Isotype Responses to Candidate Vaccine Antigens in Protective Sera Obtained from Mice Vaccinated with Irradiated Cercariae of *Schistosoma mansoni*

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In experimental schistosomiasis, sera of mice multiply vaccinated with radiation-attenuated cercariae of *Schistosoma mansoni* passively transfer resistance against cercarial challenge to naive mice. To further characterize these sera, we tested their protective capacities in two mouse strains (C57BL/6J and CBA/J) and compared the antigen-specific isotype compositions of the different protective sera by means of the enzyme-linked immunosorbent assay. By using an array of purified schistosomal antigens, the patterns of antibody titers and isotypes differed for each experimental group and antigen. In the most-protective C57BL/6J sera, high levels of immunoglobulin G1 (IgG1), IgG2a, and IgG2b bound to heat shock protein 70 and the integral membrane protein Sm23, whereas recognition of these antigens by less-protective CBA/J sera was lower. Glutathione *S*-transferase (GST) was recognized predominantly by IgM antibodies of all vaccinated groups, and a significant portion of this response was directed against carbohydrate epitopes. Antibodies specific for triosephosphate isomerase, paramyosin, and Sm32 (hemoglobinase) were present in less-protective sera and thus seem less relevant for passive transfer of resistance. The results of this study suggest a contribution of IgG antibodies specific for heat shock protein 70 and Sm23, and possibly a contribution of GST-specific IgM antibodies, to the protective effect of sera from C57BL/6J mice vaccinated with irradiated cercariae.

Vaccination with radiation-attenuated cercariae of *Schistosoma mansoni* can induce high levels of resistance against challenge infections in mice (12, 27). Humoral and cellular immune compartments participate in this protective response because μ-chain-depleted mice as well as athymic mice fail to develop resistance after vaccination (31). In addition, sera of multiply vaccinated mice are able to passively transfer resistance to naive mice (6, 15, 21, 22).

Characterization of antigens recognized by these protective sera might facilitate the development of a defined vaccine. The antigen specificity of sera from mice vaccinated with irradiated cercariae, however, varies depending on the host's genetic background and the irradiation dose given to the immunizing cercariae (29). We examined whether these parameters also influence the protective capacity of vaccine sera by comparing levels of resistance induced in two different mouse strains (C57BL/6J [C57] and CBA/J [CBA]) by transfer of homologous sera that were obtained from mice after three vaccinations with moderately (15 kilorads) or highly (50 kilorads) irradiated cercariae.

As indicated by a previous study, not only the specificity but also the amount of antibodies generated against particular antigens is influenced by cercarial irradiation dose and genetic background of the host (29). To determine whether the titers of antibodies against specific antigens correlate with the level of resistance transferred, we measured the titers of antibodies binding to purified native or recombinant schistosomal antigens by using the enzyme-linked immu-

nosorbent assay (ELISA). We tested antigens that were previously identified as candidate vaccine antigens: integral membrane protein Sm23, glutathione *S*-transferase (GST), triosephosphate isomerase (TPI), Sm32 (hemoglobinase), heat shock protein 70 (HSP70), and paramyosin (Para) (2, 11, 26, 29). In addition to the titers and antigen specificities of these antibodies, their isotypes might play a role as well, because several studies have associated particular isotype fractions of vaccine sera with protective capacity (6, 15, 21). Therefore, we compared the levels of antigen binding of immunoglobulin G1 (IgG1), IgG2a, IgG2b, IgG3, and IgM antibodies obtained from C57 and CBA mice once or multiply vaccinated with 15- or 50-kilorad-irradiated cercariae.

MATERIALS AND METHODS

Abbreviations. In addition to the previously introduced abbreviations, the following abbreviations are used throughout: CBA and C57 1-15, mice vaccinated once with moderately irradiated cercariae; CBA and C57 3-15, mice vaccinated three times with moderately irradiated cercariae; CBA and C57 1-50, mice vaccinated once with highly irradiated cercariae; CBA and C57 3-50, mice vaccinated three times with highly irradiated cercariae; rSm23, 23-kDa recombinant integral membrane protein; rTPI, recombinant TPI; rSm32, recombinant Sm32; rHSP70, recombinant HSP70; rPara, recombinant Para; PBS, phosphate-buffered saline; PBS-T 0.3, PBS containing 0.3% Tween 20; PBS-T 0.05, PBS containing 0.05% Tween 20; MAbs, monoclonal antibodies.

Host animals and parasites. Six- to 8-week-old female CBA and C57 mice were purchased from Bomholtgaard (Ry, Denmark) and Jackson Laboratory (Bar Harbor, Maine). A Puerto Rican strain of *S. mansoni* was maintained in our laboratory by passage through *Biomphalaria glabrata* snails and Swiss Webster mice (Taconic Farms, Germantown,

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N.Y.). Parasites of various stages were also obtained from F. Lewis, Biomedical Research Institute, Rockville, Md.

Exposure of mice to irradiated cercariae. At monthly intervals, groups of CBA and C57 mice were exposed to 500 irradiated cercariae of *S. mansoni*. Freshly harvested cercariae were diluted to 500 organisms per ml and were immediately attenuated with either a 15- or 50-kilorad dose of irradiation emitted by a ^{60}Co source. Mice were anesthetized with a mixture of ketamine-HCl (0.08 mg/g of body weight) (Aveco, Fort Dodge, Iowa) and xylazine (0.005 mg/g of body weight) (Haver, Shawnee, Kans.) injected intramuscularly in the thigh and were exposed to irradiated cercariae for 30 min via their shaved abdomen by the ring method (35).

