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Immune Responses Induced by Gene Gun or Intramuscular Injection of DNA Vaccines That Express Immunogenic Regions of the Serine Repeat Antigen from *Plasmodium falciparum*

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The liver- and blood-stage-expressed serine repeat antigen (SERA) of *Plasmodium falciparum* is a candidate protein for a human malaria vaccine. We compared the immune responses induced in mice immunized with SERA-expressing plasmid DNA vaccines delivered by intramuscular (i.m.) injection or delivered intradermally by Gene Gun immunization. Mice were immunized with a pcdna3 plasmid encoding the entire 47-kDa domain of SERA (amino acids 17 to 382) or the N-terminal domain (amino acids 17 to 110) of SERA. Minimal antibody responses were detected following DNA vaccination with the N-terminal domain of SERA, suggesting that the N-terminal domain alone is not highly immunogenic by this route of vaccine delivery. Immunization of mice by Gene Gun delivery of the 47-kDa domain of SERA elicited a significantly higher serum antibody titer to the antigen than immunization of mice by i.m. injection with the same plasmid did. The predominant isotype subclass of the antibodies elicited to the SERA protein following i.m. and Gene Gun immunizations with SERA plasmid DNA was immunoglobulin G1. Coimmunization of mice with SERA plasmid DNA and a plasmid expressing the hepatitis B surface antigen (pCMV-s) by the i.m. route resulted in higher anti-SERA titers than those generated in mice immunized with the SERA DNA plasmid alone. Vaccination with DNA may provide a viable alternative or may be used in conjunction with protein-based subunit vaccines to maximize the efficacy of a human malaria vaccine that includes immunogenic regions of the SERA protein.

Human malarial infections caused by *Plasmodium falciparum* claim more than 2 million lives annually (particularly children). Current strategies for the control and treatment of malaria are compromised by several factors including the resistance of mosquitoes to insecticides and the resistance of parasites to antimalarial drugs. While a human malaria vaccine is highly desirable, the current strategies for developing a malaria vaccine are complicated by the existence of multiple, potentially variable, parasite antigens and parasite developmental stages to which distinct immune responses must be targeted in order to attain clinically effective protection. Progress towards a human malaria vaccine has been slow, largely due to a lack of available information on appropriate adjuvant and parasite antigen combinations that induce protective immunity in humans.

The *P. falciparum* serine repeat antigen (SERA) is a 120-kDa protein that is highly expressed in trophozoite and schizont blood stages of the *P. falciparum* life cycle (19). SERA is also synthesized during liver stages of infection (53). This liver- and blood-stage antigen is a candidate human malaria vaccine blood-stage antigen, in part, because it is highly conserved among clinical and field isolates of *P. falciparum* (3, 7, 19, 43).

In schizont stages, SERA is abundantly synthesized and secreted into the parasitophorous vacuole (13, 37). At the time of parasite egress from infected erythrocytes, a fraction of the accumulated 120-kDa pool of SERA protein is proteolytically processed into 47-kDa (an N-terminal fragment), 50-kDa (an interior fragment that contains a strong homology to the active-site domain of cysteine/serine proteinases), and 18-kDa fragments which accumulate in culture medium (10–12, 17). A pool of full-length SERA protein (the 120-kDa form) remains unprocessed, associates as a nonintegral membrane protein with the surfaces of free infectious merozoites (45–47), and retains the capacity to bind to inner-leaflet erythrocyte plasma membrane phospholipids (47). The 120-kDa SERA protein also associates with high-molecular-weight rhoptry proteins (47). These observations have led to the dual hypothesis that the SERA protein is likely to play a role in parasite invasion and parasite egress (12, 33, 37, 38, 44, 45, 47).

Consistent with this hypothesis, rodent and goat polyclonal antibodies directed against amino acids 24 to 285 or the 47-kDa domain of the SERA protein effectively block parasite growth in vitro (1, 2, 6, 20, 27, 44, 45). The mechanism of in vitro parasite growth inhibition appears to rely on some combination of agglutination of schizonts and merozoites (45), complement-mediated lysis of late-stage schizonts (44), or inhibition of parasite invasion of erythrocytes. A mouse monoclonal antibody specific to SERA (43E5) inhibits parasite growth in vitro (1, 2, 6, 27). The SERA epitope that is reactive with the parasite-inhibitory mouse monoclonal antibody 43E5 is localized to the N-terminal SERA domain spanning amino acids 17 to 110 (20). Protective immunity to *P. falciparum* is induced in *Aotus* monkeys immunized with a recombinant SERA protein comprising amino acids 24 to 285 (28–30) or in squirrel monkeys immunized with the 47-kDa domain (52). Protection after vaccination with the SERA protein in complete Freund's adjuvant (CFA) in the primate model is nonsterile (28–30, 52). Protection in the primate model is adjuvant

