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MONOCLONAL, BUT NOT POLYCLONAL, ANTIBODIES PROTECT AGAINST Plasmodium yoelii SPOROZOITES

YUPIN CHAROENVIT,** SYLVIE MELLOUK,* CAROLE COLE,* ROSSANA BECHARA,* MARY F. LEEF,* MARTHA SEDEGAH,* LEO F. YUAN,* FRANK A. ROBEY,* RICHARD L. BEAUDOIN,* AND STEPHEN L. HOFFMAN*

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One of the primary strategies for malaria vaccine development has been to design subunit vaccines that induce protective levels of antibodies against the circumsporozoite (CS) protein of malaria sporozoites. In the Plasmodium yoelii mouse model system such vaccines have been uniformly unsuccessful in protecting against sporozoite-induced malaria. To demonstrate that antibodies to P. yoelii CS protein could provide protection we established a passive transfer model. Passive transfer of Navy yoelii sporozoite 1 (NYS1), an IgG3 mAb against the P. yoelii CS protein, protected 100% of mice against challenge with 5000 P. yoelii sporozoites. Binding of NYS1 to sporozoites was inhibited by incubation with (QGPGAP)_2, a synthetic peptide derived from the repeat region of the P. yoelii CS protein, indicating that the epitope on sporozoites recognized by this mAb was included within this peptide. The levels of antibodies to (QGPGAP)_2 by ELISA, and to sporozoites by indirect fluorescent antibody test and CS precipitation reaction were similar in sera from mice that received NYS1 in passive transfer and were protected against challenge with 5000 sporozoites, and from mice that had been immunized with subunit vaccines containing (QGPGAP)_2 but were not protected against challenge with 40-200 sporozoites. To determine if antibody avidity, not absolute concentration could explain the striking differences in protection, we established a thiocyanate elution assay. The results suggest that NYS1, the protective mAb, has a lower avidity for (QGPGAP)_2 and for sporozoites than do the vaccine-induced antibodies. Although the results of the conventional antibody assays did not correlate with protection, sera from the protected animals inhibited sporozoite development in mouse hepatocyte cultures significantly more than did the sera from the unprotected, subunit vaccine-immunized animals, correlating with protection. The data clearly demonstrate that antibodies to the CS protein can protect against intense sporozoite infection. Improved understanding of the differences between protective mAb and nonprotective polyclonal antibodies will be important in the further development of malaria vaccines.

During the past decade, the primary strategy for malaria sporozoite vaccine development has been to produce subunit vaccines that induce antibodies to the repeat region of the CS protein, antibodies that prevent effective sporozoite invasion of hepatocytes (1-4). Thus far protective immunity after immunization of humans (5, 6) and non-human primates (7) has been disappointing. Mice immunized with subunit Plasmodium berghei vaccines have been protected against moderate, but not against large sporozoite challenge doses (8-11). In contrast, mice immunized with irradiation-attenuated sporozoites have been protected against large doses of sporozoites (8, 10, 12). In the Plasmodium yoelii model system, mice have been immunized with synthetic peptides derived from the CS protein (13), (M. Sedegah, unpublished observations), Escherichia coli produced CS protein (L. F. Yuan, unpublished observations), and vaccinia (14), and pseudorabies* viruses transformed with the gene encoding the CS protein. In the majority of experiments mice developed high levels of antibodies to the CS protein, but mice have not been consistently protected against challenge with P. yoelii sporozoites.

To determine if antibodies to the repeat region of the P. yoelii CS protein could protect mice against sporozoite challenge, we conducted passive transfer studies with NYS1, the IgG3 mAb (15) used to identify the gene encoding the P. yoelii CS protein (16, 18, 19). We now report 100% protection against challenge with 5000 sporozoites in mice with serum levels of NYS1 similar to the levels of

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vaccine-induced polyclonal antibodies that did not protect against challenge with 200 sporozoites.

