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Protective Effect of rSm28GST-Specific T Cells in Schistosomiasis: Role of Gamma Interferon

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Immunization with a single dose of 50 μg of recombinant *Schistosoma mansoni* 28-kDa glutathione-*S*-transferase (rSm28GST) was able to induce a reduction in the worm burden, the number of eggs, and the degree of hepatic fibrosis as quantified by the measurement of collagen content in the liver of *S. mansoni*-infected mice. No relationship was found between anti-Sm28GST immunoglobulin G and immunoglobulin A titers and the levels of protection obtained. Adoptive transfers of Sm28GST-specific total, $CD4^+$, or $CD8^+$ T cells reproduced the protective effect obtained with the recombinant molecule. Moreover, experiments studying in vivo T-cell depletion demonstrated that anti-CD4- or anti-CD8-treated mice showed a significant decrease in the protective effect conferred, suggesting a role of the two T-cell subpopulations in the expression of Sm28GST-mediated protection against hepatic damage. Sm28GST-specific cells produced little interleukin-4 and high levels of gamma interferon. Treatment of immunized mice with anti-gamma interferon antibody totally suppressed the Sm28GST-induced protective effect and led to the rapid death of infected animals, suggesting a role for this cytokine in the expression of the protective immunity obtained after immunization with rSm28GST.

Schistosoma mansoni infection in mice and humans commonly results in a long-term, stable disease state (2). In both species, the disease is associated with the continual daily production of viable eggs by the intravascular worms. Many of the eggs do not reach the external environment and cause an inflammatory response when trapped in liver sinusoids, leading to predominantly cell-mediated granuloma formation and ultimately to hepatic fibrosis (27). This fibrosis involves the excessive deposition of newly synthesized connective tissue matrix, with collagen being the major component (35). It is clear that this pathophysiologic scenario is carefully regulated by multiple subpopulations of distinct subsets of T lymphocytes. Egg-induced granuloma formation in the liver has been characterized as a $CD4^+$ cell-mediated delayed-type hypersensitivity response, which is maximal by 8 weeks after infection (19, 20). Granulomas are then gradually down-regulated, largely by $CD8^+$ lymphocytes (4, 11, 26), so that by 16 to 20 weeks after infection, newly formed granulomas have decreased in size. The presence of $CD8^+$ T cells was observed in both early and chronic mouse granulomas, with an increased ratio of $CD8^+$ cells in chronic granulomas (29). The secretion of Th2-related cytokines (interleukin-4 [IL-4], IL-5, and IL-10) (15), which follows egg deposition, is vigorous 8 weeks after infection, whereas Th1-related responses (IL-2 and gamma interferon [IFN-γ]), which could limit worm survival, are suppressed (24). IL-10 production, which occurs simultaneously with the reduction in the IFN-γ response, may be responsible for the observed suppression of Th1 cell cytokine synthesis and so could promote parasite survival (30). Transforming growth factor β, a cytokine with potent immunosuppressive activity, is also produced during the infection and could contribute to this regulatory phenomenon (8). Later in infection, Th2 responses are themselves down-regulated (14). Previous studies have implicated $CD8^+$ cells in this suppression (5), and it is possible that IFN-γ, already shown to strongly influence Th1/Th2 ratios, is responsible for this decrease in IL-4 and IL-5 production (12, 13). Taken together, these results indicate that expression of both protective immunity and pathologic changes is regulated by a complex network of coordinated responses leading to the concept of a T cross-regulatory circuit.

Numerous efforts over the last decade have focused on the elaboration of a reliable vaccine able to protect humans against natural infection by *S. mansoni*. Although attenuated larvae can induce a significant level of immunity in various experimental models (32), it is commonly agreed that a nonliving, defined vaccine would be easier to produce on a large scale by recombinant DNA technology, as well as being ethically more acceptable in human populations (23). One of the most promising candidates, the 28-kDa *S. mansoni* glutathione *S*-transferase (Sm28GST) (33) molecule, has been the focus of several experimental studies, allowing a better understanding of its immunogenicity and its biological functions. The corresponding recombinant Sm28GST (rSm28GST) molecule induces a significant degree of protection after being inoculated into permissive hosts such as hamsters, mice, and baboons or a semipermissive host such as the rat (1, 34) and also strongly reduces the egg-induced granuloma-related pathologic changes (3). Furthermore, it has recently been suggested that a rat monoclonal antibody (MAb) selected for its inhibitory activity against the enzymatic functions of the Sm28GST could affect the biology of the parasite by inducing a reduction of both egg laying and viability after passive transfer into mice (36). Whereas protection against challenge infection is mainly

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immunoglobulin E (IgE) mediated (10), evidence has been obtained that IgA Abs play a significant role in reducing worm fecundity and egg viability (13a). These studies demonstrate that the immunization protocol is as important as the choice of antigen in eliciting optimal protection. These findings have been substantiated by the observations that the presence of an IgA anti-Sm28GST antibody in humans has a significant correlation with decreased egg output (14).

In the present work, we tested the protective potential of a single-dose immunization of rSm28GST in the presence of aluminum hydroxide (AH), a protocol able to induce Sm28GST-specific $CD8^+$ cytotoxic T cells (23a) in the mouse model. This approach was used to determine the role of Sm28GST-specific $CD4^+$ and $CD8^+$ T cells in the observed reduction in hepatic lesions after a challenge infection. We also demonstrate a likely role for IFN-γ, produced by these specific cells, in the protective immunity obtained after administration of rSm28GST.

MATERIALS AND METHODS

Host animals and parasites. Female BALB/c mice, 6 to 8 weeks old at the beginning of the experiment, were provided by Iffa Credo, L'Arbresle, France. A Puerto Rican strain of *S. mansoni* was maintained in *Biomphalaria glabrata* snails as intermediate hosts and golden hamsters as definitive hosts. Cercariae for experimental infections were used within 1 h after collection from 1-month-infected snails exposed to light and to a temperature of 30°C for 1 h and enumeration.

rSm28GST antigen. A full-length cDNA encoding the Sm28GST of *S. mansoni* was expressed in *Escherichia coli* by using a PL expression vector (6). The purified rSm28GST protein (95% pure after passage through a glutathione-Sepharose column) was provided by F. Trottein, Institut Pasteur, Lille, France.