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specific, with protection being observed with adjuvants that are not approved for use in humans (28–30). Interestingly, in the rodent model, recombinant SERA protein corresponding to the 47-kDa domain was nearly as effective at stimulating parasite-inhibitory antibodies with or without the use of adjuvant (44).

The ability to induce an immune response to a protein antigen by administration of plasmid DNA encoding the antigen has been successfully demonstrated in many different disease and animal models. For some antigens, a single immunization suffices in eliciting long-lasting immunity (8, 9, 21, 23, 34, 36, 40, 54, 57). However, for other antigens, repeated administration of DNA is required to attain either long-lasting or any detectable immune response (4, 8, 14, 39, 50). Malaria DNA vaccines based on sporozoite stage or hepatocyte stage antigens from rodent malaria have recently been evaluated (15, 16, 22, 24–26, 35, 42, 50). Immunization of mice with a plasmid encoding the circumsporozoite protein or the PyHep17 liver-stage antigen of *Plasmodium yoelii* confers protection to mice from a subsequent challenge with this rodent-specific species.

In this study, we have analyzed a potential blood-stage human malaria DNA vaccine that expresses SERA from *P. falciparum*. We chose the 47-kDa domain of SERA because amino acids 24 to 285 of this domain can confer protective immunity in the *Aotus* monkey model (28–30). In addition, we examined an N-terminal fragment consisting of amino acids 17 to 110 of the 47-kDa subunit because a parasite growth-inhibitory epitope mapped to this region (20). This is the first report of humoral immune responses elicited by a potential human malaria DNA-based vaccine derived from a malaria parasite blood-stage antigen.

MATERIALS AND METHODS

Construction of DNA vectors. The SE47′ gene was excised from the pET vector utilizing the *Nde*I site on the 5′ end and the *Bam*HI site on the 3′ end of the gene (51). It was then cloned into the pcDNA I:Neo eucaryotic expression vector by blunting the 5′ end of the gene into the blunted *Hin*dIII site of the vector, while the 3′ end was ligated to the compatible *Bam*HI site in the vector. The blunted *Nde*I site at the 5′ end of the gene and the blunted *Hin*dIII site of the vector, when ligated, regenerate a *Hin*dIII site. The SE47′ gene was excised from the pcDNA I:Neo vector by using *Hin*dIII (partial digest) and *Bam*HI and inserted into the pcdna3 vector (InVitrogen). This new plasmid construct was named pcdna3 SERA 17-382. The pcdna3 SERA 17-110 plasmid construct was generated by utilizing the *Bsp*120I sites located at amino acid 110 of SERA and in the polylinker of pcdna3. All plasmid ligation junctions were confirmed by DNA sequence analysis (5). Heather Davis supplied the pCMV-s plasmid that served as a positive control (41). Large-scale plasmid DNA preparations were purified twice with cesium chloride gradients.

Protein expression. The expression of the SE47′ gene constructs was verified in vitro, by transfection into either Cos cells or P815 HTR cells (data not shown). All of the constructs were transfected into cells with Lipofectin (GIBCO/BRL), and protein expression was measured by an immunofluorescence assay.

Animals. Female BALB/c mice 4 to 6 weeks of age, were used for all of the vaccine trials in this study. BALB/c mice have been successfully used in several protective models of DNA immunization (50, 54, 56, 57).

Immunizations. In the first study, 100 μg of DNA resuspended in 50 μl of 0.9% saline was injected in each quadriceps muscle at each time point; in total, each mouse received 600 μg of DNA. In this experiment, we followed the immunization schedule used by Sedegah et al., which includes three immunizations at 0, 5, and 8 weeks (50). Polyethylene tubing (inner diameter of 0.38 mm) was placed over the syringe needles so that only 2 to 3 mm protruded (55). This procedure helps to ensure that during the injection the needle can be inserted only 2 to 3 mm and cannot pass through the entire muscle mass. Eight mice were coimmunized with pcdna3 and pCMV-s, eight were coimmunized with pcdna3 and pcdna3 SERA 17-382, eight were coimmunized with pCMV-s and pcdna3 SERA 17-382, and the last eight were coimmunized with pCMV-s and pcdna3 SERA 17-110. The pcdna3 vector was included in order to maintain the same concentration of DNA in all of the mice. Fifty micrograms of each plasmid was combined for each injection. Mice were anesthetized with Metofane before the injections and remained anesthetized for 15 min after the injections (55).

