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Intranasal Priming with Recombinant *Bordetella pertussis* for the Induction of a Systemic Immune Response against a Heterologous Antigen

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One of the current goals in vaccine development is the noninvasive administration of protective antigens via mucosal surfaces. In this context, the gut-associated lymphoid tissues have already been extensively explored. Vaccination via the nasal route has only recently been the focus of intensive investigation, and no live vector specifically designed for the respiratory mucosa is yet available. In this study we show that intranasal administration of the recombinant *Bordetella pertussis* BPGR60, producing the *Schistosoma mansoni* 28-kDa glutathione *S*-transferase (Sm28GST) protective antigen fused to filamentous hemagglutinin, induces priming in mice for the production of serum antibodies. In addition to significant levels of anti-Sm28GST immunoglobulin A (IgA) antibodies, high levels of anti-Sm28GST serum antibodies were obtained after intranasal boost with the purified antigen or infection with *S. mansoni* following intranasal priming with BPGR60. These antibodies were of the IgG1, IgG2a, and IgG2b isotypes, suggesting a mixed immune response. No priming was observed in animals that had received nonrecombinant *B. pertussis* or purified Sm28GST, indicating specific priming by BPGR60. This priming was also evident in immune protection against *S. mansoni* challenge. Significant protection against worm burden and egg output was obtained in mice primed with BPGR60 and intranasally boosted with purified Sm28GST. A lower but still significant degree of protection against egg output was also obtained in mice infected with a single dose of BPGR60. These results indicate that intranasal administration of recombinant *B. pertussis* can prime for serum antibody responses against a foreign antigen and for heterologous protection.

One of the main objectives in the current development of new vaccines is the induction of strong mucosal and systemic immune responses against protective antigens delivered by the mucosal route. This route of vaccination has, in principle, the advantage of easy administration in a noninvasive fashion and thereby avoidance of risks of contamination caused by injection. A promising approach for the mucosal delivery of vaccines is the use of live vectors expressing genes that encode protective antigens. Various live vectors producing heterologous antigens, such as *Salmonella* and *Mycobacterium bovis* BCG, have been developed in recent years (7, 13, 29). We have recently described the use of *Bordetella pertussis* to deliver heterologous antigens to the mucosa-associated lymphoid tissue of the respiratory tract via intranasal administration (22).

B. pertussis, the etiologic agent of whooping cough, is a highly contagious microorganism that produces several adhesins, among which filamentous hemagglutinin (FHA) is the most important (for a review, see reference 15). Mature FHA is a 220-kDa protein that is both surface associated and secreted into the extracellular environment. Therefore, a foreign protein fused to FHA would be expected to be exposed on the bacterial surface and/or to be secreted. Furthermore, in view of its importance in the adherence of *B. pertussis* (12, 21, 28), FHA is included as an important antigen in the new acellular vaccines against whooping cough (9). Both mice and children infected with *B. pertussis* or immunized with whole-cell pertussis vaccines produce anti-FHA immunoglobulin G (IgG) antibodies (1, 20). High titers of anti-FHA IgA are also detected in the nasal secretions of convalescing patients after natural *B. pertussis* infections (16, 26). Moreover, the anti-FHA IgG and IgA antibody levels in the respiratory mucosa of mice infected intranasally with virulent *B. pertussis* (1) or immunized mucosally with purified FHA (24) are of long duration. The high infectivity of *B. pertussis*, the important level of secretion of FHA, and its strong immunogenicity after nasal administration have prompted us to use surface-exposed FHA for the presentation of heterologous antigens by recombinant *B. pertussis*. We have recently shown that a single intranasal administration of recombinant *B. pertussis* producing at its surface the 28-kDa glutathione *S*-transferase of *Schistosoma mansoni* (Sm28GST) genetically fused to FHA resulted in significant levels of anti-Sm28GST IgA antibodies in the bronchoalveolar lavage (BAL) fluids of mice (22). However, no serum anti-Sm28GST antibodies were detected. Here we demonstrate that in spite of the lack of anti-Sm28GST serum antibodies after intranasal administration of the recombinant *B. pertussis* strain, the mice were primed for the production of serum antibodies that were readily detectable after an intranasal boost of purified Sm28GST without adjuvant or after infection with *S. mansoni*. Sm28GST has been shown to confer protective immunity in several animal models, including primates (3–5, 10), especially against the pathology-causing egg output and egg viability. We therefore also assessed the intranasal priming effect of the administration of recombinant *B. pertussis* on protective immunity against egg output and worm burden. The results indicate that intranasal priming with the recombi-