Materials and Methods

Animals. Female, 6 to 10 wk, BALB/c Byj mice (The Jackson Laboratory, Bar Harbor, ME) were used in all experiments. Three experiments were performed with sporozoites of the 17XNL (nonlethal) strain of \textit{P. yoelii}, which were raised in \textit{Anopheles stephensi}. Salivary gland dissected sporozoites suspended in medium 199 containing 5% normal mouse serum were used for i.v. infection. Sporozoites isolated by discontinuous gradient (17) in medium 199 without serum were used in an ELISA, as whole sporozoites or as an Ag extract in 1% SDS, and in an IFAT (15).

Ag. In addition to sporozoites, three other \textit{P. yoelii} CS protein-derived Ag were used as Ag in an ELISA (Fig. 1A). The first was a synthetic peptide composed of two copies of Gin-Gly-Pro-Gly-Ala-Pro (QGPGAP), the predominant repeat of the \textit{P. yoelii} CS protein (18, 19). The second included three copies of Gin-Gly-Pro-Pro (QGPPP), the minor repeat of the \textit{P. yoelii} CS protein (18, 19). The third, designated CS 1, is a fusion protein produced in E. coli (19) containing 258 amino acids from the CS protein fused to 81 amino acids derived from a nonstructural protein of influenza A (Fig. 1B). CS 1 includes 19 copies of the major repeat (QGPGAP), 7 copies of the minor repeat (QGPP), and 45 amino acids EERKDDDP KDGNKDDLPK EERKDDDP DPKKDDPK, apparently comprising 5 copies of a degenerative sequence of 9 amino acids (repeat 3).

mAb. NYSI, an IgG3 mAb used in this study, was produced and characterized as described previously (15), and purified from ascitic fluid from a mouse inoculated with a recombinant protein (20).

Sera. Sera used in these studies were from mice immunized with (QGPGAP) conjugated to either KLH or protosomes (21), designated as (QGPGAP)-KLH and (QGPGAP)-protosomes (M. Sedegah, unpublished observations). CS 1 (L. F. Yuan et al., unpublished observations), and from mice immunized with irradiated sporozoites (14).

Passive Immunization. Groups of mice were injected in the tail vein with mAb NYSI or sera from mice that had been immunized with four doses of irradiated sporozoites. To avoid the possible interference of IgM with IgG, a mouse serum was prepared at 6, 7, 11, and 15 days for determination of parasitemia. ELISA. A total of 100 ml of 10 ml/ml (QGPGAP), (QGPP), or PY CS 1 or 100 ml whole of whole sporozoite suspension in PBS (2 x 10 sporozoite/ml) or sporozoite Ag extracted in 1% SDS (2 x 10 sporozoites/ml) for 2 h, and the sera were added to 2 h. The wells were washed three times with washing buffer before incubating for 2 h with peroxidase-labeled goat anti-mouse IgG and anti-mouse \( \mu \) chain (Tago, Burlingame, CA). The plates were washed again after incubation, and ABTS substrate (Kirkegaard and Perry Laboratories, Gaithersburg, MD) was added. Color reaction was measured in a Dynatech Micro-ELISA auto reader at OD 405 nm. The results were recorded as mean OD readings of triplicate assays ± SE.

Inhibition ELISA. In addition to a standard ELISA, an inhibition ELISA was used to characterize the NYSI epitope. NYSI (2 mg/ml) was incubated for 2 h at 37°C with increasing concentrations (0 to 160 mg/ml) of (QGPGAP), (QGPP), or with a control peptide derived from the immunodominant repeat domain of the \textit{Plasmodium} CS protein (Gly-Asp-Arg-Ala-Asp-Gly-Pro-Ala) (GRRADGQPA) (22). The NYSI-peptide mixtures were then added to plates previously coated for 24 h at 37°C with 2 x 10\(^2\) \textit{P. yoelii} sporozoites/well. The plates were further incubated at 37°C for 2 h, washed, and then incubated with HRP-labeled goat anti-mouse IgG and ABTS substrate.

