Clin. exp. Immunol. (1980) **41**, 81–90.

Direct antiglobulin reactions in Gambian children with *P. falciparum* malaria

III. EXPRESSION OF IgG SUBCLASS DETERMINANTS AND GENETIC MARKERS AND ASSOCIATION WITH ANAEMIA

CHRISTINE A. FACER *Department of Haematology, The London Hospital Medical College, London*

(Accepted for publication 24 January 1980)

SUMMARY

The IgG subclass and Gm allotype distribution of red cell-bound IgG molecules from Gambian children with past or present *falciparum* malaria has been determined. The results show that the antibody is polyclonal with some predominance of the IgG2 and IgG4 subclasses. A restriction towards IgG2 antibodies may indicate specificity for a schizont-derived antigen which is carbohydrate in nature. Not all the allotypes normally carried by the G3m(b) allele could be demonstrated on IgG3-sensitized cells, a finding which remains unexplained. Expression of G3m(10), (11) and (14) allotypes of the IgG3 molecule was noted. Sensitization of red cells with IgG1 molecules correlated with the presence of anaemia but red cells sensitized with IgG2, IgG3 or IgG4 usually came from children with haematological findings within the normal range for that population. The implications of the results are discussed with reference to the few reports on the subclass and Gm allotype of malaria-specific IgG.

INTRODUCTION

Gambian children with past or present *Plasmodium falciparum* malaria often have a positive direct antiglobulin test (DAT) with red cells sensitized with IgG with or without complement components (Facer, Bray & Brown, 1979). In most cases the IgG eluted from the sensitized cells was found to have specificity for *P. falciparum* schizont antigens. It was concluded that these positive tests were a result of a Gell and Coombs Type II or Type III cytotoxicity involving malaria antigen–antibody complexes (Facer, 1980).

One question, however, still remained unanswered. Although a positive DAT was associated with the presence of anaemia in children with acute *falciparum* infections, there were many children, particularly those with chronic low grade parasitaemias, who had sensitized red cells but no anaemia. Theoretically, IgG-sensitized red cells in the absence of concurrent complement sensitization might be removed from the circulation by binding to blood, splenic and hepatic mononuclear phagocytes which possess receptors for the Fc fragment of IgG (Kay & Douglas, 1977). In addition, Fc receptors are also present on K cells and activated T cells which may cause antibody-dependent lysis of sensitized cells (Logue & Rosse, 1976). However, in our study, many Gambian children had IgG-sensitized red cells persisting in the circulation for up to 6 weeks after initial observation (Facer *et al.*, 1979).

Correspondence: Dr Christine A. Facer, Department of Haematology, The London Hospital Medical College, Turner Street, London E1 2AD.

0099-9104/80/0700-0081$02.00

The present studies were designed to investigate the IgG component of the immune complex bound to the red cells in more detail for two main reasons. First, since the IgG subclass is important in determining the degree of haemolysis of antibody-sensitized cells (Gilliland, 1976; Lalezari, 1976), it would be of interest to define the subclass on *in vivo*-sensitized cells. Second, since specific antibodies, including red cell antibodies, often favour a single Gm allotype out of the possible Gm phenotype of any individual (Litwin, 1973; Le Petit *et al.*, 1976), Gm markers on the antibodies should also be investigated.

MATERIALS AND METHODS

Patients

Fifty-three children from three areas were investigated. Firstly, twenty-three children presenting with acute *P. falciparum* malaria at the MRC Laboratories, Fajara, during the rains of 1976 and 1977. Secondly, ten children with chronic low-grade parasitaemias from the village population of Brefet having an age range of 1–6 years. The third group consisted of twenty primary school children with an age range of 7–9 years. All the children had a strongly positive direct antiglobulin test with anti-IgG. Children younger than 8 months were excluded from the study because maternally derived IgG might interfere with the Gm phenotyping of the patient (Grubb, 1970; Morell, Skvaril & Barandun, 1976). Further details of the three groups of children have been given in previous communications (Facer *et al.*, 1979; Facer, 1980).

Subclass of IgG on in vivo-sensitized cells

The IgG subclass was determined directly on patients' red cells with specific anti-subclass sera. Sheep antisera to human IgG1 (Nos 244–211), IgG2 (No. Z524R) and IgG4 (No. Z428E) were obtained from the Department of Experimental Pathology, University of Birmingham. A rabbit anti-IgG3 was prepared at The London Hospital by injecting rabbits with 1-mg amounts of purified IgG3 myeloma protein in Freund's complete adjuvant intramuscularly at weekly intervals for 6 weeks. The antiserum was then absorbed three times with 2-mg amounts of purified IgG1, IgG2 and IgG4 proteins. All antisera were heat-inactivated at 56°C for 30 min and absorbed with blood group O RIR2 erythrocytes for 1 hr at 37°C and 4°C. Specificity of antisera was checked by agar immunodiffusion and by agglutination of red cells sensitized with purified IgG subclass proteins using the chromic chloride method (Parish & Hayward, 1974). Sensitized cells gave strong 4+ agglutination with titres exceeding 1,024 with the appropriate subclass antisera. There were no cross-reactions.

