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Helminth infection impairs the immunogenicity of a *Plasmodium falciparum* DNA vaccine, but not irradiated sporozoites, in mice

Gregory S. Noland^{1,§,#}, Debabani Roy Chowdhury^{1,#}, Joseph F. Urban Jr.², Fidel Zavala¹, and Nirbhay Kumar^{1,3,*}

¹Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA

²Diet, Genomics, and Immunology Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, United States Department of Agriculture, Beltsville, MD, USA

³Department of Tropical Medicine, Tulane University School of Public Health & Tropical Medicine, New Orleans, LA, 70112, USA

Abstract

Development of an effective vaccine against malaria remains a priority. However, a significant number of individuals living in tropical areas are also likely to be co-infected with helminths, which are known to adversely affect immune responses to a number of different existing vaccines. Here we compare the response to two prototype malaria vaccines: a transmission blocking DNA vaccine based on Pfs25, and a preerythrocytic malaria vaccine based on irradiated sporozoites in mice infected with the intestinal nematode *Heligmosomoides polygyrus*. Following primary immunization with Pfs25 DNA vaccine, levels of total IgG, as well as IgG1, IgG2a, IgG2b (all $P=0.0002$), and IgG3 ($P=0.03$) Pfs25 antibodies were significantly lower in *H. polygyrus*-infected mice versus worm-free controls. Similar results were observed even after two additional boosts, while clearance of worms with anthelmintic treatment 3 weeks prior to primary immunization significantly reversed the inhibitory effect of helminth infection. In contrast, helminth infection had no inhibitory effect on immunization with irradiated sporozoites. Mean anti-CSP antibody responses were similar between *H. polygyrus*-infected and worm-free control mice following immunization with a single dose (65,000 sporozoites) of live radiation attenuated (irradiated) *Plasmodium yoelii* sporozoites (17X, non-lethal strain), and protection upon sporozoite challenge was equivalent between groups. These results indicate that helminth infection may adversely affect certain anti-malarial vaccine strategies, and highlight the importance of these interactions for malaria vaccine development.

Keywords

malaria; *Plasmodium*; vaccine; helminths

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*Corresponding Author: Nirbhay Kumar DTM, 1440 Canal Street, Suite 2210 New Orleans, LA, 70112 phone: (504) 988-2577 nkumar@tulane.edu.

§Present address: Division of Infectious Diseases and Global Pediatrics Program, University of Minnesota School of Medicine, Minneapolis, MN, USA

#These two authors contributed equally to the work.

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1. INTRODUCTION

Helminth parasites are ubiquitous in tropical and subtropical areas, with an estimated two billion people infected [1]. It is well documented that helminth infection impairs the outcome of different vaccines, including BCG [2], yellow fever [3], tetanus [4-8], diphtheria toxoid [9], live attenuated oral cholera [10], and two HIV-1 vaccine candidates [11,12].

An estimated 500 million people in tropical areas also suffer from malaria, which claims approximately 1.3 million lives annually [13]. Faced with widespread drug resistant parasites and insecticide resistant mosquitoes, development of an effective malaria vaccine remains a public health priority. Many candidate vaccines targeting the pre-erythrocytic phase of *Plasmodium* infection, blood stage infection, or transmission blocking vaccines are currently undergoing pre-clinical or clinical development [14,15]. However the impact of helminth infection on malaria vaccine response in target populations remains poorly defined. Recent studies have found that mice infected with the intestinal nematode, *H. polygyrus*, displayed impaired anti-malaria responses following immunization with either crude blood-stage antigen [16] or long synthetic peptide of merozoite surface protein (MSP)-3 [17]. The effect of chronic helminth infection on other types of malaria vaccine strategies has not been determined.

Here, we also utilized the *H. polygyrus* model, which is ideally suited for lengthy immunization studies [18]. Adult *H. polygyrus* worms remain viable in the lumen of the rodent host's small intestine, before spontaneously resolving eight to twelve weeks post infection. During infection, eggs are continuously excreted in the host feces, and provide a convenient indication of infection status through simple microscopic examination.