In the next study, 12 mice were immunized by intradermal Gene Gun inoculation (31). Two DNA vaccine plasmids, pcdna3 SERA 17-382 and pcdna3 SERA 17-110 were coated onto gold particles. Plasmid DNA was precipitated onto 1-μm-diameter gold beads using a solution of 1.0 M $CaCl_2$ and 100 mM spermidine as previously described (18, 48). DNA-coated gold particles were delivered to the shaved abdomens of ketamine- and xylazine-anesthetized mice. Each mouse received six shots of DNA-coated gold particles with an Accell instrument (Agrecetus, Middleton, Wis.) at a helium discharge pressure of 400 to 450 lb/in^2. Mice were immunized at weeks 0 and 4. Serum was obtained from the animals at 4, 8, 12, and 23 weeks. In the third study, 70 BALB/c female mice were used. Twenty-five mice were immunized with pcdna3 SERA 17-382 by using the Gene Gun, and 25 mice were immunized with the same plasmid by intramuscular (i.m.) injection. In this experiment, small incisions were made in the skin in order to expose the quadriceps muscles before inoculation. Ten mice were immunized with pcdna3 SERA 17-110 by using the Gene Gun. Negative controls for both Gene Gun and i.m. were also incorporated. Five mice received pcdna3 vector by Gene Gun delivery, and five mice received the vector by i.m. injections. All of the mice were immunized at 0 and 4 weeks. Serum samples were taken at 6, 8, and 18 weeks.

Antibody measurements. Serum was collected from the mice by tail bleeding or by cardiac puncture at the time mice were sacrificed. Antibody responses to the different SE47′ constructs were analyzed by enzyme-linked immunosorbent assay (ELISA) as previously described (20). Briefly, 100 ng of recombinant SERA1 (2) (SERA amino acids 24 to 285 expressed in yeast)/well in 50 μl of 50 mM carbonate buffer (pH 9.6) was bound to each well of a 96-well plate overnight at 4°C. Wells were blocked with 200-μl portions of 5% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 2 h at 20°C. Serum samples were added in PBS plus 0.1% BSA plus 0.05% Tween 20, and serial dilutions were performed directly in the wells. The secondary antibody, biotinylated anti-mouse immunoglobulin G (IgG) and IgM (Pierce) was used at a 1:5,000 dilution. Biotinylated secondary antibodies (Zymed) were used to determine antibody isotype subclasses at dilutions of 1:2,500 to 1:5,000. The Vectastain Elite kit avidin-horseradish peroxidase conjugate was used in PBS plus 0.05% Tween 20. The Vector Laboratories 2,2′-azinobis(3-ethylbenzthiazoline sulfonic acid) (ABTS) detection kit reagent was used as the substrate. Antibody responses to the hepatitis B surface antigen were measured by ELISA with recombinant hepatitis B surface antigen (Genzyme). SERA antibody ELISA titers were read at 405 nm with a microtiter plate reader (Molecular Devices, Inc.). Serial dilutions were used to measure the end point-positive titers. The cutoff point for a positive ELISA value reading was set at an absorbance of 0.3. The titer of each serum sample is the inverse of the largest dilution at which the substrate colorometric development absorbance is above (>0.3) that of a negative control (<0.3). The variation in the end point-positive titers was always within 1 "twofold serial dilution." Preimmune serum titers for the SERA protein were <80 in this assay.

