The Malaria Vaccine Candidate GMZ2 Elicits Functional Antibodies in Individuals From Malaria Endemic and Non-Endemic Areas

Micha Phill Grønholm Jepsen,[1,2,3,a] Prajakta S. Jogdand,[1,2,3,a] Susheel K. Singh,[2,3] Meral Esen,[4,5] Michael Christiansen,[1] Saadou Issifou,[4,5] Aurore B. Hounkpatin,[4,5] Ulysse Ateba-Ngoa,[5] Peter G. Kremsner,[4,5] Morten H. Dziegiel,[6] Severin Olesen-Larsen,[1] Søren Jepsen,[1] Benjamin Mordmüller,[4,5] and Michael Theisen[1,2,3]

[1]Department of Clinical Biochemistry, Immunology and Genetics, Statens Serum Institut, [2]Centre for Medical Parasitology at Department of International Health, Immunology, and Microbiology, University of Copenhagen, and [3]Department of Infectious Diseases and [6]Blood Bank, Department of Clinical Immunology, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [4]Institut für Tropenmedizin, Universität Tübingen, Germany and [5]Centre de Recherches Médicales de Lambaréné, Lambarené, Gabon

Background. GMZ2 is a hybrid protein consisting of the N-terminal region of the glutamate-rich protein fused in frame to the C-terminal region of merozoite surface protein 3 (MSP3). GMZ2 formulated in $Al(OH)_3$ has been tested in 3 published phase 1 clinical trials. The GMZ2/alum formulation showed good safety, tolerability, and immunogenicity, but whether antibodies elicited by vaccination are functional is not known.

Methods. Serum samples prior to vaccination and 4 weeks after the last vaccination from the 3 clinical trials were used to perform a comparative assessment of biological activity against *Plasmodium falciparum*.

Results. We showed that the maximum level of immunoglobulin G (IgG) antibodies obtained by GMZ2 vaccination is independent of ethnicity, time under malaria-exposure, and vaccine dose and that GMZ2 elicits high levels of functionally active IgG antibodies. Both, malaria-naive adults and malaria-exposed preschool children elicit vaccine-specific antibodies with broad inhibitory activity against geographically diverse *P. falciparum* isolates. Peptide-mapping studies of IgG subclass responses identified IgG3 against a peptide derived from MSP3 as the strongest predictor of antibody-dependent cellular inhibition.

Conclusions. These findings suggest that GMZ2 adjuvanted in $Al(OH)_3$ elicits high levels of specific and functional antibodies with the capacity to control parasite multiplication.

Keywords. GMZ2; GLURP; MSP3; vaccine; ADCI; antibody; immunity; avidity; clinical trial; *Plasmodium falciparum*.

The GMZ2 malaria vaccine candidate is a recombinant fusion protein expressed in *Lactococcus lactis*. It consists of conserved fragments of the 2 *Plasmodium falciparum* antigens glutamate-rich protein (GLURP) and merozoite surface protein 3 (MSP3). $GLURP_{27-500}$ represents the R0 nonrepeat region, which is a major B-cell epitope [1]. Endogenous GLURP is expressed during preerythrocytic and erythrocytic stages of *P. falciparum* development [2]. $MSP3_{212-380}$ is a conserved part of the otherwise highly polymorphic MSP3 [3]. Numerous seroepidemiological studies have demonstrated that high titers of cytophilic antibodies (especially immunoglobulin G 3 [IgG3]) against the GLURP and MSP3 domains contained in GMZ2 are significantly associated with protection against clinical malaria [1, 4–6]. This correlation is irrespective of the study design, geographic location, and statistical methods used. Furthermore, one study showed that synergism of antibodies to GLURP and MSP3 predicts protection against malaria episodes [7]. A possible

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[a]M. P. G. J. and P. S. J. contributed equally to this study.

Correspondence: Michael Theisen, PhD, DSc, Department of Clinical Biochemistry and Immunology, Statens Serum Institute, Ørestads Blvd 5, 2300 Copenhagen S, Denmark (mth@ssi.dk).

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mechanism of action has been suggested by functional in vitro studies, whereby antiplasmodial antibodies inhibit parasite growth through monocyte-derived mediators, a process referred to as antibody-dependent cellular inhibition (ADCI). We and others have repeatedly demonstrated that IgG antibodies, affinity-purified against defined epitopes and fragments of GLURP and MSP3, from semi-immune African adults inhibit *P. falciparum* growth through ADCI [8–10]. It is hypothesized that anti-GLURP and anti-MSP3 IgG antibodies reduce parasite multiplication in vivo through cross-linking Fcγ receptors on the surface of monocytes, thereby activating these effector cells to secrete toxic substances with the capacity to inhibit *P. falciparum* growth.

The 2 components of GMZ2 have been tested individually as long synthetic peptides in clinical phase 1a studies conducted in Europe. Both vaccine candidates elicited cytophilic IgG antibody responses that showed functional activity in ADCI [11, 12]. Later on, clinical development has focused on the GMZ2 hybrid protein, which can be produced in larger quantities and is highly immunogenic in preclinical models [13]. The GMZ2 vaccine is formulated with $Al(OH)_3$ and given as 3 injections, each 1 month apart. The first-in-human trial, involving German malaria-naive adults, showed good safety, tolerability, and immunogenicity of 3 doses of 10, 30, or 100 μg of GMZ2 [14]. Second, a randomized controlled trial involving malaria-exposed Gabonese adults compared the higher dose of GMZ2 (100 μg) to a control vaccine (rabies vaccine). In this study, the vaccine was well tolerated and boosted preexisting immune responses against the vaccine antigen [15]. Third, a randomized controlled trial involving malaria-exposed African children 1–5 years of age was performed in which 3 doses of either 30 μg or 100 μg of GMZ2 was compared to a control rabies vaccine [16]. Generally, GMZ2 was immunogenic, safe, and well tolerated in all 3 clinical trials. Immune responses tended to be higher against GLURP as compared to MSP3, and antibodies were primarily of cytophilic subclasses. No functional assessment of vaccine-induced antibodies has been done so far. Here, we present a comparative assessment of IgG from 3 clinical trials, using a standardized ADCI assay.