We specifically evaluated whether *H. polygyrus* infection modulated murine responses to two different malaria vaccine candidates: a DNA transmission blocking vaccine based on *P. falciparum* surface protein (Pfs)-25, and a well known experimental vaccine based on irradiated sporozoites. Pfs25 is a 25-kDa cysteine-rich protein that is expressed on the surface of zygotes and ookinetes [19]. Previous work demonstrated that DNA immunization of mice with Pfs25 resulted in significant production of IgG1, IgG2a, and IgG2b antibodies that drastically reduced *P. falciparum* oocyst development and mosquito infectivity [20]. Likewise, irradiated sporozoite immunization is a highly effective strategy against *Plasmodium* infection [21,22], and currently forms the basis for whole parasite [23] as well as sub-unit vaccine strategies [24]. Studies have also shown that protection in mice depends on antibodies directed against the circumsporozoite protein (CSP) [25,26] and CD8+ and CD4+ T-cell populations [27,28]. In view of impairment of various vaccine elicited responses by helminths, we hypothesized that induction of immunity to both Pfs25 and irradiated sporozoite immunization would be likewise compromised by patent helminth infection and any such effect might be reversed by anthelmintic treatment prior to start of vaccinations.

2. MATERIALS AND METHODS

2.1. Animals and Parasites

For all experiments, age-matched BALB/c mice were purchased from the National Cancer Institute (Bethesda, Maryland) and maintained in a pathogen-free micro-isolation facility in accordance with the National Institutes of Health guidelines for the humane use of laboratory animals. Female mice were used for Pfs25 DNA experiments, while male mice were used for irradiated sporozoite experiments. Prior to immunizations, mice were infected orally with 200 third stage *H. polygyrus* larvae [29].

2.2. Pfs25 DNA immunizations

Beginning three weeks post helminth infection, *H. polygyrus*-infected and worm-free control mice (n=10 per group) were given three immunizations, spaced four weeks apart, of 50 µg Pfs25-containing DNA plasmid VR1020. DNA was suspended in sterile PBS and 50 µl injected intramuscularly into the right and left anterior tibialis of each mouse. Expression of the Pfs25 gene is driven by a strong eukaryotic cytomegalovirus promoter and secretion of expressed protein is facilitated by a tissue plasminogen activator signal peptide sequence upstream of the cloning site [20]. Four weeks after each immunization, blood was collected by tail bleed, and sera stored at -20°C until analyzed. An experimental replicate was performed using n=5 for all groups, with results similar to those reported here.

A similar protocol of Pfs25 DNA immunizations was followed in mice treated with the anthelmintic pyrantel pamoate to clear the adult worms. In these experiments, uninfected control mice and those infected with *H. polygyrus* were treated with a single oral dose of pyrantel pamoate 200 mg/kg body weight two weeks after nematode infection. Three weeks later, treated mice, as well as non-treated *H. polygyrus*-infected mice (n=5 per group), were given three 50 µg Pfs25 DNA immunizations as above. Immunizations and serum collection intervals were as above.

2.3. Irradiated sporozoite immunizations and challenge

Five weeks post helminth infection, *H. polygyrus*-infected and worm-free control mice were immunized intravenously with approximately 65,000 *P. yoelii* (17X non lethal strain) sporozoites exposed to 45,000 rads. Blood was collected by tail bleed from helminth infected-immunized, worm-free-immunized, and naive non-immunized mice four weeks post immunization, for determination of anti-CSP antibody responses. Five days later, cohorts of mice from each group were challenged with either 50 or 50,000 sporozoites. Mice challenged with 50 sporozoites (n≥4 mice per group) were followed for malaria parasitemia by examining Giemsa-stained blood smears commencing five days post malaria infection until resolution of infection. Mice challenged with 50,000 sporozoites (n≥3 mice per group) were sacrificed 40 hours post infection and liver-stage parasite burden determined by quantitative RT-PCR [30].

