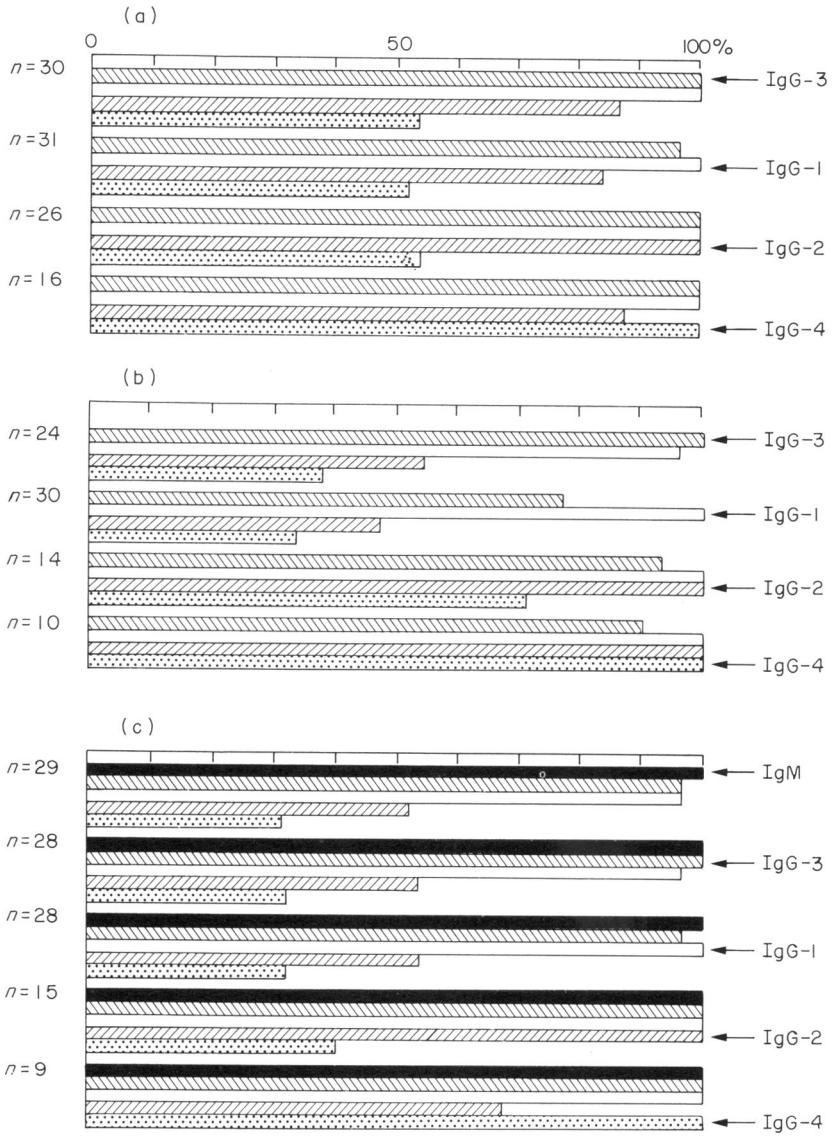

Fig. 1. Relative number of sera (%) coexpressing anti-*P. falciparum* antibodies of different IgG subclasses (in c also IgM) seen when a given isotype (←) is expressed. (a) IFA for intraerythrocytic parasites; (b) IFA for parasite antigens in the membrane of infected erythrocytes; (c) ELISA using a *P. falciparum* trophozoite-schizont antigen. n = number of sera in each group; (■) IgM; (□) IgG-1; (▩) IgG-2; (▧) IgG-3, (▨) IgG-4.

this figure the isotypes are given in the order in which the corresponding Igh-C genes are arranged on chromosome 14 (Flanagan & Rabbitts, 1982). Thus, when IgG-4 antibodies were present, there was almost 100% coexpression of the three IgG isotypes encoded 5′ to IgG-4. Similarly when IgG-2 antibodies were present IgG-1 and IgG-3 antibodies were also present. IgG-1 and IgG-3 were coexpressed in almost all sera.