Immunization procedure. On day −1, mice were given intraperitoneal injections of 50 μg of rSm28GST mixed with AH (Serva, Heidelberg, Germany). Control mice were injected with saline buffer or with bovine serum albumin (BSA) as an irrelevant protein by using the same protocol.

Infection protocols. Animals were exposed percutaneously to 50 *S. mansoni* cercariae on day 0. Mice in the different groups were bled weekly through the retroorbital sinus and sacrificed. The liver and spleen were removed and fixed in Bouin's fixative. Parasite burdens were evaluated by liver perfusion 42 days after cercarial exposure. Protection was expressed as the percent reduction of the mean worm numbers after Sm28GST or BSA immunization compared with the numbers in the control mice.

Enzyme-linked immunosorbent assay (ELISA). Microtiter plates (Dynatech, Denkendorf, Germany) were incubated overnight at 4°C with 5 μg of rSm28GST per ml in 0.1 ml of sodium carbonate buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$ [pH 9.6]). The plates were then saturated for 1 h with phosphate-buffered saline (PBS)–3% BSA. After careful washes, 0.1-ml samples of mouse sera diluted 1/50 in dilution buffer (0.15 M NaCl, 0.05% Tween 20, 10 mM PBS [pH 7.2]) were dispensed in each well and incubated overnight at 4°C. After additional washes, 0.1 ml of peroxidase-labeled anti-mouse IgG (Diagnostic Pasteur, Marnes-la-Coquette, France) was added at a dilution of 1/5,000 in the same buffer for 1 h at room temperature. After a final wash, 10 mg of substrate (orthophenyldiamine; Sigma, St. Louis, Mo.) per ml in 0.1 ml of 0.1 M sodium phosphate buffer (pH 5.5) containing H_2O_2 (1 ml/liter) was added, and the mixture was incubated for 15 min at room temperature, and the reaction was stopped by the addition of 50 μl of 1 N HCl. The optical density at 492 nm was measured in a multichannel spectrophotometer (Titertek Multiskan MCC 1340). Results are expressed as the mean of duplicate wells after subtraction of the background.

Radioimmunoassay. Microtiter plate wells (Microtest flexible assay plate; Falcon, Oxnard, Calif.) were each coated for 24 h at room temperature with 0.1 ml (containing 1 μg) of rSm28GST in 0.1 M Na_2CO_3 buffer (pH 9.6). A 2-h saturation at room temperature was then carried out by addition of 0.1 ml of 0.1 M PBS containing 3% BSA. The plates were then washed twice in PBS containing 0.3% BSA before the addition of 0.1 ml of appropriately diluted mouse sera. The plates were then incubated at room temperature for 24 h and again washed twice in PBS–0.3% BSA. A final 18-h incubation with 10^5 cpm of ^{125}I-labeled anti-mouse IgA Ab was performed to detect the presence of IgA specific for Sm28GST in the sera. After washes, as described above, the wells were cut out and counted in a gamma counter (LKB, Bromma, Sweden) with a counting efficiency of 60%.

Lymphocyte culture medium. For all cultures, RPMI 1640 (Gibco, Courbevoie, France) was supplemented with 5×10^{-5} M β-mercaptoethanol (Merck, Darmstadt, Germany), 2 mM L-glutamine (Merck), 1 mM sodium pyruvate (Gibco), antibiotics (100 IU of penicillin per ml, 100 μg of streptomycin per ml) (Specia, Paris, France), 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; Sigma), and 10% heat-inactivated fetal calf serum (Gibco) (ML10).

Lymphokine production. Spleen cells were isolated from Sm28GST-immunized mice on day 28, and lymphokines were obtained by in vitro stimulation of 5×10^6 cells with 40 μg of rSm28GST per ml. Supernatant fluids were aliquoted and frozen after collection at 24 and 48 h for determination of IL-2 and IL-4 and after 60 and 72 h for determination of IFN-γ

Lymphokine assays. Culture supernatants were assayed for IL-2 by using the ATCC CTLL-20 cell line (American Type Culture Collection, Rockville, Md.). The supernatants were serially diluted in culture medium and plated in triplicate in 96-well round-bottom plates (Nunc). ATCC CTLL-20 cells, maintained in ML10 supplemented with 5% concanavalin A-stimulated mouse spleen supernatant, were cultured in a volume of 0.2 ml per well. After 18 to 20 h of culture and a 4- to 6-h pulse with 1 μCi of [^{3}H]thymidine, incorporation of radioactivity was measured. Test supernatants were compared with a standard curve generated by using rIL-2 (Genzyme, Boston, Mass.). IL-4 was measured by using the ELISA method described above. Briefly, plates were coated for 3 h at 37°C with 1 μg of rat anti-murine IL-4 MAb (PharMingen, San Diego, Calif.) per ml in 0.1 ml of sodium carbonate buffer and then washed with PBS–1% Tween. After a blocking step of 2 h with 1% BSA, serial dilutions of test supernatants in 0.1 ml were added to the wells and incubated overnight at 4°C. After washings, 1 μg of biotin-labeled rat anti-murine IL-4 Ab (PharMingen) per ml was added for 2 h at 37°C. After additional washings, peroxidase-labeled streptavidin conjugate (PharMingen) was added at a dilution of 1/1,000 for 1 h at room temperature. This was followed by washing and substrate addition. Test supernatants were compared with a standard curve generated with rIL-4 (Genzyme). Culture supernatants were assayed for IFN-γ with an ELISA kit (Genzyme) that uses a hamster anti-mouse IFN-γ epitope-specific MAb and a goat anti-mouse IFN-γ polyclonal Ab with multiple epitope specificity. A standard curve was generated by serial dilutions of an 8,200-pg/ml solution of rIFN-γ. The kit accurately measures IFN-γ between 50 and 8,200 pg/ml.

Lymphocyte proliferation assay. Spleen cells were isolated from mice immunized with rSm28GST on day 28, enriched in

TABLE 1. Reduction of worm burdens after immunization of mice with rSm28GST[a]

Immunization status	Worm burden (no./mouse)	% Protection[b]	P[c]
Infected	30 ± 5		
Sm28GST immunized and infected	16 ± 3	46	<0.05
BSA immunized and infected	28 ± 5	6	>0.05

[a] Immunizations were performed with 50 μg of antigen (rSm28GST or BSA) in the presence of AH. Control mice received adjuvant alone. Mice were challenged with 50 cercariae 1 day after the injection, and the percent reduction of the worm burden of each mouse was estimated by liver perfusion on day 42 of infection.

