INFECTION AND IMMUNITY, Nov. 1993, p. 4919–4924
0019-9567/93/114919-06$02.00/0

Vol. 61, No. 11

Effect of Cholera Toxin on Vaccine-Induced Immunity and Infection in Murine Schistosomiasis Mansoni

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Received 6 May 1993/Returned for modification 25 June 1993/Accepted 1 September 1993

Intradermal vaccination of mice with soluble adult worm antigen (SWAP) in combination with *Mycobacterium bovis* BCG (Swedish strain) induced significant protection against subsequent infection with *Schistosoma mansoni* cercariae. When cholera toxin (CT) was used as an adjuvant in combination with SWAP or fraction A, no significant protection was observed. However, intradermal vaccination in combination with CT triggered a strong anti-SWAP antibody response and induced a strong delayed-type hypersensitivity response to schistosome antigens (SWAP or fraction A), one significantly higher than that in the SWAP-BCG group. In addition, vaccinating mice intranasally with SWAP or cercarial antigen together with CT as adjuvant failed to induce any significant protection. Surprisingly, mice given CT alone intranasally revealed a significantly enhanced worm burden. These findings suggest that mucosal application of CT may modulate the host-parasite relationship in favor of parasite survival.

Schistosomiasis mansoni is a helminth infection of humans causing a public health problem in the tropics, and development of an effective vaccine against this disease is a major challenge (14). Although high levels of protection (up to 70%) against experimental infection can be achieved after vaccination with irradiated cercariae (19), the general opinion is that a live vaccine would be impractical for use in humans (22). To date, one of the most promising vaccination procedures against schistosomiasis is intradermal injection of schistosome antigen together with *Mycobacterium bovis* BCG (bacillus Calmette-Guérin) (8, 12, 23). Among several potential vaccine antigen candidates of *Schistosoma mansoni*, a 28-kDa antigen and paramyosin (97-kDa antigen) appear to be promising (2, 23). Thus, the authors of the latter work found that mice vaccinated intradermally with schistosome paramyosin combined with BCG were significantly protected, and they suggested that the protection induced in this system was mediated by T-cell-dependent immune responses.

Several studies have demonstrated that cholera toxin (CT) greatly enhances local as well as systemic humoral immune responses when given by the oral or parenteral route in combination with the antigen material (5, 15, 16, 18, 21, 26). The results from in vitro studies also suggest that CT can affect several of the types of cells involved in an antigen-specific immune response (5, 7). In the present study, we have investigated the possible adjuvant effect of CT on protection against subsequent *S. mansoni* infection in mice vaccinated intradermally or intranasally with schistosome antigens. For comparison, intradermal vaccination was tried with BCG as adjuvant.

S. mansoni (Puerto Rican strain) adult worms and cercariae were collected as described elsewhere (1). Soluble extract of adult worms (SWAP) was prepared by freeze-thawing and homogenization in phosphate-buffered saline (PBS), pH 7.4, on ice with a glass homogenizer followed by centrifugation at 12,000 × *g* for 30 min and then by ultracentrifugation at 100,000 × *g* for 4 h. The supernatant was filtered through a 0.45-μm-pore-size membrane filter (Schleicher & Schuell, Dassel, Germany), and the protein concentration was measured according to Lowry et al. (17). The preparation was stored at −70°C until use.

For intranasal immunization, SWAP and cercarial antigen (CERC) were prepared by freeze-thawing and homogenization of adult worms and cercariae in distilled water on ice. The adult worm preparation was centrifuged at 40,000 × *g* for 30 min, and the protein content of the preparation was determined as described above. Both preparations were lyophilized and stored at +4°C until use. At the time of intranasal immunization, lyophilized SWAP was dissolved in PBS, and lyophilized cercarial antigen was suspended in PBS containing 0.1% Tween 20 (Sigma, St. Louis, Mo.).

Fraction A (FA) was prepared as described by Sher et al. (24) as the first peak fraction of SWAP isolated by size chromatography. Briefly, Sephacryl S-300 HR (Pharmacia LKB Biotechnology, Uppsala, Sweden) was packed into a column (2.6 by 70 cm) (Pharmacia). The column was then calibrated before fractionation of 5 to 6 ml of SWAP (6 to 9 mg/ml).

Purification of schistosome paramyosin was performed according to the salt extraction protocol described by Harris and Epstein (6). The concentration of paramyosin in the preparation used was 0.1 mg/ml.

Female C57BL/6 mice (ALAB, Sollentuna, Sweden) aged 6 to 10 weeks were shaved and vaccinated intradermally by injection in the thoracic region with 100 μl of schistosome antigen (1 mg of SWAP or 50 μg of FA) admixed with 5×10^6 CFU of BCG bacteria (Swedish strain, GB453) or 3 μg of CT (List Biological Laboratories, Inc., Campbell, Calif.). Control mice received only SWAP, FA, BCG, CT, or PBS. Two weeks after primary immunization, the mice were given a second intradermal injection, identical to the first. Four weeks later, the mice were challenged by percutaneous exposure with 100 to 150 *S. mansoni* cercariae. At 6 weeks after challenge, worm burden was assessed by perfusion from the portal vein as described by Smithers and Terry (25).

