Anti–MSP1 Block 2 Antibodies Are Effective at Parasite Killing in an Allele-Specific Manner by Monocyte-Mediated Antibody-Dependent Cellular Inhibition

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We investigated whether anti–merozoite surface protein-1 (MSP1) block 2 antibodies mediate the monocyte-dependent antibody-mediated cellular inhibition (ADCI) of *Plasmodium falciparum*. This study was performed because soluble molecules have been shown to trigger ADCI and because MSP1 block 2 is released following processing and is the target of cytophilic IgG3 responses in exposed populations. We assessed human anti–MSP1 block 2 antibodies against 4 *P. falciparum* strains that carry the 3 main block 2 sequence alleles. These antibodies were able to inhibit in vitro growth of *P. falciparum* only in cooperation with human monocytes, whereas no direct inhibition was observed. However, the ADCI effect was strictly allele specific. Our findings highlight a new mechanism involving MSP1 in the protection against malaria.

Acquired immunity to blood-stage malaria parasites in humans depends on antibodies that are supposed to act directly, by blocking adherence of infected red blood cells to the vascular endothelium or by preventing merozoite invasion of red blood cells, or indirectly, via merozoite activation of blood monocytes effector cells. The antigens targeted by the latter 2 mechanisms are mainly those accessible during the free-parasite (i.e., merozoite) stage. However, it was shown recently that soluble antigens released at the time of schizont rupture could also trigger monocyte-mediated antibody-dependent cellular inhibition (ADCI) of *Plasmodium falciparum*, provided they define at least 2 antigenic determinants and are targeted by cytophilic antibodies [1]. Indeed, *P. falciparum* antigens able to trigger ADCI, such as MSP3, MSP6, GLURP, and SERP-SERA [2–4], are not anchored to the membrane but are only associated with the merozoite surface.

The gene encoding merozoite surface protein-1 (MSP1) is divided into 17 discrete blocks, of which the most polymorphic is block 2 [5]. The MSP1 precursor goes through a posttranslational proteolytic process that generates 4 major fragments. One fragment, p-42, is additionally cleaved into p-33 and p-19 (the only glycosylphosphatidylinositol [GPI]–anchored fragment that remains bound to the parasite during erythrocyte invasion). The remaining 3 fragments—p-83, p-38, and p-28–30—are not released and remain noncovalently associated to the merozoite membrane (figure 1, which appears only in the electronic edition of the *Journal*).

All block 2 sequences belong to 1 of 3 main serotypes, represented by prototypical variants containing polymorphic regions originally identified in the K1, MAD20, and RO33 isolates [5]. Antibodies to MSP1 block 2 are strictly allele specific: antibodies to one allele family do not cross-react with the other families [5, 7].

Interestingly, there are differential patterns of naturally acquired responses to distinct regions of MSP1 in humans, with IgG1-type responses dominant against $MSP1_{-19}$ and IgG3 responses dominant against MSP1 block 2 [8]. Epidemiologic studies indicated an association between IgG3 directed to MSP1 block 2 and protection from clinical malaria [6, 9], reminiscent of similar findings reported for MSP3b [10].

The fact that MSP1 block 2 can be released, is the target of IgG3, and can be associated with protection against malaria led us to assess the ability of MSP1 block 2–specific human antibodies to achieve in vitro parasite killing in cooperation with blood monocytes via the ADCI mechanism. Results show that this is indeed the case, although as expected, it occurs in a strict allele-specific manner.

Materials and methods. The recombinant GST–MSP1 block 2 fusion protein from *P. falciparum* Uganda Palo Alto (K1 type) was purified by affinity chromatography on glutathione agarose by use of Triton X114 (Sigma-Aldrich) to obtain a protein with low endotoxin content, and the protein was stored in PBS at −20°C. Control GST antigen was purified from bacteria transfected by the pGEX plasmid alone. The recombinant protein MSP3-$CT_{184-354}$, which corresponds to the conserved C-terminus of the MSP3 protein, a major target of protective

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Figure 1. Schematic representation of *Plasmodium falciparum* merozoite surface protein-1 (MSP1) precursor (adapted from [6]).

The figure is available in its entirety in the online edition of the *Journal of Infectious Diseases*.

Figure 2. Immunofluorescence assay findings showing type-specific recognition of acetone-fixed *Plasmodium falciparum* schizonts by merozoite surface protein-1 (MSP1) block 2 affinity-purified antibodies.

antibodies in the ADCI mechanism, was used as positive control and was purified as described previously [11].

