Cross-protection between attenuated *Plasmodium berghei* and *P. yoelii* sporozoites

M. SEDEGAH, W. W. WEISS & S. L. HOFFMAN

1Malaria Program, Naval Medical Research Center, Silver Spring, MD, USA

**SUMMARY**

An attenuated *Plasmodium falciparum* sporozoite (*PfSPZ*) vaccine is under development, in part, based on studies in mice with *P. berghei*. We used *P. berghei* and *P. yoelii* to study vaccine-induced protection against challenge with a species of parasite different from the immunizing parasite in BALB/c mice. One-hundred percent of mice were protected against homologous challenge. Seventy-nine percent immunized with attenuated *P. berghei* sporozoite (*PbSPZ*) (six experiments) were protected against challenge with *P. yoelii* sporozoite (*PySPZ*), and 63% immunized with attenuated *PySPZ* (three experiments) were protected against challenge with *PbSPZ*. Antibodies in sera of immunized mice only recognized homologous sporozoites and could not have mediated protection against heterologous challenge. Immunization with attenuated *PySPZ* or *PbSPZ* induced CD8+ T cell-dependent protection against heterologous challenge. Immunization with attenuated *PySPZ* induced CD8+ T cell-dependent protection against homologous challenge. However, homologous protection induced by attenuated *PbSPZ* was not dependent on CD8+ or CD4+ T cells, and depletion of both populations only reduced protection by 36%. Immunization of C57BL/10 mice with *PbSPZ* induced CD8+ T cell-dependent protection against *P. berghei*, but no protection against *P. yoelii*. The cross-protection data in BALB/c mice support testing a human vaccine based on attenuated *PfSPZ* for its efficacy against *P. vivax*.

**INTRODUCTION**

In 1967 it was reported that immunization of A/J mice with radiation-attenuated *Plasmodium berghei* sporozoites (*PbSPZ*) protected the mice against malaria (1). Several years later, it was reported that humans immunized by exposure to radiation-attenuated *P. falciparum* sporozoites (*PfSPZ*) (2–4) were also protected. There now is a major effort to develop an attenuated sporozoite (SPZ) vaccine to protect humans against *P. falciparum*, the malaria parasite responsible for greater than 98% of the 1–3 million deaths caused by malaria annually (5). It is not known if immunization with attenuated *PfSPZ* will protect against exposure to *P. vivax*, *P. malariae* or *P. ovale*, the three other *Plasmodium* species that infect humans. One individual was immunized with *PfSPZ*, shown to be protected against *P. falciparum*, and then challenged with *P. vivax* SPZs, and shown not to be protected (6), raising the possibility that there may not be cross-protection. Determination of cross-protection in humans must await clinical trials. However, because of the importance murine studies have played in providing the foundation for human studies with attenuated SPZs, we have used two rodent malaria parasites, *P. berghei* and *P. yoelii*, to study cross-protection in mice. The results indicate that there is significant cross-protection, and that different immune responses may be active against closely related parasite species.

**MATERIALS AND METHODS**

**Mice**

Female, 6- to 8-week-old BALB/CByJ and C57Bl/10 mice from Jackson Laboratories (Bar Harbor, ME, USA) were used in this study.
Parasites and immunizations

Sporozoites of *P. berghei* (ANKA) (7) and *P. yoelii* (17NL, 1.1 clone) (8) were produced in laboratory-bred and infected *Anopheles stephensi* mosquitoes. Sporozoites for immunization were separated from infected mosquitoes using a renograffin discontinuous gradient as previously described (7). Sporozoites were prepared from infected mosquitoes that had been exposed to 10 000 rad from a 137Ce source. Harvested SPZs were counted, diluted to the required concentration in M199 containing 5% normal mouse serum, and a volume of 0.2 mL was injected i.v. through the tail vein. Mice received multiple doses at 2-week intervals consisting of $7 \times 10^4$ irradiated SPZs as the first dose and $3 \times 10^4$ irradiated SPZs as subsequent doses. In challenge experiments, hand-dissected, infected mosquito glands were triturated in medium to obtain SPZs. Challenge doses varied from 1 to $10 \times 10^3$ nonirradiated SPZs and were injected i.v. Minimum infective dose (ID$_{50}$) of SPZs used to challenge the immune animals were determined during most challenges by making fivefold dilutions of the challenge inocula.