To determine the IgG subclass a 2·5% suspension of the patients' red cells was added to doubling dilutions of subclass antisera in microtitre V-plates and left for 1 hr at 37°C when the presence or absence of agglutination was recorded (Facer *et al.*, 1979).

Gm allotypes on in vivo-sensitized cells

Typing was carried out for the following antigens: G1m(1), (2), (3) and G3m(5), (10), (11) and (14). The allotypes determined and the reagents used are shown in Table 1. The numerical nomenclature for genetic factors as recommended by the WHO is used (WHO, 1976). The control reference sera with known Gm phenotypes were as follows:

1/K/75: G1m(1, 2, 17): G2m(−23); G3m(21); Km(−1, −2);
2/J/75: G1m(3); G2m(23); G3m(5, 10, 11, 13, 14); Km(1, 2).

All reagents and reference sera, unless otherwise stated, were kindly provided by Diana Brazier, MRC Blood Group Reference Laboratory. Anti-Gm sera were first heat-inactivated at 56°C for 30 min and then absorbed with blood group A and B rr erythrocytes at 37°C and 4°C for 1 hr to remove any anti-A or anti-B antibodies in the donor sera.

The Gm allotypes of the antibody molecules were determined directly on the patients' red cells. Doubling dilutions of antisera in saline were made in microtitre V-plates, a 2·5% suspension of patients' washed red cells added, and plates left for 1 hr at 4°C then read for agglutination pattern.

Alternatively, agglutination was read by a tile technique using undiluted antisera. Positive control cells included blood group O RIR2 donor red cells sensitized with incomplete IgG anti-D carrying known Gm markers.

In all cases where agglutination was obtained with specific antisera (anti-subclass or Gm allotype), inhibition tests were performed using purified myeloma proteins of the four IgG subclasses. Allotypic specificity was also checked by inhibition using sera of known phenotype. Results not confirmed by these inhibitions were ignored.

Gm phenotyping

The serum Gm phenotypes were determined by the classical agglutination inhibition technique (Ropartz, Rivat & Rousseau, 1963), using blood group O RIR2 donor red cells for sensitization and undiluted patients sera for inhibition.

Haematology

Packed cell volume (PCV) and haemoglobin (Hb) were determined as described previously (Facer *et al.*, 1979).

Statistical analysis

For the association between IgG subclass and anaemia, an adaptation of the χ^2 test generalized for a five-way contingency table was used.

RESULTS

IgG subclass on the patients' red cells

The IgG subclass distribution of red cell-bound IgG was determined in fifty-one of the fifty-three children investigated. The results are shown in Tables 2, 3 and 4. There was no overall restriction to one particular IgG subclass but there did appear to be an association between the age of the child and the subclass. For example, if the children were classified into two groups according to age, the first from 8 months to 4 years (which includes the majority with acute *falciparum* malaria), and the second from 5 to 9 years (mainly the primary school children), then more in the latter group had cells sensitized with IgG2 antibodies. The reverse was true for sensitization with IgG1 (Fig. 1). There was no obvious difference between the two age groups in frequency of IgG3 and IgG4 sensitization.

Association of subclass with anaemia

Children were considered anaemic if they presented with a PCV of $< 30\%$ (Hb $< 10{\cdot}0$ g%). Table 5 shows the distribution of subclasses in relation to anaemia. Statistical analysis indicated no significant association between red cells sensitized with IgG2, IgG3 and IgG4 alone or in combination, and anaemia ($P > 0{\cdot}1$). However, a positive correlation was found between anaemia and

Table 1. Reagents used to detect immunoglobulin allotypes

Chain	Genetic marker	Coating antigen (anti-Rh)	Antiglobulin (anti-Gm)
$\gamma 1$	G1m(a) or (1)	G106 & Biotest 111116	GD27 human
	G1m(x) or (2)	G101 & Biotest 114066	GD49 human
	G1m(f) or (3)	G35	GD52 human
$\gamma 3$	G3m(b0) or (11)	G91	GD110 human
	G3m(b1) or (5)	G30	GD33 human
	G3m(b4) or (14)	G18	GD31 human
	G3m(b5) or (10)	G104	GD110 human & Biotest 128038 human