RESULTS

Immune responses induced by i.m. injection of DNA vaccines. Mice were immunized by i.m. injection in each quadriceps muscle with 100 μg of plasmid DNA and given booster injections twice at 5 and 8 weeks after the primary inoculation. As a positive control for immunization by i.m. injection, the pCMV-s DNA plasmid was coinjected with SERA DNA plasmid in some groups of mice. The pCMV-s plasmid, a successful DNA vaccine, elicits humoral and cell-mediated immune responses to the hepatitis B surface antigen (8, 9). Two SERA DNA vaccines with which we obtained good protein expression in transiently transfected Cos cells and P815 HTR cells (data not shown) were evaluated. The first pcdna3 SERA 17-382, encodes the full-length SE47′ recombinant protein, and the second expresses only the N-terminal SERA amino acids 17 to 110. Serum was collected 1 to 2 weeks after the last booster injection. Four of eight mice immunized with the pcdna3 SERA 17-382 plasmid responded to the SERA protein with end point titers ranging from 200 to 800 (Fig. 1, left panel). Five of eight mice coimmunized with pCMV-s and pcdna3 SERA 17-382 responded with titers ranging from 200 to 4800 (Fig. 1, middle panel). Seven of those eight mice also responded to the hepatitis B surface antigen (data not shown). Six of eight mice coimmunized with pCMV-s and pcdna3 SERA 17-110 responded to the SERA protein with titers ranging from 200 to 800 (Fig. 1, right panel). All of the mice in this coimmunized group also responded to the hepatitis B surface antigen, as well as mice immunized only with pCMV-s (data not shown). Mice immunized by i.m. injection with only pcdna3

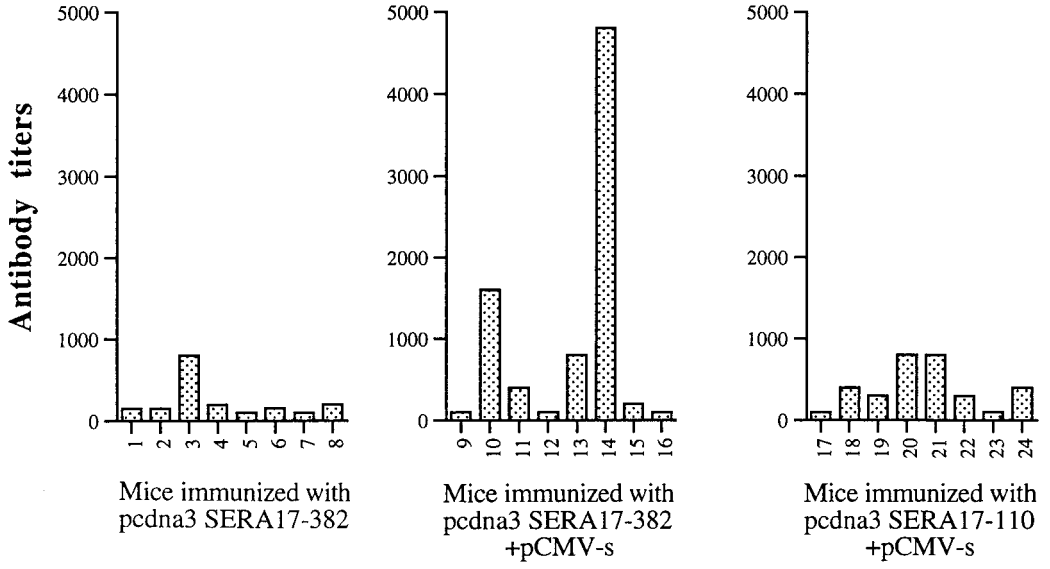

FIG. 1. Antibody titers from mice immunized with pcdna3 SERA 17-382 or pcdna3 SERA 17-110 DNA vaccines by i.m. injection. Where indicated, mice groups were coimmunized with the pCMV-s DNA plasmid, which was used as a positive control. Mice were immunized at 0, 5, and 8 weeks, and serum samples were collected at week 10. Serial dilutions were used to measure the end point-positive titers. The results presented are the averages of two separate ELISAs. The titer of each serum sample is the inverse of the largest dilution at which the substrate colorometric development is above that of a negative control. The variation in the end point-positive titers was always within one "twofold serial dilution." Preimmune serum titers for the SERA protein were <80 in this assay.

plasmid DNA or pCMV-s plasmid DNA had undetectable anti-SERA titers of <80 (data not shown).

Following i.m. injection, an antibody response to hepatitis B surface antigen was observed in 23 of 24 mice that were immunized with pCMV-s. The antibody titers generated to the hepatitis B surface antigen in pCMV-s-immunized mice were equivalent to those generated in pCMV-s-pcdna3 SERA-coimmunized mice. On the other hand, the hepatitis B surface antigen, a strong immunogen encoded on the pCMV-s plasmid, had adjuvant-like activity that resulted in the generation of higher anti-SERA antibody titers in the groups of coimmunized mice (Fig. 1).