IgG subclass antibodies to (QGPGAP). The subclass of IgG antibodies to (QGPGAP) was determined by ELISA with the use of the method previously described (23). Briefly, affinity-purified mouse myeloma IgG1, IgG2a, IgG2b, and IgG3 (Sigma Laboratories, St. Louis, MO) were tested in the ELISA assay against HRP rat anti-mouse IgG1, IgG2a, IgG2b, and IgG3 (Pharmingen Laboratories, San Diego, CA) and the standard curves of the IgG subclasses were plotted. Serial dilutions of sera from subunit vaccine-immunized mice were incubated with (QGPGAP) coated on the plates, followed by HRP rat anti-mouse IgG1, IgG2a, IgG2b, and IgG3, and ABTS substrate. The OD reading at 405 nm was measured, and the percent (QGPGAP)-specific subclass antibodies were calculated from the standard curves.

Indirect fluorescent antibody test and circumsporozoite precipitation test. IFAT and CS precipitation tests were performed as previously described (15, 24) by using \textit{P. yoelii} sporozoites.

Estimation of antibody avidity. The avidity of antibodies to CS protein and sporozoites was estimated by using the method described by Pullen et al. (25) with a slight modification. Briefly, all sera to be tested were first titrated against the Ag by using the standard ELISA. The Ag used were (QGPGAP) and PY CS 1 at a concentration of 10 ml/ml, and whole sporozoites or sporozoite Ag extract at 2 x 10 sporozoites/ml. Sera at the dilutions at which the absorbance (405 nm) in the standard ELISA was 0.8 were incubated for 2 h at 37°C in Ag-coated plates. The plates were washed, and varying concentrations (0.1 to 4.0 M) of NaSCN were added to them. The plates were then reincubated for 15 min at 37°C to disrupt cross-linking of Ag-antibody binding. The plates were washed, HRP-conjugated goat anti-mouse IgG was added to them, and they were further incubated for 2 h at 37°C. The plates were washed and HRP substrate added. Color reaction was measured at OD 405 nm. The results were depicted by plotting the log % initial OD (absorbance when no SCN was added) against the molarity of SCN used. The avidity Index was calculated as the molarity of SCN that resulted in a 50% reduction in absorbance from the reading when no SCN was added (i.e., log of 50% reduction) = 1.69 log (initial OD/OD at 50% reduction).

Inhibition of liver stage development assay (ILSDA). The effect of sera from immunized mice or mice passively transfected with NYSI on development of liver stage parasites was determined by using the ILSDA as previously described (1). Briefly, hepatocytes isolated from either rats (26) or mice (27) were seeded in eight-chamber Lab-Tek plastic slides at 1 x 10 cells/chamber. After 24 h of incubation at 37°C in an atmosphere of 5% CO\( _2 \) in air, medium was removed and 5 x 10\(^6\) salivary gland dissected sporozoites suspended in 25 ml of medium, and 25 ml of varying dilutions of sera from immunized or control mice were added. After 3 h of incubation the cultures were washed to remove sporozoites, and fresh medium was added. At 24 h the medium was changed, and at 48 h the cultures were fixed and incubated with a mAb directed against liver stage parasites of \textit{P. yoelii} (NYLS1) (Y. Charoenvit, unpublished observations) before incubating with FITC-labeled goat anti-mouse Ig. The number of liver-stage schizonts in each culture was counted by using an Olympus UV microscope. The average number of liver schizonts in duplicate cultures was recorded, and percent inhibition was calculated with the following formula:

\[
\text{% Inhibition} = \left(1 - \frac{\text{Avg. no. of parasites in cultures with immune serum}}{\text{Avg. no. of parasites in cultures with normal serum}}\right) \times 100
\]

Results

Passive Immunization. In previous studies mice immunized with vaccinia transformed with the \textit{P. yoelii} CS gene (14), PY CS 1 (L. F. Yuan, unpublished observations)
against challenge with 200 sporozoites. We therefore studied the protective efficacy of mAb NYS1 in passive transfer experiments. Previous work in our laboratory (unpublished results) indicated that the number of P. yoelii sporozoites required to infect 50% of mice ranged from 1.6 to 40 sporozoites and that 100% of mice were always infected by the inoculation of 200 sporozoites. In the first experiment, varying amounts of NYS1 (15.1 μg to 1000 μg) were given to mice before challenge with 200 sporozoites. All mice that received 500 μg NYS1, and 67% of those that received 125 μg were protected (Table I). In a subsequent experiment, mice that received 500 μg NYS1 were challenged with 200 to 25,000 sporozoites. All mice challenged with 5,000 sporozoites, 83% of those challenged with 10,000 sporozoites, and 67% of those challenged with 25,000 sporozoites were protected (Table II).