Table 2. Fajara 1976/77: genetic markers and serological data in Gambian children with acute *falciparum* malaria and a positive DAT

Patient	DAT titre IgG	DAT titre C3d	Isotype on *in vivo*-coated red cells	Allotype on *in vivo*-coated red cells
C5	128	32	IgG1, IgG3, IgG4	n.t.
C6	128	16	IgG4	n.t.
C30	256	32	IgG1, IgG3, IgG4	G1m(1); G3m(11, 14)
C49	256	16	IgG1, IgG3	n.d.
C53	256	32	IgG1, IgG3	G1m(1); G3m(10)
C66	64	16	IgG1, IgG3	G3m(10)
C71	32	8	IgG1	n.d.
C76	64	32†	IgG1	n.d.
C81	1,024	32†	IgG1, IgG3	G3m(10)
C86	32	16	n.t.	G3m(10)
C87	>2048	0	IgG1	n.d.
C98‡	1,024	128	IgG2, IgG4	0
C116	256	128	IgG2, IgG4	0
C120‡	512	128	IgG1, IgG4	G1m(1)
C130‡	64	0	IgG2	0
C148	512	256	IgG1, IgG4	G1m(1)
C151	>2,048	>2,048*	IgG2, IgG4	0
C153‡	1,024	128	IgG4	0
C154	1,024	256	IgG2, IgG4	0
C157‡	>2,048	512†	IgG3, IgG4	n.t.
C162	128	0	IgG4	0
C167	1,024	64	IgG2, IgG4	0
C171	>2,048	256	IgG3, IgG4	G3m(10, 11)

All patients had a Gm(1, −2, −3, 5, 10, 11, 14) phenotype.

0 = No marker yet discovered for negroid IgG2 and IgG4.

n.t. = Not tested.

n.d. = Not detected using antisera available.

* C3b found in addition to C3d but without concurrent C4b sensitization.

† C3b and C4b also found membrane bound.

‡ Red cell eluates made from these patients had specificity for *falciparum* schizont antigens.

For details on antisera used in the DAT see Facer *et al.*, 1979.

sensitization with IgG1 molecules ($\chi^2 = 7{\cdot}52$; $P < 0{\cdot}01$; 1 degree freedom). In addition there was a greater chance of finding a child anaemic if IgG1 was present and IgG4 absent ($P < 0{\cdot}01$). Children found with red cells sensitized with IgG2 or IgG4 alone or in combination (and without concomitant complement fixation) were never found to be anaemic.

Gm allotype on in vivo-coated red cells

Results for individuals are shown in Tables 2, 3 and 4. Allotyping was restricted to patients with red cells sensitized with IgG1 or IgG3 since, as yet, no markers for the Fc or Fd of negroid IgG2 and IgG4 have been described (van Loghem *et al.*, 1978). The distribution of IgG antibody molecules in relation to their subclass and Gm type is shown in Fig. 2. Of seventeen children with IgG1 antibodies, six showed the G1m(1) allotype and one the G1m(4). The majority of children with IgG3 antibodies showed a preferential expression of particular Gm allotypes. Seven individuals

Table 3. Brefet 1976: genetic markers and serological data in Gambian children with chronic low-grade parasitaemias and a positive DAT

Patient	DAT titre IgG	DAT titre C3d	Isotype on *in vivo*-coated red cells	Allotype on *in vivo*-coated red cells
707	64	32	IgG3	n.d.
772	>2,048	8	IgG3	G1m(1); G3m(10)
717	32	0	IgG2, IgG3, IgG4	G1m(1); G3m(11, 14)
744	32	0	IgG4	0
745	32	8	IgG3	n.d.
751	64	0	IgG4	0
799	512	32	IgG2, IgG3, IgG4	G3m(14)
807	1,024	32	n.t.	G1m(1); G3m(11)
812	64	64*	IgG1, IgG3, IgG4	n.t.
837	512	16	IgG3	G3m(11, 14)

All patients had a Gm(1, −2, −3, 5, 10, 11, 14) phenotype with the exception of 837 who had a Gm(1, −2, −3, −5, −10, 11, 14) phenotype.

For abbreviations see footnote to Table 2.

* C3b and C4b also found membrane-bound.

showed the G3m(10) allotype alone or in combination with G3m(11) and the remainder had molecules marked by the G3m(14) allotype.