Antisera. Vaccinated mice were bled from the tail 26 days after each exposure to irradiated cercariae. Sera from each experimental group of at least 10 mice were pooled. Control sera were obtained from strain- and age-matched naive mice.

Challenge infection and passive transfer of resistance. For challenge infections, mice were exposed to 130 freshly harvested, nonattenuated cercariae for 30 min by the ring method as described above (35). Mice vaccinated with irradiated cercariae were challenged 4 weeks after the last exposure. For passive transfer of resistance, groups of mice received an intraperitoneal injection of 400 μl of serum 9 days after infection with normal cercariae to target the parasite in its lung stage (21). Sera for transfer were obtained after the third and fourth vaccinations of at least two independent vaccination experiments and were pooled for each experimental group (C57 1- and 3-15, C57 1- and 3-50, CBA 1- and 3-15, CBA 1- and 3-50). Control sera were obtained as described above. Groups of mice received sera from the homologous strain. Seven weeks after the challenge infection, adult worms were recovered from the hepatic portal system by mesenteric vein perfusion (7). The degree of resistance was expressed as % resistance = [(NMS mean − VMS mean)/NMS mean] × 100, where NMS is normal mouse serum and VMS is vaccinated mouse serum. Statistical significance was assessed by Student's *t* test.

Schistosomal antigens. HSP70, GST, and Para were purified with previously reported modifications by ATP (36), glutathione (33), and collagen (19) affinity chromatography, respectively (29). Antigens were concentrated and dialyzed against PBS. rTPI was cloned and expressed as described by Shoemaker et al. (32) and was purified by using isoelectric focusing in the Rotofor cell (Bio-Rad, Richmond, Calif.). rSm32, rHSP70, and rPara as bacteriophage MS2-polymerase fusion proteins were a generous gift of M. Q. Klinkert, Istituto di Biologia Cellulare, Consiglio Nazionale delle Ricerche, Rome, Italy (16, 17, 23). The large extracellular domain of the integral membrane protein Sm23 (rSm23) was expressed as a maltose-binding fusion protein in our laboratory (28). The antigen concentrations were estimated by using the Micro-BCA colorimetric assay (Pierce, Rockford, Ill.).

ELISA. For the ELISA, 50 μl of purified antigen was dispensed into each well of a 96-well plate (Immulon 2; Dynatech, Chantilly, Va.) at a concentration of 5 μg/ml and was incubated overnight in a humid chamber at 4°C. The wells were washed at least eight times with PBS-T 0.05 and were blocked with PBS-T 0.3 for 1 h at 37°C. After several washes with PBS-T 0.05, 50 μl of antiserum diluted 1:200 in PBS-T 0.3 was added to the wells in duplicate. The plates were incubated for 2 h as described above and were washed eight times with PBS-T 0.05. Each serum sample was then probed with 50 μl of biotin-labeled rabbit anti-mouse IgG1, IgG2a, IgG2b, IgG3, or IgM antibody (Zymed, San Fran-

TABLE 1. Reduction in worm burden in C57 and CBA mice vaccinated once or multiple times with irradiated cercariae

Mouse strain	Irradiation frequency and dose (kilorads)	Hosts (*n*)	Mean worm burden ± SE	% Reduction in worm burden	*P* value
C57	Control	9	21.7 ± 3.9		
	1–15	10	7.2 ± 1.7	66.8	0.0014
	3–15	10	3.8 ± 0.8	82.5	0.0001
	1–50	9	16.8 ± 4.6	22.6	NS[a]
	3–50	10	8.4 ± 2.4	61.2	0.0047
CBA	Control	8	14.8 ± 4.3		
	1–15	6	5.0 ± 2.9	66.2	0.0532
	3–15	10	4.2 ± 1.2	71.5	0.0098
	1–50	8	14.1 ± 2.7	4.2	NS
	3–50	8	7.6 ± 1.5	48.3	NS

[a] NS, not significantly different.

cisco, Calif.) diluted 1:500 in PBS-T 0.3. After an incubation for 1 h at 37°C, the wells were washed as described above, 50 μl of strepavidin (Sigma, St. Louis, Mo.) at a dilution of 1:1,000 was added, and the wells were incubated for 1 h at 37°C. After washes with PBS-T 0.05, wells were developed with 100 μl of tetramethylbenzidine substrate (Kirkegaard and Perry, Gaithersburg, Md.). The reaction was stopped by adding phosphoric acid at a final concentration of 1:40, and the optical densities at 450 nm were measured with an ELISA reader (UVmax; Molecular Devices, Menlo Park, Calif.). Sodium metaperiodate treatment was performed according to the method of Woodward et al. (37). Briefly, wells with plated antigen were washed with PBS-T 0.05 followed by 50 mM sodium acetate buffer (pH 4.5). One hundred microliters of 20 mM sodium metaperiodate in sodium acetate buffer was incubated in each well for 1 h at room temperature in the dark. Plates were rinsed with acetate buffer and incubated with 100 μl of 50 mM sodium borohydride in PBS. After a 30-min incubation at room temperature, wells were washed, blocked with PBS-T 0.3, and treated as described above. The data displayed reflect the mean results of replicate wells of a representative assay with pooled sera.