**Epitope of NYS1.** Having established that NYS1 was highly protective, we mapped its antigenic site on sporozoites. In a standard ELISA, NYS1 recognized (QGPGAP)\textsubscript{2} but did not recognize (QGPP)\textsubscript{2} (data not shown). In an inhibition ELISA, the binding of NYS1 to whole sporozoites was completely inhibited by prior incubation with (QGPGAP)\textsubscript{2} (Fig. 2), indicating that (QGPGAP)\textsubscript{2} included the epitope on sporozoites recognized by NYS1.

**Antibody levels and CS precipitation reaction.** Because passive transfer of NYS1 conferred such impressive protective immunity against challenge with 5000 sporozoites, whereas immunization with the subunit vaccines induced excellent antibody responses but no protection against 200 sporozoites, we studied the sera taken from the mice that received 500 μg NYS1 in passive transfer and mice that were immunized with the subunit vaccines. Because we had established that mice immunized with irradiated sporozoites were no longer protected against challenge with 200 P. yoelii sporozoites after in vivo depletion of CD8+ T cells (14, 28), indicating that circulating antibodies were not responsible for the excellent protective immunity induced by immunization with irradiated sporozoites, sera from mice immunized with irradiated sporozoites were also included in these studies. All sera used were collected just before challenge. The results outlined in Table III indicated that there were no striking differences in levels of antibodies to (QGPGAP)\textsubscript{2}, the 66% of the CS protein represented by PY CS.1, and sporozoites in the sera of the protected and nonprotected animals. IgM antibody to CS protein was not detectable in the sera of these mice (data not shown).

**Avidity assays.** The above findings suggested the possibility that the protective mAb, NYS1, bound to sporozoites with higher affinity or avidity than did the nonprotective polyclonal antibodies. Studies conducted by using the thiocyanate elution assay suggest that this may not be the case (Fig. 3 A to D). The avidity index of NYS1 for (QGPGAP)\textsubscript{2}, PY CS.1, sporozoite extract, and for whole sporozoites is 5 to 10 times lower than that of the vaccine-induced IgG antibodies.

**Inhibition of liver stage development.** Having established that none of the conventional assays for measuring antibodies provided an indication of why the mice that received NYS1 in passive transfer were protected, but mice immunized with the subunit vaccines were not protected against sporozoite challenge, we studied the effect of the sera on invasion and development of liver stage parasites in vitro. There was a striking difference between sera from protected and nonprotected animals.

---

**TABLE I**

**Passive transfer of NYS1 to BALB/c ByJ mice and challenge with 200 P. yoelii sporozoites**

<table>
<thead>
<tr>
<th>Groups</th>
<th>NYS1 (μg/Mouse)</th>
<th>IFAT (Titer)</th>
<th>No. Infected/No. tested</th>
<th>%Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1000</td>
<td>8000</td>
<td>0/6</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>4096</td>
<td>0/6</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>3000</td>
<td>1/6</td>
<td>83</td>
</tr>
<tr>
<td>4</td>
<td>125</td>
<td>2048</td>
<td>2/6</td>
<td>67</td>
</tr>
<tr>
<td>5</td>
<td>62.5</td>
<td>750</td>
<td>6/6</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>30.3</td>
<td>512</td>
<td>6/6</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>15.1</td>
<td>128</td>
<td>6/6</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>PBS</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>1000 μg NYS1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;8</td>
<td>6/6</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> A mAb directed against the repeat region of the P. falciparum CS protein.

**TABLE II**

**Passive transfer of 500 μg NYS1 to BALB/c ByJ mice and challenge with varying numbers of P. yoelii sporozoites**

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. Sporozoites for Challenge</th>
<th>INFECTED/NO. TESTED</th>
<th>%Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200</td>
<td>0/6</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>1000</td>
<td>0/6</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>5000</td>
<td>0/6</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>10,000</td>
<td>1/6</td>
<td>83</td>
</tr>
<tr>
<td>5</td>
<td>25,000</td>
<td>2/8</td>
<td>67</td>
</tr>
<tr>
<td>6</td>
<td>Control&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6/6</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>b</sup> Mice that received PBS instead of NYS1 and were challenged with 200 sporozoites.