[b] The protection was expressed as the percent reduction of the mean worm burden in immunized mice (n = 10) compared with adjuvant control mice (n = 10).

[c] P was estimated by Student's t test.

T-cell populations by passage through a nylon wool column, and maintained at 37°C in a 5% CO_2 atmosphere in lymphocyte culture medium. For the in vitro assays, 5×10^5 T cells and 10^6 syngeneic irradiated splenic cells as antigen-presenting cells were cultured with rSm28GST (10 to 80 μg/ml) or BSA (50 μg/ml) in a total volume of 0.2 ml in flat-bottom microtiter tissue culture plates (Nunclon, Roskilde, Denmark). The cells were then exposed to 18.5 kBq of [^{3}H]deoxythymidine for the last 18 h of a 5-day culture period. Finally, the cells were harvested by filtration on fiberglass discs by using a multiharvester (Skatron, Lierbyen, Norway), and the amount of incorporated [^{3}H]deoxythymidine was measured by using a liquid scintillation counter (LKB, Wallac, Turku, Finland). Data are expressed as the arithmetic mean of counts per minute of triplicate cultures.

In vitro cell depletions. Spleen cells were isolated from mice immunized with Sm28GST on day 28. Lymphocyte suspensions were depleted of $CD4^+$ or $CD8^+$ T cells by incubation with rat MAb anti-CD4 (GK 1.5) or anti-CD8 (2.43), respectively, followed by complement lysis. The MAbs were obtained by growing GK 1.5 or 2.43 hybridoma cells (American Type Culture Collection) in serum-free medium and purified by ammonium sulfate precipitation and protein A adsorption chromatography. The effectiveness of depletion was monitored by fluorescence-activated cell sorter analysis of cells stained with either $CD4^+$- or $CD8^+$-specific fluorescein-conjugated Abs (Caltag, San Francisco, Calif.).

In vivo cell depletions. For in vivo depletion studies, each mouse received 1 mg (0.5 ml) of anti-CD4 (GK 1.5) or anti-CD8 (2.43) MAbs intraperitoneally daily for 7 days. Control mice were inoculated with 0.5 ml of saline intraperitoneally on the same days. The selective depletion of the cell population in question was confirmed by flow fluorometry analysis of residual cell populations within the spleens of treated animals. In untreated mice we observed 59% $CD4^+$ cells and 32% $CD8^+$ cells, whereas in mice depleted of $CD4^+$ cells we observed 22% $CD4^+$ and 75% $CD8^+$ cells and in mice depleted of $CD8^+$ cells we observed 11% $CD8^+$ and 83% $CD4^+$ cells. Animals were then immunized with rSm28GST, infected 1 day later with *S. mansoni*, and received an additional dose of 1 mg of GK 1.5 or 2.43 1 week after infection.

Adoptive transfer experiments. Total, $CD4^+$, or $CD8^+$ Sm28GST-specific T cells (15×10^6) were injected intravenously in 0.2 ml of PBS into normal mice 18 h before exposure to *S. mansoni* cercariae.

IFN-γ treatment and depletion. Treatment with murine rIFN-γ (kindly provided by Juana Wietzerbin) consisted of an intraperitoneal injection of 10,000 antiviral units per animal at 7, 14, 21, and 42 days after *S. mansoni* infection of rSm28GST-immunized mice. For IFN-γ depletion, each mouse received 10^4 neutralizing units of rabbit anti-mouse IFN-γ polyclonal Ab (Biosource International, Camarillo, Calif.) intraperitoneally at 1, 14, 21, and 42 days after *S. mansoni* infection of rSm28GST-immunized animals.

Granuloma number determination. The number of granulomas was counted on liver sections sampled at days 42, 69, and 96 after infection by *S. mansoni*. The sections, embedded in paraffin and hematoxylin-eosin stained, were examined by a Leitz Asm 68 K image analyzer (Wild Leitz, Rueil-Malmaison, France) connected to a Leitz Diaplan microscope. All the granulomas present on the section were counted and the surface of the section was measured to determine the mean number for a defined surface. Significant differences (percent reduction) in granuloma number between different groups and control mice were calculated by Student's t test.

Evaluation of fibrosis. Hepatic fibrosis, quantified by measurement of collagen and protein content, was evaluated on liver sections sampled at days 42, 69, and 96 after infection by *S. mansoni*. This method was applied to 4-μm-thick sections that were placed on slides, deparaffinized, and incubated in filtered aqueous picric acid solution containing 0.1% Fast Green FCF (Sigma), which stained noncollagenous proteins, and 0.1% Sirius red F3B (Gurr BDH Chemicals Ltd., Poole, England), which stained collagen. Sections were kept out of the light and incubated for 2 h at room temperature. They were then rinsed with distilled water until the elution fluid was completely free of color. Each slide was then covered with 1 ml of 0.1 N NaOH in absolute methanol (1:1, vol/vol) and kept until all the color was eluted from the section (usually within a few seconds). Fluids were carefully withdrawn and read in a DU 64 spectrophotometer (Beckman Instruments Inc., Fullerton, Calif.). Fast Green and Sirius red have their maximal absorbance at 630 and 540 nm, respectively. Values were obtained with the use of the formula described previously (17). Student's t test and the coefficient of correlation were used in

TABLE 2. Diminution of hepatic granulomas after immunization of mice with rSm28GST[a]

Immunization status	No. of hepatic granulomas (% reduction) on day[b]:		
	42	69	96
AH alone and infected	64 ± 09	138 ± 21	268 ± 40
Sm28GST immunized and infected	31 ± 10 (51%)	84 ± 18 (39%)	165 ± 20 (38%)
BSA immunized and infected	72 ± 05 (0%)	126 ± 30 (8%)	248 ± 37 (7%)

[a] Immunizations were performed with 50 μg of antigen (rSm28GST or BSA) in the presence of AH. Control mice received adjuvant alone. Mice were challenged with 50 cercariae 1 day after the injection.

[b] Analyses were performed on defined liver sections (n = 3 per mouse) sampled on days 42, 69, and 96 of infection, and the number of granulomas present in each section was determined. The protection was expressed as the percent reduction of the mean hepatic egg number in immunized mice (n = 10) compared with adjuvant control mice (n = 10).