RESULTS

Reduction in worm burden by vaccination with irradiated cercariae. Initial experiments were performed to compare the levels of resistance between C57 and CBA mice after single or multiple vaccination with 15- or 50-kilorad-irradiated cercariae. In both strains, mice that had been vaccinated with 15-kilorad-irradiated cercariae developed the highest levels of resistance (Table 1). After three exposures, the levels of resistance in vaccinated C57 mice were significantly higher than that in once-vaccinated mice (82.5 versus 66.8%; $P \le 0.044$). The reduction in worm burden in CBA mice, however, did not increase significantly with additional vaccinations (66.2 versus 71.5%; $P = 0.385$). Among mice that had been vaccinated with 50-kilorad-irradiated cercariae, only C57 3-50 mice exhibited significant levels of resistance (61.2%; $P = 0.005$). Thus, the reduction in worm burden was greatest in mice that had been vaccinated with 15-kilorad-irradiated cercariae in both mouse strains examined, and multiple vaccinations increased the level of resistance in C57 mice.

Passive transfer of resistance. Sera of mice multiply vaccinated with irradiated cercariae were examined for the ability

TABLE 2. Reduction in worm burden after transfer of serum obtained from mice vaccinated with irradiated cercariae[a]

Serum						
Obtained from:		Transferred to:				
Mouse strain	Irradiation dose (kilorads)	Mouse strain	Host (n)	Mean worm burden ± SE	% Reduction in worm burden	P value
		C57	7	35.3 ± 7.0		
C57		C57	5	35.2 ± 8.2		
C57	15	C57	5	12.6 ± 1.7	64.2	0.0138
C57	50	C57	7	13.7 ± 5.2	61.0	0.0210
		CBA	8	40.6 ± 5.4		
CBA		CBA	5	38.2 ± 8.1		
CBA	15	CBA	6	16.2 ± 2.8	57.7	0.0106
CBA	50	CBA	7	22.9 ± 2.5	40.2	0.0312

[a] Serum transfer was conducted 9 days after challenge.

to transfer resistance to challenged mice of the homologous strain. The levels of resistance in C57 mice treated with C57 3-15 or C57 3-50 sera did not differ significantly (64.2 versus 61.0%; $P = 0.432$), whereas those of CBA mice injected with CBA 3-15 or CBA 3-50 sera were different (57.7 versus 40.2%; $P = 0.0495$) (Table 2). All vaccine sera tested conferred significant protection against challenge infection.

Isotype distribution in vaccine sera recognizing purified schistosomal antigens. To characterize the specificities of these vaccine sera, we compared the levels of isotypes binding to purified schistosomal antigens that had previously been shown to be recognized by vaccine sera (29). In a preliminary ELISA, the specificity of the anti-isotype antibodies to be used throughout the study was confirmed. An array of murine MAbs of known isotypes was plated and probed with the anti-isotype antibodies. Nonspecific binding of these probes was generally lower than 1/10 of the positive signal (Table 3). Only the anti-IgG2a antibody bound IgG1 and IgG2b MAbs, and the anti-IgG3 antibody bound IgG1 MAbs with a nonspecific signal as strong as one-third of the specific positive signal. All sera were also tested for the presence of IgE and IgA antibodies binding to purified schistosomal antigens. However, no IgE antibodies were detected, and binding of IgA antibodies appeared to be nonspecific, because antibody levels were similar in vaccine and control sera. Thus, this study focuses on IgG and IgM isotypes.

In sera of vaccinated C57 mice, significant amounts of IgG1, IgG2a, and IgG2b were found to specifically bind to rSm23 expressed as a maltose-binding fusion protein, whereas CBA mice generated predominantly antibodies of the IgG1 and IgG2a isotypes against this protein (Fig. 1). Multiple exposures to irradiated cercariae increased the

production of these IgGs in C57 1- and 3-15 and CBA 1- and 3-15 mice by more than three times over the production observed for once-vaccinated mice. In C57 1- and 3-50 and CBA 1- and 3-50 mice, titers of IgG antibodies were lower and were only significant after multiple vaccinations. No specific IgM antibodies were detected. Amounts of antibodies binding to the maltose-binding protein control were insignificant (data not shown). Thus, the highest levels of anti-Sm23 antibodies of the IgG1 and IgG2 isotypes are generated in both mouse strains after multiple vaccinations with moderately irradiated cercariae.

In contrast to rSm23, only low levels of the IgG1, IgG2a, and IgG2b isotypes were observed to bind to GST (Fig. 2A). Of these, IgG2a seemed to be most abundant in once-vaccinated CBA mice. Interestingly, all groups of vaccinated mice generated GST-specific IgM antibodies. Multiply vaccinated C57 mice produced significantly more IgM than once-vaccinated mice. When GST was treated with sodium metaperiodate before incubation with antibody, the binding of IgM in the vaccine sera was reduced by 40 to 65%, indicating that a significant portion of these antibodies recognized carbohydrate epitopes (Fig. 2B). In contrast, IgG antibodies did not recognize periodate-sensitive epitopes (data not shown). Therefore, IgM production dominates the humoral response to carbohydrate epitopes, as well as to non-periodate-sensitive epitopes on GST, and is elevated after multiple exposures to irradiated cercariae, whereas relatively little IgG2a production directed against non-periodate-sensitive epitopes is observed after primary vaccination.