For intranasal vaccination, mice were anesthetized by inhalation of ether. The animals were then exposed by the intranasal route to 50-μl volumes of PBS containing SWAP (2 mg) or CERC (1.7×10^4 and 10^4 cercariae were used in experiments 5 and 6, respectively) combined with CT (10 μg)

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on days 1 and 14. Control mice received SWAP, CERC, CT, or PBS. The animals were then challenged by percutaneous exposure with 100 to 150 cercariae on day 15, day 18, or day 21. The worm burden was assessed at 6 weeks after the challenge infection.

The effect of immunization on worm burden was expressed as the quotient obtained after dividing numbers of worms recovered from immunized mice with those from nonimmunized control animals within the same experiment. The statistical significance of differences in mean worm burden was calculated by Student's t test.

For Western blot (immunoblot), SWAP prepared in the presence of 10 mM EDTA and purified paramyosin were diluted 1:4 with sample buffer containing 10% sodium dodecyl sulfate (SDS), 0.5 M Tris-HCl (pH 6.8), 5% 2-β-mercaptoethanol, 0.05% bromophenol, and 10% glycerol. After boiling for 4 min, the samples were electrophoretically separated by SDS-polyacrylamide gel electrophoresis on 7.5% slab gels. The separated fractions were transfected by blotting for 1 h onto nitrocellulose paper. After blocking with Tris-buffered saline, pH 7.6, containing 1% skim milk and 0.05% Tween 20, the strips were probed with antisera from the immunized mice. Bound antibodies were detected with peroxidase-conjugated rabbit anti-mouse immunoglobulin (Ig) (DAKOPATTS, Copenhagen, Denmark) followed by 0.003% H_2O_2 and 0.4 mg of diaminobenzidine per ml (Sigma) in Tris-buffered saline. Molecular weight markers (Bio-Rad Laboratories, Richmond, Calif.) were separated on the same gel.

For enzyme-linked immunosorbent assay (ELISA), microtiter plates (Nunc, Roskilde, Denmark) were coated by overnight incubation at 4°C with SWAP (100 μl per well, 5 μg/ml) in 0.1 M bicarbonate buffer (pH 9.6). After blocking with 1% bovine serum albumin (BSA) (Sigma) in PBS containing 0.05% Tween 20 (PBS-T), the plates were incubated overnight with serial dilutions of the sera to be analyzed. Then the plates were washed and incubated with heavy chain-specific peroxidase-conjugated goat anti-mouse IgG, IgG1, IgG2a, IgM, or IgA (Southern Biotechnology Associates, Inc., Birmingham, Ala.). IgE antibodies were detected by using biotin-conjugated anti-mouse IgE (The Binding Site, Birmingham, United Kingdom) and peroxidase-conjugated streptavidin (Southern Biotechnology). After washing with PBS-T, antibody binding was quantitated by the addition of 2,2′-azino-di-(3-ethylbenzthiazoline-sulfonic acid) (ABTS; Sigma) in acetate buffer containing H_2O_2. After 30 min of incubation, the A_{405} was measured by means of a multichannel spectrophotometer (Labsystems Multiskan PLUS). Results were expressed as the mean absorbance of duplicate wells after subtraction of the background. Serum antibody titers were calculated as the highest sample dilution yielding an ELISA reading twice that of the control (no sample added).

Skin test responses were assayed by injecting mice which had previously been immunized with SWAP-BCG, SWAP-CT, or SWAP alone with 25-μl volumes of PBS containing 100 μg of SWAP in the right footpad and with 25 μl of PBS alone in the left footpad. Mice vaccinated with FA-CT or FA alone were injected with 50 μg of FA in 25 μl of PBS. Footpad thickness was measured 0.5, 24, and 48 h after the injection with an Oditest spring calliper (Kröplin, Hessen, Germany). The responses were expressed as differences in thickness (in millimeters) between right and left footpads.