2.4. Antibody ELISAs

2.4.1. Pfs25—Immulon 4 microtiter plates (Thermo Electron, Milford, MA) were coated with 100 µL of recombinant Pfs25 (1.5 µg/ml) (rPfs25 was a kind gift from MVDB, NIAID, NIH [31]), in bicarbonate buffer (4 mM Na₂CO₃, 8 mM NaHCO₃, pH 9.6) overnight at 4°C. Wells were washed with PBS-0.05% Tween 20, then blocked with 5% non-fat dry milk in PBS (blocking buffer). Total IgG end-point titers were determined by serially diluting individual mouse sera in blocking buffer-0.05% Tween and adding 100 µl of samples to each well in duplicate. Plates were incubated for 1 hour at 37°C, washed, and incubated again with 100 µl (0.1 µg/ml) HRP-conjugated goat anti-mouse IgG (heavy plus light chain) (Kirkegaard & Perry Labs [KPL], Gaithersburg, MD). After further washing, plates were developed with 100 µl ABTS (2, 2'-azino-di[3-ethylbenzthiazoline-6-sulfonate]) (KPL), and absorbance read at 405 nm. Titers were defined as the reciprocal serum dilution with absorbance value greater than the mean OD plus 3 SDs for pools of pre-immune sera in each assay. Anti-Pfs25 IgG isotype responses were determined as above from 1:1000 dilutions of individual mouse sera, using 100 µl (0.1 µg/ml) HRP-conjugated goat anti-mouse IgG1, IgG2a, IgG2b, or IgG3 (Southern Biotech, Birmingham, AL) as secondary antibodies.

2.4.2. CSP—Total IgG anti-CSP response was determined by ELISA using Immulon 4 plates coated with 275 ng per well of synthetic peptide QGPGAPQGPGAP representing the *P. yoelii* B-cell epitopes of CSP repeat domain diluted in PBS. Samples were diluted from 1:25

to 1:3200 and analyzed as above, except 1% BSA-PBS was used as blocking buffer, and all incubations were carried out at RT.

2.5. Analysis of Memory B-cell

To generate Pfs25-specific memory B-cells, uninfected or *H. polygyrus* infected mice (n=5 per group) were immunized three times with Pfs25 DNA vaccine as described above and then rested for 14 weeks. By this time Pfs25-specific primary B-cells are not detectable [32]. Groups of *H. polygyrus* infected mice and uninfected controls treated with pyrantel pamoate were also immunized and rested following the same protocol. Following rest, the mice were given a boost of 10 µg rPfs25 protein formulated in incomplete Freund's adjuvant through peritoneal injection. To analyze the memory B-cell population, the mice were sacrificed 7 days post protein boost. Splenocytes were harvested from individual mice and single cell suspensions made in PBS. The splenocytes were incubated with primary mAbs CD19-FITC and CD27-biotin (BDPharmingen) diluted in FACS buffer (1% BSA in PBS) for 45 min in dark on ice. Cells were washed three times in FACS buffer and stained with streptavidin-PE (BD-Pharmingen) for another 45 min in dark on ice. Cells were washed and resuspended in PBS at 10^6 cells/ml. Fluorescence was measured by BD FACScan (BD Biosciences) and data were analyzed using CellQuest software (BD Biosciences).

2.6. Quantitative RT-PCR

2.6.1. mRNA isolation—Livers of mice challenged with 50,000 sporozoites were harvested 40 hours post infection, rinsed 2x in PBS, and homogenized in 4 ml of 4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7), 0.5% sarcosyl, 0.1 M 2-mercaptoethanol [33]. RNA was extracted from 600 µl of liver homogenate by adding 60 µl of 2 M sodium acetate (pH 4), 600 µl phenol, and 150 µl chloroform-isoamyl alcohol (24:1). After 15 min. incubation on ice, suspensions were centrifuged at 15,000 rpm for 20 min at 4°C. The aqueous phase was transferred to a fresh tube, mixed with an equal volume of isopropanol, and placed at -20°C overnight. Precipitated RNA was washed with ethanol, dissolved in TE buffer, and frozen at -20°C at a concentration of 1 µg/µl.

