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Macrophages as Effector Cells of Protective Immunity in Murine Schistosomiasis: Macrophage Activation in Mice Vaccinated with Radiation-Attenuated Cercariae

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Cell-mediated immune responses contributing to macrophage activation were compared in mice that demonstrated partial resistance to challenge *Schistosoma mansoni* infection as a result of vaccination with radiation-attenuated cercariae or of ongoing low-grade primary infection. Vaccinated mice developed significant delayed hypersensitivity reactions to soluble schistosome antigens in vivo. Splenocytes from vaccinated animals responded to in vitro culture with various specific antigens (soluble adult worm extract, living or disrupted schistosomula) by proliferation and production of macrophage-activating lymphokines as did lymphocytes from *S. mansoni*-infected animals. Macrophage-activating factors produced by spleen cells from vaccinated mice upon specific antigen stimulation eluted as a single peak on Sephadex G-100 with a molecular weight of approximately 50,000 and contained gamma interferon activity. Moreover, peritoneal macrophages with larvicidal and tumoricidal activity were recovered from vaccinated mice after intraperitoneal challenge with soluble schistosome antigens, a procedure also observed to elicit activated macrophages in *S. mansoni*-infected animals. These observations demonstrate that vaccination with irradiated cercariae stimulates many of the same cellular responses observed after primary *S. mansoni* infection, and suggest that lymphokine-activated macrophages may participate in the effector mechanism of vaccine-induced and concomitant immunity to challenge schistosome infection. This is the first demonstration of a potential immune effector mechanism in the irradiated vaccine model.

There are two methods of specific immunization against *Schistosoma mansoni* infection that reproducibly induce significant levels of resistance to challenge parasites. Both of these involve primary exposure to living infective larvae.

Primary infection of mice with low numbers of *S. mansoni* cercariae results in partial protection against homologous challenge infection (33). In this model of resistance, which has been termed concomitant immunity (33), there is evidence for the participation of both antibody and various types of effector cells (reviewed in reference 32). Recently, it has been observed that *S. mansoni*-infected mice develop activated macrophages capable of killing both nonspecific (tumor cell) and specific (schistosomula) extracellular targets in vitro (10, 14, 17). T lymphocytes from infected animals responded to stimulation with schistosome antigens by production of lymphokines (LK) that showed chemotactic-chemokinetic activity for macrophages in vitro and could activate control macrophages from uninfected mice to kill tumor cell and schistosomulum targets (9, 12). Moreover, strains of inbred mice with inherent defects in macrophage activation failed to produce larvicidal macrophages as a result of *S. mansoni* infection and showed impaired concomitant immunity in vivo (15). These observations suggest that macrophage activation is involved in the effector mechanism of concomitant immunity. However, there is growing evidence that a portion of the resistance to challenge infection shown by chronically infected mice may be due to pathology and circulatory changes resulting from cumulative deposition of eggs in the host tissues (3). The concomitant immunity model appears, therefore, to be a complex system in which both specific immune responses and nonspecific pathology contribute to the inability of challenge cercariae to survive. These observations have complicated the analysis of immune effector mechanisms of resistance in the concomitant immunity model.

Vaccination of mice by percutaneous exposure to *S. mansoni* cercariae that have been attenuated by high-dose gamma irradiation also confers partial protection against challenge infection with *S. mansoni* (19). These irradiated cercariae do not mature into egg-laying adults, so that in this model resistance develops in the absence of egg-related pathology (20). Although the mechanism of vaccine-induced resistance is unknown, it appears to have an immunological basis, since congenitally athymic and μ-suppressed mice are not protected against challenge infection by vaccination (30). Both B and T lymphocytes could potentially contribute to protection; exposure to irradiated cercariae elicits antilarval antibody production and sensitizes T lymphocytes to proliferate and produce chemotactic factors in response to in vitro challenge with schistosomulum antigens (8). Moreover, challenge infection elicits strong anamnestic cellular and humoral responses in vaccinated mice (R. Correa-Oliveira, A. Sher, and S. L. James, Am. J. Trop. Med. Hyg., in press).