Gm phenotyping

All sera taken from children included in this investigation were tested for G1m(1), (2), (3) and G3m(5), (10), (11) and (14). Gm(1, −2, −3, 5, 10, 11, 14) was the common phenotype (Table 2). However, patient 837 had a Gm(1, −2, −3, −5, −10, 11, 14) phenotype (Table 3), and patient 487 a Gm(1, −2, 3, 5, 10, 11, 14) phenotype with probable Gm(1, 5, 10, 11, 13, 14, 17) and Gm(3, 5, 13, 14, 23) haplotypes indicating mixed Caucasian ancestry (Table 4). IgG anti-IgG autoantibodies were present in many of the sera, occasionally complicating Gm typing, further details of which will be presented in a subsequent communication.

DISCUSSION

In previous papers it has been shown that Gambian children often have a positive direct antiglobulin test and that the IgG bound to the red cell has *P. falciparum* schizont specificity (Facer, 1980). Frequently, we found children without evidence of haemolysis at presentation, in whom IgG-sensitized red cells persisted in the circulation for periods of up to 6 weeks (Facer *et al.*, 1979).

The present investigations demonstrate that these observations can be partly explained by the immunochemical characteristics of the individual IgG molecules sensitizing the red cells. In agreement with Gilliland (1976), the IgG subclass appears to be an important factor in determining destruction of the red cells and subsequent anaemia. In the autoimmune haemolytic anaemias it is now evident that the red cell-bound IgG subclass has a decisive effect on both the severity and the site of *in vivo* red cell destruction (Lalezari, 1976). The *in vivo* effects have been attributed to two independent properties. The first is the efficiency with which IgG antibodies fix complement. In this function IgG3 is efficient, IgG1 has moderate activity, IgG2 slight activity and IgG4 does not bind C1q at all but may activate the alternate pathway (Hobart & McConnell, 1975). Another property influencing the *in vivo* fate of red cells coated with IgG, is related to the interaction between the Fc

Table 4. Primary school children: genetic markers and serological data on children with low-grade parasitaemias and a positive DAT

Patient	DAT titre IgG	DAT titre C3d	Isotype on *in vivo*-coated red cells	Allotype on *in vivo*-coated red cells
401	256	512	IgG1, IgG2	n.d.
403	512	512	IgG1, IgG3	G3m(14)
418	>2,048	0	IgG4	0
421	256	0	IgG4	0
428	128	0	IgG2, IgG4	0
436*	>2,048	0	IgG2, IgG4	0
441	512	64	IgG1	n.d.
444	128	0	IgG2	0
447*	512	0	IgG2	0
454	128	128	IgG3	n.d.
483	512	256	IgG4	0
486*	>2,048	32	IgG1, IgG2, IgG4	G1m(1)
487	128	128	IgG1, IgG4	G1m(1, 3)
499	512	64	IgG3	n.d.
509	>2,048	0	IgG2, IgG4	0
942	>2,048	0	IgG2, IgG4	0
944	512	0	IgG2	0
957	256	0	IgG2, IgG4	0
968	512	0	IgG2	0
1002	512	0	IgG2	0

All children had a Gm(1, −2, −3, 5, 10, 11, 14) phenotype with the exception of 487 who had a Gm(1, −2, 3, 5, 10, 11, 14) phenotype.

For abbreviations see footnote to Table 2.

* Red cell eluates made from these patients had specificity for schizont antigens.

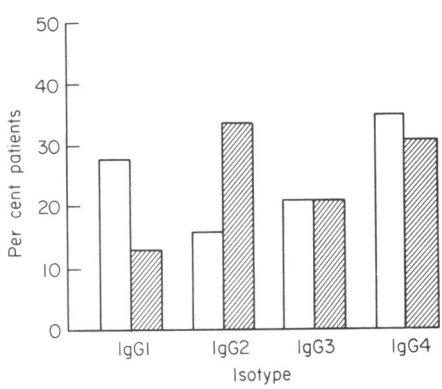

Fig. 1. Distribution of red-cell bound IgG subclass antibodies in Gambian children as related to age. (□) 1–4 years, (▨) 5–9 years.

Table 5. Distribution of IgG isotypes sensitizing erythrocytes from fifty-one Gambian children with past or present *P. falciparum* malaria. Association with anaemia

	IgG isotype sensitizing red cells												
	1	2*	3	4*	1+2	1+3	1+4	2+3	2+4*	3+4	1+ 2+4	1+ 3+4	2+ 3+4
Number of children	4	6	6	8	1	4	3	1	10	2	1	3	2
Per cent total	7·8	11·7	11·7	15·7	1·9	7·8	5·8	1·9	20	3·9	1·9	5·8	3·9
Per cent anaemic	75	0	0	0	100	75	33	100	40	100	0	33	0

* Patients with red cells sensitized with subclasses IgG2 or IgG4 (or together) *without* concomitant sensitization with C′ did not present with anaemia.