METHODS

Study Populations, Serum Samples, and IgG Purification

Serum samples obtained on day 0 (D0) prior to vaccination and on day 84 (D84; 4 weeks after the last vaccination) were collected in the 3 clinical trials of GMZ2 adjuvanted in $Al(OH)_3$. All samples were collected after informed consent was obtained. Details of the study populations and vaccinations were published previously [14–16]. Sera from German volunteers receiving 30 μg (n = 9) or 100 μg GMZ2 (n = 10), Gabonese adults receiving 100 μg GMZ2 (n = 18) or rabies vaccine (n = 18), and Gabonese children receiving 30 μg GMZ2 (n = 10), 100 μg GMZ2 (n = 10), or rabies vaccine (n = 10) were available for analysis. Total IgG was purified as previously described [17].

Multiplex Assay for Antibody Determination

The multiplex assay with recombinant proteins was performed exactly as described previously [18]. The multiplex assay with synthetic peptides was performed essentially as described for recombinant proteins [18], with the following modifications: 9 peptides, corresponding to amino acids 27–98 (MR186), 87–213 (LR67), 191–288 (LR68), 274–352 (LR129), 304–418 (LR130), and 406–500 (MR187) of GLURP and 181–276 (LR55), 264–323 (MR188), and 307–380 (LR132) of MSP3 [6], were conjugated to 1.25×10^7 microspheres, using 50 μg (LR67, LR68, LR70, and MR187) or 25 μg (LR 129, LR130, LR55, LR132, MR186, and MR188) per bead region. Numbering of peptides was according to previous convention [19]. Samples were tested in duplicate at dilutions of 1:1000 and 1:500 for total IgG and IgG subclasses, respectively. Bound IgG was detected with PE-labeled goat anti-human IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). Detection of IgG1 and IgG3 subclasses were performed after incubating samples with either mouse anti-human IgG1 or IgG3 (clones NL16 and Zg4, Skybio, United Kingdom), followed by incubation with secondary PE-labeled anti-mouse IgG (Jackson ImmunoResearch Laboratories), according to the manufacturer's instructions. Each plate contained positive, negative, and blank controls. To account for interassay variation the median fluorescence intensity (MFI) was adjusted using the positive control as calibrator. Adjusted MFI was calculated using the formula

$$\text{Adjusted MFI} = \frac{(\text{Average MFI calibrator all plates} - \text{MFI blank})}{(\text{MFI calibrator} - \text{MFI blank}) \times (\text{MFI sample} - \text{MFI blank})}$$

Determination of Antibody Avidity

Antibody avidity was estimated essentially as described [20]. Briefly, the recombinant multiplex assay was performed as described above with an additional incubation step with diethylamine (DEA). Serial dilutions (1:250 to 1:781 250) of plasma were tested in duplicate wells with an addition of either 50 μL of DEA (10 mM, pH 10.9) or 50 μL of assay buffer E (0.1% bovine serum albumin, 0.05% Tween 20, and 0.05% sodium azide in phosphate-buffered saline [pH 7.4]). Microspheres were incubated for 15 minutes on a shaker in the dark, washed thrice, and incubated with goat anti-human PE-labeled antibody as described above. An avidity index was calculated for each sample, using the following formula: [reciprocal plasma dilution at the midpoint of DEA-treated sample]/[reciprocal plasma dilution at the midpoint of buffer-treated well].

Parasite Culture and Synchronization

Laboratory-adapted *P. falciparum* isolates NF54, F32, and M7201 [21] were cultured as described previously [22]. Parasites were synchronized for ring stages by use of sorbitol, and schizonts were enriched by flotation on gelatin [23].

ADCI Assay

ADCI assays were performed with cryopreserved monocytes from 2 Danish blood donors as described previously [22]. Because of the known donor-to-donor variation in monocyte activity [22], the data presented here were measured using a mixture of monocytes from 2 donors. Samples from Gabonese children were tested at least 3 times, while German adults were tested once on *P. falciparum* NF54. In addition, D84 samples from Gabonese children and German adults were also tested once by using *P. falciparum* F32 and twice by using the *P. falciparum* M7201. Unfortunately, serum samples from Gabonese adults were not available in sufficient quantity for analysis in ADCI. ADCI assays were also performed on IgG purified from plasma samples from 2 previously published clinical trials that used GLURP [12] or MSP3 [24] as vaccine antigen. Total IgG purified from serum samples of vaccinated individuals was used at a concentration of 125 μg/mL in ADCI assays. The investigators involved in ADCI processing were blinded to treatment allocation of study participants until after measuring, to avoid possible analysis interpretation bias. Each plate contained wells with a positive control (HIG) and a negative control (NIG) as previously described [22]. Specific growth inhibition index (SGI) calculations were performed as previously described, using the formula

$$\text{SGI} = 100 \times \left(\frac{1 - (\%\ \text{parasitemia with MN and test IgG} / \%\ \text{parasitemia test IgG})}{(\%\ \text{parasitemia with MN and NIG} / \%\ \text{parasitemia NIG})} \right)$$

Statistical Analysis

Antibody levels were $\log_{10}$ transformed, and differences between D0 and D84 antibody responses were calculated using a paired *t* test and back transformed to give the ratio to the prevaccination value. Geometric mean levels were compared between vaccine groups using analysis of covariance (ANOVA) or the *t* test. The Bonferroni correction factor was used where multiple comparisons were calculated. Nonparametric tests were used to analyze SGI data. For analysis of antibody data, 0 values were considered as left censored data and replaced with a constant value equal to half of the smallest non-0 value for that antigen in the dataset. Peptide-specific IgG levels from each population were pooled because significant intergroup differences were not anticipated [14, 16]. Univariate linear regression was used to identify the antibody response pattern against single peptides that best predicted ADCI. Statistical analysis was done using GraphPad Prism software, version 5.0, or SAS software, version 9.2. A 2-tailed *P* value of $< .05$ was considered statistically significant.