The level of resistance achieved by vaccination also varies in different strains of mice (13, 21). Interestingly, in a recent survey of several inbred strains of mice, animals with genetic defects in macrophage activation (A/J and P/J [1, 2]) were found to develop little or no vaccine-induced immunity (13). This in vivo parallel with the concomitant immunity model

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again suggests a central role for macrophages in the development of resistance to schistosome infection. It was therefore of interest to examine macrophage function in vaccinated mice. In the current study, we have analyzed the development of cell-mediated immune responses leading to macrophage activation in vivo and in vitro in C57BL/6J mice, a strain that exhibits very high levels of vaccine-induced resistance to challenge *S. mansoni* infection, and we have compared the reactivity of macrophages from vaccinated mice to cells from animals with primary *S. mansoni* infection. The similarities observed suggest a possible common underlying immune mechanism of resistance in the two models, involving delayed hypersensitivity response.

MATERIALS AND METHODS

Laboratory animals and parasites. Female C57BL/6 mice were obtained from the Jackson Laboratories (Bar Harbor, Maine) at 6 to 8 weeks of age. C3H/HeN mice were obtained from the Division of Research Services, National Institutes of Health, Bethesda, Md.

S. mansoni cercariae (NMRI strain) were provided by the Biomedical Research Institute (Rockville, Md.). Skin-stage or mechanically transformed schistosomula were prepared as described previously (5, 34). A soluble adult worm antigenic preparation (SWAP) was made by homogenizing adult *S. mansoni* worms in phosphate-buffered saline (PBS) and retaining the water-soluble portion after ultracentrifugation (7). The protein content of SWAP was determined by the method of Lowry et al. (18).

Immunization. Mice were vaccinated by exposure of tail skin to cercariae that had been attenuated by receiving 50 kilorads of gamma irradiation from a ^{60}Co source by the method of Minard et al. (19). When challenged with viable cercariae at 4 to 6 weeks after vaccination, C57BL/6J mice routinely show 50 to 70% protection (8, 30). In some experiments, mice were given a primary *S. mansoni* infection by injection of 35 to 50 cercariae subcutaneously on the back (24). C57BL/6J mice challenged at 6 weeks after such a primary infection showed approximately 30% protection (15).

Measurement of delayed hypersensitivity response to SWAP. Vaccinated or control mice were injected intradermally with 25 μl of PBS in the left hind foot or 25 μl of PBS containing 100 μg of SWAP in the right hind foot. At 24 h after antigen challenge, the footpad size was measured with a dial micrometer, and the reaction is reported as the difference in swelling between right and left feet.

Measurement of cell-mediated immune response in vitro. (i) Lymphocyte proliferation. Splenic leukocytes were cultured at 10^6 cells per well with various schistosome antigens in a flat-bottomed microtiter plate in a total volume of 0.2 ml of RPMI 1640 (Biofluids, Rockville, Md.) containing 5% heat-inactivated fetal bovine serum (Sterile Systems, Inc., Logan, Utah), 0.3 mg of L-glutamine per ml, 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer, and 50 μg of gentamicin per ml. SWAP antigen was utilized at a final concentration of 250 μg/ml. Living schistosomula were included at 200 per well, and disrupted schistosomula (killed by one cycle of freezing and thawing) were used at 500 per well. [^{3}H]thymidine (0.5 μCi per well) was added at the end of day 4 of culture in assays measuring response to SWAP and at the end of day 2 in assays involving larvae. These times were shown to be optimal for these antigens in previous studies (8). After 16 h, the contents of the wells were harvested, and the counts incorporated into water-insoluble material were quantitated. Results are presented as stimulation index, representing the mean number of counts incorporated by spleen cells in the presence of antigen divided by the counts incorporated in the absence of antigen.