For assay of gamma interferon (IFN-γ), single cell suspensions were prepared by gently forcing spleen tissue, obtained from the immunized mice at 6 weeks after challenge infection, through a fine nylon net into Iscove's medium (GIBCO, Grand Island, N.Y.). After lysing erythrocytes by addition of Tris-buffered ammonium chloride solution, the cell suspensions were washed and the cells were suspended at 10^7 cells per ml in tissue culture medium (Iscove's medium supplemented with 2 mM L-glutamine, 50 μM 2-mercaptoethanol, 10 μg of gentamicin per ml, 24 mM L-asparagine, 55 mM L-arginine, and 10% heat-inactivated fetal calf serum). Incubation of cells was done with or without SWAP at 500 μg/ml in 24-well tissue culture plates (Nunc) at 37°C in an 8% CO_2 atmosphere. After 48 h, supernatants were harvested, clarified by centrifugation at 450 × g for 15 min at 4°C, and stored at −20°C until used. The amount of IFN-γ in the supernatants was assayed by two-site ELISA with immobilized anti-mouse IFN-γ (rat IgG1, no. 25001, Lee BioMolecular Research Laboratories, Inc., San Diego, Calif.) and a polyclonal rabbit anti-mouse IFN-γ (AMS Biotechnology, Burford, United Kingdom). Briefly, flexible polyvinyl chloride microtiter plates (Dynatech Laboratories, Inc., Chantilly, Va.) were coated with (per well) 100 μl of 2 μg of anti-mouse IFN-γ per ml in PBS and incubated overnight at 4°C. After blocking with 3% BSA in PBS, the plates were incubated with samples and recombinant mouse IFN-γ (Genzyme, Cambridge, Mass.) diluted in Iscove's medium with 10% fetal calf serum (50 μl per well). The plates were washed with PBS-T and incubated with 50 μl of a 1:1,000 dilution of polyclonal rabbit anti-mouse IFN-γ per well. After washing with PBS-T, incubation was performed with (per well) 75 μl of a 1:5,000 dilution of peroxidase-conjugated goat anti-rabbit Ig (Bio-Rad) in PBS-T containing 0.1% BSA. The plates were washed with PBS-T, and then sub-

TABLE 1. Effect of intradermal immunization on worm recovery[a]

Expt no.	Immunization	n	No. of worms recovered (mean ± SD)	% Protection	Statistical significance
1	SWAP-BCG	10	15.8 ± 12.6	55	$P < 0.02$
	SWAP-CT	10	22.7 ± 13	36	NS[b]
	FA-CT	10	34.5 ± 23		
	FA-BCG	10	30.7 ± 19.6		
	SWAP	10	33.5 ± 28		
	FA	9	35.5 ± 13.8		
	CT	10	33.7 ± 10		
	BCG	2	37 ± 7		
	PBS	6	35.3 ± 14.9		
2	SWAP-BCG	8	5.75 ± 6	73	$P < 0.01$
	SWAP	8	13.2 ± 7.9	38	NS
	BCG	9	29.8 ± 16.2		
	PBS	10	21.6 ± 11.4		
3	SWAP-CT	9	42.6 ± 7		
	FA-CT	10	59.5 ± 29.6		
	SWAP	11	45.3 ± 12		
	FA	11	45.4 ± 14		
	PBS	13	45.6 ± 21.6		
4	CT	8	42.7 ± 14.3		
	PBS	12	41.3 ± 32.1		

[a] Mice were immunized intradermally on days 0 and 14, and then challenge infection was performed on day 42 except for experiment 4, in which mice were challenged on day 15. Perfusion was performed 6 weeks after the challenge infection.

[b] NS, not significant at $P < 0.05$.

TABLE 2. Effect of intranasal immunization on worm recovery[a]

Expt no.	Immunization	n	No. of worms recovered (mean ± SD)	% Enhancement	% Protection	Statistical significance
5	SWAP-CT	5	25 ± 3.4			
	CERC-CT	6	23 ± 9.6			
	SWAP	10	23.5 ± 12.4			
	CERC	9	30.5 ± 11.4			
	CT	7	39.5 ± 20.5	83		$P < 0.05$
	PBS	10	21.6 ± 11.4			
6	SWAP-CT	4	49.6 ± 50.4		28.5	NS[b]
	CERC-CT	3	71.5 ± 10.6			
	SWAP	10	74.9 ± 24.3			
	CERC	9	71.3 ± 20.9			
	CT	6	87.4 ± 30.4	26		NS
	PBS	9	69.4 ± 9			
7	CT	10	70.7 ± 23.4	37.5		$P < 0.05$
	PBS	10	51.4 ± 13.7			
8	CT	8	72.6 ± 27	51		$P < 0.05$
	PBS	9	48 ± 18.5			

[a] Mice were exposed intranasally on days 1 and 14. Challenge infection was performed with 100 to 150 cercariae on day 15 (experiment 5), day 18 (experiment 7), and day 21 (experiments 6 and 8). Perfusion was performed 6 weeks after the challenge infection.
[b] NS, not significant at $P < 0.05$.

strate solution (100 μl per well) containing 1 mg of ABTS per ml in 44 mM Na_2HPO_4–28 mM citric acid–0.003% H_2O_2 was added. The A_{405} was measured with a reference of 490 nm on a multichannel spectrophotometer. The amount of IFN-γ in unknown samples was calculated by reference to a standard curve with known amounts of recombinant mouse IFN-γ.

