# REPORT DOCUMENTATION PAGE

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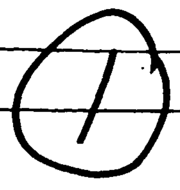

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| 1b. RESTRICTIVE MARKINGS | |
| 2b. DECLASSIFICATION/DOWNGRADING SCHEDULE | 3. DISTRIBUTION/AVAILABILITY OF REPORT<br>Approved for public release; distribution is unlimited |
| 4. PERFORMING ORGANIZATION REPORT NUMBER(S)<br>NMRI 88-108 | 5. MONITORING ORGANIZATION REPORT NUMBER(S) |
| 6a. NAME OF PERFORMING ORGANIZATION<br>Naval Medical Research<br>6b. OFFICE SYMBOL (If applicable) | 7a. NAME OF MONITORING ORGANIZATION<br>Naval Medical Command |
| 6c. ADDRESS (City, State, and ZIP Code)<br>Bethesda, Maryland 20814-5055 | 7b. ADDRESS (City, State, and ZIP Code)<br>Department of the Navy<br>Washington, D.C. 20372-5120 |
| 8a. NAME OF FUNDING/SPONSORING ORGANIZATION Naval Medical Research and Development Command<br>8b. OFFICE SYMBOL (If applicable) | 9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER |
| 8c. ADDRESS (City, State, and ZIP Code)<br>Bethesda, Maryland 20814-5055 | 10. SOURCE OF FUNDING NUMBERS |

| PROGRAM ELEMENT NO. | PROJECT NO. | TASK NO. | WORK UNIT ACCESSION NO |
|---|---|---|---|
| 61102 | 3M161102BS13 | AK-111 | DA313955 |

11. TITLE (Include Security Classification) Use of a vaccinia construct expressing the circumsporozoite protein in the analysis of protective immunity to Plasmodium Yoelii

12. PERSONAL AUTHOR(S) Sedegah M, Beaudoin RL, De la Vega P, Leef MF, Ozcel MA, Jones E, Charoenvit Y, Yuan LF, Gross M, Majarian WR, Robey FA, Weiss W, Hoffman SL

| 13a. TYPE OF REPORT | 13b. TIME COVERED | 14. DATE OF REPORT (Year, Month, Day) | 15. PAGE COUNT |
|---|---|---|---|
| Book article | FROM ____ TO ____ | 1988 | 15 |

16. SUPPLEMENTARY NOTATION in Technological advances in vaccine development: New series Vol.84 Editor: Laurence Lasky. N.Y.: Alan R. Liss. 1988. pp. 295-309

| 17. COSATI CODES | | |
|---|---|---|
| FIELD | GROUP | SUB-GROUP |
| | | |
| | | |

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number) Plasmodium Yoelii, CD8+cells, protective immunity, malaria, Vaccinia virus, circumsporozoite protein, immunogens, Murine malaria, immunization, antibody specificity

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

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| 20. DISTRIBUTION/AVAILABILITY OF ABSTRACT<br>☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS | 21. ABSTRACT SECURITY CLASSIFICATION<br>Unclassified |
| 22a. NAME OF RESPONSIBLE INDIVIDUAL<br>Phyllis Blum, Information Services Division | 22b. TELEPHONE (Include Area Code) 202-295-2188 — 22c. OFFICE SYMBOL ISD/ADMIN/NMRI |

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

Technological Advances in Vaccine Development, pages 295-309
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# USE OF A VACCINIA CONSTRUCT EXPRESSING THE CIRCUMSPOROZOITE PROTEIN IN THE ANALYSIS OF PROTECTIVE IMMUNITY TO PLASMODIUM YOELII

Martha Sedegah,[1] Richard L. Beaudoin,[1] Patricia De la Vega,[1] Mary F. Leef,[1] M. Ali Ozcel,[1,4] Elaine Jones,[2] Yupin Charoenvit,[1] Leo F. Yuan,[1] Mitchell Gross,[2] William R. Majarian,[2,5] Frank A. Robey,[3] Walter Weiss,[1] and Stephen L. Hoffman[1]