TABLE 3. Reduction of hepatic fibrosis after immunization of mice with rSm28GST[a]

Immunization status	Amt of collagen (μg)/mg of protein (% reduction) on day[b]:		
	42	69	96
AH alone and infected	8.4 ± 0.9	13.3 ± 1.4	17.7 ± 1.4
Sm28GST immunized and infected	3.8 ± 0.4 (54%)	7.7 ± 0.5 (42%)	10.7 ± 0.9 (39%)
BSA immunized and infected	9.9 ± 1.4 (0%)	12.6 ± 0.7 (5%)	19.5 ± 0.4 (0%)

[a] Immunizations were performed with 50 μg of antigen (rSm28GST or BSA) in the presence of AH. Control mice received adjuvant alone. Mice were challenged with 50 cercariae 1 day after the injection.

[b] The measurement of collagen content per milligram of protein was made in liver sections (n = 3 per mouse) sampled on days 42, 69, and 96 of *S. mansoni* infection, and final results were expressed after subtraction of values obtained in normal mice 42, 69, and 96 days after the beginning of the experiment (respectively 40.2 ± 1.4; 41.2 ± 2.1, and 41.5 ± 1.4). The protection was expressed as the percent reduction of the mean of the result in immunized mice (n = 10) compared with control mice (n = 10) that received only adjuvant.

the statistical evaluation of the results. Data are reported as mean ± standard error.

RESULTS

Effect of rSm28GST administration. As shown in Table 1, the number of worms in the liver on day 42 of infection of rSm28GST-immunized mice was significantly diminished (up to 46%) compared with that observed in BSA (as irrelevant protein)-immunized mice or control animals that received adjuvant alone. This result confirmed, with this single-dose protocol, the previously described (3, 34) protective effect of Sm28GST on the parasite burden. A visible morphological amelioration of the liver of rSm28GST-immunized mice was also observed and quantified by two different parameters. Therefore, marked reductions in the number of hepatic granulomas were obtained, in particular at day 42 of infection (51%; $P > 0.05$), whereas no effect was seen in mice immunized with BSA compared with control animals (Table 2). Variable reductions of the granuloma surface were sometimes observed, but studies are under way to confirm the role of the Sm28GST in this effect. Immunization with rSm28GST also induced marked diminutions in collagen content per milligram of protein, ranging from 54 to 39%, according to the time of infection if compared with the control group (Table 3). Results were expressed after subtraction of the collagen content per milligram of protein in normal mice 42, 69, or 96 days after the beginning of the experiment. Thus, the level of collagen in the livers of rSm28GST-immunized mice was close to that observed in normal mice. This effect was not obtained after immunization with BSA.

Immunization with Sm28GST also durably protected the mice, since the immunized animals survived 2 months longer than the control mice and were sacrificed. The mice in the other groups (BSA immunized or control) died rapidly on about day 100 of *S. mansoni* infection (data not shown).

Antibody response to rSm28GST. The production of Sm28GST-specific IgG and IgA Ab was investigated during the course of *S. mansoni* infection. As seen in Fig. 1, under our experimental conditions we observed no significant difference in Ab response between the rSm28GST- or BSA-immunized mice and the control group immunized with adjuvant alone. Therefore, no relationship was observed between anti-Sm28GST IgG or IgA titers and the levels of protection

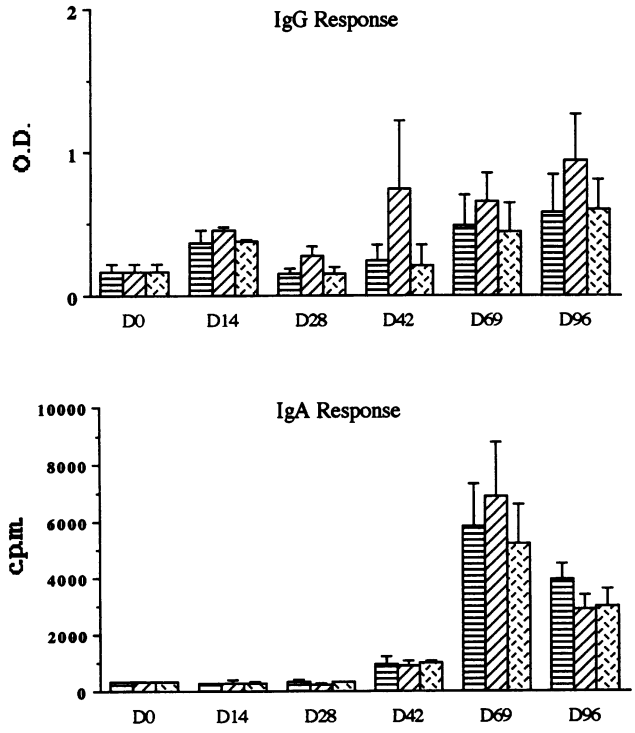

FIG. 1. IgG and IgA Ab profiles to Sm28GST in mice immunized with rSm28GST (□), BSA (▨), or AH alone (▤) during the experimental course of *S. mansoni* infection. O.D., optical density.

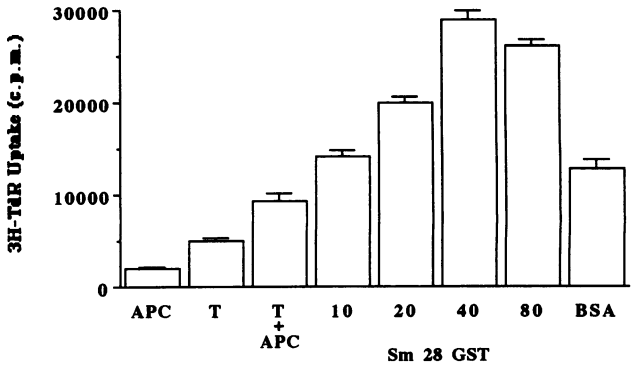

FIG. 2. In vitro proliferative response of T lymphocytes from mice immunized with Sm28GST 28 days before, after stimulation with rSm28GST (from 10 to 80 μg/ml). Results are expressed as the means of three experiments in triplicate. T, APC, and T + APC refer to the counts per minute obtained after in vitro incubation of lymphocytes alone, APC alone, and lymphocytes with APC, respectively. It represents the background proliferation of the cells, one of our negative controls (the other being the proliferative response obtained with 50 μg of BSA per ml as the irrelevant antigen).