Antibody responses

An indirect fluorescent antibody test (IFAT) was used to detect antibodies to *P. yoelii* and *P. berghei* SPZs in sera of mice by methods previously described (8). Briefly in the IFAT, diluted sera were reacted with air-dried SPZs and end point titres were detected using fluorescein isothiocyanate (FITC)-labelled rabbit anti-mouse immunoglobulin.

Protection against challenge

Two weeks after the last immunization, mice were challenged by i.v. inoculation in the tail vein with viable *P. yoelii* or *P. berghei* SPZs. Giemsa stained blood films were examined with a light microscope under an oil immersion objective (1000×) for the presence of asexual erythrocytic stage parasites. Mice showing no parasites in their blood for 15 days following challenge with infective SPZs were designated as negative.

Depletion of CD8+ T lymphocytes

To determine if CD8+ T cells were required for protective immunity, we depleted mice of CD8+ T lymphocytes just prior to challenge. Mice immunized with five doses of irradiated SPZs were given three 0.5-mg i.p. injections of the mAb 19/178 at 24-h intervals to deplete them of CD8+ T lymphocytes as previously described (9). Peripheral lymphocyte samples were prepared and analysed for depletion of the population carrying the CD8+ T cell marker in a fluorescent-activated cell sorter (FACS). The mice were challenged with infective SPZs 4 days later. Treatment with mAB 19/178 was continued every third day after challenge until patency occurred. Control immunized mice were injected with the same quantities of rat immunoglobulin.

Calculation of the ID$_{50}$ and challenge units

Because the infectivity of individual preparations of SPZs may vary, the infectivity of the SPZs used for challenge was monitored during challenge in some of the experiments (Tables 1–3). Serial dilutions of SPZs were inoculated into groups of naive mice to determine the ID$_{50}$ (the number of SPZs needed to infect 50% of injected normal mice) of the SPZs used in the challenge. For *P. yoelii* SPZ, 200, 40, 8 or 1.6 SPZs were injected i.v. into groups of six mice. For *P. berghei* SPZ, 1000, 200 or 40 SPZs were injected i.v. into groups of six mice as *P. yoelii* SPZs are more infectious than *P. berghei* SPZ. To obtain the ID$_{50}$, we took the natural logarithm (ln) of the doses tested. We used logistic regression to model the log odds of infection vs. the log-transformed doses. The ID$_{50}$ is the log

| Table 1 Cross-protection between *Py*SPZ and *Ph*SPZ in BALB/c mice |
|------------------|---------------|---------------|---------|
| Immunization     | Challenge SPZa | Experiment 1  | Experiment 2 |
| *Irradiated Py*SPZ| *P. yoelii*    | 6/6           | 7/7      |
| *Irradiated Py*SPZ| *P. berghei*   | 4/6           | 3/7      |
| *Irradiated Ph*SPZ| *P. berghei*   | 6/6           | 7/7      |
| *Irradiated Ph*SPZ| *P. yoelii*    | 5/6           | 7/7      |
| *Naive*          | *P. yoelii*    | 0/6           | 0/7      |
| *Naive*          | *P. berghei*   | 0/6           | 0/7      |

Mice were immunized i.v. with five doses of radiation-attenuated *Py*SPZ or *Ph*SPZ. Two weeks after the last immunization, mice were challenged with infective SPZ. The number of *Py*SPZ used for the challenge was $5 \times 10^3$, and for *Ph*SPZ was $10 \times 10^3$. The units of ID$_{50}$ were calculated by dividing the challenge dose by the ID$_{50}$. Although we did SPZ titrations to determine the infectivity of the SPZs used in Experiments 1 and 2, we only obtained enough data points (see Materials and methods) in one experiment for each parasite to calculate the ID$_{50}$. The ID$_{50}$ for *P. yoelii* in Experiment 1 was $3 \times 3$ SPZs (95% CI 1.2–8.8), and the number of *P. yoelii* ID$_{50}$ units in the $5 \times 10^3$ SPZs used for this challenge was 1525.7 (95% CI 569.1–4090.3). The ID$_{50}$ for *P. berghei* in Experiment 2 was 370 SPZs (95% CI 126.5–1082.1), and the number of *P. berghei* ID$_{50}$ units in the $10 \times 10^3$ SPZs used for this challenge was 27.0 (95% CI 9.2–79.1).