Immune responses induced by Gene Gun injection of SERA DNA vaccines. The same SERA-expressing plasmids were used to immunize mice via Gene Gun. Mice were immunized at 0 and 4 weeks. Serum samples collected at the 4-week time point before the booster dose showed that none of the mice responded to SERA after one immunization (data not shown). At 8 weeks, 4 weeks after the final Gene Gun injection, five of the six mice that received pcdna3 SERA 17-382 plasmid DNA had generated antibodies with end point-positive titers ranging from 1,600 to 25,600 (Fig. 2, top panel). These titers are up to 32-fold higher than the highest titer that was generated by i.m. injections of the same plasmid (compare Fig. 1 and Fig. 2). All of the mice immunized with pcdna3 SERA 17-110 by Gene Gun injection had low but detectable antibody responses to the SERA protein, and all measured responses were less than or equal to a titer of 400 at all time points analyzed. Although it has been demonstrated that parasite growth-inhibitory epitopes lie within the SERA 17-110 region (20), it appears from this data that effective T-cell helper epitopes may not. Therefore, in the absence of adequate T-cell helper epitopes and without adjuvant, the mice cannot effectively respond to the parasite growth-inhibitory epitope(s) within this region of the SERA protein. These results are also consistent with the lower anti-SERA titers observed after i.m. injection with pcdna3 SERA 17-110 plasmid DNA.

In the pcdna3 SERA 17-382 Gene Gun-injected mice, the 12-week time point titers ranged from 800 to 12,800. At the 23-week time point, the maximum titers had decreased to 4,000 (Fig. 2, top panel).

Comparative immune responses induced by i.m. injection or Gene Gun delivery of SERA DNA vaccines. The Gene Gun immunizations were repeated and directly compared with i.m. immunizations in the same experiment. In this study, like the Gene Gun group, the i.m.-vaccinated mice received only two immunizations at 0 and 4 weeks; thus, the vaccination schedules were the same for both the Gene Gun and i.m. delivery systems. In addition, the i.m. immunization protocol differed slightly from previous experiments in that small incisions were made on the back legs of the mice in order to expose the quadriceps muscles prior to injection. This experiment allowed us to more directly compare the responses generated to SERA DNA vaccine delivery by the i.m. and intradermal (Gene Gun) routes.

Serum samples were taken at 6 weeks, and ELISA analysis showed that about half of the mice immunized by i.m. injection with pcdna3 SERA 17-382 had generated low but detectable levels of anti-SERA antibodies with titers ranging from 200 to 800 (Fig. 3, right panel). Mice that were immunized by i.m. injection with pcdna3 SERA 17-110 had no detectable antibody responses (titers <80) (data not shown). In contrast, at the 6-week time point, each of the 25 mice immunized by Gene Gun with pcdna3 SERA 17-382 had generated positive titers to SERA, with 22 of 25 mice having ELISA titers between 1,000 and 8,000 (Fig. 3, left panel). At the 8-week time point, serum samples were collected from a subset of the immunized mice. The responses (titers) were not significantly different between the 6-week and 8-week time points in either the groups of mice immunized i.m. or with Gene Gun (Fig. 3).

Analysis of antibody isotype subclasses generated following i.m. injection or Gene Gun injection of pcdna3 SERA 17-382 plasmid DNA. In addition to determining the end point-positive titers, we also examined the isotype subclasses of the an-