As seen in Table 1, intradermal immunization by SWAP in combination with BCG induced a significant reduction of the worm burden in two separate experiments. No significant reduction was, however, observed for the mice immunized with SWAP, FA, BCG, or CT alone or in combination SWAP-CT, FA-CT, or FA-BCG. Table 2 shows that intranasal immunization with SWAP or CERC or these preparations in combination with CT did not significantly influence the worm burden of the mice challenged. An increase of the worm burden was, however, observed for mice pretreated with only CT, the enhancing effect being statistically significant in three out of four experiments.

Determination of the anti-SWAP antibody response in intradermally and intranasally immunized mice was performed by ELISA. As shown in Table 3, the analyses of sera obtained from mice intradermally immunized with SWAP or FA alone or in combination with BCG or CT 4 weeks after the second immunization dose revealed the presence of anti-SWAP antibodies of the IgG class, the titers of specific IgG1 antibodies being higher than those of IgG2a. The highest levels were observed where the combination with CT had been used. No anti-SWAP antibodies of the IgM class were observed except at low levels in mice immunized with SWAP or FA in combination with CT. Anti-SWAP antibodies of the IgE class were observed at low levels in mice immunized with SWAP alone or in combination with BCG or CT. As shown in Table 4, in reference to intranasally immunized mice, anti-SWAP antibodies of the IgG class were detected at a high level after immunization with SWAP alone or in combination with CT. In the CERC and CERC-CT groups, no IgG anti-SWAP antibodies were detected. Anti-SWAP antibodies of the IgG1 subclass were detected at high levels in mice immunized with SWAP-CT and at low levels in mice immunized with SWAP alone. Anti-SWAP antibodies of the IgG2a subclass were not detected except at low levels in mice immunized with SWAP alone. An IgM anti-SWAP response was observed in all groups of the mice immunized, the highest level being noted after treatment with SWAP in combination with CT. In none of the experimental groups were anti-SWAP antibodies of the IgA class detected.

The results of Western blot analyses, shown in Fig. 1 and 2, refer to sera obtained 4 weeks after the second intradermal immunization. Analyses by means of SWAP revealed in sera from the SWAP-BCG and SWAP-CT groups antibodies against several schistosome antigens in the range of 45 to 116 kDa. A notable difference between the groups was that in the latter antibodies against a 200-kDa (or higher) antigen were also demonstrated. Such antibodies were also found in sera from the FA-CT group. Antibodies against paramyosin (97 kDa) were detected in all groups of mice immunized with schistosome material with or without adjuvant, an exception being the FA-CT group.

Skin test response to schistosome antigens was performed

TABLE 3. Anti-SWAP antibody titers (log 10^{-1}) in intradermally immunized mice (experiment 1)[a]

Immunization	IgG	IgG1	IgG2a	IgM	IgE
SWAP-BCG	3.56	4.00	2.56	<2.30	2.35
SWAP-CT	4.56	5.02	3.02	2.48	2.15
FA-CT	4.46	5.27	3.18	2.40	<2.00
FA-BCG	3.06	3.59	<2.00	<2.30	<2.00
SWAP	3.30	3.68	2.45	<2.30	2.31
FA	2.48	2.89	<2.00	<2.30	<2.00
CT	<2.30	<2.00	<2.00	<2.30	<2.00
BCG	<2.30	<2.00	<2.00	<2.30	<2.00
PBS	<2.30	<2.00	<2.00	<2.30	<2.00

[a] Mice were bled 4 weeks after the second immunization (1 day before cercarial challenge). Sera were pooled from 10 mice. The results of one representative experiment of two are shown.

TABLE 4. Anti-SWAP antibody titers (log 10^{-1}) in intranasally immunized mice (experiment 6)[a]

Immunization	IgG	IgG1	IgG2a	IgM	IgE
SWAP-CT	3.78	4.45	<2.00	3.63	2.09
CERC-CT	<2.00	<2.00	<2.00	2.92	<2.00
SWAP	3.32	2.31	2.28	2.76	<2.00
CERC	<2.00	<2.00	<2.00	2.65	<2.00
CT	<2.00	<2.00	<2.00	2.49	<2.00
PBS	<2.00	<2.00	<2.00	<2.30	<2.00

[a] Mice were bled 6 days after the second immunization (1 day before cercarial challenge). Sera were pooled from at least five mice. The results of one representative experiment of two are shown.

4 weeks after the second immunization (before challenge infection), and the results are illustrated in Fig. 3 and 4. In mice immunized with SWAP-BCG, SWAP-CT, or FA-CT, a significant 24-h delayed-type hypersensitivity (DTH) response was elicited after challenge with SWAP and FA. In mice immunized as described above but without adjuvant, the 24-h DTH responses were weaker. The comparison of DTH responses as influenced by CT or BCG showed a stronger response when the former adjuvant was used. From the results concerning the 30-min skin responses, it can be noted that in mice immunized with SWAP-CT and FA-CT marked reactions were elicited by SWAP and FA, respectively.