560 Journal compilation © 2007 Blackwell Publishing Ltd, Parasite Immunology, 29, 559–565
No claim to original US government works
Groups of BALB/c mice were immunized with five doses of radiation-attenuated *P. berghei* or *P. yoelii* CD8+ T cell depletion. Two weeks after the last immunization, mice were injected with anti-CD8 mAbs or a control antibody prior to challenge with infective SPZs. The challenge dose for *P. berghei* was 4 × 10^3 SPZs, and for *P. yoelii* was 10^3 SPZs.

Table 2 Protective immunity in BALB/c mice immunized with radiation-attenuated *P. yoelii* CD8+ T cell depletion

| Immunization       | Challenge SPZs | Number protected/number challenged (%) |
|--------------------|----------------|----------------------------------------|
| Irradiated *P. yoelii* Control | *P. yoelii* | 6/6 (100) |
| Irradiated *P. yoelii* CD8+ | *P. yoelii* | 0/6 (0) |
| Irradiated *P. berghesi* Control | *P. berghesi* | 5/6 (83) |
| Irradiated *P. berghesi* CD8+ | *P. berghesi* | 0/7 (0) |
| Naive – | *P. yoelii* | 0/6 (0) |
| Naive – | *P. berghesi* | 0/6 (0) |

Table 3 Protective immunity in BALB/c mice immunized with irradiated *P. berghei* with and without CD8+ T cell depletion

| Immunization       | Challenge SPZs | Number protected/number challenged (%) |
|--------------------|----------------|----------------------------------------|
| Irradiated *P. berghei* Control | *P. berghei* | ND |
| Irradiated *P. berghei* CD8+ | *P. berghei* | 5/5 (100) |
| Irradiated *P. yoelii* Control | *P. yoelii* | 5/5 (100) |
| Irradiated *P. yoelii* CD8+ | *P. yoelii* | 0/5 (0) |
| Naive – | *P. berghesi* | 0/6 (0) |
| Naive – | *P. yoelii* | 0/6 (0) |

Table 4 Antibodies in BALB/c mice to *P. yoelii* and *P. berghei* after immunization with radiation-attenuated *P. yoelii* and *P. berghei*

| Immunization       | SPZs in IFAT | IFAT titre |
|--------------------|--------------|------------|
| Irradiated *P. yoelii* | *P. yoelii* | 2048 |
| Irradiated *P. berghei* | *P. berghei* | <8 |
| Irradiated *P. berghei* | *P. berghei* | 4096 |
| Irradiated *P. yoelii* | *P. yoelii* | <8 |

Mice were immunized i.v. with five doses of radiation-attenuated *P. yoelii* or *P. berghei*. Two weeks after the last immunization, just prior to challenge with infective SPZs, pooled sera were tested for antibodies to air-dried *P. yoelii* or *P. berghei* by IFAT.

RESULTS

Antibody responses

Table 4 shows that immunization with irradiated *P. yoelii* or *P. berghei* resulted in the production of high antibody titres to the parasite used for the immunization, but no cross-reacting antibodies to the other species.