RESULTS

Antibody Responses in 3 GMZ2-Vaccinated Populations

In previous publications, anti-GMZ2 antibody responses have been assessed in 3 populations: German adults never exposed to malaria [14], Gabonese semi-immune adults [15], and preschool children [16]. To compare antibody responses in these populations, D0 and D84 sera were reanalyzed in a direct comparative manner with a bead-based approach (Figure 1). As reported previously, GMZ2 vaccination elicited a significant increase in geometric mean GMZ2 IgG levels ranging from 2.8-fold (95% confidence interval [CI], 1.7–4.7]) in Gabonese adults to 293-fold (95% CI, 64–1344) in German volunteers receiving 100 μg GMZ2 ($P < .05$, by the paired *t* test; Figure 1). No significant increase was observed in the rabies control groups ($P = .06$, by the paired *t* test; Figure 1). At D0, there was a significant difference in baseline GMZ2 IgG levels in the 3 populations, with Gabonese adults having a 50-fold (95% CI, 12.9–194) and a 63.5-fold (95% CI, (16.1–250.1) higher level of GMZ2 IgG than Gabonese children and German adults,

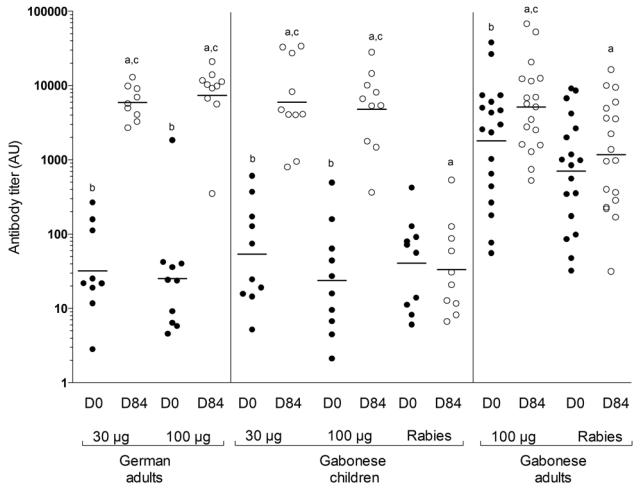

Figure 1. GMZ2 immunoglobulin G (IgG) levels. IgG antibody levels in arbitrary units (AU) against GMZ2 at baseline (day 0 [D0]; black circles) and 1 month after last vaccination (day 84 [D84]; open circles). Each circle represents a sample, the horizontal line represents the geometric mean. Data shown for populations receiving either GMZ2 or rabies vaccine. *A*, Comparison of IgG levels at D0 and D84 within each group. *B*, Comparison of IgG levels at D0 across all groups vaccinated with GMZ2. *C*, Comparison of IgG levels at D84 across all groups vaccinated with GMZ2.

respectively ($P < .0001$, by ANOVA; Figure 1). In contrast, there was no significant difference between the 3 populations at D84 ($P = .94$, by ANOVA; Figure 1). Since booster injections of GMZ2 are intended not only to increase antibody levels but also to increase antibody affinity for the pathogen, avidity maturation was measured in the same samples. At D84, the geometric mean avidity index increased 1.8-fo1d (95% CI, 1.4–2.3) in semi-immune adults and 3.3-fold (95% CI, 2.0–5.6) in children receiving 100 μg GMZ2, whereas no significant increase was observed in children receiving only 30 μg (1.9-fold increase [95% CI, 0.9–3.7]) or in any of the rabies vaccine groups. In summary, the maximum level of IgG antibodies obtained by GMZ2/Al$(OH)_3$ vaccination is independent of ethnicity, *P. falciparum* exposure, and vaccine dose.

Recognition Profiling of GMZ2 IgG

To compare the epitope-recognition pattern in the 3 populations, GMZ2-specific antibodies were measured using a panel of 9 overlapping peptides (Figure 2*A*). On D84 after vaccination, a response was observed against all peptides except MR188 (Figure 2*B*–J). Moreover, geometric mean IgG levels against all GLURP peptides except MR187 were significantly higher in vaccinated children on D84 than in semi-immune adults on D0 ($P < .005$, by the *t* test; Figure 2*B*–*F*), demonstrating that GMZ2 vaccination elicit GLURP-specific responses that exceed those obtained after years of natural exposure in this malaria-endemic area of Gabon. In contrast, MSP3-specific IgG levels on D84 were either similar (Figure 2*H* and 2*J*) or lower ($P < .002$, by the *t* test; Figure 2*I*) in children as compared to the D0 levels in Gabonese adults. Comparison of postvaccination antibody responses in Gabonese children and adults demonstrated that levels of GLURP-specific responses were comparable (Figure 2*C*–*G*) or slightly higher in children ($P < .002$, by the *t* test; Figure 2*B*), while MSP3-specific responses were generally lower in children as compared to adults ($P < .003$, by the *t* test; Figure 2 *H*–*J*), confirming that MSP3 is less immunogenic than GLURP when formulated with Al$(OH)_3$. Since baseline (ie, D0) GMZ2 IgG levels increase with exposure, the fold-increase in peptide-specific responses was plotted against D0 levels for individual volunteers. All peptide responses except that of MR188 showed a reverse correlation with the individual D0 IgG levels, demonstrating that vaccine responses are dependent on levels of preexisting antibodies (Figure 3*A*–*G* and 3*I*).

