Several antigens (Ag) of the asexual blood stages of *Plasmodium falciparum* are considered protective since they have been associated with clinical immunity in individuals living in areas of endemicity. Conversely, clinical immunity has been associated with cytophilic immunoglobulin G (IgG) antibodies (Abs) (i.e., IgG1 and/or IgG3) (4, 5, 9–11, 25, 28). The identification of certain immune parameters characteristically observed in clinically immune individuals might help to focus on critical issues for the design of vaccine subunits. Thus, the present study aimed to examine the distribution of diverse classes and subclasses of Abs specific to asexual blood stages of *P. falciparum* in individuals from areas differentially exposed to this parasite in Senegal in order to elucidate the dynamic aspects of Ig secretion and utilization.

Plasma was collected with informed consent from adult villagers over 18 years of age and with no clinical episodes of malaria for the previous 9 months. These individuals were born and live in Dielmo, Ndiop, and Barkedji, Senegal. *P. falciparum* malaria transmission is perennial and intense in Dielmo (29), seasonal and intense in Barkedji (16), and seasonal and moderate in Ndiop (29). Sampling was done before the end of the dry season, when transmission is low (Dielmo), episodic and focal (Barkedji), and virtually absent (Ndiop) (16, 20, 29). The villagers were approximately gender and age matched in each site. Most of them were found to be aparasitemic by means of the exact Fisher’s test.

To measure plasma Abs levels in plasma, we used (i) a lysate from a crude extract of schizont-enriched *P. falciparum*-infected erythrocyte culture (22) which contains epitopes from a variety of Ags but little MSP1 (23) and (ii) the first EGF-like motif of MSPp19, obtained as an *Escherichia coli*-recombinant Ag cleaved from the fusion protein (6). These Ags are subsequently referred to as Pf.sAg and MSP1, respectively. Individual plasma samples were diluted 1/200 in phosphate-buffered saline–1% bovine serum albumin–0.1% Tween-20. Unless otherwise stated, reagents were purchased from Sigma (Saint Louis, Mo.). Enzyme-linked immunosorbent assays were performed as previously described (2, 13, 21, 22) with Pf.sAg-coated (overnight; 4°C) MaxiSorp plates (Nunc, Roskilde, Denmark) and MSP1-coated (0.5 μg/ml) Immulon-4 plates (Dynatech, Springfield, Va.). Polyclonal goat anti-human IgG (1/6,000), IgG1 (1/2,000), IgG2 (1/10,000), IgG3 (1/10,000), IgG4 (1/30,000), and IgM Abs (1/4,000) were used as secondary reagents and were applied for 1 h at 37°C (Cappel; Organon-Teknika, Turnhout, Belgium). Optical densities (OD) were read at 450 nm. Results were expressed as OD ratios, deduced from OD values of a negative reference European serum pool (Institut Pasteur, Paris, France), in order to minimize the plate-to-plate variation. Statistical analysis was done by means of the exact Fisher’s test.

To determine the prevalence of antimalarial IgG in our populations we first measured anti-Pf.sAg-specific IgG levels in all the study subjects. First, almost all the study participants from the selected villages were found to have anti-Pf.sAg IgG (63 of 64; 98%), and the majority (55 of 64; 86%) also had specific IgM (Fig. 1). By comparison, 47% of individuals from Dakar had detectable anti-Pf.sAg IgG. These assays were designed to detect Abs to a wide number of Ags and to characterize Abs in populations exposed to repeated and/or recent infections. Second, we observed that a relatively high proportion of villagers had elevated levels of either IgG or IgM Abs to MSP1 (Fig. 2). The use of MSP1 was designed to characterize Abs to one of the major “protective” Ags in populations which may represent groups of potential vaccinees (1, 30). With a positive cutoff value of 1.5 for OD ratios, 15 of 25 (60%) samples in Dielmo, 15 of 25 (60%) samples in Ndiop, and 9 of 14 (64%) samples in Barkedji had anti-MSP1 Abs, but...
very few had both IgG and IgM (3 of 25, 5 of 25, and 2 of 14, respectively, in Dielmo, Ndiop, and Barkedji). Indeed, anti-MSP1 IgM predominated in Dielmo, whereas IgG was more frequent in Ndiop and Barkedji (Fig. 2). A similar pattern of IgG versus IgM was seen in Dakar (Fig. 2). The presence of specific IgG in every adult who has been exposed for years to many infective bites would have been the predicted finding (9).

In contrast, we report a relative paucity in IgG responses in the area of hyperendemicity (Dielmo), both in terms of the proportion of responders (8 of 25; 32%) and the intensity of the response (Fig. 2). A greater proportion (and possibly level) of IgG responses would be expected in such individuals. The elevated proportion of IgM responders to both Pf.sAg and MSP1, however, is even more questionable. In Dielmo, Pf.sAg- and/or MSP1-specific IgM could result from reinfection with different parasite strains or with parasites expressing allelic variations (18). Specific IgM in Barkedji and Ndiop, where plasma has been collected during the low-transmission season, might reflect antigenic crossreactions (17), the presence of surface IgM* B lymphocytes with long-lasting memory (19), or, most likely since the IgM half-life is short (5 days) (7), the occurrence of rare infections. For comparison, Pf.sAg- and/or MSP1-specific IgM were not common in Dakar (8 of 45; 18%) (Fig. 1). Of note, no Abs were found in plasma from 12 expatriates with no history of malaria infection (data not shown).

