Control of Schistosomiasis Pathology by Combination of Sm28GST DNA Immunization and Praziquantel Treatment

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Today the control of schistosomiasis infection relies only on the use of praziquantel (PZQ) chemotherapy. However, PZQ treatment cannot prevent reinfection and progressive development of the pathology. We assessed in a mouse model the efficiency of a combined therapy, based on the combination of PZQ chemotherapy with Schistosoma mansoni 28-kDa glutathion S-transferase (Sm28GST) DNA vaccination, designed to limit the pathology. Following this combined therapy, the long-term survival of the mice was significantly enhanced in comparison with the survival of mice either vaccinated only or treated with PZQ only. In addition, the development of the pathology observed in the control groups was almost completely prevented in the vaccinated–PZQ-treated mice and was associated with a dramatic reduction of egg deposition in the tissues. We showed that PZQ treatment induced the unmasking of the native GST enzyme at the surface of the worms, thus permitting its neutralization by the antibodies raised by DNA immunization. This study provides insights into the synergistic mechanisms involved in an immunointervention strategy associated with chemotherapy for the control of a chronic infection and its associated pathology.

Schistosomiasis is the second endemic parasite disease after malaria and is responsible for 500,000 deaths annually. Schistosomiasis-associated pathology is directly linked to the number of eggs laid by the worms, and, in the case of Schistosoma mansoni infection, the accumulation of eggs in the liver leads to granuloma formation and fibrosis. Thus, the ideal treatment of the disease would have to combine an effect on the infection and an effect on parasite fecundity to limit deposition of eggs in the tissues and subsequent development of the pathology. Praziquantel (PZQ) chemotherapy has been the only method widely used for controlling schistosomiasis infection [1, 2]. PZQ appears to act primarily on the structure of the tegument of the worms and thus generates a Ca\(^{2+}\) influx, which leads to muscular contraction [3]. Several reports indicate that humoral response of the host is implicated in the efficacy of PZQ. Indeed, it has been reported that the efficacy of PZQ treatment was reduced in areas where outbreaks of the infection recently occurred in patients who had not yet developed protective immunity [4]. Moreover, PZQ has a reduced potency in T-cell– [5] and B-cell– [6] deprived mice, which can be restored by the passive transfer of either homologous immune serum or purified immunoglobulins [6]. In immunologically intact mice, the efficacy of PZQ can be enhanced by the passive transfer of sera from rabbits immunized with S. mansoni adult worm antigens [7] or by vaccination with a preparation of worm membrane antigens [8]. This mechanism would result in enhanced killing of the worms through antibody-dependent cellular reactions [9].

The implication of the humoral immune response in the efficacy of PZQ should guide the development of new control strategies aimed at complementing the effects of PZQ chemotherapy. Indeed, although PZQ chemotherapy is effective against the infection, its overall efficacy is limited by the fact that it does not prevent reinfection and redevelopment of the pathology. Intensive research has led to the development of vaccine-based control strategies, and a few molecules are now approaching the clinical testing stage [10]. One of the most promising vaccine candidates against S. mansoni infection is the 28-kDa glutathione S-transferase (Sm28GST) molecule [11–13]. The advantage of GST vaccination relies on its ability to strongly reduce both worm burden and female worm fecundity [14]. This antifecundity effect appears to be related to the induction of antibodies capable of inhibiting GST enzymatic activity [15–17]. Hence, the need for a native antigen to generate neutralizing antibodies prompted us to use a DNA-based approach [18–22], which is likely to produce the antigen with the proper folding in the transfected eukaryotic host cells. We thus investigated whether the specific antibody response
generated by Sm28GST DNA immunization [23] could potentiate the effect of PZQ treatment. This report provides the first evidence that DNA vaccination can be used effectively in combination with drug treatment in multitherapy strategies. The immunotherapeutic approach used here is particularly effective for controlling *S. mansoni*-associated pathology.