Some representative staining patterns of infected erythrocytes are shown in Fig. 2. Antibodies of IgG-1, -3 and -4 isotype usually gave a brilliant staining of all parasite structures (Fig. 2a). Similar patterns were obtained with 10/26 sera when examined for staining with IgG-2 antibodies. However in the remaining 16 sera IgG-2 only stained the surface of late stage intracellular parasites when probed with the anti-IgG-2 reagent (Fig. 2b; assigned with the letter 's' in Table 1). Titration of a few

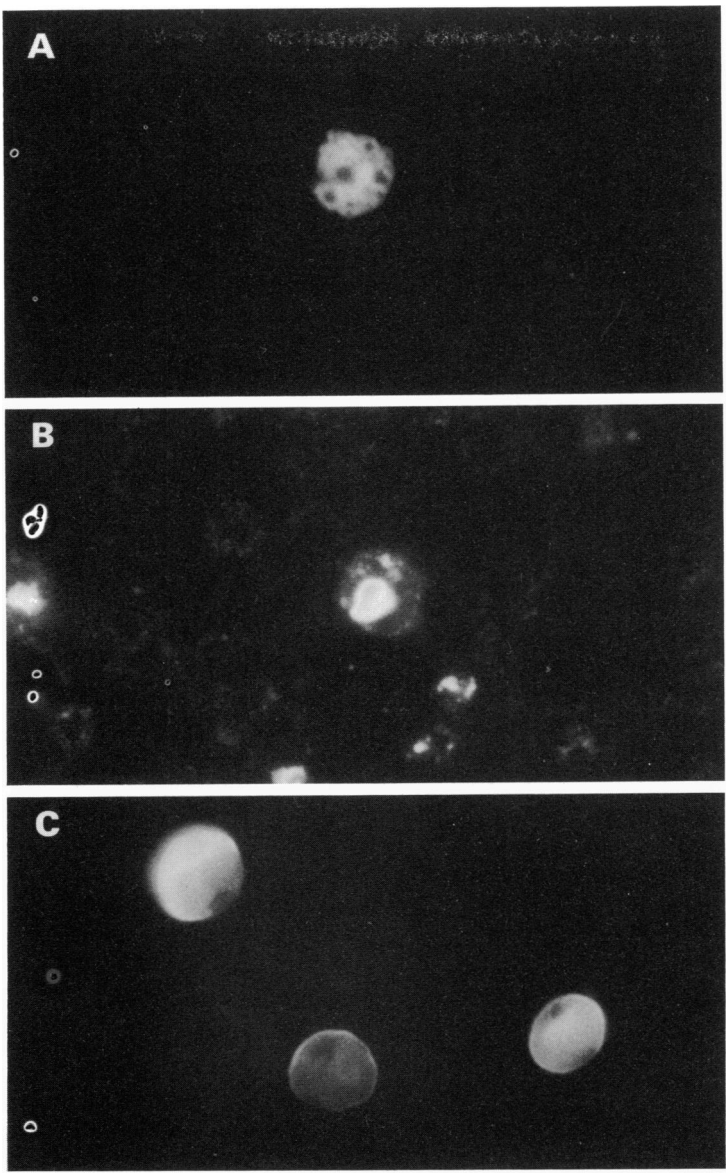

Fig. 2. Indirect immunofluorescence of (A) Intraerythrocytic *P. falciparum* stained for IgG-1 antibodies. Similar patterns were seen with all other isotypes; (B) staining of the surface of late stage asexual intracellular parasites frequently seen with IgG-2 antibodies; (C) indirect immunofluorescence of the surface of infected RBCs stained for IgG-1 antibodies similar patterns were obtained with all other isotypes.

sera with the different subclass reagents did not change the typical patterns shown in Fig. 2. Parasites freed of erythrocyte membranes by saponin lysis displayed a strong and even surface staining when probed with anti-IgG-2 antibodies. Internal parasite structures were not stained, indicating that the parasites were intact (data not shown).