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nant *B. pertussis* resulted in a significant reduction of intestinal egg output and worm burden after challenge with *S. mansoni.*

MATERIALS AND METHODS

Bacterial strains and growth conditions. Streptomycin- and nalidixic acid-resistant (Sm^r Nal^r) *B. pertussis* BPSM (18) and recombinant *B. pertussis* BPGR60 (Sm^r Nal^r) containing an in-frame *Bam*HI fragment coding for Sm28GST in the *fhaB* gene (22) were grown at 36°C on Bordet-Gengou agar (Difco, Detroit, Mich.) supplemented with 5% glycerol and 20% defibrinated sheep blood and containing 100 μg of streptomycin (Sigma, St. Louis, Mo.) per ml and 25 μg of nalidixic acid (Sigma) per ml.

Antigens. Recombinant Sm28GST (rSm28GST) was produced in *Escherichia coli* TGE901 containing pTG54 (provided by Transgène S.A., Strasbourg, France) and was affinity purified as previously described (27). FHA was purified as described elsewhere (17) from *B. pertussis* BPRA, a strain in which the pertussis toxin gene had been deleted (2).

Intranasal infection of mice. After 48 h of culture, *B. pertussis* was resuspended at a concentration of approximately 10^8 CFU/ml in sterile phosphate-buffered saline (PBS). Sublethal infections were performed by the intranasal route with 50 μl of the bacterial suspension deposited in the nostrils of 4-week-old OF1 mice (Iffa Credo, L'Arbesle, France) anesthetized with 200 μl (per 10 g of body weight) of a solution of 5% sodium pentobarbital (Sanofi, Libourne, France) given intraperitoneally. To assess the initial numbers of viable *B. pertussis* in the lungs, five infected mice were killed 3 h after exposure. The lungs were removed aseptically and homogenized in 5 ml of PBS. Serially diluted homogenates from individual lungs were plated onto Bordet-Gengou agar, and the number of CFU was counted after 3 to 4 days of incubation at 36°C.

Collection of fluids. Mice under anesthesia were bled by retro-orbital plexus puncture, the trachea was cannulated, and BAL fluids were recovered by three consecutive lavages with 0.5 ml of PBS. BAL fluids were centrifuged at 4,000 × *g* for 10 min to remove cells. Sera and BAL fluids were stored at −20°C until assayed for the presence of specific antibodies.

Serum and lung antibody determination. The level of antibodies to FHA and Sm28GST in sera and BAL fluids was determined by enzyme-linked immunosorbent assays (ELISA). Microtiter plates (Immulon III; Dynatech Laboratories, McLean, Va.) were coated for 2 h 30 min at 37°C with 50 μl of 10-μg/ml FHA or Sm28GST diluted in PBS per well. For serum antibody determinations, pooled serum samples serially diluted in PBS containing 0.1% Tween (PBS/Tw) or 0.1% Tween plus 0.5% gelatin (PBS/Tw/g) were added to the coated plates after three washes in PBS/Tw and were incubated overnight at 4°C. The plates were incubated for 30 min at 37°C and washed three times in PBS/Tw before addition of goat anti-mouse IgG–horseradish peroxidase conjugate (Southern Biotechnology Associates, Inc., Birmingham, Ala.) and incubation for 1 h 30 min at 37°C. For lung antibody determination, the BAL fluids were added to the coated plates and incubated for one night at 4°C in PBS/Tw after 30-min saturation with PBS containing 0.5% gelatin and three washes with PBS/Tw. Anti-mouse IgA–biotin conjugates (Amersham, Buckinghamshire, United Kingdom) diluted in PBS/Tw/g were added and incubated for 1 h 30 min at 37°C. After five washes with PBS/Tw, the streptavidin-horseradish peroxidase conjugate (Amersham) was added. After six washes with PBS/Tw, 1 mg of *o*-phenylenediamine (Sigma) per ml in 0.1 M sodium phosphate buffer (pH 5.5) containing 0.03% H_2O_2 was added and incubated for 30 min at 37°C. The reaction was stopped by the addition of 50 μl of 2 N HCl. The optical density (OD) was measured with a Titertek Multiscan MCC/340 reader at 492 nm, and a linear regression curve of log OD versus the serum dilution was plotted. Results were expressed in titers, reported as the reciprocal of the dilution giving an OD three times that of the conjugate control for the pooled serum samples or twice that of the conjugate control for the pooled BAL fluid samples.