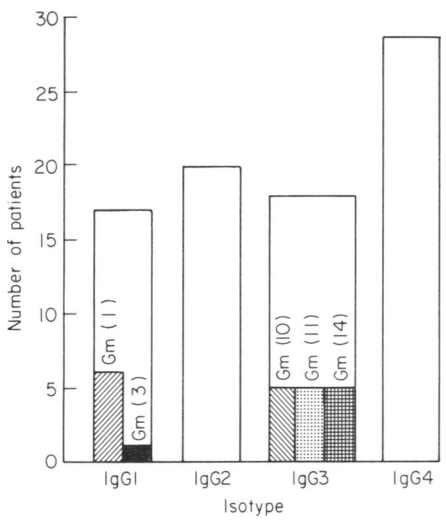

Fig. 2. Distribution of red cell-bound IgG molecules in relation to subclass and Gm allotype in children with DAT-positive red cells.

region of the cell-bound antibody and specific receptors on phagocytic and cytotoxic cells. Monocytes and tissue macrophages have receptors only for the Fc of IgG3 and IgG1 (Lobuglio, Cotran & Jandl, 1967; Abramson & Schur, 1972; Engelfriet *et al.*, 1974). The Fc receptor-mediated adherence to these cells *in vivo* plays an essential role in the destruction of IgG-sensitized red cells in the absence of complement fixation (Borne, Beckers & Engelfriet, 1977a). Thus IgG1 and IgG3 would be expected to exert a greater destructive effect on red cells than IgG2 or IgG4. Indeed, IgG3 autoantibodies invariably cause a haemolytic anaemia, while about half the patients with IgG1 alone have evidence of haemolysis (Bakemeier & Leddy, 1967; Engelfriet *et al.*, 1974; Gilliland, 1976). Conversely, the few patients with red cell-bound IgG2 antibodies do not manifest haemolytic anaemia (Bakemeier & Leddy, 1967).

The majority of Gambian children investigated had red cells sensitized with IgG2 or IgG4 alone or in combination. Only a small percentage of these children were found to be anaemic and then only if there was evidence of concurrent complement fixation on the cell membrane, possibly via the alternate pathway with IgG4 molecules (Hobart & McConnell, 1975). Evidence for this was demonstrated in one patient (C151) who had a positive DAT for IgG and C3b but was negative for

C4b. An anti-C4d antiserum might have provided further evidence for activation of the alternate pathway in many other children with red cells coated with C3d (Facer *et al.*, 1979). These results may be compared with the results from patients with autoimmune haemolytic anaemia (Borne *et al.*, 1977b) and IgG2a anti-erythrocyte antibodies in mice (Cox & Koh, 1977). The binding of antibodies *per se* does not lead to damage to the red cell, nor does it have any influence on its metabolism (Borne *et al.*, 1971).

Children with red cells sensitized with IgG1 alone were anaemic and the association between the two was found to be statistically significant. This probably relates to complement fixation by the IgG1 molecules and the interaction of IgG1 with phagocytic cells as described above. Statistical analysis also indicated that there was less chance of red cell destruction if a mixture of IgG1 and IgG4 antibodies were found. There is no obvious explanation for this, although the presence of IgG4 molecules may in some way interfere with the interaction of the Fc of IgG1 with Fc receptors on phagocytic cells.

Occasionally we found children with *falciparum* parasitaemias who had red cells sensitized with IgG3 alone, or in combination with other subclasses, who did not present with anaemia. Again the persistence of these cells in the circulation requires explanation. The Fc receptor sites for IgG3 molecules on phagocytic and mononuclear lymphoid cells may become blocked by increased circulating unbound immune complexes found in children with *falciparum* malaria (Greenwood & Mohammed, personal communication). The presence of separate cell membrane receptors for either IgM or IgG molecules distinguishes two distinct T cell populations (T_M and T_G respectively). It has recently been demonstrated that suppressor T_G cells which had reacted with IgG-containing immune complexes were not able to re-express their IgG receptors, and the lymphocytes subsequently lost most of their cytotoxic activity. Thus contact with IgG immune complexes could act as a 'switch off' signal for synthesis and/or membrane insertion of the IgG receptors by T_G (Moretta, Mingari & Romanzi, 1978). A 'switch off' mechanism of this type might not only explain the persistence of sensitized red cells in children with malaria but also might explain the high immunoglobulin levels that are characteristic of *falciparum* malaria infections (Greenwood, Odoloju & Stratton, 1977).