Material and Methods

**Plasmid purification and injection to mice.** The Sm28GST expression plasmid, pNIP28, has been described in detail elsewhere [23]. Plasmid DNA was purified by centrifugation in a discontinuous cesium chloride gradient and redissolved in filter-sterilized pyrogenic normal saline at a final concentration of 1 mg/mL. For DNA immunization, female Balb/C mice were purchased from Iffa-Credo (L’Arbresle, France); they were 6 weeks of age at the commencement of the experiments. Three successive intradermal administrations of 100 μL of the plasmid solution were performed at 2-week intervals with the indicated dosage. Injections were made into the shaved abdominal skin of the animals at 4 separate locations. Blood samples were collected from the retro-orbital sinus of the animals, and sera were stored at −20°C until required for further use.

**Parasite infection, PZQ treatment, and perfusion.** Parasite infection was performed by applying 60 furcocercariae (Guadeloupe strain) in 200 μL of mineral water (Société des Eaux de Volvic, France) to the shaved abdominal skin of anesthetized mice for 30 min. A single 300 mg/kg subcurative dose of PZQ (Sigma-Aldrich, Saint Quentin Fallavier, France) was administered orally 35 days after parasite infection. PZQ was prepared in sterile water containing 2% Cremophor EL (Sigma-Aldrich) as a 30 mg/mL solution. Deaths were recorded at regular intervals, and survival rates were compared for the whole period (days 101–274) by use of a Wilcoxon test. Worms were recovered from the surviving mice by total perfusion with a 1% heparin saline solution and were counted using optical microscopy. Eggs were recovered from the liver, intestines, spleen, and lungs after overnight digestion in a 4% KOH solution. Total egg counts correspond to the cumulative numbers of eggs in the 4 organs. Correction was made for the standardized morphologically before fixation of tissue samples in a Bouin solution (75% v/v saturated picric acid, 10% v/v formaldehyde, 5% v/v acetic acid) and were dehydrated in ethanol prior to embedding in paraffin. Sections 4 μm thick were deparaffinized in toluene, rehydrated, and stained with hematoxylin.

**Inhibition of Sm28GST enzymatic activity.** Sm28GST enzymatic activity was measured, after 100 ng of recombinant Sm28GST (Transgène, Strasbourg, France) was incubated for 1 h incubation at 37°C with 10 μL of the tested sera. Following the addition of 200 μL of reaction buffer (50 mM potassium phosphate, at pH 6.5, containing 0.36 mM 1-chloro-2,4 dinitrobenzene and 4.76 mM reduced glutathione), enzymatic activity was monitored spectrophotometrically at 340 nm every 15 s for 2 min by use of an automated microplate reader. Percentage of inhibition was calculated according to the formula: 100 − [(X − Y) × 100]/Z, where X is the activity of enzyme in the presence of tested serum, Y is the background activity of tested serum, and Z is the activity of Sm28GST in the absence of serum. Each inhibition test was performed in duplicate. For the measurement of GST activity associated with the worms (PZQ-treated and -untreated), the enzymatic assay was performed with 1 washed worm pair per well, with or without the presence of sera from immunized mice. Optical density (OD) was monitored for 2 h following the addition of substrate buffer.

**Evaluation of the pathology.** Standard colorimetric aspartate transaminase (AST) and alanine transaminase (ALT) measurements were conducted by the Service de Biologie Spécialisée of the Pasteur Institute of Lille. Liver, spleen, and lungs were examined morphologically before fixation of tissue samples in a Bouin solution (75% v/v saturated picric acid, 10% v/v formaldehyde, 5% v/v acetic acid) and were dehydrated in ethanol prior to embedding in paraffin. Sections 4 μm thick were deparaffinized in toluene, rehydrated, and stained with hematoxylin.