2.6.2. qRT-PCR—Parasite burden in livers was determined by qRT-PCR of *P. yoelii* 18S rRNA copy as described previously [30], except that a SYBR Green (Bio-Rad, Hercules, CA)-based PCR detection system was used.

2.7. Statistical analysis

Differences between antibody responses and liver stage parasite burdens were determined using the non-parametric Wilcoxon rank-sum (Mann-Whitney) test. All analysis was performed using Stata 10.0 (Stata Corporation, College Station, TX), and $P < 0.05$ was considered significant.

3. RESULTS

3.1. Effect of helminth infection on Pfs25 DNA immunization

To determine whether intestinal nematode infection affected immunization with a candidate DNA transmission blocking vaccine, titers and levels of Pfs25 antibodies were compared between worm-free and *H. polygyrus*-infected mice. A single immunization induced high titer anti-Pfs25 IgG antibodies in worm-free mice (Table 1). Consistent with previous work [20], primary immunization also resulted in significant production of IgG1, IgG2a, and IgG2b, but minimal IgG3, subclass antibodies (Fig 1a). In mice infected with *H. polygyrus*, total IgG antibody titers were more than 4-fold less than worm-free immunized mice (Table 1), while levels of antibodies (Fig 1a) were significantly less for total IgG ($P=0.0002$), IgG1 ($P=0.0002$),

IgG2a ($P=0.0002$), IgG2b ($P=0.0002$), and IgG3 ($P=0.03$). Antibody responses remained significantly lower in nematode infected mice following the second as well as the third immunization with 50 μ g Pfs25 DNA (Table 1, Fig 1b, c). As shown in Table 2, all *H. polygyrus*-inoculated mice were patently infected at the time of first immunization (3 weeks post nematode infection), with a majority remaining infected throughout the duration of three immunizations (11 weeks post infection).

To determine whether impairment of response to Pfs25 DNA immunization could be reversed through anthelmintic treatment, mice with two-week-old *H. polygyrus* infection, as well as uninfected control mice, were treated with pyrantel pamoate three weeks prior to primary immunization. As shown in Table 3 and Figure 2, anthelmintic-treated mice mounted an equivalently high-level IgG response as uninfected, anthelmintic-treated, control mice following one Pfs25 DNA immunization, while titers and levels of anti-Pfs25 antibodies were characteristically reduced in *H. polygyrus*-infected mice ($P=0.01$). Titers tended to increase in all groups of mice following the second Pfs25 DNA immunization, however titers remained lower in *H. polygyrus*-infected mice compared to other groups (Table 3). Some animals in the untreated *H. polygyrus*-infected group naturally had cleared their infection at the time of second immunization (approximately 9 weeks post helminth infections), which may have contributed to the greater degree of increase in titers between first and second immunizations in this group. Titers were equally high in all groups following three immunizations, a time at which helminth infections had been cleared or reduced to minimal levels by chemotherapy or natural elimination in all groups of mice.

In view of significantly reduced or delayed antibody responses that were sustained following multiple immunizations in mice infected with *H. polygyrus*, we investigated the possibility that *H. polygyrus* or immune changes associated with infection may impair the generation of B cell memory responses. As illustrated in Fig. 3, percentages of CD19⁺CD27^{high} and CD19⁺CD27^{low} memory B-cell populations in the spleen were similar between uninfected, untreated (mean $\pm$ SD, 1.72 ± 0.36 and 15.70 ± 1.92 , respectively), uninfected, pyrantel pamoate treated (1.66 ± 0.29 ; 16.74 ± 2.51), *H. polygyrus*-infected, untreated (1.92 ± 0.22 ; 18.32 ± 2.45), and *H. polygyrus*-infected and pyrantel pamoate treated (2.24 ± 0.26 ; 18.20 ± 3.29) Pfs25 vaccinated mice. This indicates that prior infection with *H. polygyrus* does not affect long-term immunity generated by Pfs25 DNA vaccine, although the initial antibody response was markedly diminished.