Each IgG subclass is determined at a separate gene locus and can be analysed genetically by means of the Gm markers, which are genetic determinants localized to different positions on the constant region of the γ-heavy chain of IgG (Litwin & Balaban, 1972). In rabbits, mice and man, specific antibodies often show a predominance of one or more allotypes in heterozygous subjects. For example, predominance of Gm(1) in Rh antibodies (Litwin, 1973; Le Petit *et al.*, 1976), allotypic restriction of autoantibodies in thyroid disease (Farid *et al.*, 1977), uncommon Gm phenotypes of neuroblastoma patients (Morell *et al.*, 1977), and certain allotypic restrictions of malarial IgG antibody (Curtain & Baumgarten, 1965), have all been reported.

The present investigations have similarly shown a preferential expression of certain Gm markers on the IgG sensitizing the red cells. Gm phenotyping of serum IgG, carried out to determine heterozygosity for Gm allotypes, showed the common haplotype was Gm (1, 5, 10, 11, 13, 14, 17) confirming the earlier reports of Gm phenotyping of the Gambian population (Johnson, Kohn & Steinberg, 1977). Our results demonstrated a molecular heterogeneity and probably polyclonal origin of these antibodies. There was a predominant expression of the G1m(1) allotype in children with red cells sensitized with IgG1, although it is impossible to say whether this represented preferential allotypic expression, since the negroid IgG1 molecule does not carry the G1m(2) or (4) antigens of IgG1 as in Caucasians, and the lack of anti-Gm(17) antisera prevented us typing for this IgG1 allotype. However, only G3m(10), (11) or (14) allotypes or subfactors were found expressed on IgG3 molecules sensitizing the erythrocytes. This does not represent any form of allelic exclusion since the G3m factors are not antithetic and the G3m(5), (10), (11) and (14) allotypes are all carried by the same G3m(b) allele. However, it is known that in negroes, other G3m alleles can occur when one or more of the G3m(b) allotype markers is replaced by another marker (van Loghem, personal communication). If a patient was heterozygous for such an allele and the normal G3m(b) allele, it may be that all G3m(b) subfactors were not found on sensitized cells when that particular antibody was produced by the less common allele. Recently Johnson *et al.* (1977) have provided evidence which shows that the less common Negro G3m(b) alleles do occur in the Gambian population.

The results on Gm allotypes differ from those of the earlier work of Curtain & Baumgarten (1965) who found that malaria antibody against *P. falciparum* schizonts in twenty-four Melanesian patients had G3m(5) specificity. However, this particular study was restricted in that the authors only typed for the G3m(5) and G1m(1) markers.

The results of subclassing the IgG on the same cells leads to speculation on the nature of the antigen component of the immune complex sensitizing the red cells. A variety of studies have shown that many antigens elicit antibody responses restricted to certain IgG subclasses. Subclass predominance has been reported in human IgG antibodies to diphtheria and tetanus toxin (IgG1; Yount *et al.*, 1968), measles virus (IgG1; Vandvik, Natvig & Norrby, 1977), coagulation Factor VIII (IgG4; Robboy *et al.*, 1970) and antibodies to the Rh D antigen (IgG1 and IgG3; Devey & Voak, 1974). The present study demonstrating a predominance of IgG2 and IgG4 subclasses may have certain implications. First, the predominance of IgG2 could indicate that these antibodies are directed against schizont antigens which are carbohydrate in nature. Most antibodies to carbohydrate antigens produced in man (Yount *et al.*, 1968; Riesen, Skvaril & Braun, 1976) are confined predominantly to the IgG2 subclass, one which appears by antigenic criteria to be the most primitive human subclass (van Loghem & Litwin, 1972). Wilson & McGregor (1973) have demonstrated a predominance of IgG2 antibodies to malarial 'S' antigens in adult Gambian sera. However, the 'S' antigens are thought to be proteins, although it has yet to be proved that they are parasitic in origin (Wilson, 1974; Wilson, McGregor & Williams, 1975). The other feature found in our study, namely the frequency of IgG4 antibodies, is unusual since IgG4 is normally found in low concentrations—only 0·2 mg/ml at the age of 2 years (about one-half of the adult concentration) in normal Caucasian serum (Morell *et al.*, 1976). The levels in West African negro children have not been investigated. However, IgG4 antibodies do not represent maternally derived IgG4 since all children in our study were of the age when maternally derived IgG would have been catabolized.