The humoral response to another 28-kDa antigen, TPI, that has been inconsistently detected by immunoblotting was examined. Only C57 1-15, CBA 1-15, CBA 3-15, and CBA 1-50 mice produced antibodies that bound to rTPI (Fig. 3).

TABLE 3. Specificity of anti-isotype antibodies tested on an array of MAbs

MAb	Isotype	OD$_{450}$[a] with antibody probe specific for:					Reference
		IgG1	IgG2a	IgG2b	IgG3	IgM	
M.1	IgG1	1.192	0.322	0.059	0.340	0.071	10
HB163	IgG2a	0.123	1.223	0.061	0.075	0.067	1
E1	IgG2b	0.092	0.521	1.333	0.195	0.054	18
E3	IgG3	0.120	0.073	0.062	1.248	0.056	18
HB8580	IgM	0.044	0.041	0.052	0.039	1.224	1

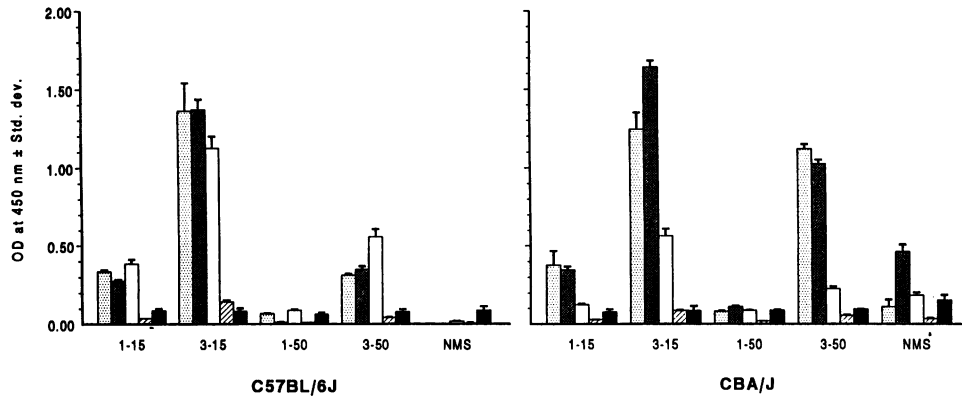[a] OD$_{450}$, optical density at 450 nm.

FIG. 1. Isotype responses of mice vaccinated with irradiated cercariae to the integral membrane protein Sm23. Shown are levels of IgG1 (▨), IgG2a (▩), IgG2b (□), IgG3 (▨), and IgM (■) antibodies in sera of C57 and CBA mice vaccinated once or three times with 15- or 50-kilorad-irradiated cercariae and in control (normal mouse serum [NMS]) sera.

These antibodies consisted of IgG1, IgG2a, IgG2b, and IgM isotypes and small but significant amounts of IgG3. Levels of the IgG2a isotype were significant in CBA 1-15 and CBA 1-50 mice but were insignificant in C57 1-15 mice. The humoral response of CBA 3-15 mice was restricted to antibodies of the IgG1 isotype. No antibodies bound to bacterial lysate that was used as control (data not shown). We concluded that the humoral response to rTPI is generally
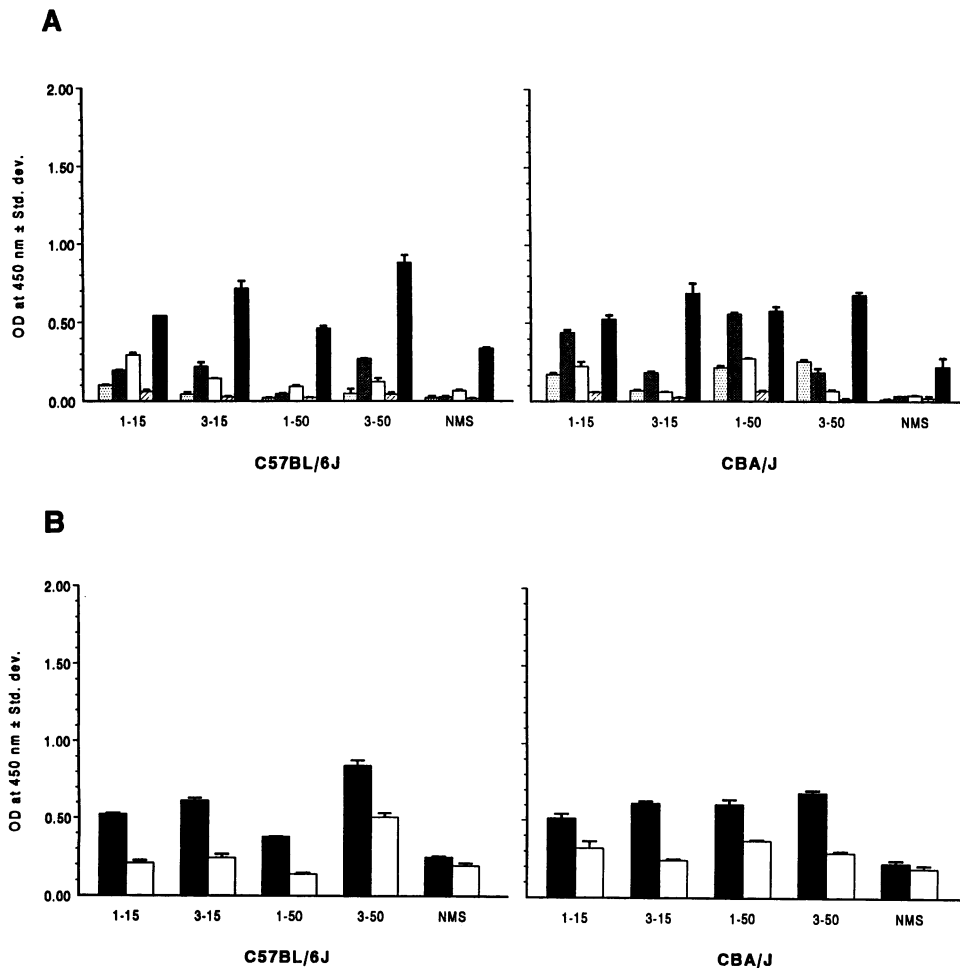