Hyperimmune serum specimens from adults in Cote d'Ivoire were screened for total IgG reactivity against recombinant GST alone and against the GST–MSP1 block 2 fusion protein to select specimens with the highest reactivity against the latter antigen and minimal reactivity toward the former. Because of the requirement for cytophilic classes of antibody in the ADCI mechanism, human IgG subclass determination was performed by an enzyme-linked immunosorbent assay (ELISA) as described previously [11], and the optical densities obtained with the control GST protein alone were subtracted.

Antibodies specific to either MSP1 block 2, MSP3 C-terminus, or GST alone were affinity purified as described elsewhere [11] on antigen-coated polystyrene beads (diameter, 10 μm [Mgenex Biosciences]), using serum specimens diluted 10-fold. The antibodies were eluted using glycine (0.2 mol/L; pH 2.5) and neutralized with Tris buffer (2 mol/L; pH 11.2), after which they were dialyzed against PBS and then RPMI, concentrated by centrifugation with Vivaspin6 (30,000-Da molecular weight cutoff polyethersulfone membrane [Sartorius Stedim Biotech]), and stored at 4°C after addition of 1% albumax. The specificity of affinity-purified antibodies was ascertained by an ELISA, and the purity was checked via SDS-PAGE. Recognition of the native parasite protein was assessed by Western blotting, using a *P. falciparum* schizont extract, and by an immunofluorescence assay (IFA) on acetone-fixed thin smears of schizonts.

IgG prepared from a pool of hyperimmune African adult serum specimens (PIAG) previously found to confer passive protection [12] was used as a positive control. Negative control IgG was prepared from samples obtained from a pool of French donors with no malaria history.

For ADCI assays, monocytes from healthy French donors were isolated by adherence as described elsewhere [1]. Anti–MSP1 block 2 affinity-purified antibodies with a 1:200 IFA titer were added at a rate of 10 μL/well, and each well was filled to a total volume of 100 μL with culture medium and infected red blood cells (final hematocrit, 2.5%, with parasitemia detected in 0.5%). Thus, the IFA-confirmed final antibody titer was 1:20. The control wells consisted of parasite culture with monocytes alone, monocytes and negative control IgG, and/or affinity-purified antibodies but without monocytes [13]. At 48 and 72 h, 50 μL of complete culture medium was added to each well. Following cultivation for 96 h, parasitemia was determined on Giemsa-stained thin smears of specimens from each well by microscopic examination of at least 50,000 erythrocytes, and the specific growth inhibitory index was calculated as described elsewhere [13]. The ADCI assays were performed in duplicate, using each of the following 4 strains expressing the 3 main block 2 sequence types: 3D7, Wellcome (kindly provided by Odile Puijalon), K1, and RO33 (MRA-159 and MRA-200, MR4 [ATCC]).

Table 1. Specificity of human anti–merozoite surface protein-1 (MSP1) block 2 as determined by enzyme-linked immunosorbent assay.

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Results. Only serum specimens in which the reactivity to GST–MSP1 block 2 was >4 times that to GST were used for affinity purification. The antibodies that were affinity purified on Uganda Palo Alto GST–MSP1 block 2 fusion protein were found to be block 2 specific: they reacted strongly to GST–MSP1 block 2 but essentially did not react with GST alone, MSP3, or MSP6 (table 1, which appears only in the electronic edition of the *Journal*). IFA revealed that these antibodies reacted with the native parasite protein (figure 2, which appears only in the electronic edition of the *Journal*), an essential requirement for ADCI, a mechanism in which the parasite triggers, through the antibody, the monocytes. They were strain-specific, as they reacted with 3D7 schizonts (which belong to the K1 family) but