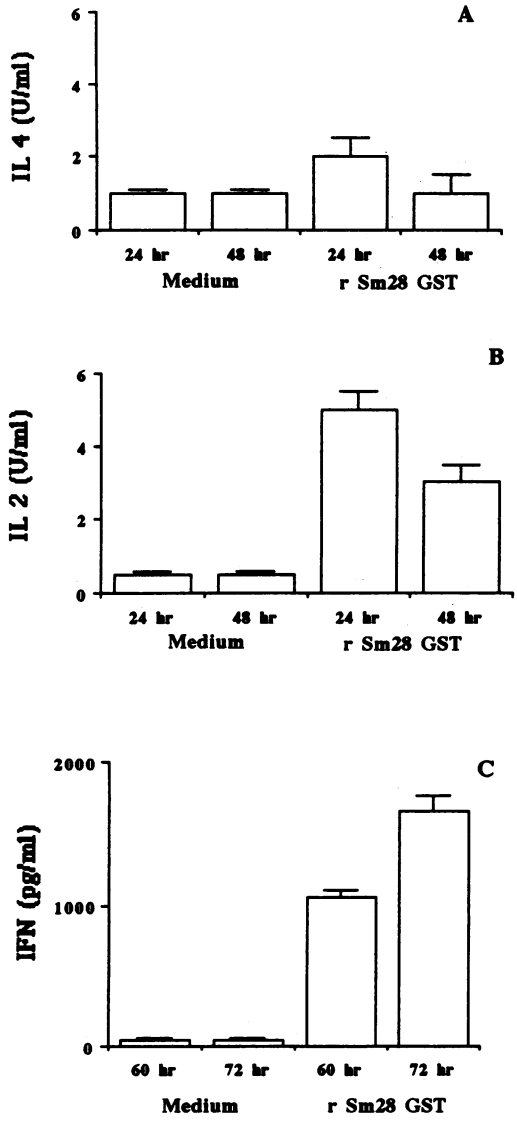

FIG. 3. Production of IL-4 (A), IL-2 (B), and IFN-γ (C) by spleen cells isolated from mice immunized with Sm28GST 28 days before. Cells were stimulated with 40 μg of rSm28GST per ml. The results shown are the means and standard errors of three experiments with five mice in each experiment.

previously evaluated. No modification of the IgG or IgA responses against schistosomulum or egg antigens was obtained (data not shown).

Cellular response to Sm28GST. At about 28 days after immunization with Sm28GST, mice, infected or not, showed a reproducible increase in the size of the spleen, which seems to be the center of a marked cellular reaction. Figure 2 shows that on day 28, T cells from these mice exhibited a dose-dependent, significant, and highly reproducible proliferative response after in vitro incubation with the rSm28GST molecule. In contrast, no stimulation was observed when BSA was used as a control Ag. Spleen cells obtained 28 days after rSm28GST immunization, stimulated with 40 μg of rSm28GST per ml, were tested for their capacity to produce IL-2, IL-4, and IFN-γ (Fig. 3). After stimulation, no significant production of IL-4 (Fig. 3A), a weak but significative production of IL-2 (Fig. 3B), and high

FIG. 4. Production of IFN-γ by spleen cells isolated from mice immunized with Sm28GST 28 days before and infected by *S. mansoni*. Immunizations were performed with 50 μg of rSm28GST per ml. One day later, mice were challenged with 50 cercariae. Control mice were given injections of AH alone and infected (Inf). IFN-γ production was evaluated after 72 h of stimulation with 40 μg of rSm28GST per ml. The results shown are the means and standard errors of three experiments with five mice in each experiment.

levels of IFN-γ ranging from 1,000 to 1,800 pg/ml (Fig. 3C) were observed. These results suggest the secretion of CD4Th1- or CD8-related cytokines (IL-2 and IFN-γ) by Sm28GST-specific cells. As seen in Fig. 4, during infection the levels of IFN-γ obtained in rSm28GST-immunized mice were superior by a factor of 2 to those observed in control mice injected with AH alone. These results raised the question of the biological role of IFN-γ synthesized during infection in rSm28GST-immunized mice.

Adoptive transfer of immunity with Sm28GST-specific T cells. As seen in Table 4, the transfer of the total T-cell population induced marked reductions in the number of hepatic granulomas and of collagen content, of 39 and 41%, respectively, on day 42 of infection when the protective effect was maximal. Protection, although less important, was also obtained with the transfer of Sm28GST-specific T-cell subpopulations, but we observed no significant difference in the levels of protection between mice given $CD4^+$ cells and mice given $CD8^+$ cells. These results show that the Sm28GST-induced reduction of hepatic damage could also be obtained with the passive transfer of Sm28GST-specific T cells and that the two subpopulations were necessary to reproduce the protection obtained with the total T-cell population.

Effect of in vivo T-cell depletion on Sm28GST-induced reduction of the hepatic damage. Compared with saline-treated mice, anti-CD4- or anti-CD8-treated animals demonstrated a marked decrease in the rSm28GST-mediated effect on both the number of hepatic granulomas and the collagen content (Table 5). This reduction in the protective effect is particularly noticeable in mice depleted of $CD8^+$ cells before immunization: protection is reduced from 46 to 11% for hepatic granulomas and from 39 to 5% for the collagen content on day 42 of infection, when the protection obtained with the Sm28GST is normally maximal. Moreover, the nonnegligible effect also induced by $CD4^+$ cell depletion suggests a role for the two T-cell subpopulations in the expression of Sm28GST-mediated protection on hepatic damage.