3.2. Effects of helminth infection on irradiated sporozoite immunization

To determine whether *H. polygyrus* infection affected generation of protective immunity following immunization with irradiated *P. yoelii* sporozoites, levels of anti-CSP antibodies were compared between worm-free and helminth-infected mice. Immunization with approximately 65,000 irradiated sporozoites resulted in significant production of anti-CSP antibodies in worm-free control mice (Fig 4). Similar levels of antibodies were also detected in the serum of immunized *H. polygyrus*-infected mice, indicating that helminth infection had no inhibitory effect on generation of anti-CSP antibodies elicited by irradiated sporozoites. To further evaluate the impact on functional immune response, *H. polygyrus*-infected and worm-free immunized mice, as well as nonimmunized controls were challenged with sporozoites four weeks following immunization. Non-immunized mice were fully susceptible to *Plasmodium* infection, with heavy liver stage parasite burden as determined by quantitative RT-PCR (mean $\pm$ SD rRNA copy number = $442,462 \pm 194,927$; Fig 5) and fulminant blood stage infections (Fig. 6). In contrast, immunized *H. polygyrus*-infected and immunized worm-free mice were resistant to challenge, with significantly reduced liver stage parasite burdens ($44,805 \pm 18,168$, $P=0.02$ and $20,478 \pm 9,759$, $P=0.03$, respectively) (Fig. 5), and transient blood stage infection in each group (Fig. 6). The comparable level of protection between helminth-infected and

uninfected mice further indicated that patent *H. polygyrus* infection had minimal effect on immunization with irradiated sporozoites.

4. DISCUSSION

Helminths are potent modulators of host immune response. Infection can result in altered response to non-parasite antigens [34], and has been shown to adversely affect a number of vaccines such as BCG [2,3,35,36], cholera [10], tetanus [4-8], and two HIV-1 candidate vaccines, including a DNA-based vaccine construct [11,12]. As the majority of individuals, particularly children, suffer from helminthiases in malaria endemic areas, it has been suggested that efficacy of candidate malaria vaccines may also be compromised [37]. Here, we document that mice infected with the intestinal nematode *H. polygyrus* displayed impaired antibody responses to a transmission blocking DNA vaccine based on Pfs25. In contrast, *H. polygyrus* infection appeared to have no inhibitory effect on irradiated sporozoite immunization in this model.

Impairment of asexual stage malarial vaccine response during helminth infection has previously been reported. Su and colleagues [16] found that levels of total IgG, IgG1, and IgG2a antibodies to crude blood stage antigen were lower in *H. polygyrus*-infected mice compared to worm-free controls following immunization. Likewise, *H. polygyrus*-infected mice produced lower levels of IgG2a following immunization with MSP-3-LSP, though no information is reported on other subclass or isotype responses [17]. Cellular and humoral immune responses during *Plasmodium* infection have also been shown to be altered by patent helminth infection [38-40]. As shown in this study and others [16,41,42] elimination of helminth parasites prior to immunization appears to restore normal vaccine responsiveness. The fine kinetics of this aspect has not been determined; however, this will be of critical logistical importance if mass chemotherapy prior to immunization is to be considered, particularly given the high rates of re-infection in helminth-endemic areas.

The success of any vaccine is directly dependent on its ability to elicit immunological memory in the individual. The reduced antibody responses observed in helminth-infected Pfs25-immunized mice naturally raised the question of whether helminth pre-infection also altered the status of memory B-cells generated in response to immunization. In this study we did not find any significant modification in total memory B-cell populations in helminth-infected Pfs25-immunized mice as compared to non-infected immunized controls. While this suggests that helminth infection does not greatly affect long-term memory B-cell responses following DNA immunization, such responses may differ depending on the type of vaccine used and timing of immunization in relation to helminth infection.