[1]Infectious Diseases Department, Naval Medical Research Institute, Bethesda, Maryland 20814

[2]Smith Kline and French Laboratories, 709 Swedeland Road, King of Prussia, Pennsylvania 19406

[3]National Institute of Dental Research, Bldg. 30, Rm. B17B, National Institutes of Health, Bethesda, Maryland 20892

ABSTRACT Antibodies to the circumsporozoite (CS) protein may play a role in the protective immunity induced by immunization with irradiated sporozoites. However, the observation that protection against a large (5000 sporozoites) inoculum is dependent on T cells of the suppressor/cytotoxic phenotype (CD8) has clearly established the importance of cellular mechanisms in this model system. In an attempt to stimulate a protective cellular immune response, Balb/CByJ mice were immunized intraperitoneally with one to 4 doses of a vaccinia recombinant construct expressing the entire CS protein of Plasmodium yoelii. The mice were challenged 2 weeks after the last dose of vaccine with 200 or 10,000 sporozoites. Mice vaccinated with irradiated

[4]Present address: Department of Parasitology, Medical Faculty, Ege University, Bornova-Izmir, Turkey
[5]Present address: Praxis Biologics, 300 E. River Road, Rochester, New York 14623

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sporozoites were protected, but all of the animals immunized with the recombinant construct became infected, even though the anti-sporozoite IFA titers of both groups of vaccinated mice were comparable. To determine if antibodies alone might be responsible for protecting mice immunized with irradiated sporozoites against a low dose challenge, we depleted them of CD8+ T cells, and found that they were no longer protected against even a 200 sporozoite challenge . It is unclear whether failure to protect mice with the vaccinia CS construct results from antigen presention inadequate to induce the required cell mediated immune response, or whether the CS protein is not the appropriate antigen. It does appear however, that antibodies induced by the irradiated sporozoite vaccine are not an adequate explanation for the protection observed against even a low dose challenge.

## INTRODUCTION

Malaria is transmitted to humans by anopheline mosquitoes that inject sporozoites while feeding. Initial attempts to develop subunit sporozoite vaccines focused on developing *immunogens* that induced high levels of antibodies to the repeat regions of the circumsporozoite (CS) protein of the sporozoite. This approach has thus far proved only marginally successful in humans (1,2). Furthermore, synthetic peptides and E. coli produced recombinant vaccines have been shown to induce protection against moderate (500-1000), but not large (10,000) sporozoite challenge in the Plasmodium berghei rodent model (3,4), but no protection in the P. yoelii system (5). This is not surprising since several studies have shown that the protective immunity developed after immunization with irradiated sporozoites can be independent of antibody (3,6,7), and is dependent on CD8+ T lymphocytes (8,9). Protective immunity after immunization with irradiated sporozoites is also dependent on immunization with live sporozoites suggesting that the sporozoites must enter cells to induce the appropriate immune response. It is not known if this immune response is directed against antigens expressed by the sporozoite or neo-antigens first expressed by the parasite within hepatocytes.

In an attempt to develop a vaccine that would induce a protective T cell response by presenting antigen expressed within host cells, we constructed a vaccinia recombinant that includes the entire gene encoding the P. yoelii CS protein. We now report the results of the first immunization experiments with this construct.

## MATERIALS AND METHODS

Construction of the vaccinia P. yoelii CS Recombinant (Vpy).