Immunofluorescence of the membrane of infected erythrocytes

To stain antigens deposited in the membrane of recently infected erythrocytes, the cells are fixed with GA and air dried as described elsewhere (Perlmann *et al.*, 1984). The major antigen responsible

Table 2. Immunofluorescence of *P. falciparum* antigens in the membrane of infected erythrocytes

Patient	Immune status	Titres	IgM	IgG-3	IgG-1	IgG-2	IgG-4
A.M. -C	A U	15,625	+	+	+	–	–
A.C. -C	A U	3,125	+	+	+	+	–
B.P. -S	A U	3,125	+	+	+	+	+
X.G. -L	– U	3,125	+	+	+	+	+
B.J. -S	A F	2,500	+	+	+	+	+
ISL1 -L	– I	625	+	–	+	+	+
ISL3 -L	– I	625	+	+	+	+	+
G.T. -S	A U	625	–	+	+	+	–
T.P. -S	A U	625	+	+	+	+	+
M.V. -C	A U	625	–	+	+	+	–
X51 -L	– U	625	–	+	+	–	–
G.G. -L	– U	625	+	+	+	+	–
A-R.F. -C	A U	250	+	+	+	+	+
B.L. -S	A F	250	–	+	+	–	–
Y11 -L	– U	250	+	+	+	+	+
ISL2 -L	– I	125	–	–	+	–	–
ISL4 -L	– L	125	+	+	+	+	+
H.M. -L	A U	125	–	+	+	+	+
B.T-W. -S	A U	125	+	+	+	–	–
J-O.R. -C	A U	125	+	+	+	–	–
Y.C. -S	A F	125	+	–	+	–	–
X29 -L	– U	125	–	+	+	–	–
H.A. -C	A U	125	–	–	+	–	–
X.R. -C	A U	50	–	–	+	–	–
J-E.C. -C	A U	25	–	+	+	–	–
K.P. -S	A F	25	–	–	+	–	–
Y1 -L	– U	25	–	+	+	–	–
12K303-L	– U	25	+	+	+	–	–
Y2 -L	– U	25	–	–	+	–	–
S.L. -S	A F	25	+	+	+	–	–
X12 -L	– U	5	+	–	–	–	–
X27 -L	– U	5	+	+	–	–	–

Indirect immunofluorescence of parasite antigens in the membrane of infected erythrocytes. Human sera were diluted 1/25. For other details see legend to Table 1.

for this staining is a merozoite derived antigen of 155,000 kD (Pf 155) (Perlmann *et al.*, 1984, Wåhlin *et al.*, 1984, Wahlgren *et al.*, 1985a). The serum titres obtained by using this procedure ranged from 1/5 to 1/15 625 (Table 2). Both IgM antibodies and antibodies of all four IgG subclasses exhibited the typical erythrocyte surface staining (Fig. 2C). As above, antibodies of IgG-2 or IgG-4 isotype were mainly found in high titred sera (> 1/250, Table 2), IgM antibodies were present in both high and low titred sera (19/32). When IgG-4 antibodies were present, antibodies of IgG-2, IgG-1 and IgG-3 isotype were found in almost every case (Fig. 1b). Similarly, when IgG-2 antibodies were expressed, IgG-1 and IgG-3 antibodies were usually also present. However—in contrast to what was seen in the IFA for intracellular parasite antigens—whilst all sera contained IgG-1 antibodies, only 75% of them appeared to be positive for IgG-3 antibodies. Of the 19 sera containing IgM antibodies, 100% coexpressed IgG-1 and 90% IgG-3 while expression of IgG-2 and IgG-4 was approximately 50% (Table 2).

Relationship between the two immunofluorescence assays

To establish whether the serum titres obtained with the two different methods could be correlated,

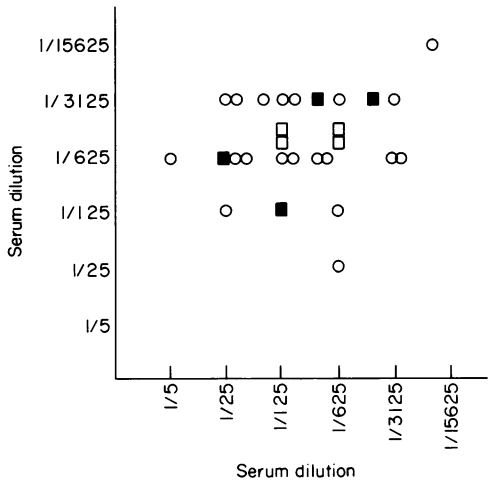

Fig. 3. Serum titres (IFA) of 28 sera to intracellular parasites (*y*-axis) and to parasite antigens in the membrane of infected erythrocytes (*x*-axis). (○) Patient with unknown previous exposure to *P. falciparum*; (■) first infection; (□) clinically immune.

the titres of individual sera were plotted as shown in Fig. 3. In general, there was no statistically significant correlation between the titres of individual sera as determined in the two assays ($r = 0{\cdot}08$, $P = 0{\cdot}688$, Spearman's rank correlation).