Functional Activity of GMZ2 Antisera in the ADCI Assay

Upon natural exposure, GLURP and MSP3 components of GMZ2 can induce IgG with ADCI activity [8, 10]. Therefore, we assessed the ability of GMZ2 to elicit similar functional activities. At D0, there was no significant difference in SGI values between GMZ2-vaccinated groups and the rabies control group ($P = .09$, by the Kruskal-Wallis test; Figure 4). In contrast, SGI values increased significantly at D84 for Germans receiving 30 μg or 100 μg of GMZ2 ($P = .01$ and $P = .009$, respectively, by the Wilcoxon matched-pairs signed rank test) and for Gabonese children receiving 100 μg of GMZ2 ($P = .003$, by the Wilcoxon matched-pairs signed rank test). A similar increase was not observed in the rabies control group, suggesting that the ADCI activity at D84 is due to anti-GMZ2 IgG (Figure 4). At D84, German adults and Gabonese children receiving 100 μg had significantly higher SGI values than Germans receiving 30 μg GMZ2 ($P < .05$, by the Kruskal-Wallis test), suggesting that vaccine dose might be an important parameter for induction of ADCI-effective antibodies. Since cytophilic IgG subclasses are the main mediators of ADCI [25], peptide-specific IgG1 and IgG3 responses (Supplementary Figure 1) and avidity indices (Supplementary Table 1) were measured, and their ability to predict ADCI was assessed by linear regression. The best-fitting model indicates that IgG3 against LR55 ($P = .0008$) can account for most of the ADCI activity of IgG from German adults (Table 1). The IgG3 response to LR55 remained significant ($P = .0005$) after including GMZ2-vaccinated Gabonese children in the model, suggesting that IgG against LR55 plays a major role in ADCI induced by GMZ2 vaccination. In contrast, the avidity of GMZ2 specific antibodies did not predict the ADCI activity (Supplementary Table 1).

GMZ2 Antisera Promote ADCI in an Isolate-Independent Manner

Antigenic variation may limit the breadth of inhibitory antibodies as demonstrated for AMA1 [26]. To assess the breadth of *P. falciparum* inhibition, purified D84 IgG from 9 German adults and 6 Gabonese children receiving 100 μg of GMZ2 were tested for ADCI activity on diverse *P. falciparum* laboratory isolates of East African (F32), European/African (NF54), and West African (7201) origin. IgG from German adults ($P = .96$, by the Friedman test) and Gabonese children ($P = .69$, by the Friedman test) promoted ADCI in an isolate-independent manner (Figure 5).

Comparison of Antibody Responses Against GMZ2 With Single-Component Vaccines

Vaccines based on GLURP (LR67) and MSP3 (LR55) were tested individually in phase 1 clinical trials involving European volunteers and were found to elicit ADCI-active antibodies [11, 12]. To compare the functional activity of antibodies generated in the GMZ2, LR67, and LR55 clinical trials, purified IgG from groups of volunteers receiving the same dose (100 μg) in the same adjuvant, Al$(OH)_3$, were assessed in a comparative manner in the ADCI assay. As for GMZ2, there was a significant increase in SGI levels after a full vaccination schedule ($P = .03$ and $P = .03$, by the Wilcoxon matched-pairs signed rank test, for LR67 and MSP3-LR55, respectively; Figure 6). No significant difference in SGI levels was found between the 3