A 1.3 Kb fragment encoding the entire P. yoelii CS protein coding region (Fig. 1) (10) was isolated by PAGE and subcloned into the Sma I site of pGS20 (11) such that the 7.5 Kb gene promoter directs transcription of the inserted DNA. Vaccinia virus recombinants were then generated as referenced previously. Briefly, monolayers of CV-1 cells were infected with wild type vaccinia virus ($TK^-$ Wyeth strain) at a multiplicity of infection of 0.05 plaque forming units (pfu) per cell. Two hours post infection, recombinant plasmid DNA was introduced by transfection of calcium phosphate precipitated DNA. Cells were harvested 48 hrs later, and virus was released by 3 freeze-thaw cycles. $TK^-$ recombinant virus was selected from cell lysates by plaque assay on $TK^-143$ cells in the presence of 25 µg/ml 5-bromodeoxyuridine (BUdR). Isolated $TK^-$ plaques wer picked and used to infect fresh monolayers of $TK^-143$ [illegible]. Forty eight to 72 hrs later, when significant cytopathic effect (cpe) was evident, cells were harvested, subjected to 3 cycles of freezing and thawing, and cell lysates were then screened for expression of the P. yoelii CS protein by immunoblot analysis. Lysates were applied to nitrocellulose sheets with the aid of a Dot-blot manifold (Minifold I; Schleicher & Schuell, Keene NH) and unoccupied sites blocked by incubation for 1 hr in RIPA buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 0.5% Triton X-100) supplemented with 3% BSA (w/v). Filters were then incubated overnight at room temperature in the presence of NYS1, a monoclonal antibody specific for the P. yoelii CS protein (12) diluted 1:1000 in RIPA buffer. These were then washed 3 times with RIPA buffer, and

further incubated for 1 hr at room temperature with RIPA containing 1 μCi $^{125}$I-protein A. Filters were again washed three times with RIPA and positive spots visualized by autoradiography. Recombinant viruses which were positive in this assay were subject to 3 rounds of plaque purification. Finally, plaque purified virus was used to infect monolayers of CV-1 cells and cell lysates were screened by western blotting to confirm the presence of the full length *P. yoelii* CS protein.

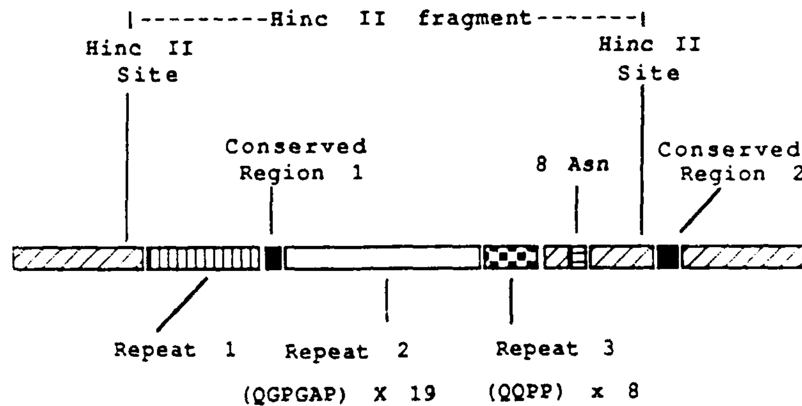


FIGURE 1. Diagramatic structure of the *Plasmodium yoelii* gene encoding the circumsporozoite (CS) protein.

Malaria Parasites.

The 17X (NL) strain of *P. yoelii yoelii* was used for all experiments. Sporozoites were isolated by discontinuous gradient centrifugation as previously described (13) and adjusted to required concentration in Medium 199 supplemented with 10% mouse serum. Irradiated sporozoites (IRRspz) used for immunization were isolated from infected mosquitoes that had been exposed to 10 Krads from a $^{137}$Cs source.

Immunogens.

The following were using as immunogens:

a) The Vpy vaccinia CS construct encoding the full length CS protein of P. yoelii ($10^8$ pfu/dose) inoculated intraperitoneally (ip).

b) A control vaccinia construct (Vgalk) encoding an unrelated E. coli galactokinase ($10^8$ pfu/dose) inoculated ip.

c) IRRspz injected intravenously (iv). The first dose was 5 x $10^4$ sporozoites, and subsequent doses were 3 x $10^4$ sporozoites.

Immunization and Challenge Schedule.