Inhibition of vaccine-elicited immune responses is generally attributed to Th2 polarization and IL-4 production associated with most helminth infections. However, the fact that some helminths like *S. mansoni* and filarial nematodes elicit mixed Th1-Th2 phenotypes [43], suggests that this view may not be adequate. Additionally, we observed that the intestinal trematode *Echinostoma caproni*, which only induces low level IL-4 production [39,44], also inhibited response to Pfs25 DNA immunization in a manner similar to the strongly Th2 biased-*H. polygyrus* infection (data not shown). Recent studies from the *H. polygyrus* model provide evidence that CD4⁺ CD25⁺ [45] and CD8⁺ [46] regulatory T-cell populations may directly mediate immune suppression, potentially in concert with sensitized dendritic cells [47]. Such a broader regulatory environment might more appropriately account for the suppression of both Th1-associated IgG2a and Th2-associated IgG1 antibody isotypes observed here and in other studies [16,34,48].

In striking contrast to the suppression observed for Pfs25 DNA immunization, helminth infection seemed to have no effect on abrogating protective immunity induced by irradiated sporozoite immunization. There are several possible explanations for these findings. First, unlike plasmid DNA or synthetic protein-based immunizations, irradiated sporozoite immunization is a whole organism vaccine. While helminths have previously been reported to not alter responses to certain live attenuated vaccines [2], impairment to other live organism vaccines is observed, suggesting that this distinction per se is not particularly relevant. A more likely explanation is that irradiated sporozoite immunization elicits a multifaceted and potent immune response, comprised of antibodies, CD4+ and CD8+ T-cells, and IFN- γ [27,28], and as such may be able to compensate for any selective helminth-induced immune modulation. Indeed, we found no difference in the magnitude of anti-CSP antibody responses and IFN- γ production from CD8+ T-cells measured by ELISPOT between *H. polygyrus* infected and worm-free animal groups (data not shown). Likewise, preliminary experiments carried out in the *E. caproni* infection model suggested that the frequency of IFN- γ producing cells (ELISPOT) and CS tetramer+/CD8+ T cells (flow cytometry) were similar between helminth-infected and worm free mice (data not shown). Another factor to consider is the relatively high immunizing dose of irradiated sporozoites used here (approximately 65,000 sporozoites), which may have induced a vigorous immune response that could not be altered by *H. polygyrus* infection. It is estimated from human experiments that protection requires a cumulative dose of 10^4 - 10^5 irradiated sporozoites given over a period in multiple doses [23, 49], and it would be interesting to evaluate whether helminth infection compromises immunity at lower doses of immunizing irradiated sporozoites.

In summary, results from these experiments demonstrate that helminths exert potent, but heterogenous and reversible, effects on various malaria vaccine strategies. These results reinforce the view that the health status of potential vaccine target populations must be taken into consideration, and further description of these interactions will be important to the successful development and deployment of an effective vaccine against malaria.

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a.

b.

c.

Figure 1.

Mean and SD total IgG, IgG1, IgG2a, and IgG2b anti-Pfs25 antibody levels determined by ELISA in 1:1000 dilutions of sera from *Heligmosomoides polygyrus* - infected (open bars) and worm-free (filled bars) mice (n = 10) following one (a), two (b), or three (c) immunizations with 50 µg Pfs25 DNA at 7, 11, and 15 weeks after inoculation with *H. polygyrus*, respectively. (**) $P < 0.001$, (*) $P < 0.05$ by Wilcoxon rank-sum test.

Figure 2.