The existence of more than one IgG subclass on the red cells of our patients suggests a number of different schizont-derived antigens are involved in the immune complexes. To confirm this one would need to elute the bound immune complex and investigate the nature and origin of the antigens in the eluate. It is proposed to continue investigations along this line particularly since defining plasmodial antigens has importance not only in the immunopathology of *falciparum* malaria, but also in the development of an effective malaria vaccine.

This investigation was supported by a grant from the United Kingdom Medical Research Council. The author expresses her gratitude to Dr R. S. Bray and the staff at the MRC Laboratories, Fajara, for their help and for the provision of facilities. Thanks are also due to Diana Brazier for instruction on Gm phenotyping and supply of reagents, to Dr Erna van Loghem for advice on Gm allotypes and to Dr P. M. Johnson for useful discussions. Finally, acknowledgment goes to Dr Tom Marshall for help with statistical analysis and Mrs Sheila Newton for typing the manuscript.

REFERENCES

ABRAMSON, N. & SCHUR, P.N. (1972) The IgG subclasses of red cell antibodies and relationship to monocyte binding. *Blood*, **40**, 500.

BAKEMEIER, R.F. & LEDDY, J.P. (1967) Structural characteristics of erythrocyte autoantibodies: recent studies and implications. *Blood*, **30**, 869.

BORNE, A.E.G.KR., BECKERS, D. & ENGELFRIET, C.P. (1977a) Mechanisms of red cell destruction mediated by non-complement binding IgG antibodies: the essential role *in vivo* of the Fc part of IgG. *Br. J. Haematol.* **36**, 458.

BORNE, A.E.G.KR., BECKERS, D., MEULEN, F.W. & ENGELFRIET, C.P. (1977b) IgG autoantibodies against erythrocytes without increased haemolysis: a case report. *Br. J. Haematol.* **37**, 137.

BORNE, A.E.G.KR., ENGELFRIET, C.P., BECKERS, D. & LOGHEM, J.J. (1971) Autoimmune haemolytic anaemias. IV. Biochemical studies of red cells from patients with autoimmune haemolytic anaemia with incomplete warm antibodies. *Clin. exp. Immunol.* **8**, 377.

COX, K.O. & KOH, L.Y. (1977) Disappearance of IgG2B autoantibodies associated with recovery from anaemia. *Clin. exp. Immunol.* **27**, 560.

CURTAIN, C.C. & BAUMGARTEN, A. (1965) The distribution of genetic factors in malaria antibodies as determined by a fluorescent antibody test. *Aust. J. Exp. Biol. Med. Sci.* **43**, 351.

DEVEY, M.E. & VOAK, D. (1974) A critical study of the IgG subclasses of Rh anti-D antibodies formed in pregnancy and in immunised volunteers. *Immunology*, **27**, 1073.

ENGELFRIET, C.P., BORNE, A.E.G.KR., BECKERS, D. & LOGHEM, J.J. (1974) Autoimmune haemolytic anaemia; serologic and immunologic characteristics of the autoantibodies; mechanism of cell destruction. *Semin. Haematol.* **7,** 328.

FACER, C.A. (1980) Direct Coombs antiglobulin tests in Gambian children with *P. falciparum* malaria. II. Specificity of erythrocyte-bound IgG. *Clin. Exp. Immunol.* **39,** 279.

FACER, C.A., BRAY, R.S. & BROWN, J. (1979) Direct Coombs antiglobulin reactions in Gambian children with *Plasmodium falciparum* malaria. I. Incidence and class specificity. *Clin. exp. Immunol.* **35,** 119.

FARID, N.R., NEWTON, R.M., NOEL, E.P. & MARSHALL, W.H. (1977) Gm phenotypes in autoimmune thyroid disease. *J. Immunogenet.* **4,** 429.

GILLILAND, B.C. (1976) Coombs negative immune haemolytic anaemia. *Semin. Haematol.* **13,** 267.

GREENWOOD, B.M., ODOLOJU, A.J. & STRATTON, D. (1977) Lymphocyte changes in acute malaria. *Trans. R. Soc. Trop. Med. Hyg.* **71,** 408.

GRUBB, R. (1970) *The Genetic Markers of Human Immunoglobulins.* Chapman & Hall Ltd, London.

HOBART, M.J. & MCCONNELL, I. (Eds) (1975) *The Immune System.* Blackwell, Oxford.

JOHNSON, W.E., KOHN, P.H. & STEINBERG, A.G. (1977) Population genetics of the human allotypes Gm, Inv, and A2m. *Clin. Immunol. Immunopathol.* **7,** 93.

KAY, N.E. & DOUGLAS, S.D. (1977) Monocyte–erythrocyte interaction *in vitro* in immune haemolytic anaemias. *Blood,* **50,** 889.