Groups of 8 female Balb/CByJ mice (Charles River Labs), 8 weeks old, received 1, 2, 3 or 4 doses of vaccine prior to challenge. Vaccine doses were administered at 2 week intervals. Two weeks after the last immunizing dose, blood for serum was obtained and the mice were challenged with infective sporozoites. Mice that received 1, 2 and 3 doses were challenged with $10^4$ sporozoites, while mice that received 4 doses of vaccine were challenged with 200 sporozoites. Mice were then monitored for parasitemia as an indicator of protective immunity.

Detection of Antibodies to Sporozoite Epitopes.

Pooled sera were analyzed for reactivity with sporozoite antigens using the following techniques:

Indirect fluorescent antibody test (IFAT). The IFAT was carried out using air dried whole sporozoites as previously described (12).

Enzyme linked immunosorbent assay (ELISA). ELISA was used to analyze the epitopes on the CS protein recognized by sera from the different groups of vaccinated mice. Briefly, the test consisted of a solid phase assay in which synthetic peptides or recombinant proteins were coated to the wells of 96 well flat bottom Immulon II microtiter plates (Dynatech Laboratories, Alexandria,VA). Wells were coated with one of the following: a) a recombinant fusion protein produced by the Hinc II fragment CS gene in E. coli that includes the 3 P. yoelii repeat domains and the conserved Region I sequence fused to 81 amino acids from the

non-structural protein of viral influenza; b) a synthetic peptide containing 2 copies of repeat #2, Gln.Gly.Pro.Gly.Ala.Pro [QGPGAP]$_2$; and c) a synthetic peptide containing 3 copies of repeat #3, Gln.Gln.Pro.Pro [QQPP]$_3$. Each well was then blocked with casein and incubated overnight. The wells were then reacted with the appropriate dilution of a test serum, washed 3x with buffer after which the revealing antibody, a horseradish peroxidase (HRP) labelled goat anti-mouse Ig was added followed by the peroxidase substrate consisting of equal volumes of $H_2O_2$ and ABTS (2.2'-azino-di[3-ethyl- benzothiazoline sulfonate]) (Kirkegaard and Perry Laboratories, Inc., Gaithersburg MD). Color development was measured photo-metrically at 405 nm using Microelisa Autoreader MR 580 (Dynatech Laboratories).

Depletion of CD8+ T Lymphocytes.

Mice immunized with 4 doses of irradiated sporozoites and shown to be protected against challenge with P.yoelii sporozoites 17 days previously were given two 1 mg ip injections of monoclonal antibody 19/178 at 24 hour intervals to deplete them of CD8+ T lymphocytes as previously described (8). They were challenged with either 200 or 10,000 sporozoites 48 hours later. Treatment with Mab 19/178 was continued every third day after challenge until blood parasites appeared. Blood films were examined for parasites until patency or for 14 days, whichever occurred sooner. When parasites were detected in the blood, mice were killed and spleen cells prepared and analysed for depletion of the subpopulation carrying the CD8 marker by fluorescent activated cell sorter. (FACS).

## RESULTS

Protection Against Challenge.

Results of the challenge of mice immunized with Vpy are summarized in Table 1. Forty percent of the mice that received a single dose of irradiated sporozoites were protected against challenge with $10^4$ sporozoites, and this level of protection increased to 100% in the

three groups of mice that received 2, 3, or 4 doses of irradiated sporozoites. In contrast, all mice immunized with the recombinant Vpy construct became infected after challenge including the group that had received 4 doses of the construct and was challenged with only 200 sporozoites.

TABLE 1
Results of challenging mice immunized with vaccine preparations Vpy, Vgalk and IRRspz.

| Expt | No.of Doses | Vaccine Preparation | IFAT Titer | Ratio of Mice Prot./Chall. |
|---|---|---|---|---|
| Challenged with $10^4$ sporozoites/mouse. | | | | |
| A | 1 | Vpy | 32 | 0/8 |
| | | Vgalk | <8 | 0/8 |
| | | IRRspz | 16 | 3/8 |
| | | Control | <8 | 0/8 |
| B | 2 | Vpy | 512 | 0/8 |
| | | Vgalk | <8 | 0/8 |
| | | IRRspz | 256 | 8/8 |
| | | Control | <8 | 0/8 |
| C | 3 | Vpy | 2,048 | 0/8 |
| | | Vgalk | <8 | 0/8 |
| | | IRRspz | 2,048 | 8/8 |
| | | Control | <8 | 0/8 |
| Challenged with 200 sporozoites/mouse. | | | | |
| D | 4 | Vpy | 1,024 | 0/8 |
| | | Vgalk | <8 | 0/8 |
| | | IRRspz | 2,048 | 8/6 |
| | | Control | <8 | 0/8 |

Antibody levels.