Effect of Ab to IFN-γ on immunity conferred by rSm28GST administration. Since Sm28GST-specific cells produced IFN-γ and since IFN-γ is a potent inhibitor of collagen synthesis both in vitro and in vivo (27, 28), we envisaged a role for this cytokine in the protective effect of the rSm28GST on hepatic damage. In control mice we observed a major aggravation of the damage at day 96 of infection, in particular in terms of the

TABLE 4. Reduction of hepatic damage after adoptive transfer of T cells from Sm28GST-immunized mice[a]

T-cell type	No. of hepatic granulomas (% reduction) on day[b]:			Amt of collagen (μg)/mg of protein (% reduction) on day[b]:		
	42	69	96	42	69	96
None	64 ± 09	138 ± 21	288 ± 16	6.8 ± 0.1	13.0 ± 1.4	21.0 ± 1.4
Total	39 ± 05 (39%)	88 ± 08 (36%)	202 ± 11 (29%)	4.0 ± 0.8 (41%)	8.1 ± 1.1 (37%)	14.6 ± 0.9 (30%)
$CD4^+$	51 ± 05 (20%)	111 ± 15 (19%)	245 ± 07 (15%)	5.4 ± 0.1 (20%)	10.6 ± 1.8 (18%)	18.1 ± 0.8 (13%)
$CD8^+$	49 ± 03 (23%)	105 ± 09 (23%)	235 ± 0.5 (18%)	5.1 ± 0.2 (25%)	10.0 ± 0.1 (23%)	17.0 ± 0.8 (19%)

[a] Total, $CD4^+$, or $CD8^+$ T cells from mice immunized with Sm28GST 28 days before were passively transferred to syngeneic mice 1 day before *S. mansoni* infection.
[b] Analysis was performed on defined liver sections (n = 3 per mouse) sampled on days 42, 69, and 96 of infection. The number of granulomas present and the collagen and protein content in the sections were determined. The protection was expressed as the percent reduction of the mean of the result in treated mice (n = 10) compared with control mice (n = 10) that received only medium.

collagen content in the liver, after treatment by an anti-IFN-γ Ab (Table 6). In immunized animals, a significant ($P < 0.05$) inhibition of the rSm28GST-induced protective effect in terms of both the number of hepatic granulomas (29%) and the collagen deposition (34%) was obtained. In both cases, we observed reproducible and rapid death of the treated mice, often before day 96 of infection (data not shown).

The injection of IFN-γ into mice after infection resulted in a marked diminution of the number of hepatic granulomas (40%) and of collagen deposition (33%) (Table 6) and also resulted in prolonged survival of the animal beyond 150 days of infection (data not shown). These preliminary results, taken together, strongly suggest a role for IFN-γ in the protective immunity induced by rSm28GST.

DISCUSSION

In the present work, we observed a marked reduction in the number of granulomas and of fibrosis in the liver of *S. mansoni*-infected mice after immunization with a single dose of rSm28GST. We also confirmed the previously described reduction in the worm burden (3). Moreover, immunized mice seemed durably protected, since they survived more than 2 months after the death of control animals. We observed no relationship between anti-Sm28GST IgG and IgA Ab titers and the levels of protection obtained by this immunization protocol, so we tested the hypothesis of a role of Sm28GST-specific T cells in the reduction of hepatic lesions. In support of a cell-mediated mechanism of protection, we observed that at day 28, Sm28GST-immunized mice showed a reproducible increase in the size of the spleen, which seemed to be the center of an important cellular reaction. This increase was also obtained if mice were infected with *S. mansoni* after the immunization. The adoptive transfer of the Sm28GST-specific total T-cell population, isolated at this period, reproduced the protective effect obtained. Protection, although less marked, was also obtained after transfer of Sm28GST-specific T-cell subpopulations, but no significant difference in the levels of protection between mice given $CD4^+$ T cells and mice given $CD8^+$ T cells was observed, suggesting that both subpopulations were involved in the protection conferred by the total T-cell population. Moreover, in vivo T-cell depletion experiments showed that anti-CD4 or anti-CD8 treatment of mice led to an important decrease in the Sm28GST-mediated effect. Mathew et al. observed that depletion in the number of $CD4^+$ T cells decreased the level of resistance to challenge as well as of IL-2 production and of vigorous granuloma formation (19, 20) but increased morbidity of mice. Conversely, depletion of $CD8^+$ T cells reduced susceptibility, indicating that this lymphocyte subset had a suppressive effect on $CD4^+$ cells (28). Our results suggest that the two T-cell subpopulations have a role in the expression of Sm28GST-mediated protection against hepatic damage. In the mouse, the division of $CD4^+$ T-cell clones into an IL-2- and IFN-γ-producing Th1 subset and an IL-4- and IL-5-producing Th2 subset is now well established (21). The tendency of rSm28GST for preferential elicitation of IFN-γ production by splenocytes at day 28 of infection may indicate a role for this antigen in directing the type of CD4 Th1 but also CD8 (12) T-cell response observed in early stages of infection. In the context of the vaccination of

TABLE 5. Effect of in vivo T-cell depletion on protection induced by Sm28GST administration[a]

Depletion	Protection[b] based on:					
	No. of hepatic granulomas on day:			Amt of collagen (μg)/mg of protein on day:		
	42	69	96	42	69	96
None	46%	36%	31%	39%	31%	30%
$CD4^+$ cells	22%	18%	15%	19%	14%	16%
$CD8^+$ cells	11%	9%	6%	5%	2%	2%

[a] Mice were depleted of $CD4^+$ or $CD8^+$ T cells by injection of MAbs. Control mice received saline buffer under the same experimental conditions. Immunizations were performed with 50 μg of rSm28GST in the presence of AH, and mice were challenged with 50 cercariae 1 day after the injection.
[b] Analysis was performed on defined liver sections (n = 3 per mouse) sampled on days 42, 69, and 96 of infection. The number of eggs present and the collagen and protein contents in the sections were determined. The protection was expressed as percent reduction of the mean of the results in depleted and nondepleted mice (n = 10) before Sm28GST administration and infection compared with only infected mice (n = 10).

TABLE 6. Ab to IFN-γ abrogates immunity to *S. mansoni* conferred by immunization with rSm28GST[a]

Immunization status	No. of hepatic granulomas	Amt of collagen (μg)/mg of protein
Infected	268 ± 40	17.7 ± 1.4
IFN-γ treated and infected	160 ± 05	11.8 ± 0.9
Anti-IFN-γ treated and infected	305 ± 10	25.5 ± 1.4
Sm28GST immunized and infected	166 ± 17	9.4 ± 0.9
Sm28GST immunized + anti-IFN-γ and infected	242 ± 23	15.4 ± 1.0

[a] Immunizations were performed with 50 μg of rSm28GST, and mice (n = 10) were challenged 1 day later with 50 cercariae of *S. mansoni*. Control mice (n = 10) received adjuvant alone before infection. The mice were then treated or not treated with Ab to IFN-γ on days 7, 14, 21, and 42 after infection, and analysis was performed on defined liver sections (n = 3 per mouse) sampled on day 96 of infection. The number of eggs present and the collagen and protein content in the section were determined.