LALEZARI, P. (1976) Serologic profile in autoimmune disease: pathophysiologic and clinical interpretation. *Semin. Haematol.* **13,** 277.

LE PETIT, J.C., RIVAT, L., FRANCOIS, N., ROPARTZ, C. & BRIZARD, C.P. (1976) Expression of genetic markers of erythrocyte immunoglobulin G autoantibodies in autoimmune haemolytic anaemia. *Vox Sang.* **31,** 183.

LITWIN, S.D. (1973) Allotype preference in human Rh antibodies. *J. Immunol.* **110,** 717.

LITWIN, S.D. & BALABAN, S. (1972) A quantitative method for the determination of human γG allotype antigens (Gm). II. Differences in Gm gene expression for γG1 and γG3 H chains in sera. *J. Immunol.* **108,** 991.

LOBUGLIO, A.F., COTRAN, R.S. & JANDL, J.H. (1967) Red cells coated with immunoglobulin G: binding and sphering by mononuclear cells in man. *Science,* **158,** 1582.

LOGUE, G. & ROSSE, W. (1976) Immunologic mechanisms in autoimmune haemolytic anaemia. *Semin. Haematol.* **13,** 277.

MORELL, A., KASER, H., SCHERZ, R. & SKVARIL, F. (1977) Uncommon Gm phenotypes in sera from neuroblastoma patients. *J. Immunol.* **118,** 1083.

MORELL, A., SKVARIL, F. & BARANDUN, S. (1976) Serum concentrations of IgG subclasses. *Clin. Immunobiol.* **iii,** 37.

MORETTA, L., MINGARI, M.C. & ROMANZI, C.A. (1978) Loss of Fc receptors for IgG from human T lymphocytes exposed to IgG immune complexes. *Nature,* **272,** 618.

PARISH, C.R. & HAYWARD, J.A. (1974) The lymphocyte surface. I. Relation between Fc receptors, C3 receptors and surface immunoglobulin. *Proc. R. Soc. Lond.* **187,** 47.

RIESEN, W.F., SKVARIL, F. & BRAUN, D.G. (1976) Natural infection of man with group A streptococci. Levels, restriction in class, subclass and type, and clonal appearance of polysaccharide group-specific antibodies. *Scand. J. Immunol.* **5,** 383.

ROBBOY, S.J., LEWIS, E.J., SCHUR, P.H. & OLIVERA, B. (1970) Circulating antibody to factor VIII. Immunochemical studies and clinical response to factor VIII concentrates. *Am. J. Med.* **49,** 742.

ROPARTZ, C., RIVAT, L. & ROUSSEAU, P.-Y. (1963) Le Gm(b) et ses problemes. *Vox Sang.* **8,** 717.

VANDVIK, V., NATVIG, J.B. & NORRBY, E. (1977) IgG1 subclass restriction of oligoclonal measles virus-specific IgG antibodies in patients with subacute sclerosing panencephalitis and in a patient with multiple sclerosis. *Scand. J. Immunol.* **6,** 651.

VAN LOGHEM, E. & LITWIN, S.D. (1972) Antigenic determinants on immunoglobulins of non-human primates. *Transplant. Proc.* **4,** 129.

VAN LOGHEM, E., SALIMONU, L., WILLIAMS, A.I.O., OSUNKOYA, B.O., BOYD, A.M., LANGE, G. & NIJENHUIS, L.E. (1978) Immunoglobulin allotypes in African populations. I. Gm-Am haplotypes in a Nigerian population. *J. Immunogenet.* **5,** 143.

WHO (1976) Review of the notation for the allotypic and related markers of human immunoglobulins. *J. Immunogenet.* **3,** 357.

WILSON, R.J.M. (1974) Soluble antigens as blocking antigens. In *Parasites in the Immunised Host: Mechanisms of Survival,* pp. 185–203. Ciba Foundation Symposium 25. Elsevier Excerpta Medica, Amsterdam.

WILSON, R.J.M. & MCGREGOR, I.A. (1973) Immunoglobulin characteristics of antibodies to malarial S-antigens in man. *Immunology,* **25,** 385.

WILSON, R.J.M., MCGREGOR, I.A. & WILLIAMS, K. (1975) Occurrence of S-antigens in serum in *Plasmodium falciparum* infections in man. *Trans. R. Soc. Trop. Med. Hyg.* **69,** 453.

YOUNT, W.J., DORNER, M.M., KUNKEL, H.G. & KABAT, E.A. (1968) Studies on human antibodies. VI. Selective variations in subgroup composition and genetic markers. *J. exp. Med.* **127,** 633.