Anti-sporozoite antibody titers as measured by IFAT were comparable in the groups that were vaccinated with IRRspz and those that received the Vpy construct. Results obtained by ELISA using the Hinc II fusion protein containing all the repeat domains were also consistent with the IFAT findings. However, although both groups had made comparable levels of antibodies to Hinc II peptide (Fig.2), there was a significant difference in the epitopes recognized by each at the endpoint titer.

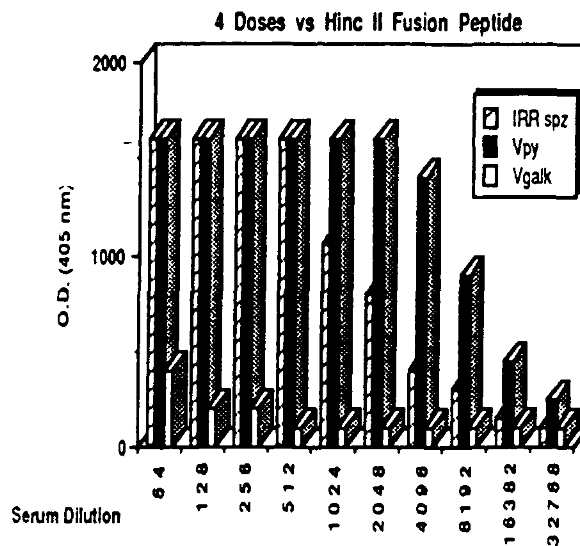


FIGURE 2. ELISA determination of the antibody response to the Hinc II fusion peptide of mice immunized with vaccine preparations IRRspz, Vpy and Vgalk.

Mice immunized with irradiated sporozoites produced significantly higher levels of antibodies to repeat #2 ([QGPGAP]$_2$) while mice immunized with the Vpy construct made higher levels of antibodies to repeat #3 ([QQPP]$_3$) (Figs.3 and 4 respectively).

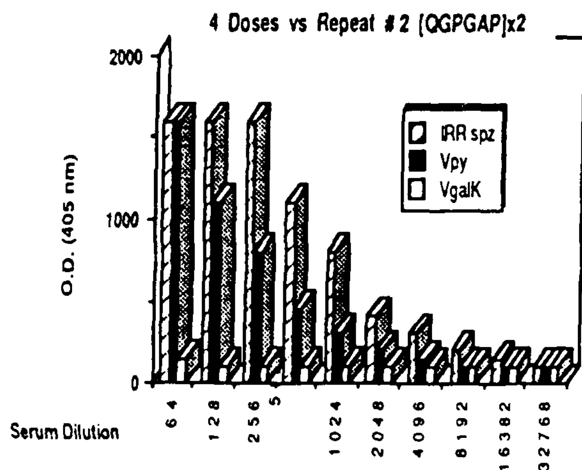


FIGURE 3. ELISA determination of the antibody response to Repeat #2 $[QGPGAP]_2$ of mice immunized with vaccine preparations IRRspz, Vpy and Vgalk.