**Results**

**Antibody response.** After DNA vaccination, the intensity of the specific IgG antibody response at day 65 was proportional to the amount of Sm28GST-encoding plasmid pNIP28 administered, with no detectable specific antibody response in mice receiving either <10 μg of pNIP28 or the control plasmid pRe-CMV (figure 1A). The parasite infection through the skin systematically induced a rapid anamnestic response on the specific Sm28GST DNA-primed IgG antibody response. No specific antibody response was detected in infected mice that had received 100-μg doses of pRe-CMV. At day 101, all groups of mice, including the control group, presented, as a consequence of the ongoing infection, a notable IgG antibody response specific to Sm28GST. However, the groups immunized with 10-, 50-, or 100-μg doses of pNIP28 had a higher level of IgG antibodies than the groups that had received 5-μg doses of pNIP28 or control plasmid. Following the administration of PZQ treatment on day 101, an increase in the IgG antibody response was
observed in immunized animals, especially in mice that were administered the highest doses of plasmid. In addition, in mice immunized with 10-, 50-, or 100-μg doses of plasmid, PZQ treatment induced the appearance at day 126 of a specific IgA response that was undetected before the treatment (figure 1B). By day 274, the specific IgA antibody response had further increased in a dose-dependent manner in the treated animals. The rapid increase of the specific IgG response and the progressive appearance of a specific IgA response were directly related to PZQ treatment, because IgG titers progressively declined after day 101 in mice immunized with 100-μg doses of pNIp28 and not receiving PZQ treatment after infection, and no IgA could be detected (data not shown). During the course of the experiment, the dominant IgG isotype was IgG1, whereas neither IgM nor IgE could be detected (data not shown). Thus, PZQ treatment induced both an amplification and a maturation of the specific antibody response, presumably as a result of parasite killing.

**Inhibition of Sm28GST enzymatic activity by immune sera.** We have observed in vitro that mammalian cells transfected with pNIp28 produced an Sm28GST protein with glutathione S-transferase activity (M.H., L.D., and G.R., unpublished data). This attested to the proper folding of the produced GST and led to the prediction that in vivo administration of pNIp28 would produce inhibitory antibodies. In effect, the sera of mice immunized with 100 μg of pNIp28 plasmid were able to notably inhibit Sm28GST enzymatic activity (table 1). After parasitic infection, neutralizing activity increased further in the sera of animals that received 100-μg doses of immunizing plasmid and was also detectable in sera of animals that received 50-μg doses. The inhibitory capacity of sera from animals immunized with plasmid DNA was again significantly enhanced after PZQ treatment. This neutralizing activity was still significant after 274 days. At this latest time point, neutralizing capacity toward Sm28GST activity could also be measured in the sera of mice that received as little as 5- or 10-μg doses of pNIp28. In contrast, in vaccinated–PZQ-untreated animals, the neutralizing activity levels reached just after infection (day 79) did not further increase during the time course of the infection (data not shown). Thus, the combination of DNA vaccination and PZQ treatment induced a long-lasting antibody response with strong inhibitory capacity toward Sm28GST activity.

**Survival of infected mice.** To assess the efficacy of the specific immune response obtained by the combination of Sm28GST DNA immunization and PZQ treatment, the survival time course of the infected mice was recorded (figure 2). In the group of nonimmunized and non–PZQ-treated animals, death occurred as early as day 108 (which corresponds to 42 days after parasite infection). By day 143, all the animals of this group had died of the infection. Mice immunized with 100-μg doses of pNIp28 that received no PZQ treatment had a slightly prolonged survival in comparison with the control animals, since 3 of 16 mice survived the infection until day 206. The group injected with 100-μg doses of pRc/CMV and receiving PZQ treatment presented a significant survival rate at

<table>
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<tr>
<th>pNIp28 dose (μg)</th>
<th>Day 0</th>
<th>Day 65</th>
<th>Day 79</th>
<th>Day 101</th>
<th>Day 126</th>
<th>Day 274</th>
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<td>100</td>
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**Table 1.** Capacity of pooled sera from Sm28GST DNA–immunized mice to inhibit Sm28GST enzymatic activity.