(ii) Production of LK-containing supernatant fluids. LK were generated from *Mycobacterium bovis* BCG-immune spleen cells by purified protein derivative of tuberculin (PPD) stimulation in vitro as previously described (25). Schistosome-related LK were prepared by in vitro stimulation of 20 × 10^6 spleen cells from vaccinated or *S. mansoni*-infected mice with (i) 1,000 μg of SWAP protein, (ii) 4,000 living skin-stage schistosomula, or (iii) 10,000 dead schistosomula (killed by one cycle of freezing and thawing) in a total volume of 2 ml of RPMI medium in wells of Costar Cluster[24] tissue culture plates (Costar, Cambridge, Massachusetts) and harvested after 48 h of incubation.

LK from coculture of spleen cells from vaccinated mice with SWAP were fractionated over Sephadex G-100 as previously described (11, 16). Cytochrome *c*, chymotrypsin, ovalbumin, albumin, and immunoglobulin G were utilized as molecular weight standards.

Interferon assays were initially performed by Meloy Laboratories (Springfield, Va.). The levels of alpha and gamma interferon were quantified by comparing the ability of original supernatant fluids and acid-treated (pH 2) supernatant fluids to inhibit vesicular stomatitis virus plaque formation on L929 cells, defining the titer as the reciprocal of the log dilution resulting in 50% reduction of viral cytopathic effect. Interferon titers of the Sephadex G-100 and anti-interferon eluates were determined as previously described (31). Murine recombinant gamma interferon was provided by Genentech Inc., South San Francisco, Calif.

Rabbit anti-mouse gamma interferon was generously provided by Howard M. Johnson (University of Texas Medical Branch, Galveston) (4, 23). An immunoglobulin fraction of the antiserum, prepared by ammonium sulfate precipitation, was equilibrated with columns of protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, N.J.). Control protein A columns were prepared likewise with the immunoglobulin fraction of normal rabbit serum. After the columns were washed, 1-ml samples of macrophage-activating mouse LK were added to the columns. Eluates were quantitatively collected and assayed for gamma interferon and macrophage activation.

Macrophage-mediated cytotoxicity assays. Peritoneal cells (PC) were collected from *S. mansoni*-infected or vaccinated mice or from uninfected mice that were untreated (resident cells) or that had been injected intraperitoneally 4 days previously with 1 ml of 1.2% sodium caseinate (Difco Laboratories, Detroit, Mich.) or 16 h previously with 250 μg of SWAP in 1 ml of PBS. Cells were harvested by peritoneal lavage with cold Dulbecco modified Eagle medium (GIBCO Laboratories, Grand Island, N.Y.) containing 5% fetal bovine serum and 5 U of heparin (Abbott Laboratories, North Chicago, Ill.) per ml.

Total leukocyte numbers were determined by hemocytometer counts, and differential cell counts were made on Wright-stained cell smears (10). Casein-elicited cells consisted of approximately 70% macrophages and 30% lymphocytes. SWAP injection resulted in cell populations containing 40 to 50% macrophages and 15 to 30% lymphocytes in uninfected or 6-week-vaccinated mice. The remainder of the cells recovered after SWAP injection were mostly neutrophils (25 to 30%). Vaccinated mice developed a mean of 11 ± 6% (standard deviation) eosinophilia at 16 h after SWAP injection, and control animals showed 2 ± 1% eosinophils under the same conditions.

TABLE 1. Delayed hypersensitivity response to SWAP in vaccinated C57BL/6J mice

Expt	24-h footpad response (mm × 10^{-2})		
	Control mice[a]	Vaccinated mice[b]	P
1	9 ± 2 (5)	43 ± 15 (5)	<0.005
2	4 ± 4 (3)	28 ± 9 (3)	<0.02
3	3 ± 3 (3)	29 ± 4 (3)	<0.001

[a] Nonimmunized C57BL/6J mice were injected with 25 μl of PBS in the left hind foot or 25 μl of PBS containing 100 μg of SWAP in the right hind foot. Values represent mean difference in footpad thickness at 24 h between right and left feet ± standard deviation. Values within parentheses represent the numbers of mice per group.