***S. mansoni* challenge.** The life cycle of the Guadeloupe *S. mansoni* strain (kindly provided by C. Combes) was maintained by using hamsters as definitive hosts and *Biomphalaria glabrata* snails as intermediate hosts. Mice were exposed percutaneously (25) to 70 *S. mansoni* cercariae. Whole-body perfusion was carried out on day 42 or 49 after infection, and the worm burden was determined by counting with a light microscope. At the time of perfusion, the small intestine and the liver were collected and digested with 4% potassium hydroxide to estimate the numbers of eggs deposited in these tissues. The percentage of protection was calculated by the formula $(A - B/A) \times 100$, where A is the average parasite burden of the control group of 14 to 16 mice and B is that of the experimental group of 9 to 12 mice. Student's t test was used for the statistical analysis.

RESULTS

Induction of anti-Sm28GST immune response after priming with recombinant *B. pertussis* and intranasal boosting with purified Sm28GST. The mucosal immune response obtained after a single nasal administration of *B. pertussis* BPGR60 producing the FHA-Sm28GST hybrid protein has been previously evaluated (22). Mice receiving BPGR60 produced high levels of anti-FHA serum antibodies but insignificant levels of anti-Sm28GST serum antibodies (see Table 1) that did not increase even 3 months after bacterial infection. However, as previously shown (22), the BAL fluids contained significant levels of anti-Sm28GST IgA and IgG that lasted for more than 1 month after infection.

Although serum anti-Sm28GST antibody levels were insignificant after intranasal administration of BPGR60, we wished to investigate whether infection with this strain could nevertheless prime mice for a secondary serum antibody response when boosted with the purified antigen. Two months after the primary immunization with BPGR60, when clearance of bacteria was completed (22), the mice were boosted by a single intranasal administration of 20 μg of purified rSm28GST without adjuvant. Control mice were either not preinfected or preinfected with wild-type *B. pertussis* BPSM. Two weeks after the boost, a strong antibody response to Sm28GST was detected in the serum of only those mice that had previously received BPGR60 (Fig. 1a). No anti-Sm28GST antibody response was detected in the serum of naive or BPSM-infected mice after a single administration of rSm28GST. Furthermore, a second intranasal administration of the purified rSm28GST to uninfected mice did not result in the production of detectable serum anti-Sm28GST antibodies (titer < 20). These results indicate that the administration of BPGR60 is able to prime the mice for a strong anti-Sm28GST-specific immune response in the serum after intranasal boosting with purified antigen without adjuvant.

The isotypic distribution of the antibody response against Sm28GST and against FHA was also evaluated. In the serum of mice that received BPGR60 and then rSm28GST, the anti-Sm28GST response showed a high level of specific IgG1, IgG2a, and IgG2b isotypes (Table 1). In addition, a significant level of serum anti-Sm28GST IgA which was as high as the level of anti-FHA serum IgA was detected. The isotypic pattern of the anti-FHA response showed high levels of IgG1, IgG2a, and IgG2b isotypes. A comparable anti-FHA antibody response was obtained after administration of wild-type *B. pertussis*, with or without administration of rSm28GST. Furthermore, no significant change in the level of anti-FHA serum antibodies was observed after the boost with rSm28GST.

The level of anti-Sm28GST IgA in the BAL fluids also increased in BPGR60-primed mice boosted with 20 μg of rSm28GST, with maximal specific IgA production 2 weeks after the boost (Fig. 1b).

Previous results (22) have shown that BPGR60 was undetectable in the lungs 5 weeks after infection. However, it has been shown that *B. pertussis* can be internalized and survive within resident macrophages (8, 23). Therefore, the stress of intranasal administration might restore bacterial growth. To test the possible impact of intranasal administration, the lungs of mice intranasally boosted 2 months after infection with BPGR60 were removed 1 week later, and no viable bacteria were detected. Since *B. pertussis* is known to cause a strictly localized respiratory-tract infection, it is unlikely that BPGR60 disseminates at distal sites. The increase in the antibody level against Sm28GST therefore appears to result from the boost with rSm28GST following priming with BPGR60.