mice with attenuated larval stages, it was shown that vaccinated animals responded primarily with Th1 lymphokines and that, in contrast to the situation in infected mice, larval schistosome antigens tend to stimulate stronger Th1 cell responses than egg antigens did (18). Moreover, Sm28GST, expressed at different developmental stages of the parasite, could maintain a constant stimulation to elicit a permanent anti-Sm28GST response. The protective function of $CD8^+$ T cells in experimental murine cutaneous leishmaniasis might be explained in part by their ability to secrete IFN-γ (22). We are now attempting to establish Sm28GST-specific CD8 T-cell lines or clones in order to determine whether these cells could be responsible for the production of IFN-γ. The adoptive transfer of such cells before infection by *S. mansoni* will be of considerable interest for the understanding of the Sm28GST-induced protective mechanism. At day 42 of infection, we observed a reduction in worm burden, but we cannot conclude, at this stage of our work, that this decrease was responsible for the reduction of the hepatic lesions observed at day 42 of infection. IFN-γ, produced by Sm28GST-specific cells, could act either in regulating antibody response (25) or in activating effector cells such as macrophages (16). Since IFN-γ inhibits proliferation of mouse Th2 cells and IL-10 reciprocally inhibits Th1 cells, it has been postulated that the expression of resistance in mice might reflect the balance in $CD4^+$ Th1 and Th2 cell clones and that this balance could be modified not only by their reciprocal regulatory influences but also directly by the parasite itself. The production of IL-10, an inhibitor of the IFN-γ response, will also be evaluated in our model of immunization by rSm28GST. IFN-γ is a potent inhibitor of collagen synthesis both in vitro and in vivo (7, 9), and we envisaged a role for this cytokine secreted by Sm28GST-specific cells in the observed reduction of the hepatic damage. Treatment of immunized mice with anti-IFN-γ antibody suppressed the Sm28GST-induced protective effect and led to the rapid death of anti-IFN-γ-treated animals. This result could be related to that obtained by Smythies et al., who demonstrated that administration of a MAb to IFN-γ modified pulmonary inflammatory responses and abrogated immunity in mice vaccinated with attenuated cercariae (31). Thus, the neutralization of IFN-γ during challenge responses may shift the Th balance toward domination by the Th2 subset and promote parasite survival. Moreover, the use of IFN-γ under our experimental conditions of infection resulted in a marked decrease in the hepatic damage and in survival of the treated mice. This observation was in accord with the results of Cazja et al. (9), who demonstrated that in murine schistosomiasis there was a profound inhibition of collagen deposition accompanied by reductions in mRNA content for types I and III procollagen after treatment by IFN-γ.

In conclusion, rSm28GST, a molecule known for its capacity to elicit antibody response and antibody-dependent cellular mechanisms, is also able to induce, under particular experimental conditions, cell-mediated mechanisms leading to a reduction of the hepatic damage of murine schistosomiasis. Moreover, these results confirm the role of activated T cells throughout the production of IFN-γ in the control of *S. mansoni* infection.

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REFERENCES

1. **Balloul, J. M., J. M. Grzych, R. J. Pierce, and A. Capron.** 1987. A purified 28,000 dalton protein from *Schistosoma mansoni* adult worms protects rats and mice against experimental schistosomiasis. J. Immunol. **138**:3448–3453.
2. **Boros, D. L.** 1989. Immunopathology of *Schistosoma mansoni* infection. Clin. Microbiol. Rev. **2**:250–269.
3. **Boulanger, D., G. D. F. Reid, R. F. Sturrock, I. Wolowczuk, J. M. Balloul, D. Grezel, R. J. Pierce, M. F. Otieno, S. Guerret, J. A. Grimaud, A. E. Butterworth, and A. Capron.** 1991. Immunization of mice and baboons with the recombinant Sm28GST affects both worm viability and fecundity after experimental infection with *Schistosoma mansoni*. Parasite Immunol. **13**:473–490.
4. **Chensue, S. W., S. R. Wellhausen, and D. Boros.** 1981. Modulation of granulomatous hypersensitivity. II. Participation of Ly 1^+ and Ly 2^+ T lymphocytes in the suppression of granuloma formation and lymphokine production in *Schistosoma mansoni*-infected mice. J. Immunol. **127**:3630–3635.
5. **Colley, D. J.** 1981. T lymphocytes that contribute to the immunoregulation of granuloma formation in chronic murine schistosomiasis. J. Immunol. **126**:1465–1468.
6. **Courtney, M., A. Buchwalder, L. H. Tessier, M. Jaye, A. Benavente, A. Balland, V. Kolhi, R. Lathe, P. Tolstoshev, and J. P. Lecocq.** 1984. High level production of biologically active human-antitrypsin in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **81**:669–674.
7. **Czaja, M. J., F. R. Weiner, and M. Eghbali.** 1987. The differential effects of gamma-interferon on collagen and fibronectin gene expression. J. Biol. Chem. **262**:13348–13351.
8. **Czaja, M. J., F. R. Weiner, K. C. Flanders, M. A. Giambrone, R. Wind, L. Biempica, and M. A. Zern.** 1989. *In vitro* and *in vivo* association of transforming growth factor-1 with fibrosis. J. Cell Biol. **108**:2477–2486.
9. **Cazja, M. J., F. R. Weiner, S. Takahashi, M. A. Giambrone, P. Van der Meide, H. Schellekens, L. Biempica, and M. A. Zern.** 1989. γ-Interferon treatment inhibits collagen deposition in murine schistosomiasis. Hepatology **5**:795–800.
10. **Dunne, D. W., A. E. Butterworth, A. J. C. Fulford, H. Curtis Kariuki, J. G. Langley, J. M. Ouma, and A. Capron.** 1992. Immunity after treatment of human schistosomiasis mansoni: association between IgE antibodies to adult worm antigens and resistance to reinfection. Eur. J. Immunol. **22**:1483–1494.
11. **Fidel, P. L., and D. L. Boros.** 1990. Regulation of granulomatous inflammation in murine schistosomiasis. IV. Antigen-induced suppressor T cells down-regulate proliferation and IL-2 production. J. Immunol. **145**:1257–1264.
12. **Fong, T. A. T., and T. R. Mossmann.** 1990. Alloreactive murine $CD8^+$ T cell clones secrete the Th1 pattern of cytokines. J. Immunol. **144**:1744–1752.
13. **Gajewski, T. F., and F. W. Fitch.** 1988. IFN-γ inhibits the proliferation of Th2 but not Th1 murine helper T lymphocyte clones. J. Immunol. **140**:4245–4252.