FIG. 2. Isotype responses of mice vaccinated with irradiated cercariae to GST. (A) Levels of IgG1 (▨), IgG2a (▩), IgG2b (□), IgG3 (▨), and IgM (■) antibodies in sera of C57 and CBA mice vaccinated once or three times with 15- or 50-kilorad-irradiated cercariae and in control (normal mouse serum [NMS]) sera binding to native GST. (B) Levels of IgM antibodies binding to untreated (■) or periodate-treated (□) GST.
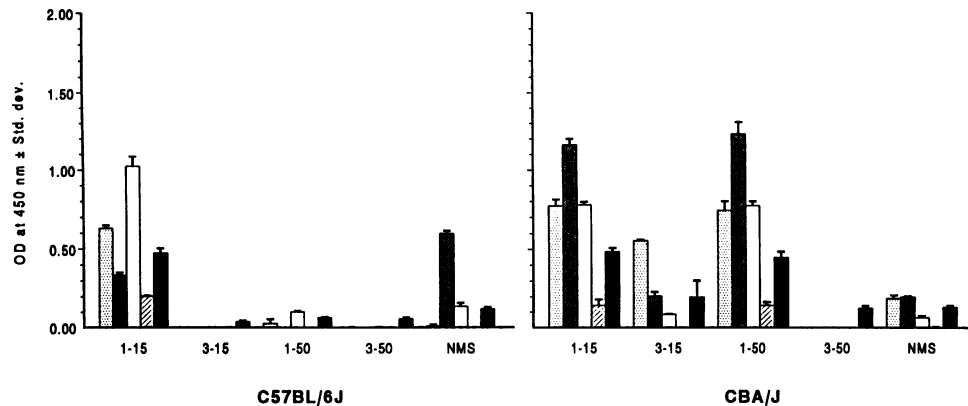
FIG. 3. Isotype responses of mice vaccinated with irradiated cercariae to TPI. Shown are levels of IgG1 (▦), IgG2a (▨), IgG2b (□), IgG3 (▧), and IgM (■) antibodies in sera of C57 and CBA mice vaccinated once or three times with 15- or 50-kilorad-irradiated cercariae and in control (normal mouse serum [NMS]) sera.

restricted to once-vaccinated mice and that this response is dominated by IgG2a in CBA mice.

In a previous study, immunoblotting indicated that vaccinated mice recognize two enzymes of the schistosome's digestive tract, Sm32 and cathepsin B (29). We examined the humoral response to rSm32 and found that it was restricted to vaccinated CBA mice (Fig. 4). Once-vaccinated CBA mice (CBA 1-15 and CBA 1-50) produced IgG2a and IgG2b, whereas CBA 3-15 mice generated large amounts of IgG1. Thus, only vaccinated mice of the CBA strain recognize rSm32.

The titers of antibodies against both native HSP70 and rHSP70 were examined. Levels of HSP70-specific IgG1 antibodies increased with multiple exposures in all experimental groups (Fig. 5A). Vaccinated C57 mice, in particular C57 1-15 and C57 3-15, produced the highest titers of this isotype; lower IgG2a and IgG2b levels and a slightly elevated IgG3 level were also detected. In all vaccinated CBA mice, low but significant titers of IgG2a were measured, but in CBA 3-15 mice, a relatively large IgG1 response was also detected. The ratios of the various isotypes in the experimental groups generally were similar for the native and recombinant antigen, except that IgM bound only to native HSP70 and the IgG1 level in CBA 3-15 mice was higher against the recombinant antigen (Fig. 5B). We concluded

that HSP70 is predominantly recognized by IgG1 antibodies of vaccinated C57 mice.

Para was also probed in the native and rPara forms. All vaccinated CBA mice produced IgG1 and IgG2a antibodies specific for native Para (Fig. 6A). The titer of IgG1 against native Para increased significantly with additional exposures and higher irradiation dose (in the order CBA 1-15 < CBA 3-15 < CBA 1-50 < CBA 3-50). CBA 3-50 mice produced about twice as much specific IgG1 as CBA 3-15 mice did. In vaccinated C57 mice, only low levels of these antibodies were detected to bind to native Para and none bound to the rPara. With rPara, only the IgG1 response of CBA 3-50 mice was as high as that observed with the native antigen (Fig. 6B). In addition to IgG1, CBA 1-15 and CBA 1-50 mice produced IgG2a and IgG2b antibodies to rPara. (A nonspecific IgG2b signal in the presence of the native Para was observed throughout all sera, including normal mouse sera.) Thus, Para is detected predominantly by the IgG1 antibodies of vaccinated CBA mice and, in particular, the IgG1 antibodies of CBA 3-50 mice.