Secondary immune response after infection with *S. mansoni*. In mice a serum antibody response against Sm28GST during experimental *S. mansoni* infection can be detected only 9 weeks after infection (8a). In order to evaluate the capacity of the parasite to boost an immune response to Sm28GST in BPGR60-primed animals, mice were percutaneously infected with 70 *S. mansoni* cercariae 2 months after administration of BPGR60. Twenty days later, we detected low levels of serum

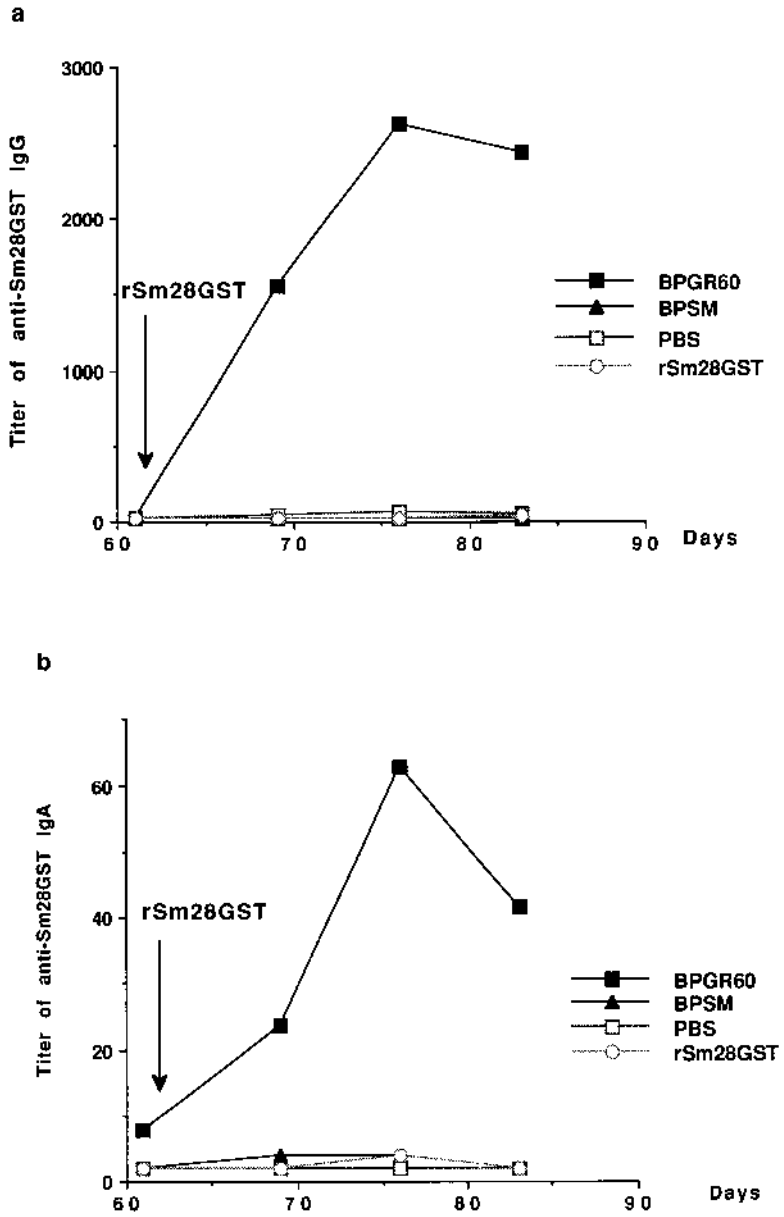

FIG. 1. Anti-Sm28GST antibody response after intranasal administration of BPGR60 and intranasal boost with rSm28GST. (a) Serum IgG antibody response; (b) IgA antibody response in the BAL fluids. OF1 mice were intranasally immunized with either 20 μg of purified rSm28GST, $5 \cdot 10^6$ CFU of BPGR60 or wild-type parent strain BPSM, or PBS and intranasally boosted 2 months later with 20 μg of purified rSm28GST (as indicated by the arrow). Pooled serum samples of five OF1 mice per time point were assessed for the presence of anti-Sm28GST antibodies by ELISA.

anti-Sm28GST IgG which gradually increased up to the titer of 843, 42 days after parasite infection (Fig. 2a). The isotypic profile of this immune response was found to be similar to that obtained after the intranasal boost with rSm28GST (data not shown). In control mice that received either PBS or *B. pertussis* BPSM prior to infection with *S. mansoni*, no antibody response against Sm28GST was observed in the serum up to 42 days after *S. mansoni* infection.