Cross-protection in BALB/c mice

Two weeks after the last immunization with radiation-attenuated SPZs, mice were challenged by i.v. injection of infective *P. yoelii* or *P. berghei*. In two challenge experiments, the number of *P. yoelii* and *P. berghei* used for challenge was 5 × 10^3 and 10 × 10^3, respectively. In all challenges, 100% of naive mice became infected. In these two challenges, protection against challenge with *P. yoelii* or *P. berghei* was 100% in groups
that received the same parasite species for immunization and challenge (homologous immunization and challenge) (13 out of 13 mice protected for both parasites) (Table 1). However, BALB/c mice immunized with radiation-attenuated SPZs of either parasite strain showed different levels of cross-protection against the heterologous parasite species challenge. In two experiments, the level of cross-protection obtained when mice immunized with attenuated \( P.\) \textit{bberghei} SPZs were challenged with \( P.\) \textit{bberghei} SPZs was greater (12 out of 13 mice protected, 92.3\%) than when mice immunized with attenuated \( P.\) \textit{yoelii} SPZs were challenged with \( P.\) \textit{yoelii} SPZs (7 out of 13 mice protected, 54\%); \( P = 0.073, \) Fisher's exact test, two-sided. This difference occurred despite significantly higher infectivity of the \( P.\) \textit{yoelii} SPZ (mean \( ID_{50} = 10^{-6} \)) vs. \( P.\) \textit{bberghei} SPZ (mean \( ID_{50} = 571.1 \)); \( P = 0.0047, \) independent samples t-test.

**Mechanisms of cross-protection in BALB/c mice**

In BALB/c mice, CD8\(^+\) T cells have been shown to be responsible for the immunity against \( P.\) \textit{yoelii} SPZ after immunization with radiation-attenuated \( P.\) \textit{yoelii} SPZ (8–10), while protection induced in BALB/c mice after immunization with irradiated \( P.\) \textit{bberghei} SPZ is independent of CD8\(^+\) T cells (11). To determine whether the same immune mechanisms were responsible for the cross-protection between \( P.\) \textit{yoelii} and \( P.\) \textit{bberghei}, we injected immunized mice with an anti-CD8 mAb and then challenged the mice with infective SPZs. The FACS analysis confirmed that injection of the mAb resulted in depletion of more than 99\% of the CD8\(^+\) T cells (data not shown). Depletion of CD8\(^+\) T cells in BALB/c mice immunized with attenuated \( P.\) \textit{yoelii} SPZ eliminated protection against challenge with homologous \( (P.\) \textit{yoelii}) and heterologous \( (P.\) \textit{bberghei}) SPZs (Table 2). In mice immunized with attenuated \( P.\) \textit{yoelii} SPZs, treatment with anti-CD8 antibodies had no effect on challenge with homologous \( (P.\) \textit{bberghei}) SPZs, but it eliminated protection against heterologous \( (P.\) \textit{yoelii}) SPZs (Table 3).

Since CD8\(^+\) T cell depletion did not affect protective efficacy in BALB/c mice immunized with radiation-attenuated \( P.\) \textit{bberghei} SPZ and challenged with \( P.\) \textit{yoelii} SPZs (Table 3), we assessed the effect of CD4\(^+\) T cell depletion in these mice (Table 5). Depletion of CD4\(^+\) T cells had no effect on protection (Table 5), but depletion of both CD4\(^+\) and CD8\(^+\) T cells had a modest effect on protective efficacy (Table 5). CD4\(^+\) T cell depletion reduced, but did not eliminate protection against challenge with \( P.\) \textit{yoelii} SPZ.

**Cross-protection between the two parasite species in C57BL/10 mice**

In BALB/c mice immunized with radiation-attenuated \( P.\) \textit{bberghei} SPZ, CD8\(^+\) T cells are not required for protection against \( P.\) \textit{yoelii} SPZ challenge (11,12). However, in \( A/J \) (H-2a) mice immunized with radiation-attenuated \( P.\) \textit{bberghei} SPZ, CD8\(^+\) T cells are required for protection (12,13). Since the mechanism of protection against \( P.\) \textit{bberghei} varied among different mouse species, we wanted to determine if cross-protection extended to another mouse strain. We immunized C57BL/10 (H-2b) mice with irradiated \( P.\) \textit{bberghei} to determine the mechanism of protection induced in this mouse strain against homologous challenge and also to determine if mice immunized with irradiated \( P.\) \textit{bberghei} showed cross-protection against \( P.\) \textit{yoelii} SPZ. In this mouse strain, C57BL/10 (H-2b), protection against the homologous \( P.\) \textit{bberghei} challenge was dependent on CD8\(^+\) T cells (Table 6). Furthermore, C57BL/10 (H-2b) mice immunized with irradiated \( P.\) \textit{bberghei} did not show cross-protection against \( P.\) \textit{yoelii} SPZ (Table 6).