Mean and SD total IgG anti-Pfs25 antibody levels determined by ELISA in 1:1000 dilutions of sera from worm-free pyrantel pamoate-treated mice ((-)/drug), mice infected with *Heligmosomoides polygyrus* for 2 weeks, then drug treated (Hp/drug), and non-treated *H. polygyrus*-infected mice (Hp) (n = 5) following each of three Pfs25 DNA immunizations at 9, 13, and 17 weeks after inoculation with *H. polygyrus*, respectively.

Figure 3.

Analysis of memory B-cells from Pfs25 DNA immunized mice. Spleen cells from *H. polygyrus* uninfected (top panels) or infected (bottom panels) and pyrantel pamoate (PP) untreated (left panels) or treated (right panels) mice harvested at 15 weeks post third DNA immunization were analyzed using flow cytometry as described. CD19⁺CD27^{high} cells were gated as R1, and CD19⁺CD27^{low} cells were gated as R2. The data from one representative mouse from each group is demonstrated (n = 5 mice per group).

Figure 4.

Mean $\pm$ SD anti-CSP antibodies in serum 4 weeks post immunization of worm-free – irradiated sporozoite immunized ($\square$), *Heligmosomoides polygyrus*-infected – immunized (Δ) or non-immunized controls ($\times$) BALB/c mice. n = 5 mice per group.

Figure 5.

Mean + SD *Plasmodium yoelii* liver stage parasite burden in non-immunized control mice (filled bar), worm-free – irradiated sporozoite immunized (open bar), or *Heligmosomoides polygyrus*-infected – irradiated sporozoite immunized (hatched bars) mice determined by real time qRT-PCR for 18S rRNA 40 hours after challenge with 50,000 sporozoites. Asterisks (*) indicate significantly less ($P < 0.05$) than control mice. $n \geq 3$ mice per group.

Figure 6.

Mean $\pm$ SD malaria parasitemia in non-immunized control (x), worm-free-irradiated sporozoite immunized (□), or *Heligmosomoides polygyrus*-infected – immunized (Δ) BALB/C mice, after challenge with 50 *P. yoelii* 17X NL sporozoites. n = 5 mice per group.

Table 1

Geometric mean (SD) anti-Pfs25 total IgG antibody titers in worm-free (-) and *Heligmosomoides polygyrus*-infected (Hp) mice 4 weeks after each of three immunizations with 50 µg Pfs25 DNA, representing weeks 7 (1st), 11 (2nd), and 15 (3rd) after inoculation with *H. polygyrus*.

Group (n = 10)	Pfs25 DNA Immunization		
	1 st	2 nd	3 rd
(-)	17,148 (5,060)	16,000 (5,903)	55,715 (13,492)
Hp	4,000 (0)	4,595 (1,687)	19,698 (15,457)

Table 2*Heligmosomoides polygyrus* infectivity status.

	Pfs25 DNA Immunization (weeks post <i>Hp</i> inoculation)		
	1 st (3 wks.)	2 nd (7 wks.)	3 rd (11 wks.)
No. of <i>Hp</i> -infected mice / total	10 / 10	8 / 10	6 / 10
Mean (SD) fecal egg count (epg)	14,250 (1,800)	7,600 (1,100)	2,300 (300)

Table 3

Geometric mean (SD) anti-Pfs25 total IgG antibody titers in worm-free pyrantel pamoate-treated mice ((-)/drug), mice infected with *Heligmosomoides polygyrus* for 2 weeks, then drug treated (Hp/drug), and non-treated *H. polygyrus*-infected mice (Hp) 4 weeks after each of three Pfs25 DNA immunizations, representing weeks 9 (1st), 13 (2nd), and 17 (3rd) after inoculation with *H. polygyrus*.

Group (n=5)	Pfs25 DNA Immunization		
	1 st	2 nd	3 rd
(-) / drug	16,000 (0)	21,112 (8,764)	64,000 (0)
Hp / drug	16,000 (0)	16,000 (8,764)	111,430 (28,622)
Hp	4,000 (0)	10,556 (4,382)	111,430 (28,622)