[b] C57BL/6J mice were vaccinated by percutaneous exposure to 500 irradiated cercariae 4 weeks (experiment 1), 6 weeks (experiment 2), or 8 weeks (experiment 3) before testing. Statistical comparisons were made by the Student *t* test.

Macrophage-mediated larvicidal and tumoricidal assays have been described in detail previously (10, 14). PC (8×10^5 macrophages per 0.5 ml) were added to 16-mm Cluster[24] culture wells and incubated for 2 h at 37°C in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum, 1% L-glutamine, and 50 μg of gentamicin per ml for 48 h at 37°C. Nonadherent PC were removed by washing with cold medium. To measure macrophage-mediated tumor cytotoxicity, [^{3}H]thymidine-labeled L929 tumor cells were added to culture wells with adherent PC at 4×10^4 target cells per well. Labeled tumor cell monolayers digested with 0.5% sodium dodecyl sulfate in water were used to estimate total incorporated counts. Cytotoxicity was determined by measuring release of incorporated [^{3}H]thymidine from prelabeled tumor cells in triplicate cultures at 48 h and expressed as the percentage of sodium dodecyl sulfate total counts (25).

To measure larvicidal activity, 80 schistosomula were added to each well. In some experiments, the larvae had previously been incubated for 45 min at 37°C in 5 ml of a 20% solution of heat-inactivated (56°C, 45 min) normal mouse serum, serum from C57BL/6J mice vaccinated 6 weeks previously, or serum from outbred mice infected with 35 cercariae 12 weeks previously. After 48 h of incubation, macrophage-mediated larval death was evaluated microscopically by the criteria of loss of motility, granularity, and loss of internal structure. Cytotoxicity was expressed as the mean percent dead larvae in duplicate cultures.

In experiments involving in vitro activation, cells were either exposed to LK-containing supernatant fluids for the entire 48-h incubation period with targets or were pretreated with LK for 5 h and then washed three times before the addition of targets.

RESULTS

Specific delayed hypersensitivity response to schistosome antigen by vaccinated mice in vivo. To determine whether vaccination with irradiated cercariae sensitizes T_{DH} cells to recognize challenge schistosome antigens in vivo, 24-h hypersensitivity response to SWAP was measured in mice that had been exposed to attenuated cercariae 4 to 8 weeks previously. After intradermal injection with 100 μg of SWAP, vaccinated animals showed significant ($P < 0.02$) delayed footpad swelling as compared with similarly treated control mice (Table 1). The degree of 24 h response by vaccinated mice was comparable to that demonstrated by infected animals (23 ± 9 in experiment 3; data not shown).

In vitro reactivity of T cells from vaccinated mice toward schistosome antigens. Spleen cells from vaccinated mice responded to various schistosome antigens (SWAP, disrupted or living larvae) in in vitro lymphocyte proliferation assays by increased uptake of [^{3}H]thymidine (Table 2). Moreover, spleen cells from immunized mice produced macrophage-activating lymphokines upon in vitro exposure to these same antigens, as measured by the ability of culture supernatant fluids to activate control macrophages to kill schistosomula in an in vitro assay (Table 2). Control spleen cells showed no reactivity in the same experiments. Both proliferation and LK production in response to SWAP and schistosomulum antigens have been shown to be T cell dependent in previous studies utilizing lymphocytes from *S. mansoni*-infected mice (7, 9, 12).

Characterization of macrophage-activating LK produced by vaccinated mice. The reactivity of macrophage-activating LK produced by spleen cells from vaccinated C57BL/6J mice in response to SWAP stimulation was compared with that of LK prepared from spleen cells of *S. mansoni*-infected mice under the same conditions (Fig. 1). The ability of both types of LK to activate control macrophages to lyse tumor cells or to kill skin-stage schistosomula in vitro was titrated by

TABLE 2. In vitro responsiveness of spleen cells from vaccinated mice to schistosome antigens

Source of spleen cells[a]	Antigen[b]	Lymphocyte proliferation[c] (SI)	P	LK production[d] (% larvicidal activity)	P
Control mice	SWAP	1.1 ± 0.2		2 ± 1	
	Live schistosomula	1.0 ± 0.3		3 ± 3	
	F-T schistosomula	1.4 ± 0.9		5 ± 5	
Vaccinated mice	SWAP	4.8 ± 1.6	<0.02	80 ± 28	<0.05
	Live schistosomula	3.8 ± 1.0	<0.05	30 ± 2	<0.005
	F-T schistosomula	6.3 ± 0.6	<0.005	80 ± 29	<0.05

[a] Spleen cells were obtained from C57BL/6J mice 4 to 6 weeks after vaccination with irradiated cercariae or from age-matched control mice.