**DISCUSSION**

There were several major new findings in these studies. There was significant cross-protection between \( P.\) \textit{yoelii} and \( P.\) \textit{bberghei} in BALB/c mice (Tables 1–3 and 5), antibodies against SPZs did not play a role in the cross-protection and

---

**Table 5** Protective immunity in BALB/c mice immunized with radiation-attenuated \( P.\) SPZ with and without CD8\(^+\) and/or CD4\(^+\) T cell depletion

| Immunization | T cell depletion | Challenge SPZs | Number protected/number challenged | Total (% protected) |
|--------------|------------------|----------------|------------------------------------|--------------------|
| Irradiated \( P.\) SPZ | Control | \( P.\) \textit{berghei} | 7/7 | 14/14 (100) |
| Irradiated \( P.\) SPZ | CD4\(^+\) | \( P.\) \textit{berghei} | 7/7 | 7/7 (100) |
| Irradiated \( P.\) SPZ | CD4\(^+\) and CD8\(^+\) | \( P.\) \textit{berghei} | 5/7 | 9/14 (64) |
| Irradiated \( P.\) SPZ | Control | \( P.\) \textit{yoelii} | 4/7 | 9/14 (64) |
| Irradiated \( P.\) SPZ | CD4\(^+\) | \( P.\) \textit{yoelii} | 2/7 | 4/14 (29) |
| Naive | – | \( P.\) \textit{berghei} | 0/7 | 0/14 (0) |
| Naive | – | \( P.\) \textit{yoelii} | 0/7 | 0/14 (0) |

Groups of BALB/c mice were immunized with five doses of radiation-attenuated \( P.\) SPZ and depleted of either T cells expressing CD4\(^+\) or T cells expressing CD4\(^+\) and CD8\(^+\) markers, and challenged with \( 7 \times 10^3 \) \( P.\) SPZ or \( 1 \times 10^3 \) \( P.\) \textit{yoelii} SPZ.
the immune mechanisms required for protection against homologous challenge, but not heterologous challenge, in BALB/c mice were apparently different for *P. yoelii* (Table 2) and *P. bergheri* (Tables 3 and 5).

The potentially most important finding was the cross-protection. One-hundred percent of mice were protected against homologous challenge. Thirty-one of 39 (79%) BALB/c mice immunized with attenuated *PbSPZ* were protected against challenge with *PySPZ*, and 12 of 19 (63%) BALB/c mice immunized with attenuated *PySPZ* were protected against challenge with *PbSPZ* (Tables 1–3 and 5).

Mice immunized with *PySPZ* did not produce antibodies that recognized *PbSPZ*, and mice immunized with *PbSPZ* did not produce antibodies that recognized *PySPZ* (Table 4). Thus, antibodies against SPZs could not have been responsible for the protection against heterologous challenge.

The protection against heterologous challenge in mice immunized with either radiation-attenuated *PySPZ* or *PbSPZ* was dependent on CD8$^+$ T cells (Tables 2 and 3). When mice were immunized with radiation-attenuated *PySPZ* and challenged with nonirradiated *PbSPZ*, or immunized with radiation-attenuated *PbSPZ* and challenged with nonirradiated *PySPZ*, the protective immunity was eliminated by treatment of the mice before challenge with an antibody to CD8$^+$ T cells. These data demonstrated that CD8$^+$ T cells were required for the cross-protection. The requirement for T cells to provide protection against heterologous parasites was consistent with the finding that these mice made no antibodies against the heterologous SPZs (Table 4).