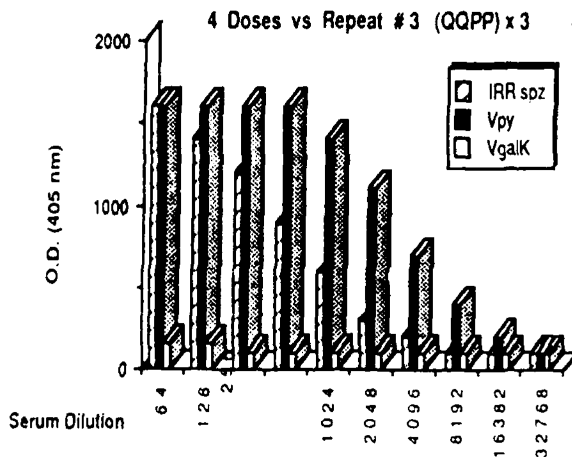


FIGURE 4. ELISA determination of the antibody response to Repeat #3 $(QQPP)_3$ of mice immunized with vaccine preparations IRRspz, Vpy and Vgalk.

Protection After CD8 T Cell Depletion.

We knew that a Mab to repeat #2 protected mice in passive transfer experiments, whereas a second Mab reacting with repeat #3 did not (Charoenvit, in preparation) and we wondered if the difference in antibody specificity between the IRRspz and Vpy immunized groups might account for the differences in protection observed in each, especially for low challenge doses.

TABLE 2
Results of rechallenging protected mice depleted of CD8+ T-lymphocytes

| Mouse No. | IFAT Titer at challenge | Day after Challenge Blood Infection Detected | | |
|---|---|---|---|---|
| | | +3 | +4 | +5 |
| <u>Challenged with 200 sporozoites/mouse</u>. | | | | |
| CD8+ T cell depleted. | | | | |
| 1 | 512 | - | - | + |
| 2 | 1,024 | - | - | + |
| 3 | 512 | - | - | + |
| 4 | 2,048 | - | - | + |
| Challenge controls. | | | | |
| 1 | <8 | - | + | |
| 2 | <8 | - | + | |
| 3 | <8 | - | + | |
| 4 | <8 | - | + | |
| <u>Challenged with 10,000 sporozoites/mouse</u>. | | | | |
| CD8+ T cell depleted. | | | | |
| 5 | 1,024 | - | + | |
| 6 | 2,048 | + | | |
| 7 | 512 | + | | |
| 8 | 1,024 | + | | |
| Challenge controls. | | | | |
| 5 | <8 | + | | |
| 6 | <8 | + | | |
| 7 | <8 | + | | |
| 8 | <8 | + | | |

However, when mice immunized with 4 doses of irradiated sporozoites were depleted of CD8+ T cells (verified by FACS analysis; data not shown) and challenged with either 200 or $10^4$ sporozoites, they all became infected indicating that the antibodies induced by immunization with IRRspz were not sufficient to protect these mic against challenge with $10^4$ sporozoites or even the very low challenge dose of 200 (Table 2).

## DISCUSSION

Observations in the rodent malaria model systems have provided the foundation for development of human malaria sporozoite vaccines. Murine studies now clearly demonstrate the importance of cell-mediated immune mechanisms in the protective immunity elicited by immunization with irradiated sporozoites (3,7,8,9). In an attempt to induce a protective cellular immune response directed against the CS protein, we constructed a P. yoelii-CS vaccinia recombinant. The failure of this vaccine to protect against even a minimal sporozoite challenge indicates that either the CS protein is not the target of such immunity, or that intraperitoneal immunization with Vpy did not induce the required cellular immune response. Recent studies in the P. berghei system (Sadoff J; personal communication) indicate that the CS protein may be the target of such an immune response. If so, this vaccinia recombinant may be an inadequate vector for properly delivering the immunogen, or the route of administration may have been inappropriate for induction of the cytotoxic T cells that we believe are involved in a protective response. Further studies are in progress to explore these possibilities.

