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Humoral Immune Responses of Solomon Islanders to the Merozoite Surface Antigen 2 of *Plasmodium falciparum* Show Pronounced Skewing towards Antibodies of the Immunoglobulin G3 Subclass

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The immunoglobulin G (IgG) subclass distribution of antibodies to merozoite surface antigen 2 of *Plasmodium falciparum* in Solomon Islanders showed marked skewing towards the IgG3 subclass. This was not observed with crude *P. falciparum* schizont antigen. IgG3 responses may be short-lived and require repeated restimulation for their maintenance. This may be provided by persistent infection (premunition) or new infections.

Several antigens of asexual blood-stage *Plasmodium falciparum* have been identified as vaccine candidates because of their ability to protect against malaria in animal model systems and/or because antibodies to them inhibit parasite multiplication in vitro (14). Identifying the immune responses to such "protective" antigens following natural infections can aid understanding of the host-parasite relationship and provide information beneficial to the development of malaria vaccines. We have had a long-term interest in merozoite surface antigen 2 (MSA2; also called merozoite surface protein 2) of *P. falciparum* (19, 20). MSA2 has undergone a phase I clinical trial (22) and is undergoing further phase I testing in preparation for a phase 2 trial. There are two major allelic families of this antigen, represented by the parasite strains FCQ-27 and 3D7. Immunity based on MSA2 can be envisaged to involve antibodies or antibody-mediated mechanisms (5, 12, 15).

Isotype or subclass specificities of antibodies elicited to defined malarial antigens can be expected to have a marked bearing on the effectiveness of the immune response. Immunoglobulin G1 (IgG1) and IgG3 subclass antibodies are cytophilic and show extensive complement fixing ability. They promote phagocytosis, antibody-dependent cell-mediated cytotoxicity, and antibody-dependent cellular inhibition (ADCI); this is not the case with IgG2 or IgG4 antibodies, which are not cytophilic or complement fixing. Indeed, IgG2 or IgG4 may adversely affect these mechanisms by competing with IgG1 and IgG3 for the antigen (5, 10). In Thai adults infected with *P. falciparum*, the predominance of antimalarial antibodies of the IgG1 and IgG3 subclasses was associated with protection whereas a predominance of IgG2 and IgG4 subclasses or IgM or low levels of antibodies were associated with susceptibility (5). Sera from protected individuals mediated ADCI in vitro (5). Elevated levels of malaria-specific IgE antibodies have been detected in the sera of Papua New Guineans, Africans, and Asians (16). The significance of these IgE antibodies is unclear. There is no evidence that such antibodies are protective.

A high proportion of villagers in the Solomon Islands and in Papua New Guinea (PNG), where *P. falciparum* is prevalent, have antibodies to MSA2 (2, 20). This study presents data on the distribution of IgG subclass antibodies to the FCQ-27 and 3D7 forms of the MSA2 protein. Both these allelic forms occur in PNG (8) and the Solomon Islands (17).

Sera were collected with informed consent from villagers (14 to 48 years old) living in Guadalcanal, Solomon Islands. Antibodies of the various IgG subclasses were measured by enzyme-linked immunosorbent assay (7, 9). Antigens used were (i) a recombinant MSA2 (r-MSA2) protein with the 3D7 sequence (22), (ii) a comparable r-MSA2 protein with the FCQ-27 sequence (22), and (iii) a crude antigen preparation of late-asexual-stage *P. falciparum* parasites (schizont antigen) made by repeated freeze-thawing of schizont- and late-trophozoite-enriched cultures of the FCQ-27 strain. Flat-bottomed microtiter plates were coated with 2 μg of r-MSA2 protein (FCQ-27 or 3D7) per ml or with schizont antigen equivalent to 5×10^4 parasites/well. After overnight incubation at 4°C, wells were washed with phosphate-buffered saline–Tween, and then twofold dilutions of the human serum samples were added before a further overnight incubation at 4°C. After being washed, plates were incubated with monoclonal antibody to either IgG1 (NL-16; Oxoid, Basingstoke, Hampshire, United Kingdom), IgG2 (HP6014; Sigma Chemical Co., St. Louis, Mo.), IgG3 (SJ33; Sigma Chemical Co.), or IgG4 (SK44; Biomakor, Rehovot, Israel). The antisubclass antibodies were selected for their high affinity and because they were not restricted to specific allotypes (7, 9). After incubation for 3 h at 37°C, affinity-purified sheep anti-mouse immunoglobulin tagged with horseradish peroxidase conjugate was added. The plates were further incubated at 37°C for 1 h, and after a washing, 10 μl of the substrate ABTS (2,2-azino-di [3-ethylbenzthiazolone sulfanate-6) (Boehringer, Mannheim, Germany) was added. The absorption was read at 410 nm following a 1-h incubation at 37°C. The enzyme-linked immunosorbent assay endpoint titer for each serum was defined as the reciprocal dilution corresponding to half the value of the difference be-