DISCUSSION

The irradiation dose of attenuated cercariae, the host's genetic background, and the numbers of vaccinations are
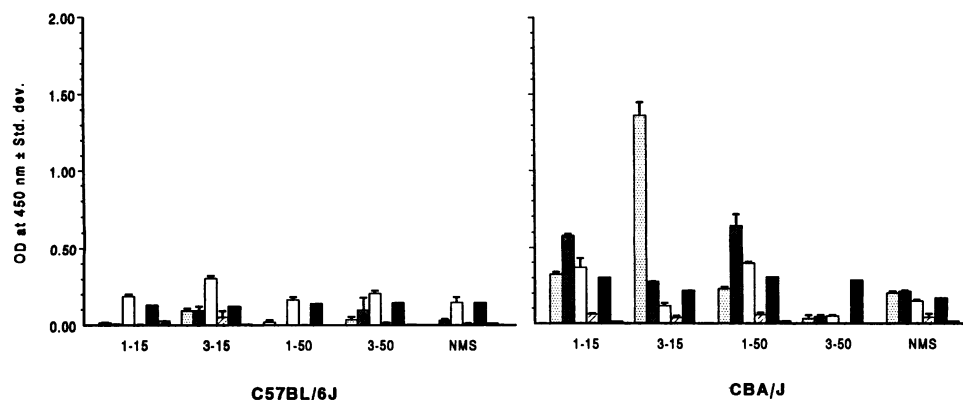

FIG. 4. Isotype responses of mice vaccinated with irradiated cercariae to Sm32 (hemoglobinase). Shown are levels of IgG1 (▦), IgG2a (▨), IgG2b (□), IgG3 (▧), and IgM (■) antibodies in sera of C57 and CBA mice vaccinated once or three times with 15- or 50-kilorad-irradiated cercariae and control (normal mouse serum [NMS]) sera.
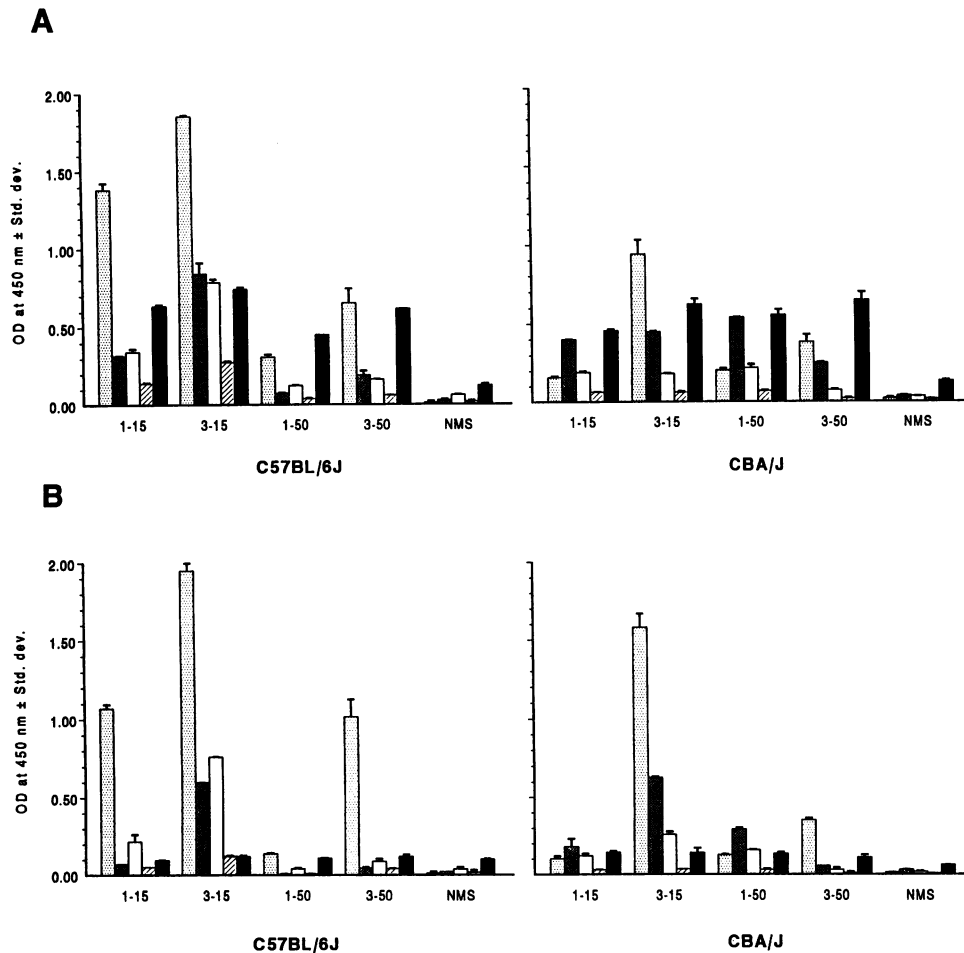
A

B

FIG. 5. Isotype responses of mice vaccinated with irradiated cercariae to native HSP70 (A) or rHSP70 (B). Shown are levels of IgG1 (▨), IgG2a (▦), IgG2b (▢), IgG3 (▨), and IgM (■) antibodies in sera of C57 and CBA mice vaccinated once or three times with 15- or 50-kilorad-irradiated cercariae and in control (normal mouse serum [NMS]) sera.