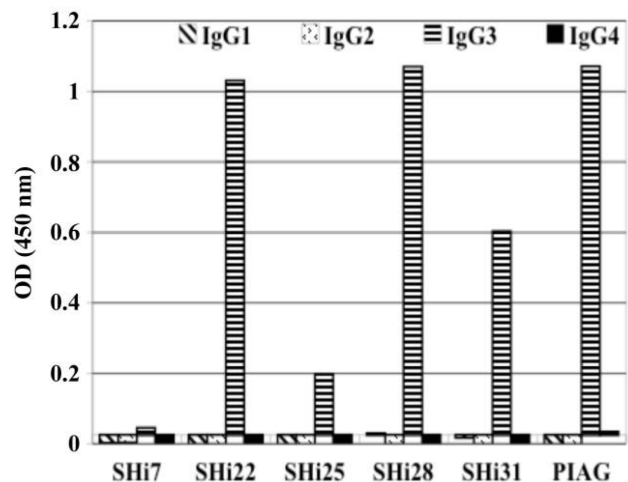

Figure 3. Pattern of anti–merozoite surface protein-1 (MSP1) block 2 IgG subclass distribution. *Bars* represent the optical density (OD) after subtracting the mean OD of the control serum specimens + 3 SDs. SHi are hyperimmune serum specimens from protected adults living in area of endemicity. PIAG is a pool of hyperimmune African adult serum specimens.

Table 2. Distribution pattern of human IgG subclasses in anti–merozoite surface protein-1 block 2 antibodies prepared by affinity purification.

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not with the Wellcome (figure 2) or R033 strains. Western blot that used total schizont extract from the 3D7 strain showed that these antibodies labeled the MSP1 precursor, the MSP1 N-terminal fragment (p-83), which comprises the block 2 sequence (data not shown).

The isotype distribution of specific antibodies among 5 African adult serum specimens and the PIAG shows an outstanding dominance of antibodies belonging to the IgG3 isotype, whereas other subclasses were essentially absent (figure 3 and table 2, which appears only in the electronic edition of the *Journal*).

In the ADCI assay, affinity-purified anti–MSP1 block 2 antibodies, in cooperation with blood monocytes, showed a potent effect against parasite strains K1 and 3D7. In contrast, no direct inhibition of parasite growth in the absence of monocytes was observed at the tested concentration. The ADCI effect was as high as that obtained with the PIAG or with anti–MSP3 C-terminal antibodies (figure 4). GST-specific control antibodies were ineffective.

The monocyte-dependent inhibition of parasite growth was K1 type specific since, under the same conditions, anti–MSP1 block 2 antibodies showed no effect whatsoever on either the Wellcome or the R033 strains. In contrast, the latter parasite strains were strongly inhibited by the PIAG and MSP3 C-terminal antibodies (i.e., when using antibodies directed to multiple targets and a nonpolymorphic target). Thus, in agreement with data from the literature and from our IFA studies, the antibodies specific for the UPA strain MSP1 block 2 acted, during ADCI, in a strong but strictly allele-specific manner.

Discussion. MSP1 was one of the first malaria molecules characterized and has been the object of a very large number of biological studies and vaccine trials. The vast majority of those investigations have focused on the C-terminal part of the molecule, $MSP1_{-19}$ or $MSP1_{-42}$, which contains EGF-like domains and is anchored by GPI [14].

Although the 190L vaccine candidate derived from the p-83 N-terminal fragment was the first derived from MSP1 and showed substantial promise in *Aotus* monkeys [15], these early hopes were not confirmed when using GMP-grade material, and this part of the molecule was subsequently minimally studied. Our finding of a strong, monocyte-mediated ADCI effect of anti–block 2 antibodies, although in allele-specific manner, should contribute to revived attention to regions of MSP1 other than $MSP1_{-19}$, which has been the most studied.

The characteristics of $MSP1_{-19}$ and block 2 are very different. First, $MSP1_{-19}$ is strongly anchored to the merozoite surface, whereas block 2 is released into the microenvironment. Second, $MSP1_{-19}$ is the target of antibodies inhibiting parasite invasion of the erythrocyte, whereas block 2 is now understood to be the target of antibody-mediated parasite killing through monocytes. Third, $MSP1_{-19}$ is predominantly targeted by IgG1, whereas block 2 is the target of IgG3, possibly the most critical class of cytophilic antibodies.