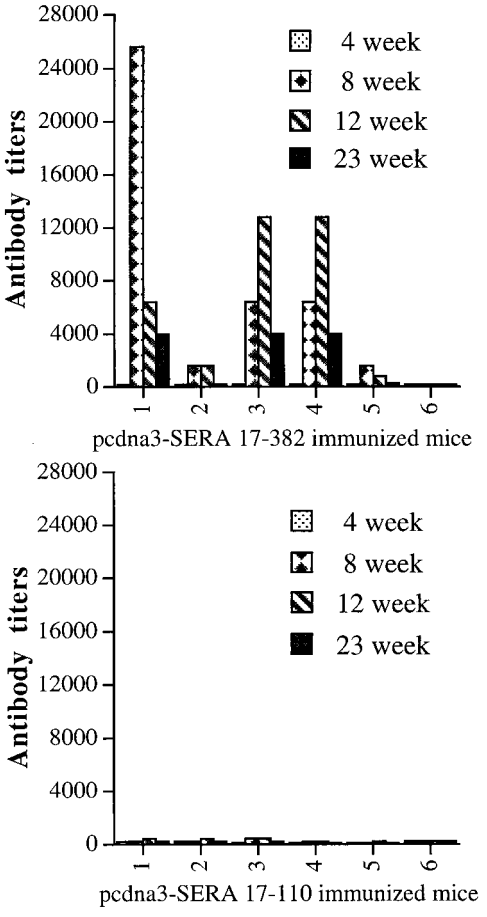

FIG. 2. Antibody titers from mice immunized with pcdna3 SERA 17-382 or pcdna3 SERA 17-110 DNA vaccines by intradermal Gene Gun injection. Serum samples were collected at weeks 4, 8, 12, and 23 from mice immunized at weeks 0 and 4. Serial dilutions were used to measure the end point-positive titers. The results presented are the averages of two separate ELISAs. The titer of each serum sample is the inverse of the largest dilution at which the substrate colorometric development is above that of a negative control. The variation in the end point-positive titers was always within one "twofold serial dilution." Preimmune serum titers for the SERA protein were <80 in this assay.

tibodies that were generated following vaccination. Twelve mice of each group (i.m. or Gene Gun) with variable end point-positive ELISA titers to SERA (Fig. 3) were assessed to determine the relevant antibody isotypes. The predominant isotype subclass of the antibodies generated in response to the Gene Gun immunizations was IgG1 (Table 1). The predominant subclass generated in response to the i.m. immunizations was IgG1 for the SERA protein, but there were also significant levels of IgM even 4 weeks after the last immunization. The ratio of isotypes was consistent in all of the mice evaluated, regardless of the overall level of the total IgM and IgG anti-SERA titer. Therefore, both methods of immunization using the SERA DNA vaccine stimulated Th2-type responses to the SERA protein. In contrast, IgG2a was the predominant subclass of the anti-hepatitis B surface antigen antibodies generated in response to the pCMV-s positive-control vector injected by the i.m. route in both mice immunized with pCMV-s alone and mice coimmunized with pcdna3 SERA 17-382. Injection of pCMV-s by the i.m. route also elicited significant levels of IgM (Table 1).

DISCUSSION

The anti-SERA antibody responses generated to the SERA DNA vaccines are moderate compared to the antibody responses that are generated with recombinant protein and adjuvant (2, 20, 44, 51). The highest anti-SERA titer generated via i.m. injection, 4,800, was generated in a mouse that was coimmunized with the hepatitis B DNA vaccine. This titer is about 50-fold lower than antibody titers generated to the corresponding glutathione *S*-transferase (GST)-SERA fusion protein delivered together with CFA to mice intraperitoneally (titers were generated in the same ELISA). Even when mice were vaccinated by Gene Gun, which is optimal for generating high antibody titers in DNA-mediated immunization systems, the highest anti-SERA titer was 25,600, a titer that is significantly lower than we observed previously with recombinant protein antigen and adjuvant (20).

The predominant isotype subclass of the antibodies generated to the SERA DNA vaccines by Gene Gun immunization is IgG1. Gene Gun immunizations commonly elicit Th2-type responses of which IgG1 is a hallmark (18, 42). Surprisingly, IgG1 was the predominant subclass from immunization with SERA DNA vaccines by i.m. injection. To date, there are no published reports of a dominant Th2-type response being generated from immunization of mice with any DNA vaccine by i.m. injection. Immunization of mice with the SERA 17-382 DNA vaccine by i.m. injection also produced a moderate level of IgM antibodies. This Th2-type response is specific to the SERA protein, because IgG2a is the predominant isotype subclass of the antibodies generated to the hepatitis B surface antigen from the pCMV-s positive-control DNA vaccine in the coimmunized mice. This indicates that the immune response to the hepatitis B surface antigen was mediated by a Th1-type and not by Th2-type helper T-cell response. These mice also had significant levels of anti-hepatitis B surface antigen IgM antibodies. It would be interesting to determine whether the Th2 response to the SERA protein is specific to BALB/c mice or if i.m. immunization of other mouse strains also results in the induction of Th2-type responses. It is interesting to note that IgG2a antibodies to SERA can be generated in BALB/c mice when they are immunized with recombinant SERA protein and CFA (44, 45). Furthermore, anti-SERA IgG2a, IgG2b, and IgG3 are significantly more potent in in vitro parasite growth inhibition assays than is anti-SERA IgG1 (44, 45). It is not known what type of response is protective during a human malaria infection; hence it will be valuable to determine the isotypes of the anti-SERA antibodies generated during a human infection.