NOTE: Sm28GST, *Schistosoma mansoni* 28-kDa glutathione S-transferase. Day 0, immunization; day 65, infection; day 101, praziquantel treatment. –, 0%–20% inhibition; +, 20%–40% inhibition; ++, 40%–60%; and ++++, >60% inhibition.
Figure 2. Survival rate of infected mice. Groups of 7 mice were immunized on days 1, 14, and 28 with 5, 10, 50, or 100 μg of pNIp28, and 1 group of 17 mice received 100 μg of pRc/CMV on days 1, 14, and 28. Single 300 mg/kg dose of praziquantel (PZQ) was administered orally 35 days after parasite infection. Untreated control groups included mice (n = 16) injected with 100 μg of pNIp28 or injected with 100 μg of pRc/CMV. Parasite infection was performed with 60 furcocercariae, 66 days after first DNA injection. Death was monitored at regular intervals from day 101 (which corresponds to day 35 after infection) until day 274. Statistical analyses were proceeded using a Wilcoxon test.

day 248. This effect was partial, however, since only 8 of 17 mice survived the long-term infection. Very clearly, animals immunized with 10-, 50-, and 100-μg doses of pNIp28 and then treated with PZQ displayed a significantly enhanced survival rate in comparison with the animals receiving control plasmid and PZQ (P < .05, Wilcoxon test). Only 1 animal from each of these vaccinated–PZQ-treated groups of 7 animals died during the time course of the experiment. Therefore, PZQ treatment efficacy was potentiated by prior immunization with Sm28GST DNA.

Egg and parasite loads in surviving mice. Eggs were systematically recovered from the liver, intestines, lungs, and spleen of the mice at day 274. Immunization with 100 μg of plasmid DNA induced a significant (P < .01) 86% reduction in the number of eggs in tissues when compared with the control mice (figure 3A). None of the immunized mice had eggs entrapped in the lungs or spleen. Of particular interest was the fact that 3 out of 6 mice exhibited no egg change (even though 2 of the mice still harbored live parasite pairs). There was also a 30%–49% reduction of egg loads in the groups receiving pNIp28 plasmid doses of 10 and 50 μg, although none of the mice were completely egg-free. The group corresponding to the smallest dose of pNIp28 plasmid (5 μg) exhibited egg loads at a very similar level to the controls. Examination of the morphology of tissue eggs indicated that there were more black (nonviable) eggs in mice receiving the highest dosage of pNIp28 (50%) than in mice receiving either the intermediate dosages of pNIp28 (30%) or the lowest dosage of pNIp28 or the control plasmid (<20%) (data not shown). Reduction of the egg load was not merely due to parasite death, since no significant difference was found at this latest time point in the number of worm pairs (average of 1) that were recovered from the surviving mice in the different groups (figure 3B). Independent experiments in mice have shown that Sm28GST DNA immunization performed without additional PZQ treatment induced a partial reduction of worm burden and egg load in equal proportions (data not shown). Thus, our results strongly support the hypothesis that the parasite ability to lay eggs would be affected by the Sm28GST DNA immunization when it is used in combination with PZQ treatment.

Pathological state of surviving mice. The ratio of serum transaminases (AST/ALT) can be used as a biochemical marker for evaluation of schistosomiasis-induced pathology in humans [25]. As shown in table 2, there was a substantial increase in the AST/ALT ratio from the beginning of the experiment to day 274 for the control group, as well as for the groups immunized with only 5 or 10 μg doses of pNIp28 plasmid. In contrast, the AST/ALT ratio was either unchanged or only modestly affected for the groups immunized with the 2 highest doses of pNIp28 plasmid. The levels of the AST/ALT ratio were in accordance with macroscopic and microscopic observations of the pathology. Indeed, immunized mice exhibited no obvious macroscopic signs of pathology, whereas control mice had a severe hepatosplenomegaly with signs of granuloma for-
mation in the lungs (figure 4). At the histological level, organs from the mice immunized with 100-μg doses of pNIP28 presented very limited pathology, with relatively few egg-induced granulomas in the liver (figure 5A) or any histopathological disorder in the lungs (figure 5D). In contrast, the lungs and liver of only PZQ-treated mice were the sites of extensive fibrosis around considerable numbers of eggs associated with cellular infiltrates (figure 5D). Thus, combined therapy led not only to a better survival rate but also to a dramatic reduction of S. mansoni-associated pathology, when compared with chemotherapy only.