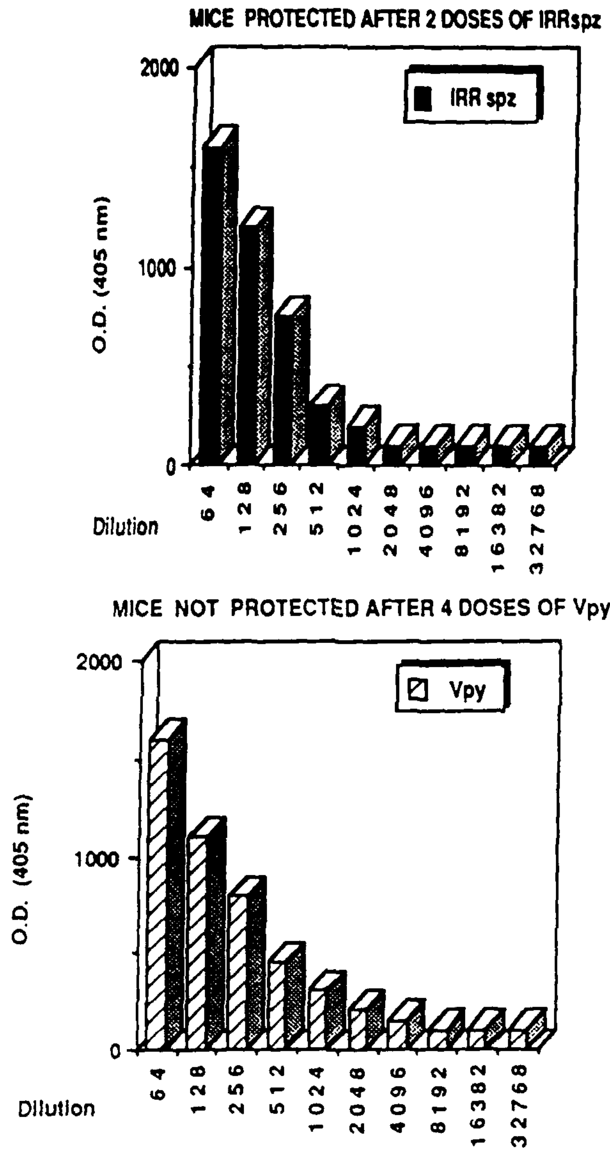


FIGURE 5. Comparison of the ELISA response to Repeat #2 [QGPGAP]$_2$ in mice vaccinated with the IRRspz and Vpy vaccine preparations. The IRRspz group was completely protected after 2 vaccine doses, whereas the Vpy group was totally suscpetible after 4 doses of Vpy.

Mice immunized with 4 doses of Vpy were not protected against challenge with even 200 sporozoites, although they had levels of antibody against CS protein and sporozoite epitopes comparable to the levels in mice immunized with 2 doses of IRRspz that were protected against challenge with 10,000 sporozoites (Fig.5).

The results of the CD8+ depletion study (Table 2) demonstrate that circulating antibody levels in mice immunized with 4 doses of irradiated sporozoites are not adequate to protect against even a low dose challenge. Protective immunity in IRRspz immunized mice appears to be wholly dependent on CD8+ T cells regardless of the size of challenge.

Although IRRspz immunized mice are not protected by antibodies, we have shown that passive transfer of a monoclonal antibody, NYS1 directed against repeat #2 (QGPGAP) can protect against challenge with a large inoculum of sporozoites (Charoenvit,in preparation). It is therefore theoretically possible that vaccines eliciting antibodies against the CS antigen will still prove protective, if the levels of antibody they achieve exceed those reached with the IRRspz and Vpy preparations used in this study. Our data provide the first indication that the immunodominance of B cell epitopes on the CS protein may be dependent on how the immunogen is delivered. Antibodies measured by IFAT were similar in the IRRspz and Vpy immunized groups. However, immunization with the IRRspz vaccine induced the highest levels of antibodies against [QGPGAP] in contrast to immunization with Vpy which produced higher levels to [QQPP]. The protective Mab, NYS1, is directed against an epitope in the [QGPGAP] domain, whereas a second Mab, NYS3 reacting with a different epitope contained in the [QQPP] domain is not protective (Charoenvit, in preparation). It would appear that future studies of subunit vaccines in this model system would be well advised to consider the epitope specificity of vaccine induced antibodies and not rely on antibodies to the entire sporozoite as the sole measure of immunogenicity.