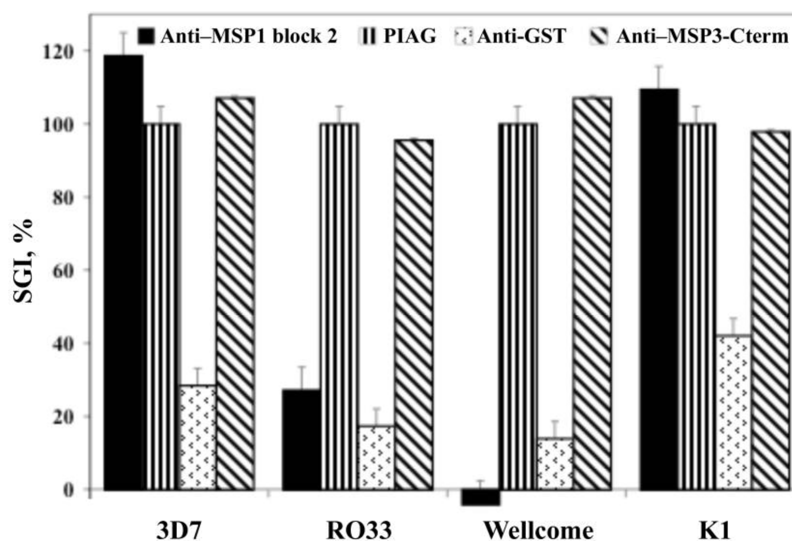

Figure 4. Antibody-dependent cellular inhibition effect of anti–merozoite surface protein-1 (MSP1) block 2 affinity-purified antibodies on 4 *Plasmodium falciparum* strains (3D7, RO33, Wellcome, and K1). Shown are the mean SGI values for each *P. falciparum* strain, obtained in 2 independent experiments performed in duplicate (±SD). PIAG stands for IgG prepared from the pool of hyperimmune African adults serum specimens previously found to confer passive protection when transferred to Thai patients. Anti-GST and anti–MSP3 C-terminus are antibodies affinity-purified on recombinant GST and MSP3 C-terminus, respectively. The values of specific growth inhibitory index obtained with affinity-purified antibodies were adjusted to the effect of PIAG (100%)

The reason why the vast majority of studies concentrated only on the C-terminus of $MSP1_{-19}$ is controversial. It may be because it is the only GPI-anchored part, the majority of monoclonal antibodies produced were directed to the EGF-like domains, and several of them were reported to inhibit erythrocyte invasion by the merozoite [14]. The main aim of MSP1-based vaccines is thus to induce such inhibitory antibodies, even though a human recombinant antibody was shown not to have this effect but to depend on Fc-γ receptors (FcγRI), albeit in a rather artificial model of *P. falciparum msp1*–transfected *Plasmodium berghei* in human Fc-γRI–transfected mice [16].

Interest in block 2 reemerged more recently because of a detailed genetic study of parasite populations combined with the analysis of immune responses to this region, which provided the basis for the concept of polymorphism driven by immune pressure [9]. Indeed, cohort studies found some degree of association between clinical protection against malaria and antibody responses to MSP1 block 2 [7–9].

Our study confirms the strict allele specificity of antibodies directed to MSP1 block 2, both by IFA and, for the first time, by functional ADCI assays. They also confirm the remarkable dominance of the IgG3 subclass described earlier [7, 8].

Our interest in evaluating the second main mechanism of defense against MSP1 block 2, namely ADCI, is partly derived from this observation of a majority of IgG3 responses. We and others found that either total IgG3 against whole parasite extract and antibodies against vaccine targets such as the MSP3b peptide [10] and the MSP3c and MSP3d peptides [11], as well as anti–GLURP R0 antibodies of the IgG3 class, were strongly associated with clinical protection [4]. The present results confirm the importance of the IgG3 subclass in protection against malaria, although it is the subclass with the shortest half-life.

Our finding that anti–MSP1 block 2 antibodies mediate ADCI in an allele-specific manner has several consequences: (1) it adds a new target for antibodies mediating ADCI in addition to the other antigens described earlier, such as MSP3, MSP6, GLURP, and SERP [2–4]; (2) it complements the MSP1-induced effects in addition to invasion of inhibitory antibodies targeting the $MSP1_{-19}$ or $MSP1_{-42}$ fragment, and thereby justifies a posteriori the need to produce full-length MSP1; (3) it confirms our previous report that released antigens can indeed trigger ADCI [1]; and (4) it raises concerns about vaccine-based induction of antibodies to all possible versions of the 3 known alleles, with the added technical difficulty of inducing antibodies of cytophilic classes to each allele and in an equilibrated manner quantitatively.

The reason why some malarial antigens are polymorphic and others are conserved is poorly understood. Therefore, the reasons for choosing as a vaccine candidate one over the other remain debatable. Some may argue that polymorphisms are evidence for immune pressure and hence activity, whereas others defend the point of view that nonpolymorphic candidates, such as the MSP3 C-terminus, are more practical for vaccine development, particularly when both candidates elicit the same mechanism to the same extent.

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