The IFN-γ production elicited through the stimulation by SWAP was measured by ELISA at 6 weeks after challenge infection (Fig. 5). High levels of IFN-γ were detected in cultures of splenocytes isolated from mice previously immunized intradermally with schistosome antigens in combination with adjuvant BCG or CT. It should be mentioned that a considerable production of IFN-γ was also observed in the group of mice treated only with CT.

Analysis of the effector mechanisms involved in protection in the mouse model pointed to the significance of T-cell-mediated immunity (9). Antibody-dependent cell-mediated cytotoxicity, on the other hand, was not considered to play any role in mice in contrast to rats or to humans (3, 4). In the present study, using a mouse model, we have examined the possible adjuvant effect of CT combined with

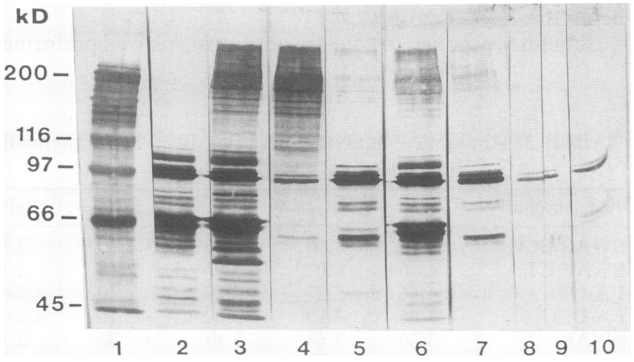

FIG. 1. Western blot analysis by means of SWAP (experiment 1). Results are for sera obtained at the time of challenge infection. All serum samples analyzed were pooled from 10 mice intradermally immunized. Lanes: 1, protein markers; 2 to 10, sera from mice immunized with SWAP-BCG, SWAP-CT, FA-CT, FA-BCG, SWAP, FA, CT alone, and BCG alone and from nonimmunized mice, respectively.

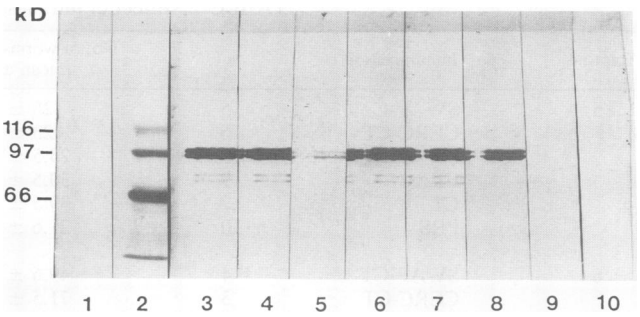

FIG. 2. Western blot analysis by means of purified paramyosin (experiment 1). Results are for sera obtained at the time of challenge infection. All serum samples analyzed were pooled from 10 mice intradermally immunized. Lanes: 1, nonimmunized mice; 2, protein markers; 3 to 10, sera from mice immunized with SWAP-BCG, SWAP-CT, FA-CT, FA-BCG, SWAP, FA, CT alone, and BCG alone, respectively.

schistosome antigens with regard to influence on worm burden. In order to relate protection to possible immune effector mechanisms induced by vaccination, cell-mediated immunity and humoral immunity were assessed at the time of challenge infection.

We observed that intradermal immunization of mice with adult worm antigen in combination with BCG induced significant protection, which is in accord with the results of others (11, 12, 23, 24). The results of these authors showed that the adjuvant effect of BCG of increasing protection against *S. mansoni* infection varied somewhat between different strains of BCG (11), and our study shows that administration of the Swedish BCG strain (GB453) displayed a significant adjuvant effect. It should be noted that no

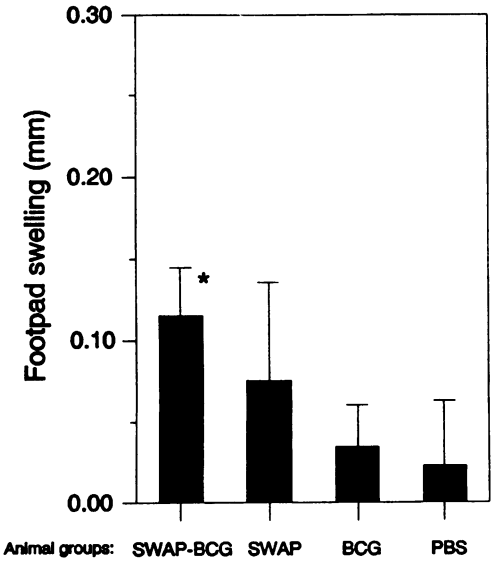

FIG. 3. Twenty-four-hour SWAP skin test response of mice immunized intradermally with SWAP with or without BCG (experiment 2). Immunized mice were tested by footpad injection of SWAP 4 weeks after the second immunization (see text). The results shown are means and standard deviations (bars) of measurements from four mice. The results of one representative experiment of two are shown. The value significantly different ($P < 0.01$) from those of the BCG control mice is indicated by an asterisk.