In BALB/c mice immunized with radiation-attenuated *PySPZ*, protection against challenge with *P. bergheri* was not eliminated by depletion of either CD8$^+$ (Table 3) or CD4$^+$ T cells (Table 5) and only modestly reduced by depletion of both (Table 5). The finding of lack of dependence on CD8$^+$ T cells in BALB/c mice immunized by i.v. injection of irradiated *PbSPZ* model was first reported more than 16 years ago (11) Interestingly this did not occur in mice immunized by the bite of irradiated, *PbSPZ*-infected mosquitoes (14). Regardless, the other results have not been previously reported. There are several possible explanations for why T cell depletion did not reduce protection against *P. bergheri* in mice immunized with irradiated *PbSPZ*. One possibility is that non-T cell immunity is more effective against *PbSPZ* infection than it is against *P. yoelii*. This could be because the infectivity (ID$_{50}$/) of *PbSPZ* is lower than that of *PySPZ* (Table 1 and see below), and the parasites are easier to eliminate by non-T cell mechanisms because fewer of them are infective. This differential infectivity could explain why, in contrast to *PbSPZ*, the protection against *PySPZ* in these same mice was eliminated by depletion of CD8$^+$ T cells. Another explanation for why T cell depletion of mice immunized with irradiated *PbSPZ* did not eliminate protection is that the effect of in vivo T cell depletion on effector T cells could have been less in mice immunized with irradiated *PbSPZ* as compared to after immunization with irradiated *PySPZ*. Although the efficiency of in vivo depletion of total splenic CD8$^+$ and CD4$^+$ T cells was 99% in these experiments, we did not measure antigen-specific T cell populations responding to *P. bergheri* or *P. yoelii* antigens after depletion. Thus, there remains a possibility that sufficient effector cells remained in the residual 1% of undepleted total T cells to protect mice against *PbSPZ* infection, but not *P. yoelii* infection. There is a region, amino acids 281–289 on the *PyCSP* (SYVPSEAEQ1) and *PbCSP* (SYIPSEAEKI), which contains a protective cytotoxic T lymphocytes (CTL) epitope (15).

### Table 6 Protective immunity in C57BL/10 mice immunized with radiation attenuated *PbSPZ*.

| Immunization | T cell depletion | Challenge SPZs | Number protected/number challenged (% protected) |
|--------------|------------------|----------------|-----------------------------------------------|
| **Experiment 1** |                  |                |                                               |
| Irradiated *PbSPZ* | None            | *P. bergheri*  | 9/9 (100)                                     |
| Irradiated *PbSPZ* | None            | *P. yoelii*    | 1/9 (11)                                      |
| Naive         | –                | *P. bergheri*  | 0/8 (0)                                       |
| Naive         | –                | *P. yoelii*    | 1/9 (11)                                      |
| **Experiment 2** |                  |                |                                               |
| Irradiated *PbSPZ* | Control          | *P. bergheri*  | 5/5 (100)                                     |
| Irradiated *PbSPZ* | CD8$^+$          | *P. bergheri*  | 0/5 (0)                                       |
| Naive         | –                | *P. bergheri*  | 0/5 (0)                                       |

Groups of C57BL/10 mice were immunized with five doses of irradiated *PbSPZ* and challenged with either 5 × 10$^3$ *PbSPZ* or *PySPZ* (Experiment 1). In a second experiment (Experiment 2), C57BL/10 mice similarly immunized were depleted of their CD8$^+$ T cell subpopulation and challenged with *PbSPZ*.
In mice immunized with irradiated \textit{Pb}SPZ and challenged with \textit{Py}SPZ, CD4$^+$ T cell depletion modestly reduced protection (Table 5), indicating that CD4$^+$ T cells play a role in this heterologous protection. Interestingly, this effect was not seen with homologous challenge, but depletion of both CD8$^+$ and CD4$^+$ T cells reduced protection against homologous (\textit{P. berghei}) challenge, again showing that CD4$^+$ T cells do play some role in protection. This finding also raises the possibility of the complementary effects of CD8$^+$ and CD4$^+$ T cells working together. Nevertheless, our findings and more than 20 years of research on mice immunized with irradiated SPZ have demonstrated that CD8$^+$ T cells are the major effector arm of the immune system, but that CD4$^+$ T cells also play a role, albeit minor role, and that in some cases, antibodies can be the major effector immune response (16).