[b] Splenocytes were cultured with SWAP, live skin-stage schistosomula or larvae that had been disrupted by one cycle of freezing and thawing (F-T).

[c] Lymphocyte proliferation was measured by uptake of [^{3}H]thymidine and is expressed as the mean stimulation index (SI), obtained by dividing the number of counts incorporated by cells in the presence of antigen by the number incorporated in the absence of antigen ± standard deviation in three experiments. Comparisons with control values were made by the Student *t* test.

[d] LK production was measured as the ability of culture supernatant fluids to activate casein-elicited macrophages to kill schistosome larvae in vitro and is expressed as mean percent dead larvae ± standard deviation in two experiments.

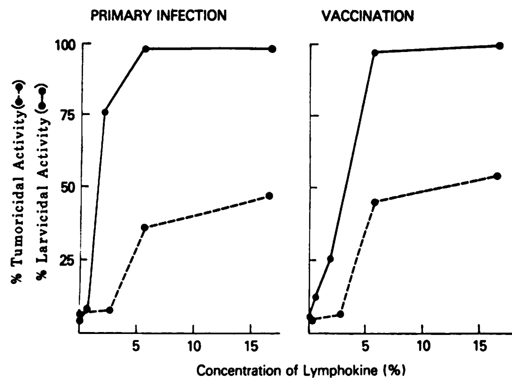

FIG. 1. Comparison of the reactivity of LK prepared by SWAP stimulation of spleen cells from C57BL/6J mice that had been infected or vaccinated 6 weeks previously. The ability of various concentrations of LK to activate resident peritoneal macrophages from uninfected C3H/HeN mice was assayed as enhanced lysis of [^{3}H]thymidine-labeled L929 cells (tumoricidal activity) or killing of skin-stage schistosomula (larvicidal activity). Macrophages were treated with LK for 5 h and then washed before the addition of targets.

exposing macrophages from uninfected mice to dilutions of lymphocyte culture supernatant fluids. As shown in a representative experiment (Fig. 1), the patterns of activation for tumor cell and larval cytotoxicity were similar for LK prepared from both cell sources. In three experiments with different LK preparations, the titration patterns of LK from vaccinated and infected mice were always comparable in these assays. The LK effect was absolutely dependent on the presence of macrophages in the cultures, since LK alone had no toxic activity against larvae or tumor cells when utilized at the same concentrations.

The reactivity of culture supernatant fluids from SWAP challenge of cells from infected or vaccinated mice was equivalent to that of positive control supernatant fluids from culture of BCG-immune spleen cells with PPD (98% larval killing and 55% tumoricidal activity in the experiment depicted in Fig. 1 with a 1:6 dilution). The macrophage-activating factors in supernatant fluids from SWAP stimulation of cells from vaccinated mice were heat labile, indicating that they were independent of contamination with bacterial lipopolysaccharide; heating to 100°C for 10 min reduced the activity of the highest concentration of LK to background levels (2% larvicidal activity, 10% tumor cell lysis). Furthermore, the addition of polymyxin B at 1 μg/ml to the macrophage activation assays did not decrease the reactivity of LK from vaccinated mice. Under these conditions, a 1:6 dilution of vaccine-derived LK, containing less than 0.25 ng endotoxin per ml as detected by the *Limulus* amoebocyte lysate assay, induced control macrophages to exhibit 100% larvicidal and 52% tumoricidal activity. Endotoxin concentrations of $\geq$ 20 ng/ml were previously found to be required for direct effect on macrophage larvicidal activity in these assays (11). These supernatant fluids also exhibited significant levels of gamma interferon activity (1,920 reference units per ml), but no alpha or beta interferon activity as distinguished by differential acid sensitivity.