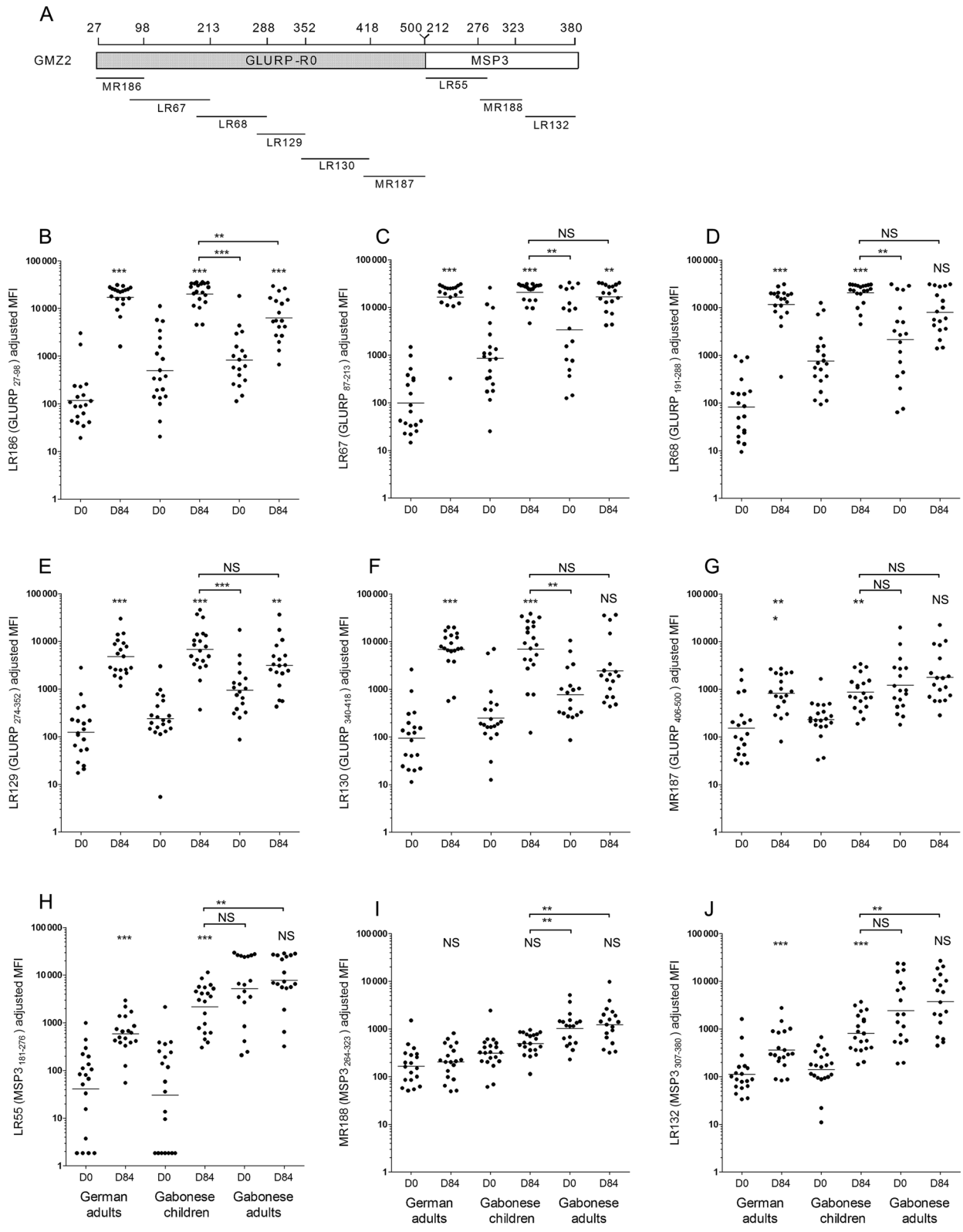

Figure 2. Recognition profile of GMZ2 immunoglobulin G (IgG). Schematic representation of GMZ2 peptides covering GMZ2 (MR186, LR67, LR68, LR129, LR130, MR187, LR55, MR188, and LR132). *A*, Antibody levels (adjusted mean fluorescence intensity [MFI]) on day 0 (D0) and day 84 (D84), displayed according to populations vaccinated with GMZ2 against 9 overlapping peptides (*B–J*), covering GMZ2. For populations receiving 30 or 100 μg of GMZ2, the antibody responses are pooled because differences were small and because, on the basis of previous results, no significant effect was anticipated. Horizontal lines represent geometric means. Because of the number of comparisons, the Bonferroni correction factor was applied, and only *P* values of < .005 are considered statistically significant. *** $P < .0001$ and ** $P < .005$. Abbreviation: NS, not significant.