Despite the excellent cross-protection in BALB/c mice, there was no cross-protection in C57BL/10 mice. It is possible that the immune response induced with the irradiated \textit{Pb}SPZ was not strong enough to overcome the highly infectious \textit{Py}SPZ challenge in the C57BL/10 mice. The high susceptibility of C57BL/10 mice to \textit{Py}SPZ may also explain why in some experiments not all mice immunized with irradiated \textit{Py}SPZ become protected after challenge with \textit{Py}SPZ (12,17).

It is generally thought that optimally radiation-attenuated SPZs pass through the bloodstream to the liver, invade hepatocytes and partially develop expressing proteins not expressed in hepatocytes. Many malarialologists believe the CD8$^+$ T cell-dependent protective immunity elicited by immunization with radiation-attenuated SPZs is primarily directed against these ‘new’ proteins first expressed in hepatocytes (10,16,17) This view has been based on a report that ‘over-irradiated’ SPZs do not provide protection even though they invade hepatocytes (18), and killed SPZs do not provide high level of protection (19). Thus, despite the fact that immunization with \textit{Py}CSP and \textit{Pb}CSP (11,20–24) based vaccines elicit CD8$^+$ T cell-dependent protection against homologous challenge, and transfer of an anti-\textit{Py}CSP T cell clone protects against \textit{P. yoelii} and \textit{P. berghei} challenge (15,25), it is thought that these SPZ-derived proteins are not adequate for the high level of protective immunity seen after immunization with radiation-attenuated SPZs. These conclusions are supported by a recent study with mice transgenic for the \textit{Py}CSP, and therefore tolerant to the \textit{Py}CSP (26,27). When these mice received two doses of irradiated \textit{Py}SPZ, there was only minimal protection, indicating that \textit{Py}CSP was important in protective immunity. However, when these mice received a full immunizing regimen of three doses of irradiated \textit{Py}SPZ, they were fully protected against challenge with infectious \textit{Py}SPZ, indicating that in this model system, immune responses against the \textit{Py}CSP were not required for protection. While the data reported herein do not indicate what antigens are responsible for the protection, the findings that a \textit{Py}CSP T cell clone was protective against challenge with the heterologous \textit{Pb}SPZ (15) possibly through the mediation of antigen-specific induction of IFN$\gamma$ is significant. The availability of the genomic sequences of \textit{P. yoelii} (28) and \textit{P. berghei} (29), and gene expression and proteomic analyses should facilitate the discovery of other antigens involved.

The data presented in this paper re-emphasize the differences in infectivity of \textit{Py}SPZ and \textit{Pb}SPZ (30–32). The mean ID$\text{so}$ of \textit{P. yoelii} was 10·6 (95% CI 6·1–18·3) while that of \textit{P. berghei} was 571·1 (249·9–1304·8), indicating that far less SPZs are needed to produce a \textit{P. yoelii} infection.

The finding of cross-protection in mice between \textit{P. yoelii} and \textit{P. berghei} after immunization with radiation-attenuated SPZs is provocative, but cannot be used to predict what will occur in humans immunized with radiation-attenuated SPZs. However, when the radiation-attenuated \textit{Py}SPZ vaccine is developed and tested (33), it will be important to determine if immunization with this vaccine protects against \textit{P. vivax}. The data presented herein suggest that cross-protection could occur. Comparative analyses of the genomes of \textit{P. falciparum}, \textit{P. vivax}, \textit{P. yoelii} and \textit{P. berghei} may provide more insight into the likelihood of cross-protection. However, there will be no substitute for carefully executed clinical trials to determine if there is cross-protection.

ACKNOWLEDGEMENTS

This work was supported by the Naval Medical Research and Development Command work units 3 M61102BS13AK111.

REFERENCES

1 Nussenzweig RS, Vanderberg J, Most H & Orton C. Protective immunity produced by the injection of X-irradiated sporozoites of \textit{Plasmodium berghei}. Nature 1967; 216: 160–162.

2 Clyde DF, Most H, McCarthy VC & Vanderberg JP. Immunization of man against sporozoite-induced falciparum malaria. \textit{Am J Med Sci} 1973; 266: 169–177.