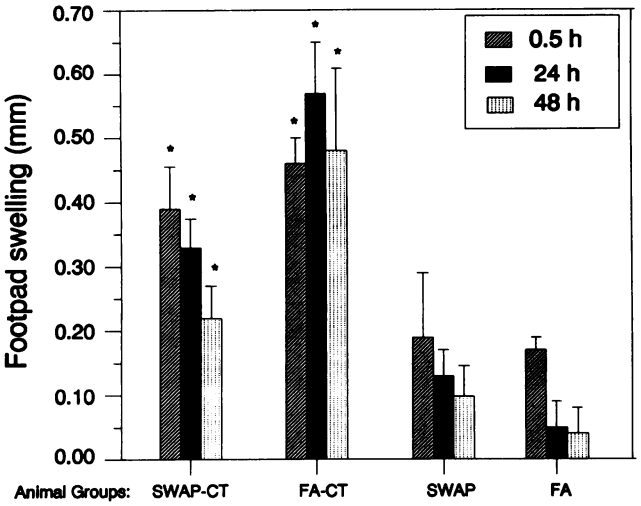

FIG. 4. Skin test responses of mice immunized intradermally with SWAP or FA with or without CT (experiment 1). Immunized mice were tested by footpad injection of SWAP or FA 4 weeks after the second immunization (see text). Footpad thickness was measured 0.5, 24, and 48 h later. The 24-h DTH responses to FA were 0.035 ± 0.02 mm and 0.026 ± 0.03 mm in the CT and nonimmunized groups, respectively. The results shown are means and standard deviations (bars) of measurements from five mice. The results of one representative experiment of two are shown. The values significantly different ($P < 0.01$) from those of SWAP- or FA-immunized mice are indicated by asterisks.

protection was observed in mice vaccinated intradermally with SWAP or FA in combination with the potential adjuvant CT. The findings of Sher et al. (24) that intradermal vaccination of mice with FA in combination with BCG induced significant protection could not be reproduced in our study. The reason for this failure in induction of protection may be the low dose of the antigen administered. It should be mentioned, however, that the dose of FA used in combination with CT was sufficient to induce strong antibody as well as DTH responses.

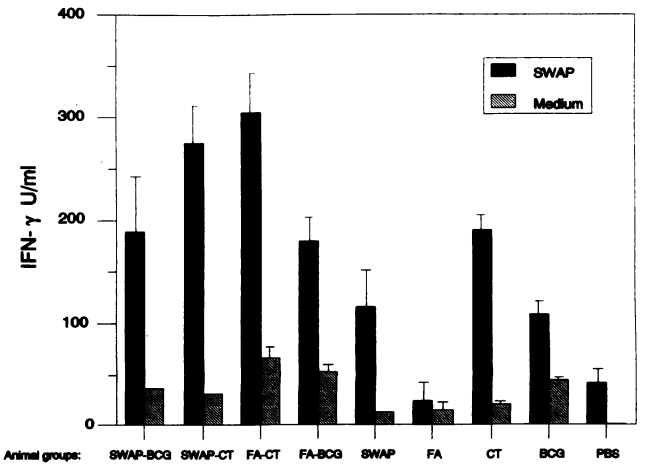

FIG. 5. IFN-γ production by splenocytes from immunized mice (experiment 1). Pooled splenocytes from the immunized mice were incubated alone (medium) or with SWAP. The results are given as mean units per milliliter ± standard deviation of duplicate samples analyzed in four different dilutions. The mice were analyzed in groups of five, and the results of one representative experiment of two are shown.

We also employed intranasal administration of antigen, since the lungs may represent an important site of elimination of schistosomulae (28). In our study, however, intranasal immunization of mice with adult worm antigen or CERC in combination with CT had no significant effect on worm burden. Similar findings were reported by Mitchell et al. (20), who found that intranasal administration of crude adult worm homogenates of either adult male or adult female schistosomes in combination with *Bordetella pertussis* did not induce protection. It should be added that the dose of CT used by us is sufficient to induce adjuvant effects in the murine mucosal immune responses (18, 21). An interesting observation is the enhanced worm burden that we observed in three out of four experiments after intranasal administration of CT alone. It should be mentioned, however, that in one experiment enhancement of the worm burden observed was not significant, a possible explanation being the high worm burden of the control group in this particular experiment.