known to affect the level of resistance conferred by irradiated cercariae (5, 12, 14, 20, 27, 34). In this study, the influences of these parameters on reduction in worm burden were compared side by side between various experimental groups. In agreement with observations by Reynolds and Harn (27) as well as Simpson et al. (34), moderately (15 kilorads) irradiated cercariae conferred significantly more resistance than highly (50 kilorads) irradiated cercariae. Although a boosting effect trend was seen in CBA mice, the number of vaccinations correlated significantly with the level of protection in C57 mice, corresponding with observations by Reynolds and Harn (27) as well as Hsü et al. (12). Several studies have demonstrated that resistance resulting from multiple vaccinations with irradiated cercariae can be partially transferred with vaccine serum (6, 15, 21, 22). In this study, we compared the protective capacities of sera from two mouse strains. All vaccine sera provided significant levels of resistance to mice of the homologous strain. Although the reduction in worm burden was generally lower than that resulting from active vaccination with irradiated cercariae, the host's genetic background and irradiation dose similarly influenced the levels of resistance. Interestingly, C57 3-15 serum also transferred resistance to mice of the heterologous strain, whereas CBA sera did not (28b).

Therefore, sera of vaccinated C57 mice might contain antibodies of different quantity or quality than those in sera of vaccinated CBA mice. By means of immunoblotting, we have previously demonstrated that intensity of antigen detection varies between these experimental groups and that recognition of certain antigens is strain specific (29). To further characterize the vaccine sera, we analyzed differences in levels of specific isotypes binding to purified schistosomal antigens by using ELISA. Although certain limitations of this assay are to be considered (i.e., differences in avidity of antibodies and competition between different isotypes for the same epitope), we compared these results between the various experimental groups.

rTPI was the antigen that was detected most strongly after primary vaccination. It elicits a similarly restricted cellular response from such vaccinated mice (30). The IgG2a isotype, induced by interleukin 2 of Th1 cells (4), dominated the humoral response of once-vaccinated CBA mice. Correspondingly, Caulada-Benedetti et al. observed that the immune response to crude schistosomal antigens is predominantly associated with Th1 cells after primary exposure to irradiated cercariae (3). Generally, additional vaccinations enhance the Th2 response (3). In response to TPI, however, Th2-induced IgG1 and IgM levels were minimal after multi-
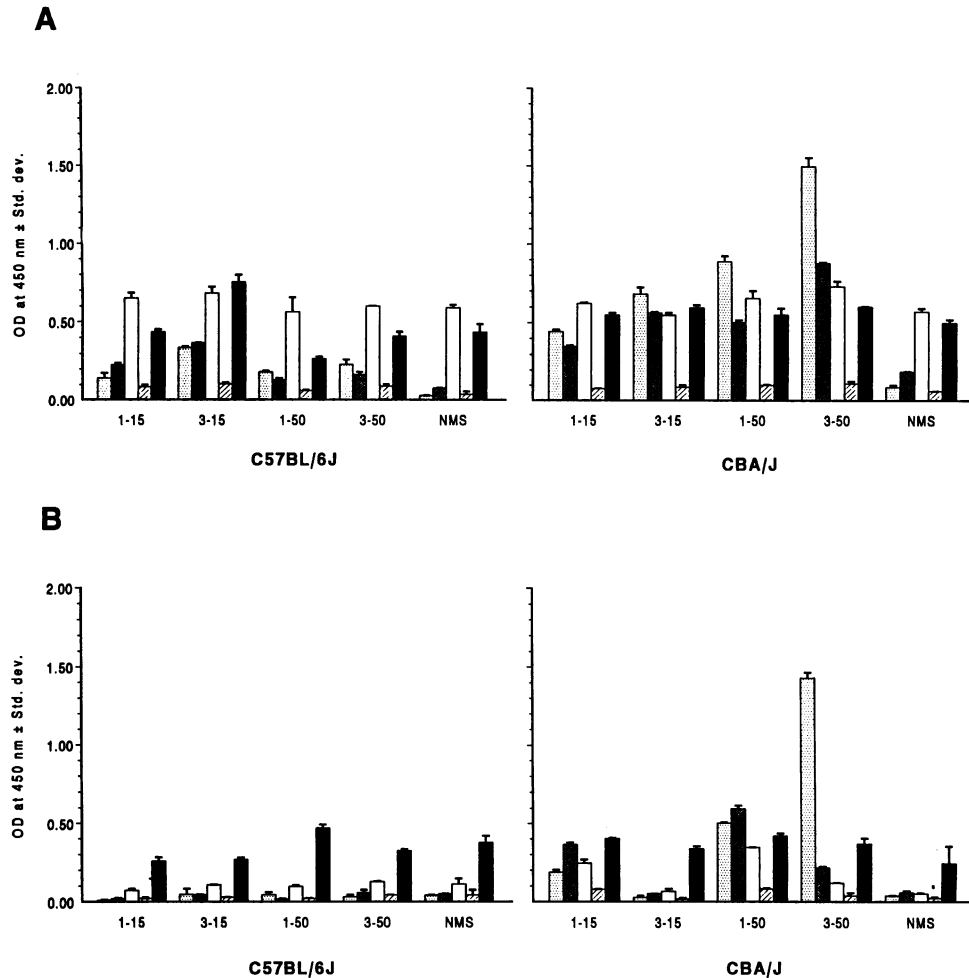
A

C57BL/6J   CBA/J

B

C57BL/6J   CBA/J

FIG. 6. Isotype responses of mice vaccinated with irradiated cercariae to native Para (A) or rPara (B). Shown are levels of IgG1 (▧), IgG2a (▨), IgG2b (□), IgG3 (▨), and IgM (■) antibodies in sera of C57 and CBA mice vaccinated once or three times with 15- or 50-kilorad-irradiated cercariae and in control (normal mouse serum [NMS]) sera.

ple vaccinations. Although it remains to be confirmed that the humoral response to native TPI is identical, production of antibodies against epitopes on rTPI appears to be down-regulated after boosting vaccination. Therefore, TPI-specific antibodies seem unlikely to contribute to the protective capacity of sera of multiply vaccinated mice.