Ten days after cercarial exposure, we also observed an increase in the level of anti-Sm28GST IgA in the BAL fluids of mice primed with BPGR60 (Fig. 2b). The level decreased 28 days after the parasite infection. No detectable antibody to Sm28GST was found in infected control mice. The challenge infection with the parasite thus appeared to boost both serum and pulmonary immune responses to Sm28GST in mice primed with the recombinant *B. pertussis* strain.

Protection against *S. mansoni* after intranasal infection with BPGR60. It has previously been shown that immunization with Sm28GST can result in significant protection against *S. mansoni* challenge in several animal models (3–5, 10). It is of particular interest that such immunization may reduce parasite fecundity and egg viability (4). Since granuloma formation around the deposited eggs is the major pathogenic trait of *S. mansoni* infection, this property of Sm28GST immunization may not only limit the spread of the infection but also lessen the pathological features. Even a relatively modest decrease in egg output may have a significant impact in the decrease of pathology. Since maximal protection conferred by Sm28GST

TABLE 1. Serum antibody titers specific for FHA and Sm28GST after intranasal immunization with recombinant or wild-type *B. pertussis*

Immunization protocol[a]	Antibody type	Titer in serum of:				
		IgG1	IgG2a	IgG2b	IgG3	IgA
BPSM-PBS	FHA	610	1,790	1,540	40	120
	Sm28GST	<20	40	<20	<20	40
BPGR60-PBS	FHA	740	2,440	1,670	40	140
	Sm28GST	40	40	40	<20	80
BPSM-rSm28GST	FHA	570	1,660	740	50	200
	Sm28GST	<20	<20	<20	<20	30
BPGR60-rSm28GST	FHA	1,030	2,670	1,080	80	70
	Sm28GST	3,000	2,250	2,150	40	130

[a] OF1 mice were intranasally immunized with $5 \cdot 10^6$ CFU of recombinant BPGR60 or wild-type BPSM and intranasally boosted 2 months later with 20 μg of rSm28GST or with PBS. The sera were collected 14 days later. The isotypic distribution of the antibody responses in the pooled serum samples was evaluated by ELISA.

in the mouse usually does not exceed 40 to 50%, the mouse model may not be the most appropriate one to evaluate protective efficacy of Sm28GST-based vaccines. We nevertheless wanted to assess whether intranasal administration of BPGR60 and subsequent intranasal boosting with purified rSm28GST have any protective effect in mice. A challenge with 70 cercariae was therefore performed 1 week after the boost when the levels of antibodies to Sm28GST in the serum and the BAL fluids were high (Fig. 1). A significant reduction in worm burden (about 33%) (Table 2) was obtained in the group of these mice. In addition, a significant reduction of the number of tissue eggs (42%) was obtained. In mice that had received only BPGR60, the worm number was slightly reduced (about 16%), although this was not statistically significant. However, in this group, a weak reduction in tissue egg numbers was recorded (28%, $P = 0.027$). A single intranasal administration of rSm28GST or wild-type *B. pertussis* did not show any significant protective effect. However, the nasal administration of the wild-type *B. pertussis* followed by a boost with rSm28GST was associated with a small but nonsignificant reduction in worm burden and egg output. Pooled sera of mice immunized with BPGR60 alone or with BPGR60 followed by rSm28GST and collected 3 days before perfusion contained anti-Sm28GST IgG titers of 815 and 1,128, respectively. Pooled sera from the other groups of mice showed titers lower than 160.