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TABLE 1. Endpoint titers of IgG subclass antibodies to malarial antigens in the sera of Solomon Islanders

Antigen	No. of serum samples	Antibody titer (mean ± SE)			
		IgG1	IgG2	IgG3	IgG4
r-MSA2 (3D7)	52	226 ± 53	63 ± 9	960 ± 107	52 ± 8
r-MSA2 (FCQ-27)	20	376 ± 83	201 ± 52	4,968 ± 994	160 ± 17
Schizont (FCQ-27)	20	1,998 ± 362	614 ± 101	1,651 ± 431	905 ± 215

tween the highest optical density (OD) value for the serum and the baseline value. The baseline value was the OD value obtained with pooled sera from healthy Australians with no history of malaria exposure at a dilution of 1/100. Baseline OD values between 0.065 to 0.08 were obtained. OD values for Solomon Island sera with the highest titers were either slightly above or slightly below 2.0.

We found that a high proportion of the Solomon Island sera contained antibodies to MSA2, which is consistent with previous findings (20). Of the 20 sera examined for IgG subclass antibodies to the FCQ-27 form of MSA2, all had titers of >100 for at least one of the four subclasses measured and 17 showed titers of >500. Comparable analyses with the 3D7 MSA2 found that 48 of 52 serum samples had titers of >100 and 21 of these had titers of >500. A striking feature of these analyses was the predominance of IgG3 antibodies, with antibodies to IgG1, IgG2, and IgG4 being only minor components of the response (Table 1). In all instances except one (namely, a serum sample with a low-titer anti-3D7 MSA2 response), the titers for IgG3 were found to exceed those for IgG1, which was unexpected under the conditions of repeated exposure to the parasite that occur in the Solomon Islands (11, 13). This is highlighted in Table 2, where individual titers for IgG1 and IgG3 antibodies to FCQ-27 MSA2 are presented for the 20 subjects screened. In contrast, IgG antibody responses to the FCQ-27 schizont antigen in the same population gave a more expected subclass distribution, with IgG1 and IgG3 subclasses dominant and with an increased representation of IgG2 and IgG4 antibodies (Table 1).

Skewing of the IgG antibody subclass response to MSA2 towards IgG3 has also been reported in the Gambia, where malaria transmission is seasonal (24). Thus, the IgG3 bias is seen in geographically widely separated populations of different ethnic backgrounds. It was concluded in the Gambian study that the anti-MSA2 IgG3 may be a very effective antiparasite response because these antibodies are cytophilic and complement fixing and may also be able to block the invasion of merozoites into erythrocytes. Such a view is consistent with the data from PNG in which antibodies to MSA2 were associated with protection (2).