Preliminary data suggest that a single injection of recombinant SERA protein can only boost anti-SERA titers in Gene Gun-immunized mice. One mouse originally immunized via Gene Gun received a booster dose with recombinant GST-SERA 17-382 protein in incomplete Freund's adjuvant at 18 weeks. The mouse generated antibody titers to SERA which were 15- to 20-fold higher than the preboost titer. The final titer of 51,200 was four- to fivefold higher than the original titers found in that same mouse 2 to 4 weeks after immunization with the SERA DNA vaccine via Gene Gun. Even later, at 26 weeks, another Gene Gun pcdna3 SERA 17-382-immunized mouse received one protein booster injection; the titer before the booster injection of 800 rose 40-fold to 32,000 2 weeks after the booster injection (data not shown). In contrast, no increase in anti-SERA antibody titers was observed in three i.m. pcdna3 SERA 17-382 immunized mice, and no antibody response was generated in pcdna3 plasmid vector-immunized mice or naive mice. Although these data are generated from a

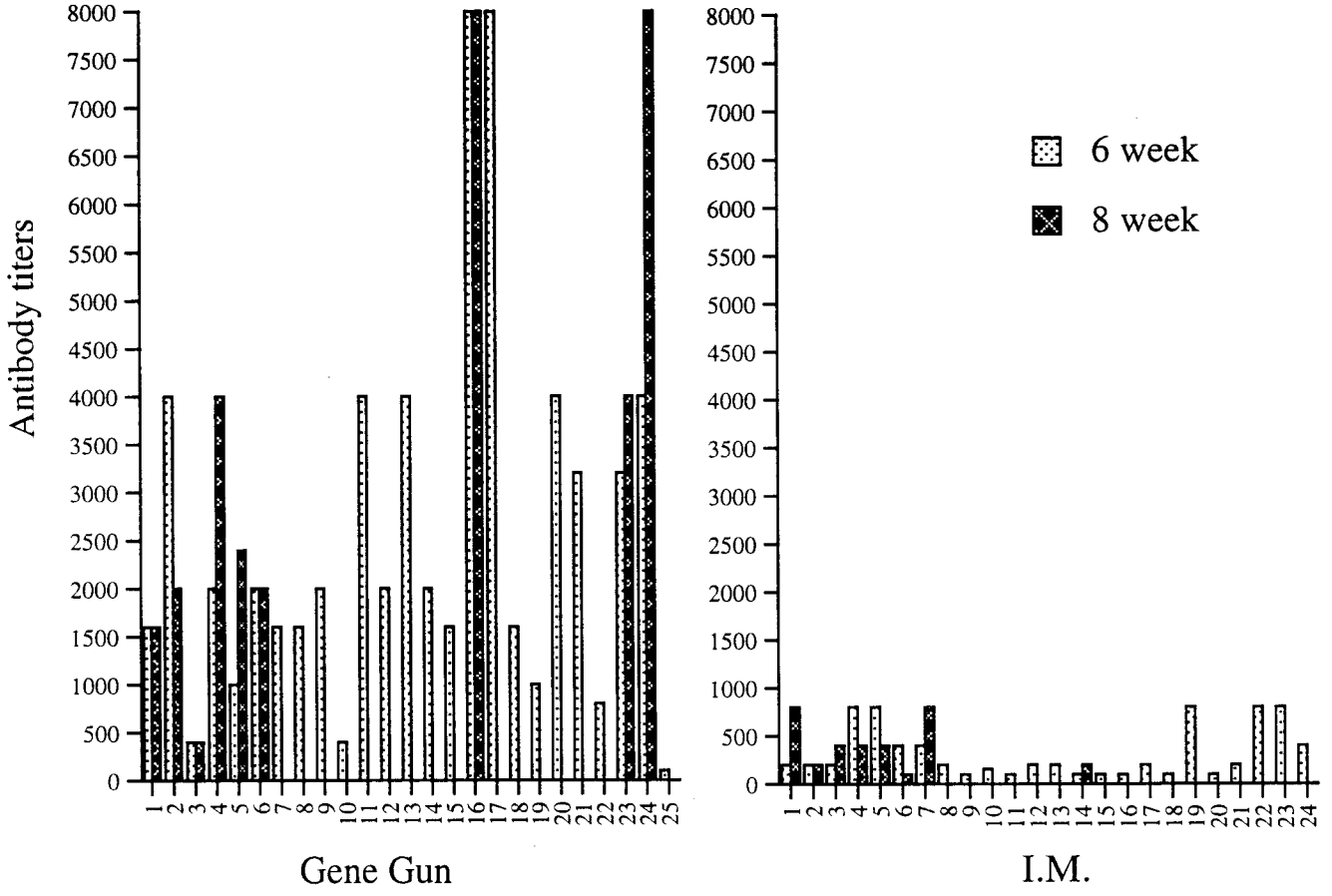

FIG. 3. Antibody titers from mice immunized with the pcdna3 SERA 17-382 DNA vaccine. Mice were immunized by i.m. injection or Gene Gun, as indicated. Serum was collected at weeks 6 and 8; all mice were immunized at weeks 0 and 4. Serial dilutions were used to measure the end point-positive titers. The results presented are the averages of two separate ELISAs. The titer of each serum sample is the inverse of the largest dilution at which the substrate colorometric development is above that of a negative control. The variation in the end point-positive titers was always within one "twofold serial dilution." Preimmune serum titers for the SERA protein were <80 in this assay.

small group of animals, they do suggest that for the SERA protein, Gene Gun delivery is more successful than i.m. delivery, especially at stimulating memory responses to the SERA protein. Our observation that antibody titers are significantly boosted by a single delivery of protein antigen in Gene Gun-immunized mice several months after DNA vaccination is significant for two reasons. First, natural exposure to malaria antigens may boost immune responses following Gene Gun DNA vaccination. Second, these data suggest that improved malaria vaccines may be developed by further exploring combined DNA and protein vaccination protocols.

TABLE 1. Anti-SERA antibody isotypes generated by i.m. and Gene Gun DNA immunizations

Immunization (*n*)	Distribution of antibody isotypes generated by immunization[a]				
	IgM	IgG1	IgG2a	IgG2b	IgG3