**Recognition of parasite Sm28GST by specific antibodies.** To assess the mechanisms involved in the synergy between the DNA vaccine and PZQ therapy, we set up an independent experiment, in which we studied the effect of this drug on antigen expression on the parasite. In immunostaining experiments, worms collected from PZQ-treated mice were positively stained by anti-Sm28GST antibodies, whereas worms collected from untreated mice displayed only a background level of staining (figure 6A). The specific labeling was clearly predominant on the tubercles of the male parasites. In order to confirm the increase in surface expression of Sm28GST, the enzymatic activity was assayed on the whole worms in vitro. We showed that the rate of hydrolysis of 1-chloro-2, 4 dinitrobenzene was more than twice as high in worms harvested from PZQ-treated mice (6.3 × 10⁻³ OD/min) compared with the worms from untreated mice (2.7 × 10⁻³ OD/min; figure 6B). In addition, the results of RT-PCR experiments conducted on RNA extracted from treated and untreated worms showed that there was no up-regulation of Sm28GST mRNA levels 90 min after PZQ treatment (figure 6C). Thus, the presence of the Sm28GST en-

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<th>Dose of pNIP28 (μg)</th>
<th>AST/ALT ratio</th>
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<td>100</td>
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<td>0</td>
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**Table 2.** Evolution of transaminase AST/ALT ratio in pooled sera before (day 0) and after (day 274) combined therapy and *Schistosoma mansoni* infection.

NOTE. AST, aspartate aminotransferase; ALT, alanine aminotransferase.

zyme at the surface of adult worms was an immediate consequence of drug-induced exposure of concealed enzyme rather than a rapid transcriptional regulation and a de novo synthesis. We then tested whether the antiserum generated by Sm28GST DNA immunization could inhibit the enzymatic activity of the disclosed GST on the worm surface. The antibodies generated by Sm28GST DNA immunization were found to inhibit the worm-associated enzymatic activity from PZQ-treated mice from 6.3 × 10⁻³ OD/min to 4.1 × 10⁻³ OD/min (figure 6B). Taken together, these results indicate that the unmasking of surface Sm28GST by PZQ allowed the binding of DNA-raised neutralizing antibodies.

**Discussion**

PZQ chemotherapy applied in human populations has been suggested to be subcurative, since cure rates are often reported to be <100% [26]. Incomplete efficacy of chemotherapy could thus lead to the selective transmission of resistant strains of
parasites, as has been recently described elsewhere [27–31]. Because the full potency of PZQ relies on the concomitant action of adapted immune responses [32], we developed a combined therapy, consisting of Sm28GST plasmid DNA vaccination and subcurative PZQ chemotherapy, and evaluated its efficacy in the context of *S. mansoni* infection in mice.

We have reported here that the survival rate was significantly increased among those animals treated with the combined therapy, compared with animals treated with PZQ only. The efficacy of this novel therapeutic approach was found to be related to the dose of immunizing plasmid administered. Indeed, >85% of the animals vaccinated with the 3 highest doses of plasmid (100, 50, or 10 μg) and then treated with PZQ survived a 30-week infection, whereas <50% of the animals survived when they were injected either with the lowest dose of plasmid encoding Sm28GST (5 μg) or with 100 μg of the control plasmid and then treated with PZQ. The mechanisms involved in our experiment seem to be synergistic, since Sm28GST DNA immunization alone had no significant effect on animal survival. The potency of the combined therapy suggests that the specific immune response induced by Sm28GST DNA immunization brought about the killing of the worms in conjunction with the drug and thereby limited the lethal pathology.

The etiology of schistosomiasis pathology is linked to an accumulative process of egg deposition, mainly in the liver.

Figure 4. Pathological state of organs collected from surviving mice 274 days after first DNA injection. Livers (*A*), lungs (*B*), and spleens (*C*), representative of those observed in animals from 100 μg pNlp28 group (left panels) and 100 μg pRe/CMV group (right panels), are shown.