The predominance of antibodies of the IgG3 subclass suggests that there may be a defect in the downstream switching of the antibody response to IgG1. IgG1 and IgG3, while similar in their functional characteristics, differ in that IgG1 responses persist for longer periods whereas IgG3 responses are normally transient (10). We have proposed that while antibodies of the IgG3 subclass may be efficient in clearing parasites, their effectiveness may be limited in situations where boosting is infrequent because they are unlikely to persist (13). Hence, in areas such as PNG and the Solomon Islands, where malaria transmission rates are among the highest in the world and transmission occurs year-round, repeated reexposure to the antigen would ensure that the IgG3 antibody responses are maintained and immunity dependent on these antibodies is not impaired. However, in situations where transmission is interrupted, e.g., in areas of seasonal transmission or in association with migration away from areas of endemicity, it might be expected that IgG3 antibodies to MSA2 would rapidly wane, and this may compromise host immunity if MSA2, as is believed, is a target of protective immunity.

Based on our proposal, in the Gambia, where malaria is seasonal, IgG3 titers should wane in the dry season, when transmission is minimal. While this has not yet been directly addressed, recently published findings from the Gambia (23) showed that total IgG antibodies to MSA2 (taken here to be mostly IgG3 according to previous Gambian data [24]) did not significantly fluctuate between transmission seasons in clinically immune adults, although there was evidence that seasonal fluctuations in antibody levels consistent with malaria transmission were evident in semi-immune children.

The findings in adults, which appear to be inconsistent with our original proposal that IgG3 antibodies will wane in areas of seasonal transmission, could be explained if it were to be demonstrated that there is chronic persistence of low-grade parasitemias in this clinically immune group of adults in the Gambia and that these parasites provide the boosting necessary to maintain the IgG3 antibody levels. We consider that persistence of parasites through at least part of the dry season is very likely to occur in the Gambia based on other data. For instance, by using sensitive PCR technology in addition to microscopy, the persistence of parasites asymptomatically in individuals for several months in the dry season, often as multiclonal infections, has been shown in Sudan (3). Other recent research also supports the idea of malaria chronicity (4, 18).

Premunition, also known as concomitant immunity, is characterized by an equilibrium in the host-parasite response with the parasite not eliminated but persisting at low densities and conferring some protection against subsequent infections (1, 6, 21). This immune state may well provide a mechanism by which immunity dependent on IgG3 subclass responses may be maintained. Premunition has been reported to be IgG dependent, with the IgG proposed to mediate ADCI, which requires cytophilic antibodies like IgG3 (6). Moreover, premunition

TABLE 2. Comparison of IgG1 and IgG3 endpoint antibody titers to r-MSA2 (FCQ-27) in individuals

Serum sample no.	Antibody titer	
	IgG1	IgG3
1	99	8,000
2	234	2,435
3	308	7,192
4	103	522
5	757	5,367
6	816	11,504
7	114	234
8	84	488
9	191	1,818
10	282	4,098
11	438	12,800
12	156	472
13	403	1,368
14	1,600	9,564
15	873	12,800
16	214	4,583
17	237	970
18	92	469
19	290	691
20	312	8,288

appears to be independent of transmission levels provided that transmission occurs at least once per year (6).

The findings that there were seasonal fluctuations in anti-MSA2 antibody titers in children in the Gambia (23) are consistent with our expectations (13). However, observed differences between children and adults in the Gambia have yet to be explained. A possible answer is that the semi-immune children may have required and received treatment for their malaria infections. Treatment with the consequent loss of parasites would then be reflected in a sharp decline in antibody levels, as we have proposed and as was observed. It may also be that semi-immune children have not yet developed immune responses leading to a state of premunition (6), which may be necessary for the maintenance of the IgG3 antibodies in the absence of new infections.

To evaluate further the *in vivo* significance of our findings, it will be important both to demonstrate directly that anti-MSA2 IgG3 titers wane rapidly in the absence of regular boosting, either as a result of new infections or by chronic infections, and to compare responses in children and in adults. It will be also important to determine whether the atypical subclass distribution seen with MSA2 is also manifested with other antigens and whether there is any evidence that such abnormal antibody responses are preferentially associated with antigens which are considered targets of protective immunity. We believe that our findings have potentially important implications for vaccines based on MSA2 and possibly other malarial antigens since induction by vaccination of antibodies primarily comprised of IgG3 may provide only short-term protection if mechanisms for regular boosting of these responses are not in place.

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