Taken together, it can be seen from Fig. 2 that there is either an IgG or an IgM response to MSP1. Anti-MSP1 IgG may thus have been utilized in preference to IgM (the functional role of which remains uncertain). It cannot be excluded, however, that some low-affinity IgG Abs may not be detectable due to competition by IgM Abs for the same epitope in individuals from Dielmo; this may explain the absence of detectable IgG subclass Abs in some individuals with high positive total IgG or vice versa (Fig. 3). Further, although there was a fair proportion of anti-Pf.sAg IgG of all four subclasses in each positive individual, anti-MSP1 responses were strictly restricted to IgG1 and IgG3 subclasses (not shown).

Protection to the blood stages of P. falciparum is often considered to be associated with cytphilic IgG1 and/or IgG3 Abs (4, 5, 9, 14, 26). In support of this notion, there have been several reports showing that the presence of anti-MSP1 IgG1, rather than IgG3, was associated with clinical protection to P. falciparum malaria (10, 11, 27) in contrast to Ags such as MSP2 (25, 28). We now report a balance between IgG1 and IgG3 responses which appears to depend mostly on the study site. In the one setting where P. falciparum transmission is perennial (Dielmo), 2 of 25 immune adults had detectable anti-MSP1 IgG1 and 10 of 25 had IgG3 (Fig. 3). Under the conditions prevalent in Ndiop, 8 of 25 individuals were positive for anti-MSP1 IgG1 and 16 of 25 were positive for IgG3 (Fig. 3). There were thus a greater proportion of individuals that were anti-MSP1 IgG3 positive than anti-MSP1 IgG1 positive in both Dielmo (P <0.01) and Ndiop (P <0.05). A different subclass balance was observed in Barkedji, where 7 of 14 and 5 of 14 individuals had MSP1-specific IgG1 and IgG3, respectively (Fig. 3), and in Dakar (Fig. 4) (P = 0.7). The measure of Abs in body fluids actually reflects a steady-state condition, i.e., Abs that are present in this fluid at the time of the sampling. The paucity or absence of specific Ab of one (or more) IgG subclass(es) suggests either that these molecules are not being produced or that these molecules are being utilized. Since MSP1 preferentially elicits IgG1 responses, and since IgG1 Abs are long-lived compared to IgG3 Abs (half-lives being 11 to 23 versus 7 to 8 days, respectively [7]), it can be further suggested that anti-MSP1 IgG1 may have been utilized in pref-

FIG. 1. Relationship between IgG and IgM specific to a crude extract of schizont-enriched P. falciparum-infected erythrocyte culture (Pf.sAg). The levels of P. falciparum-specific IgG and IgM were measured in plasma from subjects living in Dielmo, Ndiop, or Barkedji and in individuals living in Dakar who had donated blood and are plotted against each other. The inset in the Dakar panel shows data with enlarged scaling. Results are expressed as OD ratios over negative controls. The dotted lines represent the arbitrary cutoff values for the assignment of positive status.

FIG. 2. Relationship between IgG and IgM specific to the EGF1 motif of the C-terminal part of MSP1 (MSP1p19). The levels of anti-MSP1 IgG and IgM in plasma were measured for each subject from Dielmo, Ndiop, and Barkedji, and for those who had given blood donations in Dakar and are plotted against each other. The inset in the Dakar panel shows data with enlarged scaling. Results are expressed as OD ratios over negative controls. The dotted lines represent the arbitrary cutoff values for the designation of positive status.
ference to IgG3, e.g., to achieve parasite clearance in areas where transmission is perennial compared to areas where it is seasonal. To our surprise, however, there was little IgG1 compared to IgG3 detected in immune adults from Ndiop (similar to the case in Dielmo) (Fig. 3); a possible explanation would be that rare transmission of malaria does occur during the dry season in this village.

The mechanisms that are intimately involved in the secretion of an Ig of a precise class or subclass directed to a nominal protective epitope are not yet fully understood, although research to address this question is in progress (12). It is thus of major importance to evaluate the capacity of the immune system to select the appropriate mechanisms (e.g., preferential production of IgG1 and/or IgG3 Abs in response to particular Ags) which lead to a protective response to major vaccine candidates.

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REFERENCES


FIG. 3. Levels of IgG and IgG1 and IgG3 subclass antibodies specific to MSP1 in villages with different degrees of endemic P. falciparum. The individual levels of plasma anti-MSP1 total IgG, IgG1, and IgG3 are indicated for subjects from Dielmo, Ndiop, and Barkedji. Results are expressed as OD ratios over negative controls. The dotted lines represent the arbitrary cutoff values for positive plasmas.

FIG. 4. Levels of IgG and IgG1 and IgG3 subclass antibodies specific to MSP1 in Dakar (blood bank donors). The individual levels of plasma anti-MSP1 total IgG, IgG1, and IgG3 are indicated. Results are expressed as OD ratios over negative controls. The dotted lines represent the arbitrary cutoff values for positive plasmas.


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