The factors in vaccine-derived LK that were reponsible for activation of macrophages to kill both schistosomula and tumor cells eluted from Sephadex G-100 as a single peak with an apparent molecular weight range of 42,000 to 58,000 (Fig. 2). The elution pattern is comparable to that obtained for macrophage activation factors induced by mitogen or PPD stimulation of mouse spleen cells (11, 16). Interferon activity was also eluted in the same region (Fig. 2). Fractions with no LK activity (molecular weight, 136,000 and 20,000) had no interferon activity as well.

To determine whether macrophage tumoricidal and larvicidal activity induced by vaccine-derived LK was caused at least in part by interferon, we passed samples of LK over protein A-Sepharose columns coupled with either anti-mouse gamma interferon or a control gamma globulin. The interferon titer in the eluate from the antiinterferon column was less than one-fourth of the control column eluate titer, and the capacity to activate macrophages was likewise reduced (Table 3). Whereas a 1/50 dilution of untreated LK or LK eluate from the control column had potent macrophage-activating capacity, the eluate from the antiinterferon column was inactive. That gamma interferon alone could activate macrophages to kill schistosomula was shown in experiments in which mouse recombinant gamma interferon at concentrations of 35 and 7 U/ml stimulated macrophages to kill 100 and 67% of larvae, respectively.

Recruitment of activated macrophages to the site of specific antigen challenge in vivo in vaccinated mice. Initial experiments revealed that activated (tumoricidal) cells were not present in the peritoneal cavities of vaccinated mice, nor could they be elicited by intraperitoneal injection of the sterile irritant sodium caseinate (Table 4). However, within 16 h after intraperitoneal injection of 250 μg of SWAP, cells capable of lysing tumor cell targets appeared in the peritoneal cavities of vaccinated mice. The tumoricidal activity of these cells was comparable to that of control cells that had been activated in vitro by continuous exposure to PPD-BCG LK in the same experiments. Further exposure of the

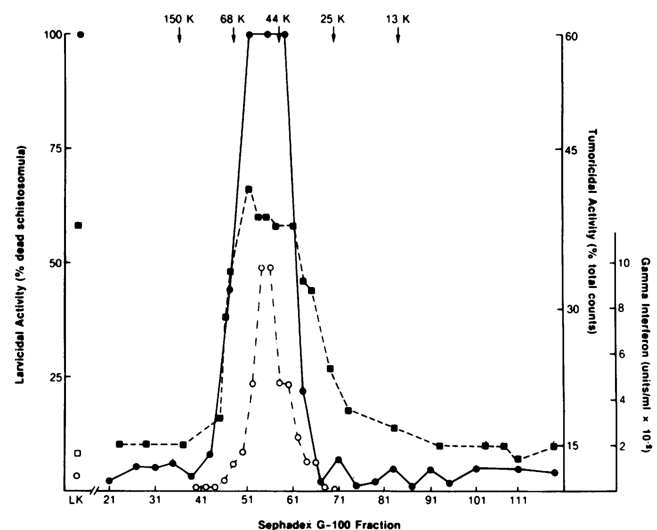

FIG. 2. Development of cytotoxic activity against schistosomula or tumor cells by macrophages treated with vaccine-derived LK fractionated by Sephadex G-100 gel filtration. Larvicidal activity (●) was assessed 48 h after culture of casein-elicited C3H/HeN macrophages with schistosomula in the presence of a 1:20 dilution of original LK or LK fractions. Tumoricidal activity (■) was assessed as percent maximal ^{3}H release from L929 cells after 48 h of incubation with macrophages and LK at a 1:10 dilution. Macrophage activity in the absence of LK (○, □) and the interferon titer (– · · · –) are also indicated.