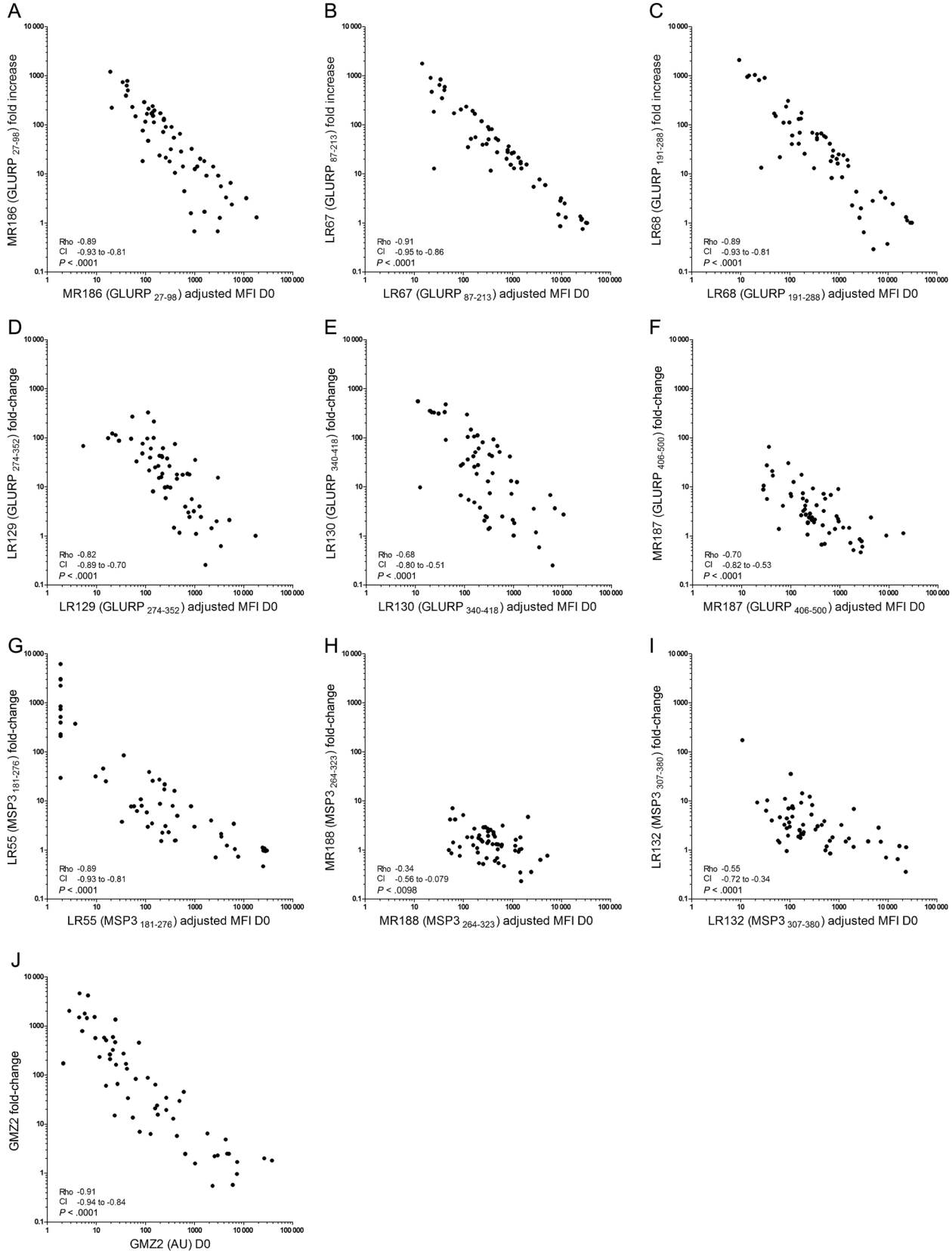

Figure 3. Correlation between day 0 (D0) antibody level and the change from D0 to day 84 (D84). Data from German adults (n = 19), Gabonese children (n = 20), and Gabonese adults (n = 18) vaccinated with GMZ2 are shown. The *x*-axis represents antibody levels on D0, and the *y*-axis represents the fold-change in antibody level from D0 to D84 on a logarithmic scale. Spearman rho values, confidence intervals (CIs), and *P* values are shown in each panel. Because of the number of comparisons the Bonferroni correction factor was applied, and only P values of P < .005 is considered statistically significant.

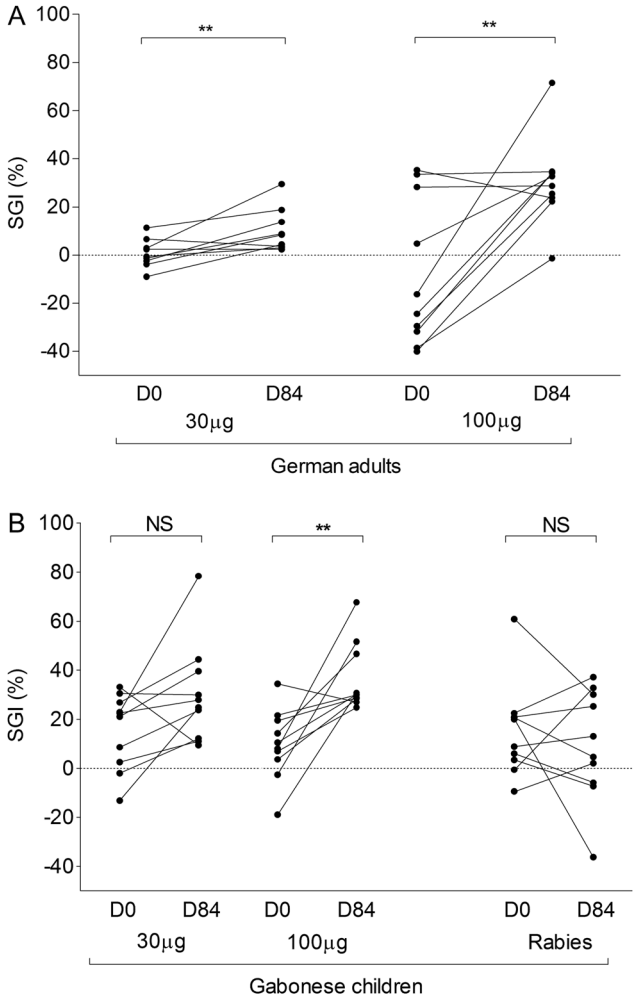

Figure 4. Antibody-dependent cellular inhibition (ADCI) activity of immunoglobulin G (IgG) from vaccinated individuals. ADCI activity was measured as specific growth inhibition (SGI), using laboratory line NF54 in unexposed German adults receiving 30 μg of GMZ2 (n = 9) or 100 μg of GMZ2 (n = 10; *A*) and exposed Gabonese children receiving 30 μg of GMZ2 (n = 10), 100 μg of GMZ2 (n = 10), or rabies vaccine (n = 10; *B*). Results from a representative experiment are shown. ** $P < .01$. Abbreviation: NS, not significant.

groups before vaccination ($P = .11$, by the Kruskal-Wallis test) or after vaccination ($P = .67$, by the Kruskal-Wallis test), suggesting no major difference in the ability to elicit ADCI-effective antibodies between GMZ2, GLURP, and MSP3.

DISCUSSION

In summary, we showed that GMZ2 elicits high levels of functionally active IgG antibodies. Previous work reported humoral responses in GMZ2-vaccinated volunteers, but whether antibodies induced by vaccination were inhibitory was not known [14–16]. We found that both malaria-naive adults and malaria-exposed preschool children elicit vaccine-specific antibodies with broad inhibitory activity against geographically diverse *P. falciparum* isolates.