ACKNOWLEDGMENTS

We wish to thank Ms. B.J. Leckey for editing the manuscript. M. Sedegah and P.de la Vega were supported in part by ONR Grant No. N00014-85-G-0255 to the Pan American Health Organization. This project was supported in part by NMR&DC work unit 3M161102BS10AK111.

The opinions and assertions herein are the private ones of the authors and are not to be construed as official or as reflecting the views of the Navy Department or the naval service at large. The experiments reported herein were conducted according to the principles set forth in the current edition of the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, DHHS Pub. No. (NIH) 86-23.

REFERENCES

1. Ballou WR, Hoffman SL, Sherwood JA, Hollingdale MR, Neva FA, Hockmeyer WT, Gordon DM, Schneider I, Wirtz RA, Young JF, Wasserman GF, Reeve P, Diggs CL, Chulay JD (1987). Safety and efficacy of a recombinant DNA Plasmodium falciparum sporozoite vaccine. Lancet i:1277.
2. Herrington DA, Clyde DF, Losonsky G, Cortesia M, Murphy JR, Davis J, Bagar S, Felix AM, Heimer EP, Gillesen D, Nardin E,Nussenzweig RS, Nussenzweig V, Hollingdale MR, Levine MM (1987). Safety and immunogenicity in man of a synthetic peptide malaria vaccine against Plasmodium falciparum sporozoites. Nature (London) 328:257.
3. Egan JE, Weber JL, Ballou WR, Hollingdale MR, Majarian WR, Gordon DM, Maloy WL, Hoffman SL, Wirtz RA, Schneider I, Woolett GR, Young JF, Hockmeyer WT (1987). Efficacy of murine malaria sporozoite vaccines:Implications for human vaccine development.Science (Wash DC) 263:453.
4. Zavala F, Tam JP, Barr PJ, Romero PJ, Ley V, Nussenzweig RS, Nussenzweig V (1987). Synthetic peptide vaccine confers protection against murine malaria. J Exp Med 166:1591.
5. Lal AA, de la Cruz V, Good MF, Weiss WR, Lunde M, Maloy W, Welsh JA, McCutchan TF (1987). In vivo

testing of subunit vaccines against malaria sporozoites using a rodent system.Proc Natl Acad Sci 84:8647.

6. Spitalny GL, Nussenzweig RS (1973). Relationship between protective immunity and antisporozoite (CSP) antibody in mice. Exp Parasitol 33:168.
7. Chen DH, Tigelaar RE, Weinbaum FI (1977). Immunity to sporozoite-induced malaria infection in mice. I. The effect of immunization of T and B cell deficient mice. J Immunol 118:1322.
8. Weiss WR, Sedegah M, Beaudoin RL, Miller LH, Good MF (1988). CD8+ T cells (cytotoxic/suppressors) are required for protection in mice immunized with malaria sporozoites. Proc Natl Acad Sci USA 85:573.
9. Schofield L, Villaquiran J, Ferreira A, Schellekens H, Nussenzweig R, Nussenzweig V (1987). y Interferon, CD8+ T cells and antibodies required for immunity to malaria sporozoites. Nature 330:664.
10. Theisin T, Gross M, Young JF, Charoenvit Y, Beaudoin RL (1987). Cloning, sequencing and expression of the Plasmodium yoelii circumsporozoite gene. Fed Proc 46:2185.
11. Mackett M, Smith GL, Moss B (1984). General method for production and selection of infectious vaccinia virus recombinants expressing foreign genes. J Virol 49:857.
12. Charoenvit Y, Leef MF, Yuan LF, Sedegah M, Beaudoin RL (1987). Characterization of Plasmodium yoelii monoclonal antibodies directed against stage-specific sporozoite antigens. Inf & Imm 55:604.
13. Pacheco ND, Strome CPA, Mitchell F, Bawden MP, Beaudoin RL (1979). Rapid, large-scale isolation of Plasmodium berghei sporozoites from infected mosquitoes. J Parasitol 65:414.