TABLE 3. Absorption of vaccine-derived LK with antibody to mouse gamma interferon removes the macrophage-activating capacity

LK absorption[a]	Interferon titer[b] (U/ml)	% Larvicidal activation[c]
None	38	93
Rabbit anti-mouse gamma interferon	5	8
Normal rabbit immunoglobulin	19	84

[a] LK prepared by SWAP stimulation of spleen cells from vaccinated C57BL/6J mice was assayed at a 1:50 dilution, before or after passage over a column of rabbit antibody to mouse gamma interferon or normal rabbit immunoglobulin bound to protein A-Sepharose.

[b] Interferon titer was determined as the ability of lymphokine to inhibit viral cytopathic effects.

[c] Larvicidal activity was determined as the ability of the LK to induce control macrophages from normal C3H/HeN mice to kill schistosomula over a 48-h culture period.

macrophages from vaccinated and SWAP challenged mice to LK in vitro had no enhancing effect, suggesting that these cells had achieved maximum activation in vivo. The effect of SWAP challenge was specific, since SWAP injection had little or no effect in control C57BL/6J mice.

Likewise (Table 5), intraperitoneal SWAP injection elicited a population of plastic-adherent cells with significant in vitro larvicidal activity in vaccinated, but not control, mice. In four experiments, PC from vaccinated mice killed approximately three times more larvae than did cells from similarly treated control mice (mean larvicidal activity of 9% with control cells versus 27% with cells from vaccinated animals, $P < 0.05$). Pretreatment of larvae with immune sera obtained from either vaccinated or chronically infected mice enhanced killing by the in vivo activated cells, but had no effect with control cells (comparison of mean larvicidal activity by control versus vaccinated cells showed $P < 0.01$ with vaccine serum and $P < 0.001$ with chronic serum).

In contrast to previous studies with cells from *S. mansoni*-infected mice (14), adherent cell monolayers were superior to suspension cultures in these larvicidal assays. Larval killing by total cell populations from vaccinated, SWAP-challenged mice in polypropylene tube cultures was erratic and, when observed, was consistently lower than that in monolayer cultures in the same experiments. The PC populations obtained from vaccinated mice contained high percentages of polymorphonuclear cells due to the recent intraperitoneal stimulation (mean of 25% neutrophils and 11% eosinophils in the four experiments shown). However, the majority of granulocytes (>80%) were not adherent after monolayer preparation and extensive washing, and plastic

TABLE 5. In vitro killing of schistosomula and tumor cells by macrophages elicited by SWAP challenge in vaccinated mice

Expt	Source of PC[a]	Tumoricidal activity[b] (%)	% Activity against larvae treated with[c]: Normal sera	Vaccine sera	Chronic sera
1	Control mice	15	7	9	11
	Vaccinated mice	48	20	26	33
2	Control mice	12	4	7	9
	Vaccinated mice	40	18	30	37
3	Control mice	12	9	10	7
	Vaccinated mice	32	22	24	34
4	Control mice	ND[d]	14	10	ND
	Vaccinated mice	ND	49	52	ND

[a] Peritoneal cells were taken from control or vaccinated C57BL/6J mice 16 h after intraperitoneal injection of 250 μg of SWAP. Plastic-adherent monolayers were prepared by using 8×10^5 macrophages per well.

[b] Tumoricidal activity was determined as mean percent lysis of [^{3}H]thymidine-labeled L929 cells after 48 h of incubation with an effector/target ratio of 20:1.

[c] Larvicidal activity was determined as mean percentage of skin-stage schistosomula exhibiting loss of motility and internal granularity at 48 h with an effector/target ratio of 10^4:1. Larvae were preincubated for 45 min in a 20% concentration of serum from 6-week vaccinated mice or chronically infected mice and washed before addition to cultures.

[d] ND, Not determined.