Figure 5. Histological analysis of livers and lungs from surviving mice. Paraffin-embedded sections were prepared from standardized organ biopsy samples from surviving mice, 274 days after first DNA injection. Hematoxylin-stained sections of liver from 1 representative mouse from 100 μg pNlp28 group (*A*) and 1 representative mouse from 100 μg pRe/CMV group (*B*) are shown. Also shown from same animals in (*C*) and (*D*), respectively, are sections of lung tissue. Arrows indicate egg-induced granulomas or cellular infiltrations. Magnification is ×200.
Figure 6. *Schistosoma mansoni* 28-kDa glutathion S-transferase (Sm28GST) exposure at surface of worms, following praziquantel (PZQ) treatment and its recognition by neutralizing antibodies. A. Indirect immunofluorescence was performed either on worms (35 days postinfection) harvested from untreated mice (left panel) or on worms (35 days postinfection) harvested from mice 90 min after PZQ administration (right panel). Sm28GST surface expression was revealed using a relevant rabbit antiserum and a fluorescein isothiocyanate-labeled goat anti-rabbit antibody (magnification ×400). B. Measurement of GST enzymatic activity was assessed directly on worm pairs harvested in same conditions as for indirect immunofluorescence (□, PZQ treated; ○, untreated). In addition, GST enzymatic activity was assessed on PZQ-treated worms in presence of 10 μL of pooled sera from mice immunized with 100 μg of pNi28 collected just before PZQ treatment (♦). C. In parallel, mRNA levels were determined by reverse transcription polymerase chain reaction (PCR) on worm pairs harvested under same conditions as for indirect immunofluorescence experiments.
associated with inflammatory granuloma formation. An examination of mice that had died during the experiment revealed that they had developed acute hepatosplenic schistosomiasis and severe lung granulomas. Furthermore, it was striking to note that among the mice that had survived the infection, those animals immunized with the highest pNIP28 plasmid doses (100 or 50 \( \mu \)g) showed only mild pathological signs, if any, whereas all mice from the groups immunized with the 2 lowest pNIP28 plasmid doses (10 or 5 \( \mu \)g) or with the control plasmid developed signs of hepatomegaly and splenomegaly. The severity of the pathology, as determined on the basis of histological observations, was related to the biochemical parameter of the AST/ALT ratio. This marker of chronic schistosomiasis in humans [25] was found to be constant over the course of infection in the sera of mice immunized with 50 or 100 \( \mu \)g of pNIP28 plasmid, whereas it increased strongly in the sera of mice from the other groups. The etiology of hepatosplenic schistosomiasis is linked to an accumulative process of egg deposition in the tissues. We found that the number of eggs recovered from the tissues of the surviving mice was correlated with the severity of the pathology observed. Indeed, the mice vaccinated with the highest plasmid dose had little or no sign of egg deposition, compared with mice in the control group.

Nevertheless, it was surprising that the number and morphological aspect of worms harvested from surviving mice were not significantly different from one group to another and thus did not correlate with the number of eggs recovered from the tissues. This discrepancy could be explained by either a delay in the oviposition or an antifecundity effect, both due to the associated treatment. However, the fact that 2 vaccinated-PZQ-treated mice presented no egg in the tissues, although they harbored viable worm pairs, could reflect an antifecundity effect of the Sm28GST DNA immunization (doses of 100 \( \mu \)g) when used in combination with PZQ chemotherapy. This type of protective effect has been shown to rely on a mechanism dependent on the presence of antibodies with a neutralizing effect toward Sm28GST enzymatic activity [15].