Isotype expression determined by anti- P. falciparum ELISA

The sera from 49 children and adolescents living in a holoendemic area of Liberia (Björkman *et al.*, 1985) were tested in ELISA (Wahlgren *et al.*, 1985b). These serum donors constituted a homogenous group with regard to *P. falciparum* malaria exposure but differed in age distribution (i.e. in duration of exposure) (Björkman *et al.*, 1985). All sera were tested at three different dilutions as described. At dilutions assuring optimal anti-*P. falciparum* reactivity under the experimental conditions applied (Wahlgren *et al.*, 1983a) a good correlation was found between the total IgG values and the sum of the values for the four IgG subclasses ($r = 0{\cdot}79$, correlation analysis). The mean OD_{405} values were for IgG antibodies (total) $0{\cdot}44 \pm 0{\cdot}35$ for IgM $0{\cdot}34 \pm 0{\cdot}29$, IgG-3 $0{\cdot}46 \pm 0{\cdot}44$, IgG-1 $0{\cdot}36 \pm 0{\cdot}27$, IgG-2 $0{\cdot}21 \pm 0{\cdot}21$ and IgG-4 $0{\cdot}20 \pm 0{\cdot}35$ (39 donors available for testing). Sera which had IgG-anti- *P. falciparum* reactivities above the mean also had elevated IgM antibody reactivity and were frequently positive for IgG antibodies of all four isotypes. In contrast, sera with IgG reactivity below average frequently lacked or displayed very low reactivities for IgG-2 or IgG-4 antibodies (Table 3). In Fig. 1c, the relative number of sera giving positive ELISA values in the different isotype tests were plotted in the same fashion as for the IFA analysis in Fig. 1a, b. The 5′ located genes coding for IgM, IgG-3 and IgG-1 appear to be expressed together as a group at significantly higher frequencies than the downstreams located genes coding for IgG-2 and IgG-4 antibodies. The reduced incidence of IgG-2 antibodies in the IgG-4 containing sera may reflect the relatively slow postnatal development of this isotype (Oxelius, 1979).

DISCUSSION

The monoclonal antibodies used to assay for anti-malarial antibodies of different IgG isotypes have earlier been shown to be subclass specific both in ELISA, haemagglutination inhibition and indirect immunofluorescence (Wahlgren *et al.*, 1983b; Lowe *et al.*, 1982; Mayumi *et al.*, 1983). As the different monoclonal reagents differed in their binding capacities, the sensitivities of the assay for different subclasses varied so that the least common subclasses (IgG-4 & IgG-3) (Wahlgren *et al.*, 1983b) were the most easily detected. On the other hand, in the immunofluorescence assays,