No correlation was observed between anti-SWAP antibody levels and the levels of worm burden in intradermally or intranasally immunized mice. No antibody specificities as indicated by the immunoblotting analyses were found to be characteristic for mice displaying protection. It should be noted that SWAP or FA in combination with CT but not with BCG induced production of antibodies against 200-kDa (or greater) antigens of SWAP, indicating that humoral immune responses to this group of antigens are not related to protection. Our findings are in agreement with those of James and Pearce (11), who showed that there was no consistent correlation between humoral reactivity and protection in mice immunized with BCG, *B. pertussis*, or saponin in combination with adult worm antigen or frozen and thawed larvae. It is of interest to note that these authors also showed that protection can be transferred to naive recipients with B-cell-depleted spleen cell populations, but not with sera from intradermally immunized animals (13). Our study on the immunization effects of SWAP-CT or FA-CT also revealed that this procedure induced strong immediate hypersensitivity responses as measured by footpad swelling 30 min after injection of SWAP or FA (Fig. 4). Since such immediate hypersensitivity responses largely depend on specific IgE antibodies, the present findings do not indicate a protective role for such antibodies in the mouse model employed.

James and colleagues (9–11) showed that induction of resistance to challenge infection in mice immunized intradermally with schistosome antigen in combination with adjuvant correlated with stimulation of antigen-specific cell-mediated immunity including DTH response, IFN-γ production, and macrophage activation. It was also shown that T cells and lymphokine-activated macrophages play an important role in the effector phase of immunity (11). They also showed that P-strain mice, displaying deficient macrophage function, failed to demonstrate resistance following intradermal immunization with dead larvae in combination with BCG (10). On the other hand, Vignali et al. (27) found that mice vaccinated with irradiated cercariae and subsequently whole-body irradiated displayed significant protection despite abrogation of the DTH response to schistosome antigen. In the present study, intradermal immunization of mice with SWAP or FA in combination with CT induced strong DTH responses which were significantly higher than those in the protected SWAP-BCG group. It should be noted, how-

ever, that the DTH response at the time of cercarial challenge in this study was evaluated as footpad swelling 24 and 48 h after injection of schistosome antigen. Because of the experimental design, IFN-γ production by splenocytes was not determined until 6 weeks after infection. These findings, however, paralleled the DTH responses as evaluated by footpad swelling. Further studies, including, e.g., determination of IFN-γ production at or around the time of challenge infection, analysis of macrophage activation, and depletion of T-cell populations, are clearly needed in order to establish more firmly the suggested relationship in mice between cell-mediated immune mechanisms and protection against schistosome infection. Such experiments are in progress.

Our observation of an increase of worm burden after administration of CT alone indicates that this phenomenon seems to be mediated through the lung mucosal system since intranasal but not intradermal administration resulted in a significant increase of worm burden. No increase of worm burden was noted when CT-SWAP was administered. These observations suggest that CT may modulate immune responses against the parasite in different ways.

The results presented in our study substantiate the idea that partial protection can be achieved in mice after intradermal immunization with adult worm antigen in combination with BCG. However, when BCG as adjuvant was replaced by CT, no protection could be demonstrated despite induction of a strong DTH response as evaluated by 24- and 48-h footpad swelling and of a strong antibody response to schistosome antigen. Of particular interest is the finding that mice intranasally exposed only to CT displayed an enhanced worm burden. This finding needs further investigation.

This study was supported by grants from the Swedish Agency for Research Cooperation with Developing Countries (SAREC); the Ellen, Walter, and Lennart Hesselman Foundation; and the Faculty of Medicine, University of Göteborg, Göteborg, Sweden.