---

**TABLE III**

**Antibodies to CS protein and sporozoites in mice that received NYS1 and in mice immunized with subunit vaccines**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sporozoites (IFAT)</th>
<th>PY CS.1 (ELISA)</th>
<th>(QGPGAP)\textsubscript{2} (ELISA)</th>
<th>Sporozoites (CSP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NYS1 passive transfer</td>
<td>4.096 6.000 16.000</td>
<td>5.92 4.000 8.000</td>
<td>2/25</td>
<td>5.92 4.000 8.000</td>
</tr>
<tr>
<td>(QGPGAP)\textsubscript{2}-KLH</td>
<td>8.192 4.000 8.000</td>
<td>2/25</td>
<td>8.192 4.000 8.000</td>
<td></td>
</tr>
<tr>
<td>(QGPGAP)\textsubscript{2}-proteosomes</td>
<td>8.192 2.000 8.000</td>
<td>0/25</td>
<td>8.192 2.000 8.000</td>
<td></td>
</tr>
<tr>
<td>PY CS.1</td>
<td>4.096 120.000 8.000</td>
<td>7/25</td>
<td>4.096 120.000 8.000</td>
<td></td>
</tr>
<tr>
<td>irradiated sporozoites</td>
<td>2.048 2.000 4.000</td>
<td>2/25</td>
<td>2.048 2.000 4.000</td>
<td></td>
</tr>
<tr>
<td>Normal mouse serum</td>
<td>&lt;8</td>
<td>&lt;200</td>
<td>&lt;200</td>
<td>0/25</td>
</tr>
</tbody>
</table>

<sup>a</sup> Serum antibody level is recorded as end point titer for both IFAT and ELISA. In the IFAT, sporozoites were used as Ag, and in the ELISA, PY CS.1 and (QGPGAP)\textsubscript{2} were used as Ag.

<sup>b</sup> CS precipitation (CSP) results are recorded as no. positive/no. sporozoites counted at a 1/2 dilution of serum.
immunized with four doses of irradiated sporozoites. These sera have no inhibitory activity in the inhibition of liver stage development assay (30). Furthermore, we have previously shown that when such mice are treated with antibodies to CD8 they are no longer protected against malaria (14, 28); the polyclonal antibodies in the sera are not protective. There was no blocking activity (Table IV), and although the differences did not reach the level of statistical significance (p = 0.07, Fisher’s exact test, two-tailed), there was a trend toward an increase in protection.

**DISCUSSION**

These studies clearly demonstrate that a monoclonal antibody directed against the repeat region of the CS protein can protect animals against a very large P. yoelii sporozoite challenge. In the P. yoelii system the ID₅₀ is almost always less than 10 sporozoites, and often 1 to 2 sporozoites, yet NYS1 can protect 100% of mice against challenge with 5000 sporozoites (500 to 5000 times the ID₅₀). It is, however, still unclear why apparently comparable levels of vaccine-induced antibodies to the CS protein provide little, if any protection against sporozoite challenge.

NYS1 was made by immunizing mice with irradiated sporozoites, the native protein. An initial hypothesis was that although the binding of NYS1 to sporozoites could be inhibited by [QGPAP]₂. NYS1 bound to sporozoites with highest efficiency at an assembled site that reflected the tertiary structure of the native protein, not the primary structure reflected by the deduced amino acid sequence. Peptides are often disordered structures in water: thus, if the synthetic peptide [QGPAP]₂ or the E. coli-produced PY CS.1 did not assume the structure of the native CS protein, immunization with these subunit vaccines would not elicit the appropriate protective antibodies, antibodies with the high degree of complementarity for the native protein required to produce the highly efficient interaction that protects against challenge with live sporozoites. One cannot draw definitive conclusions from comparisons of mAb and polyclonal antibodies in affinity or avidity assays, but the results of the thiocyanate elution studies (Fig. 3) suggest that this initial interpretation is incorrect. The apparent avidity of NYS1 for peptides and native protein is 5 to 10 times lower than that of the vaccine-induced polyclonal antibodies. Further studies are required to define the kinetics of antibody binding, the nature of the epitope(s) recognized by NYS1, and its mechanism of disease protection.