Table 3. Anti-*P. falciparum* reactivity in different sera determined by ELISA

Patient	IgG	IgM	IgG-3	IgG-1	IgG-2	IgG-4
64	1·40	1·30	>2·00	0·68	0·59	0
63	1·30	0·34	1·35	1·00	0·67	nt
L6	1·30	0·52	0·50	0·74	0·95	0·30
L7	1·18	0·75	0·60	0·73	0·35	0
K1	1·10	0·85	0·80	0·96	0·39	0·07
94	1·10	0·74	1·79	0·59	0·22	0
L1	0·88	0·72	1·63	0·60	0·33	0·41
83	0·75	0·13	0·45	0·50	nt	0
74	0·73	0·12	0·82	0·46	0·13	0·59
13	0·70	0·20	1·10	0·86	0·10	1·00
73	0·60	0·23	0·54	0·14	nt	0·86
L4	0·58	0·75	0·50	1·02	0·44	0·13
61	0·56	0·17	0·62	0·27	0·08	0·05
L3	0·47	0·85	0·47	0·55	0·41	0·09
69	0·46	0·47	0·54	0·17	0·32	0·05
29	0·46	0·15	0·31	0·30	0·04	0·19
96	0·45	0·50	0·38	0·22	nt	0
27	0·44	0·06	0·25	0·25	nt	0·33
18	0·40	0·13	0·51	0·31	0	1·20
34	0·40	0·34	0·66	0·10	0·19	0
24	0·38	0·23	0·50	0·11	nt	0·75
22	0·36	0·11	0·60	0·33	0·07	0
67	0·36	0·18	0·52	0·24	0·08	0·32
30	0·34	0·12	0·26	0·15	0	0
L2	0·31	0·60	0·26	0·31	0·26	0·09
26	0·31	0·18	0·40	0·19	nt	0
80	0·29	0·05	0·35	0·44	0·25	nt
85	0·28	0·05	0·17	0·53	0·26	nt
39	0·25	0·64	0·08	0·26	0	0
79	0·24	0·23	0·07	0·08	0	0
L8	0·23	0·69	0·21	0·54	0·39	1·20
78	0·23	0·20	0·35	0·10	0	0·05
72	0·23	0·16	0·32	0·54	0·22	nt
L5	0·22	0·74	0·17	0·46	0·40	0·05
19	0·21	0·20	0·24	0·10	0	0
35	0·21	0·30	0·16	0·13	0·05	0
37	0·20	0·11	0·25	0·19	0	0
84	0·20	0·32	0·31	0·10	0	0
93	0·20	0·66	0·16	0	nt	0
68	0·20	0·15	0·16	0·52	0·26	nt
75	0·19	0·18	0	0·10	nt	0
71	0·17	0·24	0·19	0	nt	0
36	0·17	0·20	0·13	0·60	0·18	nt
15	0·15	0·50	0·40	0·09	0	0
31	0·13	0·13	0·27	0·12	0	0
12	0·11	0·10	0·18	0·30	0·07	nt
70	0·07	0·10	0·13	0·10	0·10	nt
21	0·06	0·03	0·05	0·24	0·12	nt
65	0·05	0·05	0·05	0·35	0·10	nt

ELISA reactivities (OD, 405 nm) of sera from Liberian children and adolescents (diluted: 1/1000 for IgM, 1/5000 for IgG and 1/200 for IgG-1 to -4) with *P. falciparum* trophozoite; schizont antigen. nt Not tested.

antibodies of these rare IgG subclasses, as well as antibodies of low affinity, would be expected to be underestimated because of competition at the antigen level with more common isotypes. Moreover the shorter half-life of IgM and IgG-3 as compared with IgG-1, -2 or -4 (Spiegelberg, 1974) could also result in low estimates of these two isotypes, particularly in low titred sera or in some sera collected late after exposure of the donor to the disease. Although it cannot be ruled out that these factors may have influenced some of the results, they can only account for minor distortions. Thus IgM or IgG-3 antibodies were present in both high and low titred sera without preference. Moreover, titrations of a few selected sera in the IFA for total anti- *P. falciparum* antibodies of each of the isotypes gave the same relative subclass ratios as testing them under standard conditions (data not shown). Similar patterns were also obtained when anti-*P. falciparum* isotypes were estimated by ELISA (Wahlgren *et al.*, 1983b). As this assay is performed in antigen excess, errors due to antibody competition at the antigen level are unlikely to occur (Wahlgren *et al.*, 1983b). Finally, as only two of the sera tested (IFA) herein contained significant levels of rheumatoid factor when tested at low dilutions, the results record anti- *P. falciparum* isotypes rather than those of anti-immunoglobulins (data not shown).

There was no obvious correlation between the immune status (acute infection or clinically immune) of the serum donors and the overall IF titres in the assay for intracellular parasite antigen. Antibodies of each of the isotypes were found to react with all parasite structures as well as with the membranes of infected erythrocytes. However, antibodies staining the surface of the parasites were frequently of IgG-2 isotype. This IgG-2 staining was usually confined to late stage parasites and was well correlated to the surface immunofluorescence of parasites isolated by saponin lysis of the infected erythyrocytes. This staining probably reflects a preferential reactivity of IgG-2 antibodies with the major surface glycoprotein of relative molecular mass 195,000 present on schizonts (Holder & Freeman, 1982, Wahlgren *et al.*, in preparation).