DISCUSSION

We have previously shown that intranasal infection with a recombinant *B. pertussis* strain producing Sm28GST fused to FHA at the surface of the microorganism resulted in significant anti-Sm28GST IgA in the BAL fluid without detectable levels of specific serum antibodies (22). The aim of this study was to evaluate the priming potential of intranasal administration of this strain for serum antibodies and protection against infection by *S. mansoni*. This parasite model was chosen because the mucosal route of immunization may be most appropriate for vaccination in developing countries particularly affected by parasitic diseases. Here, we found that BPGR60-primed mice intranasally boosted with purified rSm28GST produced high titers of anti-Sm28GST serum antibodies. These serum antibodies against Sm28GST were still found 3 months after the boost, whereas no antibody response to Sm28GST was detected in the sera of mice even after two nasal immunizations with rSm28GST after a one-month interval or after priming with the nonrecombinant *B. pertussis* parent strain, BPSM. The strong specific antibody response observed after boosting with rSm28GST can thus be attributed to a priming effect of infection with BPGR60. In addition, anti-Sm28GST IgA antibodies were found in BAL fluids of BPGR60-immunized mice, which may trap the antigen as IgA-antigen complexes. It has been shown that such IgA-antigen complexes adhere to M cells of Peyer's patches much more efficiently than do the uncomplexed antigens (30). M cells are involved in the transcytosis of antigens across the mucosal epithelium. Since they are found in mucosal surfaces of both the respiratory tract and the gut-associated lymphoid tissue (14, 19), this uptake mechanism of IgA-antigen complexes could also occur with M cells in bronchus-associated lymphoid tissue and may increase M-cell transcytosis of the antigen, enhance antigen presentation via IgA-mediated Fcα receptor interaction on the surface of cells present in the mucosa-associated lymphoid tissue (14), and therefore considerably contribute to the secondary immune response.

The anti-Sm28GST isotype profile showed the presence of IgG1, IgG2a, and IgG2b as well as IgA in the sera of mice infected with BPGR60 and then boosted with rSm28GST, suggestive of a mixed Th1-Th2-type response. In order to confirm the involvement of the two T-cell subpopulations, studies to investigate the cytokine secretion profiles of antigen-stimulated cells are under way. The isotype distribution of anti-FHA antibodies in the sera of BPGR60-infected mice showed slight differences compared to the anti-Sm28GST response but also corresponded to a mixed profile as defined by the presence of IgG1, IgG2a, and IgG2b. A significant amount of serum anti-FHA IgA was also detected in these mice. The antibody response to FHA was quantitatively and qualitatively similar to that found in mice infected with the wild-type *B. pertussis*, indicating that the fusion of Sm28GST to FHA did not modify the immunogenicity of the FHA.

However, serum antibodies to Sm28GST were found only after intranasal boost with recombinant antigen, whereas serum anti-FHA antibodies were detected after a single BPGR60 administration. This discrepancy could be the result of the intrinsic characteristics of the FHA-Sm28GST hybrid protein. FHA (220 kDa) is a much larger molecule than Sm28GST (28 kDa), suggesting a higher number of epitopes on FHA compared to Sm28GST. In addition, FHA seems to be a much more immunogenic protein than Sm28GST, since a similar level of serum antibody response specific for each antigen is obtained after nasal administration of both purified antigens associated in a liposome in an FHA/Sm28GST ratio of 1 mol/100 mol (19a).

When the mice were challenged with *S. mansoni* cercariae after a single infection with BPGR60, a serum immune response against Sm28GST was detectable 3 weeks after challenge, and its level increased thereafter continuously for at least 42 days. This is in sharp contrast to the lack of detectable anti-Sm28GST antibodies in parasite-infected mice that had previously received either PBS or BPSM and again confirmed that BPGR60 is able to prime mice for a specific serum anti-Sm28GST antibody response occurring after challenge. The slow release of Sm28GST by the parasite, as well as the antigens released by killed worms, might explain the immune response observed in these BPGR60-primed mice infected by *S. mansoni.*

Previous experiments (3, 4) in the mouse model have shown that two subcutaneous immunizations of rSm28GST with either aluminum hydroxide or complete Freund's adjuvant as the adjuvant resulted in a reduction in worm burden and egg counts after experimental *S. mansoni* infection. However, in