Gene Gun pcdna3 SERA 17-382 (12)	−	++++	−	−	−
i.m. pcdna3 SERA 17-382 (12)	+	+++	−	−	−
i.m. pCMV-s (4)	+	+/−	+++	−	−

[a] End point-positive titers were compared. The total amount of immunoglobulin detected is represented by +. A single + indicates that antibody isotype represented approximately 25% of the total reactive IgM and IgG in each mouse. The isotype ratios were nearly identical for every mouse in each group.

A SERA DNA vaccine could be further improved by the addition of immunostimulatory CpG motifs to the plasmid. CpG motifs preferentially stimulate the production of Th1 cytokines such as interleukin 12 and gamma interferon (32, 49). The pcdna3 plasmid does contain some CpG motifs, but those motifs do not provide immunostimulatory activity in our system. The SE47′ gene itself does not contain any CpG motifs. Addition of optimal motifs to the plasmids may favor Th1-type responses and may also improve the efficacy of the vaccine.

The diminution of responses generated to the SERA 17-110 fragment, found only in DNA immunizations that, by definition occur without adjuvant, may indicate that this fragment does not contain the adequate helper T-cell epitopes required to stimulate a strong antibody response. This N-terminal domain, when delivered as a recombinant GST-SERA 17-110 fusion protein in CFA, elicits significantly higher ELISA titers against SERA 24-285 than those observed following immunization with a GST-SERA 17-382 protein, containing the complete SE47′ domain (18a, 20). These data suggest that this region of SERA contains relevant B-cell epitopes, but the use of adjuvant would obviate the need for helper T-cell epitopes

(20). Additional experiments are required to delineate the helper T-cell epitopes in the 47-kDa domain of the SERA protein. Known T-helper cell epitopes could be incorporated into the SERA 17-110 DNA vaccine. This is potentially an important avenue for vaccine discovery because a major parasite-inhibitory epitope maps to the N-terminal amino acids 17 to 110 of SERA (20).

Our results suggest that the SERA protein is a viable candidate as a component of a DNA-based human malaria vaccine. Primate studies are currently in progress to evaluate other malaria DNA vaccine antigens (22). Further studies in primates are planned to examine protective immune responses to the 47-kDa subunit of the SERA protein elicited by DNA vaccination and to evaluate whether combined immunization protocols employing both SERA plasmid DNA and recombinant SERA protein improve the efficacy of a SERA malaria vaccine.

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