Effective human immunity to blood forms of *Plasmodium falciparum* involves the acquisition of anti-parasite antibodies (Abs) of the cytophilic immunoglobulin G (IgG) subclasses 1 and 3, targeted at surface antigens of the invasive extracellular merozoite stage of *P. falciparum* (3, 4). The absence of parasite-specific IgG3 Abs can be associated with poor clinical prognosis of malaria (32). Although IgG1 and/or IgG3 Abs specific for merozoite and/or schizont antigens are relevant to clinical protection, the identity of the target antigens remains to be elucidated.

Merozoite surface protein 1 (MSP-1) is the precursor of most merozoite surface antigens (20, 27). The MSP-1 gene encodes conserved, dimorphic, and polymorphic regions of the protein (28, 35). There are two major families of MSP-1, based on the dimorphic sequences (35). Polymorphism in the Block 2 region is more extensive, but all Block 2 sequences belong to one or another of only three main families of MSP-1, based on the dimorphic sequences (35). Nine different recombinant glutathione S-transferase fusion proteins of MSP-1 Block 2, representative of all three known Block 2 types, were used (7, 8). Sequences of all these proteins have been published (7, 8). MSP-119 fusion protein was as described previously (6). Plasma samples were tested by enzyme-linked immunosorbent assay (ELISA) for total IgG and all IgG subclasses able to recognize the 10 different recombinant MSP-1 antigens as described elsewhere (7, 8, 16). The antigens were coated separately onto parallel columns of wells on 96-well plates (Immunlon 4; Dynex Technologies) at 50 ng per well. Each plasma sample, diluted 1:500, was added to two rows of antigen-coated wells on a plate and held at 4°C overnight. Thus, any one plasma sample was tested in duplicate against all different antigens on the same plate at the same time. Identical sets of antigen-coated plates were prepared for the determination of total IgG, IgG1, IgG2, IgG3, or IgG4 in assays performed with any one plasma sample in parallel on the same day. Plasma samples from any one location were all tested on the same day. Next, wells were incubated for 3 h with 100 μl per well of horse-radish peroxidase (HRP)-conjugated...
levels are shown for Block 2- or MSP-1 19-specific Abs in separate panels. Individuals had IgG subclasses recognizing Block 2 antigens in a type-specific manner, i.e., most samples contained IgG specific for only one of the three Block 2 types (data not shown). The levels of Block 2-specific IgG and IgG3 from any one individual are shown as a single data point representing the maximum detected with any one of the antigens used. Results for all three Block 2 types were similar and are combined in Fig. 1 for simplicity. In each donor cohort, the predominant response to Block 2 was of the IgG3 subclass, with a few individuals having both IgG1 and IgG3 (Fig. 1). In contrast, in the same donors, the predominant response to the MSP-1,19 region was of the IgG1 subclass, with some samples also containing IgG3. No IgG2 or IgG4 to any Block 2 type or to MSP-1,19 were detected in any donor (data not shown). Each cohort illustrated the same striking bias toward IgG3 responses to Block 2 and towards IgG1 responses to MSP-1,19. These distinct subclass biases of the responses to different regions of MSP-1 were statistically significant in all three cohorts of donors when analyzed by the two-tailed Wilcoxon matched-pairs ranked sign test. IgG3 was the predominant subclass against Block 2 at all three sites (z = −3.319, P < 0.0001 [Kilifi]; z = −2.5048, P < 0.0124 [Daraweesh]; and z = −4.552, P < 0.00006 [Koka]). In contrast, IgG1 was the predominant Ab subclass directed against MSP-1,19 (z = −2.9948, P < 0.0028 [Kilifi]; z = −3.2335, P < 0.0014 [Daraweesh]; z = −3.254, P < 0.0014 [Koka]).

Plasma samples from individuals in Daraweesh were from a longitudinal study of immune responses to malaria conducted since 1990 (7, 18). Therefore, we tested whether changes in IgG subclass response profiles occurred in individuals over 3 to 4 years. Longitudinal series of plasma samples from eight individuals (5 to 11 samples each) were assessed for the IgG subclass composition of Abs to Block 2 and MSP-1,19 in successive transmission seasons. Seven of the individuals consistently produced IgG3 to Block 2 and, equally consistently, IgG1 to MSP-1,19 in response to their clinical malaria infections. IgG subclass profiles of individuals A3, X7, and F11 are shown as examples in Fig. 2A to C. Among the eight individuals tested longitudinally, the one notable exception to the general pattern was the response of individual 2J8 to MSP-1,19, which shifted from the usual IgG1 subclass in 1993 to the IgG3 subclass following a clinical infection in 1994 (Fig. 2D). The anti-Block 2 response of this individual was IgG3, as usual.