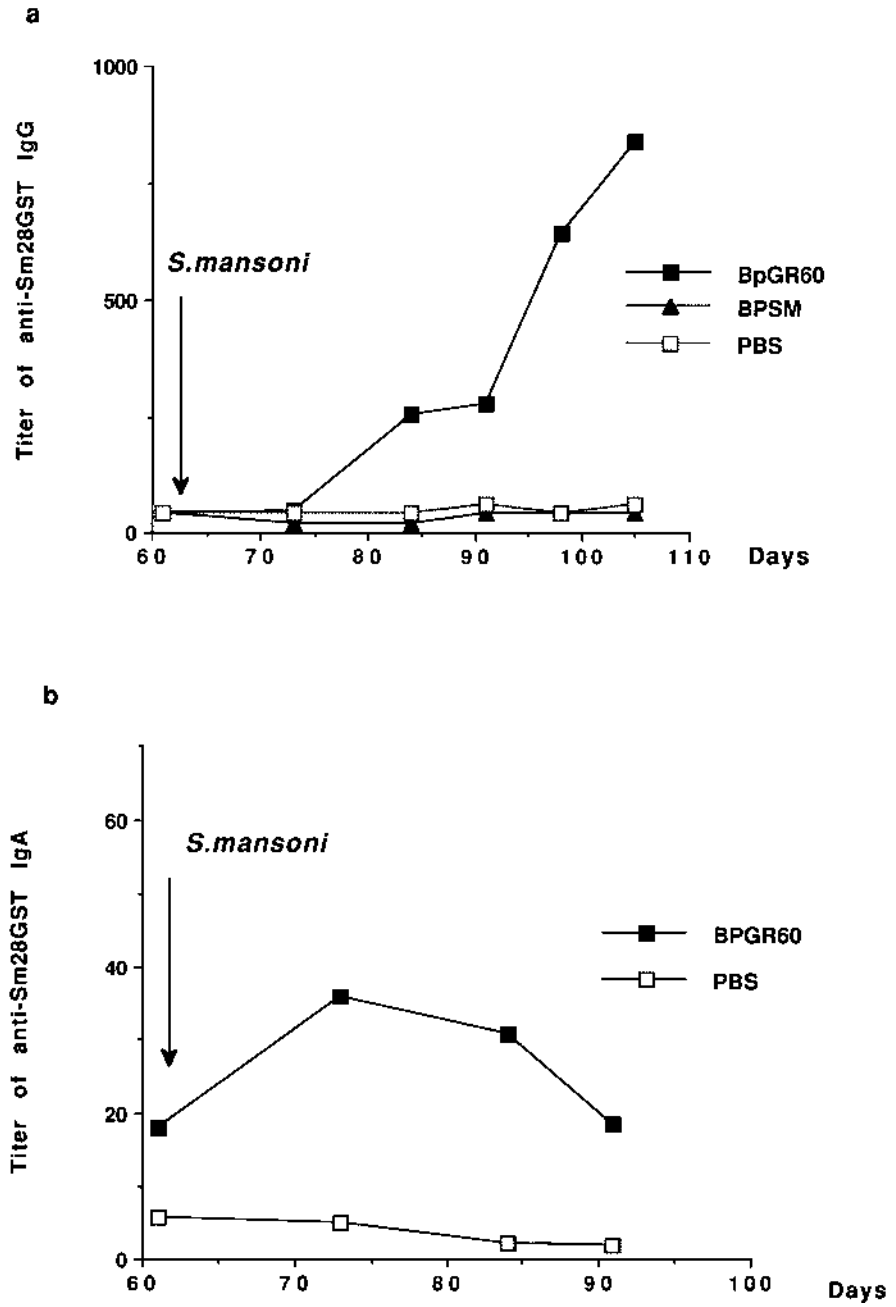

FIG. 2. Anti-Sm28GST antibody response after intranasal administration of BPGR60 and challenge with *S. mansoni* infection. (a) Serum IgG antibody response; (b) IgA antibody response in the BAL fluids. OF1 mice were immunized with $5 \cdot 10^6$ CFU of BPGR60 or BPSM or with PBS and infected 2 months later with 70 *S. mansoni* furcocercariae (as indicated by the arrow). Pooled serum samples of five OF1 mice per time point were assessed for the presence of anti-Sm28GST antibodies by ELISA.

this model the levels of protection were at best in the range of about 40%. Here, we found that intranasal immunization of mice with BPGR60 followed by an intranasal boost with purified rSm28GST resulted in a similar level of protection against challenge with *S. mansoni* performed 1 week after the boost. This protection resulted in the reduction of worm burden as well as of egg charge. It appears therefore that the mucosal administration of an Sm28GST-based vaccine using priming by recombinant *B. pertussis* may protect as effectively as subcutaneously administered vaccines. In addition, we observed 34.2% protection ($P = 0.0048$) when the animals were challenged 14 weeks after the boost, at a time when the level of anti-Sm28GST antibodies had strongly decreased (titer of 258). In contrast, a single nasal administration of purified rSm28GST did not show any protective effect, nor did it induce priming for a secondary response with either rSm28GST or *S. mansoni* infection.