13a. **Grezel, D.** Unpublished data.

14. **Grzych, J. M., D. Grezel, C. B. Xu, J. L. Neyrinck, M. Capron, J. H. Ouma, A. E. Butterworth, and A. Capron.** 1993. IgA antibodies to a protective antigen in human schistosomiasis mansoni. J. Immunol. **150**:527–535.
15. **Grzych, J. M., E. Pearce, A. Cheever, Z. A. Caulada, P. Caspar, S. Henry, F. Lewis, and A. Sher.** 1991. Egg deposition is the major stimulus for the production of Th2 cytokines in murine schistosomiasis mansoni. J. Immunol. **146**:1322–1327.
16. **James, S. L.** 1986. Activated macrophages as effector cells of protective immunity to schistosomiasis. Immunol. Res. **5**:139–146.
17. **Lopez De Leon, A., and M. Rojkind.** 1985. A simple method for collagen and total protein determination in formalin-fixed paraffin-embedded sections. J. Histochem. Cytochem. **33**:737–743.
18. **Lukacs, N. W., and D. L. Boros.** 1991. Identification of larval cross-reactive and egg-specific antigens involved in circumoval granuloma formation in murine *Schistosoma mansoni*. Infect. Immun. **59**:3237–3242.
19. **Mathew, R. C., and D. L. Boros.** 1986. Anti-L3T4 antibody treatment suppresses hepatic granuloma formation and abrogates

antigen-induced interleukin-2 production in *Schistosoma mansoni* infection. Infect. Immun. **54:**820–826.
20. **Mathew, R. C., S. Ragheb, and D. L. Boros.** 1990. Recombinant IL-2 therapy reverses diminished granulomatous responsiveness in anti L3T4-treated, *Schistosoma mansoni*-infected mice. J. Immunol. **144:**4356–4361.
21. **Mosmann, T. R., H. Cherwinski, M. W. Bond, M. A. Giedlin, and R. L. Coffman.** 1986. Two types of murine helper T cell clones. I. Definition according to profiles of lymphokine activities and secreted proteins. J. Immunol. **136:**2348–2357.
22. **Müller, I., P. Kropf, R. J. Etges, and J. A. Louis.** 1993. Gamma interferon response in secondary *Leishmania major* infection: role of $CD8^+$ T cells. Infect. Immun. **61:**3730–3738.
23. **Murray, P. K.** 1989. Molecular vaccines against animal parasites. Vaccine **7:**291–299.
23a. **Pancré, V.** Unpublished data.
24. **Pearce, E. J., P. Caspar, J. M. Grzych, F. A. Lewis, and A. Sher.** 1991. Down-regulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth, *Schistosoma mansoni*. J. Exp. Med. **173:**159–166.
25. **Pene, J., F. Rousset, F. Briere, I. Chretien, J. Y. Bonnefoy, H. Spits, T. Yokota, N. Arai, J. Banchereau, and J. deVries.** 1986. IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons γ and α and prostaglandin E2. Proc. Natl. Acad. Sci. USA **85:**6880–6884.
26. **Perrin, P. J., and S. M. Phillips.** 1988. The molecular basis of granuloma formation in schistosomiasis. I. A T cell-derived suppressor effector factor. J. Immunol. **141:**1714–1719.
27. **Phillips, S. M., and P. Lammie.** 1986. Immunopathology of granuloma formation and fibrosis in schistosomiasis. Parasitol. Today **2:**296–302.
28. **Phillips, S. M., G. P. Linette, B. L. Doughty, J. E. Byram, and F. Von Lichtenberg.** 1987. *In vivo* T cell depletion regulates resistance and morbidity in murine schistosomiasis. J. Immunol. **139:** 919–926.
29. **Ragheb, S., and D. L. Boros.** 1989. Characterization of granuloma T lymphocyte function from *Schistosoma mansoni*-infected mice. J. Immunol. **142:**3239–3246.
30. **Sher, A., D. Fiorentino, P. Caspar, E. Pearce, and T. Mosmann.** 1991. Production of IL-10 by $CD4^+$ T lymphocytes correlates with down-regulation of Th1 cytokine synthesis in helminth infection. J. Immunol. **147:**2713–2716.
31. **Smythies, L. E., P. S. Coulson, and R. A. Wilson.** 1992. Monoclonal antibody to IFN-γ modifies pulmonary inflammatory responses and abrogates immunity to *Schistosoma mansoni* in mice vaccinated with attenuated cercariae. J. Immunol. **149:**3654–3658.
32. **Taylor, M. G., and Q. D. Bickle.** 1986. Toward a schistosomiasis vaccine: irradiated schistosome vaccines. Parasitol. Today **2:**132–134.
33. **Taylor, M. G., A. Vidal, G. Torpier, D. J. Meyer, C. Roitsch, J. M. Balloul, C. Southan, P. Sondermeyer, S. Premble, J. P. Lecocq, A. Capron, and B. Ketterer.** 1988. The glutathione transferase activity and tissue distribution of a cloned M_r 28 K protective antigen of *Schistosoma mansoni*. EMBO J. **7:**465–472.
34. **Wolowczuk, I., C. Auriault, H. Gras-Masse, C. Vendeville, J. M. Balloul, A. Tartar, and A. Capron.** 1989. Protective immunity in mice vaccinated with the *Schistosoma mansoni* P-28-1 antigen. J. Immunol. **142:**1342–1350.
35. **Wyler, D. J., H. P. Ehrlich, A. E. Postlethwaite, R. Raghow, and M. M. Murphy.** 1987. Fibroblast stimulation in schistosomiasis. VII. Egg granulomas secrete factors that stimulate collagen and fibronectin synthesis. J. Immunol. **138:**1581–1586.
36. **Xu, C. B., C. Verwaerde, J. M. Grzych, J. Fontaine, and A. Capron.** 1991. A monoclonal antibody blocking the *Schistosoma mansoni* 28 kDa glutathione S-transferase activity reduces female worm fecundity and egg viability. Eur. J. Immunol. **21:**1801–1807.