REFERENCES

1. **Akhiani, A. A., L.-Å. Nilsson, and Ö. Ouchterlony.** 1991. Comparative antigen analysis of different life stages of *Schistosoma mansoni* by crossed immunoelectrophoresis. Int. Arch. Allergy Appl. Immunol. **95:**266–272.
2. **Balloul, J. M., J. M. Grzych, R. J. Pierce, and A. Capron.** 1987. A purified 28,000 daltons protein from *S. mansoni* adult worms protects rats and mice against experimental schistosomiasis. J. Immunol. **138:**3448–3453.
3. **Capron, A., and J. P. Dessaint.** 1985. Effector and regulatory mechanisms in immunity to schistosomes. A heuristic view. Annu. Rev. Immunol. **3:**455–476.
4. **Capron, A., J. P. Dessaint, M. Capron, and R. J. Pierce.** 1992. Vaccine strategies against schistosomiasis. Immunobiology **184:** 282–294.
5. **Elson, C. O., and W. Ealding.** 1984. Generalized systemic and mucosal immunity in mice after mucosal stimulation with cholera toxin. J. Immunol. **132:**2736–2741.
6. **Harris, H. E., and H. F. Epstein.** 1977. Myosin and paramyosin of *Caenorhabditis elegans*: biochemical and structural properties of wild-type and mutant proteins. Cell **10:**709–719.
7. **Hörnqvist, E., and N. Lycke.** 1993. Cholera toxin adjuvant greatly promotes antigen priming of T cells. Eur. J. Immunol., **23:**2136–2143.
8. **James, S. L.** 1985. Induction of protective immunity against *Schistosoma mansoni* by a nonliving vaccine is dependent on the method of antigen presentation. J. Immunol. **134:**1956–1960.
9. **James, S. L.** 1986. Induction of protective immunity against *Schistosoma mansoni* by a nonliving vaccine. III. Correlation of resistance with induction of activated larvacidal macrophages. J. Immunol. **136:**3872–3877.
10. **James, S. L., and L. A. DeBlois.** 1986. Induction of protective immunity against *Schistosoma mansoni* by a non-living antigen. II. Response of mouse strains with selective immune defects. J. Immunol. **136:**3864–3871.
11. **James, S. L., and E. J. Pearce.** 1988. The influence of adjuvant on induction of protective immunity by a non-living vaccine against schistosomiasis. J. Immunol. **140:**2753–2759.
12. **James, S. L., E. J. Pearce, and A. Sher.** 1985. Induction of protective immunity against *Schistosoma mansoni* by a nonliving vaccine. I. Partial characterization of antigens recognized by antibodies from mice immunized with soluble schistosome extracts. J. Immunol. **134:**3432–3438.
13. **James, S. L., and A. Sher.** 1990. Cell-mediated immune response to schistosomiasis. Curr. Top. Microbiol. Immunol. **155:**21–31.
14. **Kolata, G.** 1985. Avoiding the schistosome's tricks. Science **227:**285–287.
15. **Liang, X., M. E. Lamm, and J. G. Nedrud.** 1988. Oral administration of cholera toxin-Sendai virus conjugate potentiates gut and respiratory immunity against Sendai virus. J. Immunol. **141:**1495–1501.
16. **Lindholm, L., J. Holmgren, S. Lange, and I. Lönnroth.** 1976. Interaction of cholera toxin and toxin derivatives with lymphocytes. Int. Arch. Allergy Appl. Immunol. **50:**555–573.
17. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
18. **Lycke, N., and J. Holmgren.** 1986. Strong adjuvant properties of cholera toxin on gut mucosal immune responses to orally presented antigens. Immunology **59:**301–308.
19. **Minard, P., D. A. Dean, R. H. Jacobsen, W. E. Vannier, and K. D. Murrell.** 1978. Immunization of mice with cobalt-60 irradiated *Schistosoma mansoni* cercariae. Am. J. Trop. Med. Hyg. **27:**76–86.
20. **Mitchell, G. F., K. M. Davern, S. M. Wood, M. D. Wright, V. P. Argyropoulos, K. S. McLeod, W. U. Tiu, and E. G. Garcia.** 1990. Attempts to induce resistance in mice to *Schistosoma japonicum* and *Schistosoma mansoni* by exposure to crude schistosome antigens plus cloned glutathione-S-transferases. Immunol. Cell Biol. **68:**377–385.
21. **Nedrud, J. G., X. Liang, N. Hague, and M. E. Lamm.** 1987. Combined oral/nasal immunization protects mice from Sendai virus infection. J. Immunol. **139:**3484–3492.
22. **Pearce, E. J., S. L. James, J. Dalton, A. Barrall, C. Ramos, M. Strand, and A. Sher.** 1986. Immunochemical characterization and purification of Sm-97, a *Schistosoma mansoni* antigen monospecifically recognized by antibodies from mice protectively immunized with a nonliving vaccine. J. Immunol. **137:** 3593–3600.
23. **Pearce, E. J., S. L. James, S. Hieny, D. E. Lanar, and A. Sher.** 1988. Induction of protective immunity against *Schistosoma mansoni* by vaccination with schistosome paramyosin (sm97), a nonsurface parasite antigen. Proc. Natl. Acad. Sci. USA **85:** 5678–5682.
24. **Sher, A., E. Pearce, S. Hieny, and S. James.** 1986. Induction of protective immunity against *Schistosoma mansoni* by a nonliving vaccine. IV. Fractionation and antigenic properties of a soluble adult worm immunoprophylactic activity. J. Immunol. **136:**3878–3883.
25. **Smithers, S. R., and R. J. Terry.** 1965. The infection of laboratory hosts with cercariae of *Schistosoma mansoni* and the recovery of the adult worms. Parasitology **55:**695–700.
26. **Tamura, S., H. Kurata, H. Funato, T. Nagamine, C. Aizawa, and T. Kurata.** 1989. Protection against influenza virus infection by a two-dose regimen of nasal vaccination using vaccines combined with cholera toxin B subunit. Vaccine **7:**314–320.
27. **Vignali, D. A. A., Q. D. Bickle, and M. G. Taylor.** 1988. Studies on immunity to *Schistosoma mansoni* in vivo: whole-body irradiation has no effect on vaccine-induced resistance in mice. Parasitology **96:**49–61.
28. **Wilson, R. A., P. S. Coulson, and B. Dixon.** 1986. Migration of the schistosomula of *Schistosoma mansoni* in mice vaccinated with radiation-attenuated cercariae, and normal mice: an attempt to identify the timing and site of parasite death. Parasitology **92:**101–116.