The design of GMZ2 is based on studies of humans exposed to malaria. These studies led to the identification of B-cell epitopes that are targeted by potentially protective antibodies [8, 9]. Subsequent experiments revealed that affinity-purified IgG against these epitopes can promote strong inhibition of parasite growth in the ADCI assay [6, 27]. Thus, the aim is to design a GMZ2-based vaccination strategy that mimics natural immunity by induction of antibodies that can control parasite multiplication in vivo. It is therefore important to demonstrate that GMZ2 vaccination produces human antibodies that are functionally competent irrespective of prior exposure. Purified IgG from German adults and Gabonese children displayed strong and isolate-independent ADCI activity. A mean increase in ADCI-effective IgG of 9% and 51% was observed in German adults receiving 30 and 100 μg of GMZ2, respectively, whereas children receiving 100 μg of GMZ2 had a 20% increase in ADCI activity. In contrast, the 30-μg dose in children did not result in a significant increase in biological activity. The reason for this observation is unknown but may be related to the finding that 1 of 10 children had lower ADCI activity at D84 (SGI, 9%) as compared to D0 (SGI, 33%). This peculiarity, which was confirmed by retesting the samples in a blinded manner against *P. falciparum* NF54, could not be explained by an inefficient GMZ2 IgG response in terms of quantity, peptide recognition profile, or IgG subclass profile. However, if this aberrant sample is omitted from the comparison, there is a significant difference between D0 and D84 ($P = .0078$).

Since ADCI depends on IgG antibodies [25], it was important to determine whether GMZ2-specific antibodies could explain the observed ADCI activity of serum IgG. Unexpectedly, neither levels nor avidity of GMZ2 IgG were associated with ADCI. However, detailed peptide-mapping studies of IgG subclass responses identified IgG3 against LR55 as the strongest predictor of ADCI, suggesting that a major epitope for ADCI-effective antibodies is located within the MSP3 portion of GMZ2. This finding is in agreement with previous studies of MSP3-LR55 vaccinations, in which LR55 IgG3 was associated with ADCI [11]. GLURP-LR67 vaccinations have also been found to produce ADCI-effective IgG [12], and since anti-GMZ2 IgG reacted strongly with this peptide in all 3 populations, it was unexpected that anti-LR67 IgG did not predict ADCI activity well. Future studies in larger groups of GMZ2-vaccinated children will have to assess the degree to which epitopes within the GLURP portion of GMZ2 elicit ADCI-effective IgG. It has previously been demonstrated that the avidity of vaccine-specific antibodies may affect their functional activity in ADCI [11]. A similar effect of GMZ2 IgG was not observed. Analysis of samples from the ongoing GMZ2 phase 2 efficacy trial may help to ascertain the role of GMZ2 IgG avidity in ADCI.

Table 1. Associations Between Specific Immunoglobulin G (IgG) Antibody Levels and Antibody-Dependent Cellular Inhibition (ADCI) Activity

	Malaria-Naive Adults		Pooled[a]	
Antibody, Variable	Coefficient (95% CI)[b]	*P*	Coefficient (95% CI)[b]	*P*
IgG				
GMZ2	0.001 (−.005 to .003)	.15	0.0003 (−.0003 to .001)	.26
IgG1				
MR186	3.98 (−7.6 to 15.5)	.48	4.54 (−5.3 to 14.4)	.35
LR67	1.00 (−7.8 to 9.8)	.81	2.41 (−5.6 to 10.5)	.54
LR68	0.84 (−7.8 to 9.5)	.84	2.79 (−4.8 to 10.5)	.46
LR129	1.29 (−9.3 to 11.9)	.80	1.89 (−5.9 to 9.7)	.62
LR130	2.59 (−6.5 to 11.7)	.56	−2.14 (−8.4 to 4.1)	.49
MR187	0.71 (−11.9 to 13.3)	.91	−2.62 (−13.9 to 8.7)	.64
LR55	5.17 (−2.5 to 12.9)	.18	3.28 (−1.9 to 8.5)	.21
MR188	1.41 (−14.2 to 17.0)	.85	3.10 (−10.6 to 16.9)	.65
LR132	−2.83 (−15.3 to 9.7)	.64	−0.15 (−9.8 to 9.5)	.97
IgG3				
MR186	9.45 (−3.1 to 22.0)	.13	10.71 (3.3–18.1)	.0057
LR67	3.51 (−4.8 to 11.8)	.39	6.10 (−.1 to 12.3)	.05
LR68	0.36 (−9.2 to 9.9)	.94	5.35 (−1.2 to 11.9)	.10
LR129	3.27 (−4.1 to 10.7)	.36	4.71 (−1.5 to 11)	.13
LR130	1.30 (−7.1 to 10.5)	.77	−1.06 (−7.3 to 5.1)	.72
MR187	7.75 (−9.7 to 25.2)	.36	5.49 (−5.6 to 16.6)	.32
LR55	11.66 (5.6–17.7)	.0008[c]	7.48 (3.5–11.4)	.0005[c]
MR188	4.80 (−9.2 to 18.8)	.48	2.99 (−6.8 to 12.8)	.53
LR132	4.12 (−6.6 to 14.8)	.43	7.39 (.8–13.9)	.02

Abbreviation: CI, confidence interval

[a] Pooled data for malaria-naive subjects from the trial in Germany and the pediatric trial in Gabon. ADCI data from the Gabonese samples are the same as those presented in Figure 4.

[b] By univariate linear regression. Given the number of statistical tests carried out, the Bonferroni correction factor was used to determine the level of significance, and only *P* values of < .002 were considered significant.

[c] Statistically significant.