3 Rieckmann KH, Beaudoin RL, Cassels JS & Sell DW. Use of attenuated sporozoites in the immunization of human volunteers against falciparum malaria. \textit{Bull World Health Organ} 1979; 57: 261–265.

4 Hoffman SL, Goh LM, Luke TC, \textit{et al.} Protection of humans against malaria by immunization with radiation-attenuated \textit{Plasmodium falciparum} sporozoites. \textit{J Infect Dis} 2002; 185: 1155–1164.

5 Breman JG, Egan A & Keusch GT. The intolerable burden of malaria: a new look at the numbers. \textit{Am J Trop Med Hyg} 2001; 64: iv–vii.

6 Clyde DF, McCarthy VC, Miller RM & Hornick RB. Specificity of protection of man immunized against sporozoite-induced falciparum malaria. \textit{Am J Med Sci} 1973; 266: 398–401.

7 Pacheco ND, Strome CPA, Mitchell F, Bawden MP & Beaudoin RL. Rapid large-scale isolation of \textit{Plasmodium falciparum}...
berghel sporozoites from infected mosquitoes. J Parasitol 1979; 65: 414–417.

8 Sedegah M, Beaudoin RL, De la Vega P, et al. Use of a vaccinia construct expressing the circumsporozoite protein in the analysis of protective immunity to Plasmodium yoelii. In Lasky L (ed.): Technological Advances in Vaccine Development. New York, Liss, 1988: 295–309.

9 Weiss WR, Sedegah M, Beaudoin RL, Miller LH & Good MF. CD8+ T cells (cytotoxic/suppressors) are required for protection in mice immunized with malaria sporozoites. Proc Natl Acad Sci USA 1988; 85: 573–576.

10 Doolan DL & Hoffman SL. IL-12 and NK cells are required for protective immunity to malaria by immunization with plasmid DNA encoding circumsporozoite protein. Proc Natl Acad Sci USA 1994; 91: 9866–9870.

11 Aggarwal A, Kumar S, Jaffe R, Hone D, Gross M & Sadoff J. Oral Salmonella: malaria circumsporozoite recombinants induce specific CD8+ cytotoxic T cells. J Exp Med 1990; 172: 1083–1090.

12 Weiss WR. Host–parasite interactions and immunity to irradiated sporozoites. Immunol Lett 1990; 25: 39–42.

13 Schofield L, Villaquiran J, Ferreira A, Schellekens H, Nussenzweig RS & Nussenzweig V. Gamma-interferon, CD8+ T cells and antibodies required for immunity to malaria sporozoites. Nature 1987; 330: 664–666.

14 Seguin MC, Klotz FW, Schneider I, et al. Induction of nitric oxide synthase protects against malaria in mice exposed to irradiated Plasmodium berghei infected mosquitoes: involvement of interferon γ and CD8+ T cells. J Exp Med 1994; 180: 353–358.

15 Weiss WR, Berzovsky JA, Houghten RA, Sedegah M, Hollingdale M & Hoffman SL. A T cell clone directed at the circumsporozoite and Plasmodium berghei. J Immunol 1992; 149: 2103–2109.

16 Hoffman SL & Doolan DL. Malaria vaccines-targeting infected hepatocytes. Nat Med 2000; 6: 1218–1219.

17 Cross-protection between Plasmodium species

18 Silvie O, Semblat JP, Franetich JF, Hannoun L, Eling W & Mazier D. Effects of irradiation on Plasmodium falciparum sporozoite hepatic development: implications for the design of pre-erythrocytic malaria vaccines. Parasite Immunol 2002; 24: 221–223.

19 Nussenzweig RS, Vanderberg JP, Spitalny GL, Rivera CIO, Orton C & Most H. Sporozoite-induced immunity in mammalian malaria. A review. Am J Trop Med Hyg 1972; 21: 722–728.

20 Sedegah M, Hedstrom R, Hobart P & Hoffman SL. Protection against malaria by immunization with plasmid DNA encoding circumsporozoite protein. Proc Natl Acad Sci USA 1994; 91: 9866–9870.