IFA performed on monolayers of GA fixed and air dried erythrocytes selectively stains the membrane of infected red cells; most conspicuously in early stages of infection (Perlmann & Wahlgren, 1983; Perlmann *et al.*, 1984; Wahlgren *et al.*, 1985a). Although this staining is confined to a small number of parasite antigens, antibodies of all isotypes were seen in approximately 25% of the sera. The overall IF titres obtained in this assay were not correlated with those obtained in the conventional assay. This was in line with the results of a previous study of the sera from children and adolescents living in a *P. falciparum* holoendemic area (Wahlgren *et al.*, 1985b).

We have previously shown that the concentrations of anti-*P. falciparum* antibodies of different IgG isotypes in malarial sera parallel those of the total subclass concentrations (IgG-1 > IgG-2 > IgG-3 > IgG-4) (Wahlgren *et al.*, 1983b). In this study we have investigated the expression in individual sera of anti-*P. falciparum* antibodies of different isotypes. Similar results were obtained both in the IFAs applied to a serum material from donors who were heterogenous with regard to malaria exposure and in an anti-*P. falciparum* ELISA of sera that were homogeneous with regard to the donors' malarial experience (Wahlgren *et al.*, 1985b). Although high titred sera in both the two of the IFAs and in ELISA contained IgG antibodies of all four isotypes, a majority of the low titred sera contained primarily IgM, IgG-3 and IgG-1 antibodies. As shown elsewhere, this also holds true when the sera are analysed for isotype reactivity at the level of many individual parasite antigens (Wahlgren *et al.*, unpublished). Generally, most sera containing IgG-4 antibodies also contained antibodies of the other three IgG isotypes and IgM. Similarly, sera containing IgG-2 antibodies usually also contained IgG-1, IgG-3 and IgM antibodies. The latter three were frequently coexpressed. Thus, although there were some exceptions, the overall expression of antibodies of these isotypes in individual sera seems to parallel the 5′ to 3′ sequence of the Igh-C genes (5′-μ-δ-γ_3-γ_1-α_1-ψ-γ_2-γ_4-ε-α_2-3′) (Flanagan & Rabbitts, 1982). This suggests that isotype expression, on average, reflects a sequential downstreams activation of Igh-C genes in parasite specific B cell clones, as previously reported for various mouse models (Mongini, Paul & Metcalf, 1982; Gearhart, Hurwitz & Cebra, 1980). Isotype patterns suggesting such mechanisms have also been reported for several human systems (Scott & Nahm, 1984; Le thi Bich-Thuy & Revillard, 1984; Hammarström, Persson & Smith, 1984). Thus a downstreams activation of Igh-C genes in specific B cell clones may be an important feature in the course of natural immunization associated with infection and this may be related to the intensity of immunization. It remains to be established whether gene

activation occurs sequentially in a stepwise fashion or comprises coordinated expression of neighbouring genes (Shimizu & Honjo, 1984). Furthermore, the important question of how isotype switching in malaria infection is regulated by the T-cell system remains to be elucidated. Obviously, these results are the overall reflection of what is going on at the molecular level. As the underlying immunizations include a large number of different antigen specificities there may be a significant frequency of isotype restrictions (Yount *et al.*, 1968) occurring at the level of individual immunogens or antigenic epitopes. To approach these problems, we are now studying antibodies specific for some of the repeated peptide epitopes constituting an important feature of many plasmodial antigens (Cowman *et al.*, 1985).

This work was supported by grant no. B85-16X-00148-21A from the Swedish Medical Research Council, the Rockefeller Foundation, Great Neglected Diseases Network, New York, the Swedish Agency for Research Cooperation with Developing Countries (SAREC), the Swedish Medical Society and the Epidemiology Component of the UNDP/World Bank WHO Special Programme for Research and Training in Tropical Diseases. M. Wahlgren is holder of fellowship no. B85-16P-6892-O2B from the Swedish Medical Research Council.

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