The exclusive recognition of rSm32 and Para by the antibodies of CBA mice (CBA 3-15 and CBA 3-50, respectively) observed in a previous study (29) was confirmed by ELISA. Experimental groups with the lowest levels of resistance (CBA 1-50 and CBA 3-50) produced the highest levels of anti-Para antibodies. Similarly, another study with a nonliving vaccine has demonstrated that levels of resistance do not correlate with titers of anti-Para antibodies (13). Thus, the humoral recognition of Sm32 and Para might be less relevant to protection.

In contrast to Para and rSm32, HSP70 had been shown in immunoblots to be strongly and exclusively recognized by sera of vaccinated C57 mice (29). In this study with ELISA, C57-15 mice appeared to produce the highest levels of anti-HSP70 antibodies. Although IgG2a and IgG2b antibodies were detected, more than twice as many antibodies of the IgG1 isotype bound to HSP70 in the sera of C57 1-15 and C57 3-15 mice. Some binding of CBA antibodies, particularly

IgG1 of CBA 3-15 mice, was also observed (perhaps below the detection level of immunoblots). Interestingly, when rHSP70 was tested, the recognition by antibodies of CBA 3-15 mice was significantly increased. The recombinant protein consists of 230 amino acids at the carboxyl terminus (23), lacking the ATP-binding region at the opposite end which is most conserved across different species (8). Thus, an explanation for the reduced antibody binding to the native protein in CBA mice might be downregulation of the humoral response to minimize production of autoreactive antibodies. The lack of IgM binding to the recombinant protein might suggest that antibodies of this isotype are directed towards epitopes not present on the rHSP70, i.e., carbohydrate epitopes on native HSP70. Although vaccinated CBA mice generate some HSP70-specific antibodies, vaccinated C57 mice, particularly those with the most protective vaccine sera, produce the highest levels of antibodies to this protein.

As previously observed in immunoblots (29), GST was predominantly recognized by antibodies of the IgM isotype in all vaccinated groups; only once-vaccinated CBA mice produced detectable levels of GST-specific IgGs. Interestingly, about half of the IgM antibodies were directed against carbohydrate epitopes, whereas IgG antibodies recognized

solely non-periodate-sensitive epitopes. This result and another observation that IgM antibodies of vaccine sera bind to the oligosaccharide lacto-*N*-fucopentaose III (12a) differ from reports by Omer-Ali et al.; they detected few or no anti-carbohydrate antibodies in vaccine sera (24, 25). In our study, not only vaccine sera but also the protective MAb E1 and the nonprotective MAb E3 recognized carbohydrate epitopes on GST (28a); the MAbs are known to bind an abundant, yet unidentified oligosaccharide epitope and an epitope on keyhole limpet hemocyanin, respectively (18). The GST-specific IgM antibodies might be relevant to the protective capacity of vaccine sera, because the IgM fraction of vaccine sera and two distinct carbohydrate-specific IgM MAbs have been shown to confer resistance upon passive transfer (9, 15, 18).

Previously, Sm23 has been identified as one of the major antigens recognized by the antibodies of vaccinated C57 and CBA mice (29). Similarly, levels of IgG antibodies detected in this study were relatively high compared with those of other antigens. These titers were measured in sera from multiply vaccinated mice and thus were measured in sera with protective capacity (21). Generally, anti-Sm23 antibody levels were similar for both strains, but that of the IgG2b isotype was twice as high in C57 3-15 serum. Perhaps this difference contributes to the enhanced capacity of C57 3-15 sera to transfer resistance.

Taken together, the results show that the serum of C57 mice multiply vaccinated with irradiated cercariae transferred the highest level of resistance. Analysis of this serum's antigen-specific isotype composition and comparison with less-protective sera might serve to distinguish antigens with protective capacity for the humoral arm of the immune response. However, the cellular response plays an important role in the vaccine model (27). This study demonstrated that TPI-specific antibodies, present solely in once-vaccinated mice, do not seem to be relevant for passive transfer of resistance. Para and Sm32 were exclusively recognized by sera conferring less resistance. In contrast, GST-specific IgM antibodies were present in all vaccinated groups, and antibodies of this isotype are known to be relevant in passively transferred resistance (15). The humoral response to HSP70, however, was distinctly stronger in C57 3-15 mice than in any other experimental group and was dominated by IgG1, an isotype that is protective in passive transfer experiments (6). High anti-Sm23 antibody responses coincided with multiple vaccinations in both strains, and IgG2b antibodies were particularly abundant in C57 3-15 serum. Therefore, passive transfer of vaccine sera depleted of antibodies specific for either Sm23, GST, or HSP70 may further elucidate the relevance of these antibodies for protection and might allow definition of vaccine antigens that would stimulate the humoral immune response against a challenge infection with *S. mansoni*.

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