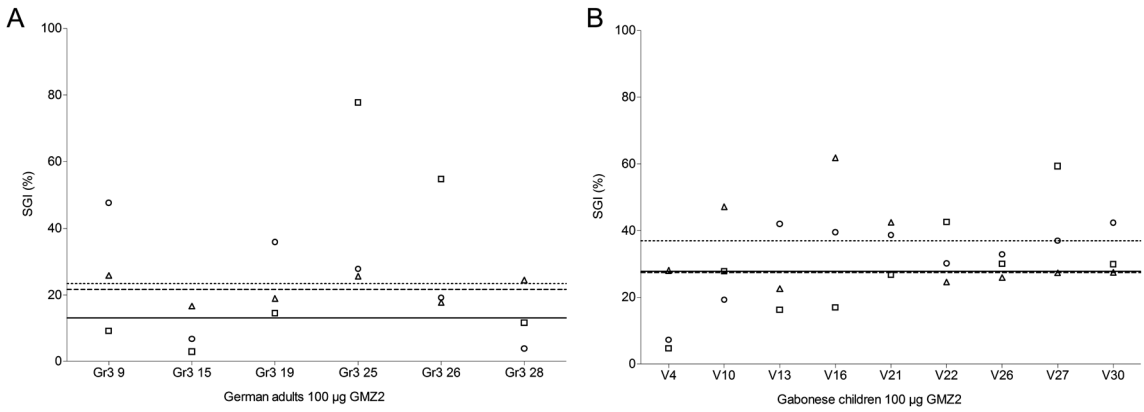

Figure 5. Antibody-dependent cellular inhibition (ADCI) activity, using different laboratory lines. ADCI assay was conducted using laboratory lines F32 (squares), NF54 (triangles), and 7201 (circles) tested on day 84 (D84) samples from German adults (n = 6; *A*) and Gabonese children (n = 9; *B*). Both groups were vaccinated with 100 µg of GMZ2. Horizontal lines denotes median specific growth inhibition index (SGI) for F32 (solid line), NF54 (dashed line), and 7201(dotted line).

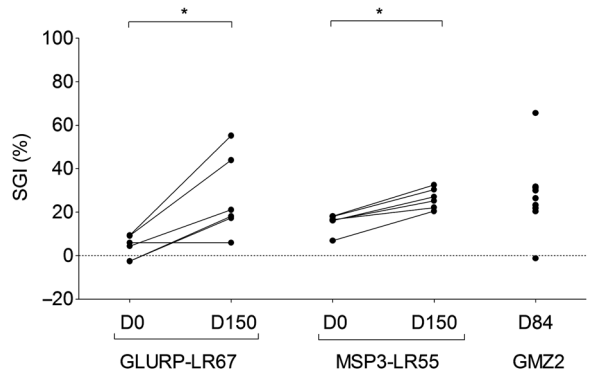

Figure 6. Comparison of GMZ2 to the GLURP-LR67 and MSP3-LR55 vaccines. Antibody-dependent cellular inhibition activity induced by 100 µg of GMZ2 in German adults (n = 10), 100 µg of GLURP-LR67 in Dutch adults (n = 6), and 100 µg of MSP3-LR55 (n = 6) before vaccination (day 0 [D0]) and 1 month after the last vaccination (day 150 [D150] or day 84 [D84]). Abbreviation: SGI, specific growth inhibition index.

The rationale for creating the GLURP-MSP3 chimera was to obtain an immune response against both domains that was stronger than that of the single-component vaccines [19]. Human vaccine trials of the individual GLURP and MSP3 components have been performed, but whether the antibodies elicited by vaccination promote the same level of ADCI is unknown [11, 12]. Here, we demonstrated that GMZ2 vaccinations promote antibodies with ADCI activity similar to that of the single components. Of importance, retesting of samples from the LR55 and LR67 clinical trials confirmed the significant increase in functional activity of antibodies produced by vaccination. Thus, human clinical trials confirm results of the initial mouse studies.

The relatively limited antigenic variation in the GLURP and MSP3 regions included in GMZ2 may facilitate the generation of broadly neutralizing antibodies. Here, we demonstrated that GMZ2 IgG was equally inhibitory against 3 geographically and genetically diverse *P. falciparum* isolates. Whether, GMZ2 IgG also inhibits parasite multiplication in infected children from geographically different regions of Africa remains to be investigated.

Preexisting antibodies have been demonstrated to modify vaccine responses in mice [28]. We found a strong correlation between levels of specific antibodies after vaccination and the prevaccination level at the individual level. As expected, individuals with low prevaccination levels, such as malaria-naive Germans and African preschool children, responded strongly to vaccination, whereas Gabonese adults with years of exposure to *P. falciparum* showed a smaller boost of naturally acquired antiparasitic responses. The peptide-mapping studies revealed that antibody responses were mainly directed against epitopes in the GLURP part of GMZ2 and less so against the MSP3 part. In fact, only German adults and Gabonese children showed an increase in MSP3 titers. This is in accordance with our previous findings [14–16] and suggests that prior exposure to *P. falciparum* might diminish subsequent boosting by vaccination. The missing boosting effect against the MSP3 part of the vaccine in the semi-immune adults has also been observed in Burkinabe adults vaccinated with MSP3-LR55 [29], indicating that this is a property of MSP3 rather than a result of its coexpression with GLURP. Alternatively, it might also be related to the adjuvant used. $Al(OH)_3$ is recognized as a particularly safe but comparatively mild adjuvant. Indeed, alternative delivery systems [30] or new and stronger adjuvants [31] can increase the overall immunogenicity of GMZ2 in mice and, in particular, the MSP3 part. Whether these findings also apply to humans remains to be investigated. Other factors, such as genetic background, age, nutrition status, and chronic parasitic infections, have been shown to alter the host immune response to vaccination, and we cannot exclude the contribution of these factors [32–36].

In conclusion, we found that GMZ2 elicits high levels of functional antibodies in both nonexposed and malaria-exposed volunteers with broad neutralizing activity against asexual stages of geographically diverse *P. falciparum* strains and that the magnitude and fine specificity of the antibody response may vary with exposure.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflict of interest. All authors: No reported conflicts.

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