This study presents the first direct evidence for strikingly distinct subclass preferences of Ab responses to two different regions of one protein, the Block 2 and the MSP-1,19 regions of MSP-1. It poses general questions about the regulation of human isotype responses and specific questions about the consequences of such responses in malaria. Strong IgG subclass biases to either IgG1 or IgG3 are a feature of human responses to different protein antigens of P. falciparum merozoites. Similar to the IgG3 bias of responses to Block 2, IgG3 is the main subclass in response to another merozoite surface protein (MSP-2) in Gambians (36) and in Solomon Islanders (31). Similar to the IgG1 bias to MSP-1,19 (13), responses to RAP-1, another antigen of P. falciparum merozoites, are also biased to the IgG1 subclass (16).

Protein antigens of most bacteria and viruses induce both IgG1 and IgG3 antibodies, with IgG1 generally being the dominant antiviral subclass (15, 19, 25). Virus-specific IgG3 correlates with current infection by nonlatent viruses and is normally a short-lived response (26). Persistence of IgG3 is usually seen only in asymptomatic latent viral infections, indicating that the presence of viral Ag is required for maintenance of the IgG3 responses (24). Like these antiviral responses, the generalized bias to IgG3 of Block 2-specific responses could be because most of the donors tested in this study were infected at the time of or shortly before plasma sample collection. However, an IgG3 bias would also be expected in the responses of these donors to MSP-1,19, but this was not observed. Thus, there must be other explanations for the differential subclass bias of responses to different regions of MSP-1.

Responses to multimeric antigens can be distinctly different from responses to less organized, soluble proteins. Repetitiveness of antigen structures on surfaces of cells or viral particles is sensed by the degree of cross-linking of surface Ig on B cells, which are thought to recognize antigen organization as a general marker of foreignness (2). Epitopes from viral proteins can elicit rapid T-cell-independent Ab responses, if expressed as part of a surface protein on recombinant bacteria, whereas the same epitopes elicit delayed, T-cell-dependent Ab responses when expressed as soluble, internal antigens (23). MSP-1,19, believed to be attached to the merozoite surface, could be recognized as a repetitively arranged surface antigen on the relatively large merozoite (1 μm). In contrast, Block 2 of MSP-1 is located on the MSP-1,19 fragment, a component of a soluble protein complex shed from the surface of the parasite. The uptake and processing by antigen-presenting cells of Block 2 and MSP-1,19 may therefore be different and is likely to
influence the type of antibody produced. MSP-1<sub>19</sub> on merozoites probably would be phagocytosed for presentation by macrophages. In comparison, the soluble Block 2 is likely to be taken up by different antigen-presenting cells, e.g., follicular dendritic cells.

The significance of the IgG3 bias in the response to the polymorphic Block 2 of MSP-1 in malaria immunity is not yet known. Merozoite-specific antibodies of the IgG1 and IgG3 subclasses can trigger the release of soluble anti-parasite mediators from monocytes in vitro (12), but whether there are...
differences in the effectiveness of these two subclasses is not known. Soluble immune complexes of antigen and specific IgG3 interact differently with immune effector cells than do complexes formed by other IgG subclasses (22, 37). IgG3-containing complexes could mediate beneficial effects, such as Ab-dependent cellular cytotoxicity and phagocytosis of the parasite, or pathological inflammation. Kelly proposed the hypothesis that in a population with a high frequency of a mutant allotype of IgG3, a chronic presence of circulating immune complexes of unknown malaria antigens with IgG3 contributes to hyperreactive malarious splenomegaly, a chronic complication of malaria associated with up to 60% mortality (21). However, anti-Block 2 Abs, shown here to be mostly IgG3, are strongly associated with protection against clinical malaria, with protective efficacy in the range of 22 to 46% for type-specific Abs against types of Block 2 that are most prevalent in natural populations of P. falciparum (10). Further work is necessary to elucidate the protective and/or pathological potential of different IgG subclass responses against distinct portions of P. falciparum MSP-1.

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FIG. 2. Longitudinal patterns of IgG subclass responses to Block 2 and MSP-19 in four donors from Daraweesh (Sudan). Solid symbols indicate IgG1 responses, and open symbols represent IgG3 responses. Squares indicate responses to MSP-19, while circles indicate responses to Block 2. Arrows indicate documented clinical malaria episodes. (A) Individual A3 (IgG3 response to MAD20 Block 2 and IgG1 response to Block 2). (B) Individual X7 (IgG3 response to MAD20 type Block 2 and IgG1 response to MSP-19). (C) Individual F11 (IgG3 response to K1 type Block 2 and IgG1 response to MSP-19). (D) Individual 2J8 (IgG3 response to K1 type Block 2 and a shift from IgG1 to IgG3 in response to MSP-19).

REFERENCES


occurring gene encoding the major surface antigen precursor p190 of *Plasmodium falciparum* lacks tripeptide repeats. EMBO J. 6:4137–4142.


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