These observations indicate that even at low levels of antibodies, BPGR60-primed mice may be at least partially protected. This contention was further supported by the finding that although a single nasal administration of BPGR60 did not induce an antibody response to Sm28GST in the serum before

TABLE 2. Protective effect of immunization against challenge with *S. mansoni* cercariae

Immunization protocol[a]	Worm recovery[b] (mean ± SD)	% Protection[c] (*P*)	Egg charge[d] (mean ± SD)	% Reduction[e] (*P*)
PBS	27.5 ± 1.7		17,535 ± 973	
PBS-rSm28GST	27.2 ± 3.2	1.02 (0.46)	15,997 ± 776	8.77 (0.17)
BPGR60	23.2 ± 2.3	15.64 (0.13)	12,974 ± 1,674	27.72 (0.016)
BPGR60-rSm28GST	18.3 ± 3.6	33.35 (0.0087)*	10,109 ± 1,466	42.35 (0.0001)*
BPSM	26.4 ± 2.1	4.00 (0.35)	14,806 ± 1,479	15.56 (0.06)
BPSM-rSm28GST	24.5 ± 1.9	10.77 (0.15)	14,495 ± 850	17.34 (0.027)

[a] OF1 mice were intranasally immunized with $5 \cdot 10^6$ CFU of recombinant BPGR60 or wild-type BPSM and boosted or not 2 months later with 20 μg of rSm28GST.
[b] Mice were infected with 70 *S. mansoni* furcocercariae. The number of adult worms was determined by portal perfusion 6 weeks later.
[c] Calculated by comparison with numbers of worms recovered from the control group. *, significant at $P < 0.01$ (Student's *t* test).
[d] Expressed for each mouse as the number of eggs per gram of intestine and liver combined.
[e] Calculated by comparison with numbers of eggs recovered from the control group. ∗, significant at $P < 0.01$ (Student's *t* test).

S. mansoni infection, a reduction of the egg charge was nevertheless observed, accompanied by a slight but nonsignificant reduction in worm burden. The difference in the effect on worm burden and on egg output after only priming with BPGR60 may be the result of a differential effect of boosting by infection with *S. mansoni* on these two parameters. It is tempting to speculate that infection with *S. mansoni* boosted the anti-Sm28GST antibody level in such a manner that it had a more profound bearing on fecundity than on worm viability. This may be particularly interesting since pathology in schistosomiasis is mainly associated with inflammation around the parasite eggs deposited in the host tissues. Even a decrease in egg number by a factor of 2 would significantly decrease pathology (6). This suggests that noninvasive intranasal priming by live vectors such as *B. pertussis* has some potential in diminishing pathology and perhaps transmission. We showed thus for the first time that it is possible to induce a protective immune response against *S. mansoni* after nasal immunization using Sm28GST, one of the candidate molecules against schistosomiasis selected by the World Health Organization (31). In addition, the presence of specific serum anti-Sm28GST IgA in BPGR60-immunized mice is in favor of a protective efficacy of our strategy. Indeed, it has recently been found that the acquisition of resistance to reinfection in human populations is closely associated with the presence of anti-Sm28GST IgA (11).

In conclusion, this study indicates that intranasal immunization with a recombinant *B. pertussis* strain primes mice both for a specific serum immune response against a heterologous protein fused to FHA and for protection. The genetic stability of the chromosomal construction used here, as well as its potential to deliver other foreign antigens, makes recombinant *B. pertussis* a novel promising candidate as a live vector for nasal vaccination. Of course, since BPGR60 is a fully virulent *B. pertussis* strain, it cannot be administered as a live vaccine to humans. We are therefore currently developing new, attenuated strains of *B. pertussis* with the hope of maintaining immunogenicity against foreign antigens while eliminating the pathogenicity of the vector.

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