<table>
<thead>
<tr>
<th>Group</th>
<th>NYS1</th>
<th>Serum</th>
<th>IFAT (Titre)</th>
<th>No. Infected/No. Treated</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>250</td>
<td>Normal</td>
<td>2048</td>
<td>7/10</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>125</td>
<td>Normal</td>
<td>1024</td>
<td>10/10</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>PBS</td>
<td>Normal</td>
<td>&lt;8</td>
<td>10/10</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>250</td>
<td>Immune</td>
<td>4096</td>
<td>2/10</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>125</td>
<td>Immune</td>
<td>2048</td>
<td>8/10</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>PBS</td>
<td>Immune</td>
<td>512</td>
<td>10/10</td>
<td>0</td>
</tr>
</tbody>
</table>

*In this experiment 8/8 control mice receiving 200 or 40 sporozoites became infected, and 4/8 mice that received 8 sporozoites became infected.

* Mice received by tail vein injection of 0.2 ml of serum from normal mice or from mice that had received four doses of irradiated sporozoites (immune).

**Figure 3.** Estimation of antibody avidity. Sera from mice immunized with [QGPAP]₁-KLH (B), [QGPAP]₂-proteosomes (D), PY CS.1 (C), and irradiated sporozoites (A), and sera from mice that received NYS1 in passive transfer (E) were tested in the SCN elution assay to estimate antibody avidity to: A. [QGPAP]₁, B. PY CS.1, C. sporozoite extract, and D. whole sporozoites. The avidity index was arbitrarily considered to be the concentration of SCN required to reduce the initial absorbance by 50% (log % initial absorbance = 1.699), and is designated by the point where the dotted line crosses the elution curve.

**Figure 4.** Inhibition of P. yoelii liver stage development in vitro. Sera from mice that received 500 μg of NYS1 in passive transfer (B) and from mice immunized with either [QGPAP]₁-proteosome (D) or PY CS.1 (B) were tested in the ELISA at dilutions of 1:10 to 1:200.

at a serum dilution of 1/100 (Fig. 4).

IgG subclasses against [QGPAP]₁. Because the protected mice had received an IgG3 mAb in passive transfer, we addressed the possibility that subclass of antibody may influence the protection. We measured the levels of IgG subclass antibodies against [QGPAP]₁. All vaccines induced primarily IgG1 antibodies against [QGPAP]₁. For the [QGPAP]₁-proteosomes vaccine 92% were IgG1, 5% IgG2a, 3% IgG2b, and 0% IgG3. For the [QGPAP]₁-KLH vaccine the respective percentages were 84, 10, 6, and 0, and for PY CS.1 they were 60, 4, 2, and 34.

Mixing experiment. To determine if sera from mice immunized with irradiated sporozoites actually reduced the protective activity of NYS1 in vivo, we passively transferred both NYS1 and sera from mice that had been
thermore, we have recently produced protective mAb by immunizing with the synthetic peptide (QQPGAP), (M. Ak, unpublished observations).

It is also possible that the vaccine-induced polyclonal antibodies contain subpopulations of nonprotective antibodies that compete with the protective antibodies for binding to sporozoites. However, our data from experiments conducted with nonprotective sera from mice immunized with irradiated sporozoites provide no evidence for this speculation (Table IV), and in fact suggest that these sera do have inhibitory activity.

Another possibility is that the ELISA and IFAT are too crude to distinguish between protective and nonprotective antibodies to sporozoites, and that the absolute concentration of the "correct" antibody is much lower in the sera from the actively immunized animals. The results of the ILSDA support the latter interpretation. There is an enormous difference in the inhibitory activity of the sera from the actively immunized animals. The results of these sera do have inhibitory activity.

Moreover, we have recently produced protective mAb against Plasmodium falciparum sporozoites. R. S. Nussenzweig, and V. Nussenzweig. 1985. Rationale for development of a synthetic vaccine against Plasmodium falciparum malaria. Science 228:1436.