In this study, we were able to demonstrate that Sm28GST DNA immunization induced Sm28GST-specific antibodies with enzyme-neutralizing capacities, which appeared to be correlated with the high potency of the combined therapy. In accordance with other models reported in the literature [20, 33], the level of antibody response, which was proportional to the neutralizing capacity of the antibodies elicited by intradermal DNA immunization, was related to the amount of injected plasmid DNA. The ability of these antibodies to neutralize the enzymatic activity of Sm28GST highlights an underestimated advantage of DNA-based strategies. Indeed, antibodies generated by DNA immunization might recognize native epitopes with a better affinity [34] because of the in situ synthesis of an optimally folded protein. The IgG antibody response induced by DNA immunization was highly specific to the native antigen, because the parasite infection induced a rapid and strong amnestic effect on this response. This observation, which confirms similar results obtained in the rat model [23], is an indication that the skin immune system would be strongly involved in the reaction of the memory immune responses while the parasite larvae, which naturally express the Sm28GST antigen, penetrate into the organism [35]. However, we observed that the antibody response slightly decreased as the time post-infection progressed. This observation could be related to the maturation process, leading to the masking of numerous antigens, including Sm28GST, that the parasites undergo. The antibody response induced by DNA immunization was again recalled following PZQ treatment, which is known to lead to degradation of the parasite tegument [3], and thus generated a second presentation of native Sm28GST antigen and provoked a second boosting of the specific antibody response.

Because the full potency of the commonly used drug PZQ relies on the concomitant action of adapted immune responses [32], we investigated the possible involvement of synergistic mechanisms in the therapy combining Sm28GST DNA immunization and PZQ treatment. The main effector mechanism contributing to the synergy with the drug is dependent on the presence of antibodies capable of recognizing cryptic antigens at the surface of the worm that become exposed as a consequence of the drug action. Synergistic antibodies identified thus far (reviewed in [9]) include a 27-kDa esterase [36], a 200-kDa tubercle glycoprotein [37], and an alkaline phosphatase [38]. We found here that, although Sm28GST was concealed in intact worms, it became exposed 90 min after PZQ treatment at the level of the male tubercles, which correspond to one of the major localizations of the protein [35, 39]. Moreover, this rapid exposure was accompanied by an increased GST enzymatic activity associated with the worms collected from treated mice. We showed that the presence of the enzyme at the surface of the worms following PZQ treatment was due to an unmasking and not due to a rapid up-regulation of its synthesis, since no increase in Sm28GST mRNA levels were observed. Thus, through the action of PZQ, Sm28GST became an accessible target for the antibodies generated by vaccination.

Our findings could be completed by serum transfer experiments in unvaccinated infected animals at the time of PZQ treatment, in order to document more precisely the in vivo mechanisms involved. Our main hypothesis is that antibodies would kill treated worms via antibody-mediated cellular reactions, as has already been demonstrated in the context of Sm28GST DNA immunization in the rat model [23]. Furthermore, the fact that the antibodies were able to neutralize newly exposed GST enzymatic activity at the surface of the treated worms indicates that they could impede GST-mediated repairation and detoxification processes. Indeed, GST enzymes play a determinant role in both the repair of PZQ-damaged worms [40] and the defense of the worms against immune attack [41]. The combined therapy clearly appeared to induce an anti-
fecundity effect, as well as an effect on egg viability. In the present work, the treatment with PZQ was performed 6 weeks after the infection and thus corresponded to the period of ovi-position and a state at which parasite susceptibility had reached a plateau phase [42–44]. This antifecundity effect is thought to be mainly due to the presence of neutralizing Sm28GST antibodies, but it could also be enhanced via the action of PZQ, as the drug has been shown to be responsible for changes in the female reproductive system [45] at the level in which Sm28GST localization has been documented [24, 35]. Alternatively, the antifecundity effect observed here could be a consequence of a deficient feeding of the female worms by the damaged male worms.

It has been suggested recently from field studies [46] that PZQ treatment can have an immunizing effect by accelerating the development of the naturally acquired immune response toward whole parasite antigens. In the case of natural immune responses to Sm28GST, a close association between specific IgA antibodies after PZQ treatment and resistance to reinfection has been reported [47]. We confirm here that PZQ treatment induced a shift in the specific antibody response elicited by the DNA immunization, leading to a heightened production of specific IgA. Thus, our study provides evidence that PZQ chemotherapy can act as a modulator of a vaccine-induced specific immune response. These observations strongly support the concept that vaccine development against schistosomiasis should be used in association with chemotherapy treatment.

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