TABLE 4. Elicitation of tumoricidal macrophages in vaccinated mice by in vivo challenge with schistosome antigens

Expt	Source of PC[a]	Injected with[b]	Tumoricidal activity (%) after exposure to[c]: Media	Lymphokine
1	Control mice	Casein	393 (14)	1,235 (43)
	Control mice	SWAP	430 (15)	1,051 (37)
	Vaccinated mice	Casein	225 (8)	1,309 (46)
	Vaccinated mice	SWAP	1,434 (52)	1,385 (49)
2	Control mice	SWAP	1,152 (7)	3,325 (20)
	Vaccinated mice	Casein	1,900 (12)	5,325 (32)
	Vaccinated mice	SWAP	6,536 (40)	5,544 (34)
3	Control mice	Casein	750 (12)	4,300 (66)
	Control mice	SWAP	1,725 (27)	3,574 (55)
	Vaccinated mice	Casein	736 (12)	3,710 (57)
	Vaccinated mice	SWAP	4,288 (66)	4,752 (73)

[a] C57BL/6J mice were used 4 to 6 weeks after vaccination or maintained as untreated controls.

[b] Peritoneal cells were collected 16 h after intraperitoneal injection of 1 ml of 1.2% sodium caseinate or 1 ml of PBS containing 250 μg of SWAP.

[c] Tumoricidal activity of PC was measured by lysis of [^{3}H]thymidine labeled L-929 cells at 48 h and is expressed as mean counts per minute released in triplicate samples. Values within parentheses indicate the percentage of total sodium dodecyl sulfate counts released. PC were incubated with tumor cells in the presence of media only, or of a 10% concentration of LK prepared from PPD stimulation of spleen cells from BCG-immune mice.

adherence was found to be as effective as density gradient sedimentation at separating macrophages from contaminating cell types (data not shown). All larvicidal studies shown in Table 5 were therefore performed in monolayer cultures. That granulocytes or lymphocytes did not contribute to larval killing was indicated by the observation that recovered nonadherent cells, comprising 100% of the eosinophils, 63% of the neutrophils, and 65% of the lymphocytes in the original population, showed no larvicidal activity against even antibody-coated targets (4% larval death with chronic serum-treated schistosomula).

DISCUSSION

Significant protection against challenge *S. mansoni* infection in mice is achieved as a result of ongoing low-grade primary infection (concomitant immunity) or pervious exposure to radiation-attenuated infective larvae (vaccination). Previous results suggest that resistance in both models involves an immune component (15, 30, 32, 33). Hypothetically, those antischistosome responses generated in common between both the concomitant immunity and vaccination models of resistance would be most likely to be of protective value. Recent in vivo studies measuring concomitant and vaccine-induced immunity in several strains of inbred mice have shown that strains with genetic defects in macrophage activation, such as A/J and P/J, develop little or no resistance to challenge infection in either model (13, 15). These observations have turned our attention to the role of macrophages in protective immunity against schistosome infection.

The current study shows that vaccination, like primary infection, sensitizes for delayed hypersensitivity (type IV) response to schistosome antigens as demonstrated by the 24-h skin reaction in vivo and production of macrophage-activating LK in vitro. Vaccine-derived LK activated control macrophages to kill both skin-stage schistosomula and tumor cell targets. As previously observed with LK produced by PPD stimulation of BCG-immune spleen cells (11, 16), fractionation of vaccine-derived LK by Sephadex G-100 gel filtration showed that activities responsible for induction of cytotoxic capacity against both types of extracellular targets cochromatographed as a single peak in the 50,000-molecular-weight region. Vaccine-derived LK fractions active in both the larvicidal and tumoricidal assays also contained the potent immune mediator gamma interferon. Furthermore, absorption of vaccine-derived LK with anti-gamma interferon antibody substantially lowered its macrophage-activating potential in the larvicidal assay, suggesting functional identity. Thus, vaccination with irradiated cercariae sensitizes mice to respond to specific antigen challenge by production of a LK proven to exert powerful anti-tumoricidal and anti-microbicidal effects in other systems (22, 28, 29).

In conjunction with delayed hypersensitivity responsiveness, macrophages activated to kill extracellular targets, notably schistosomula, are recruited to the site of specific antigen challenge in vaccinated mice. A requirement for immune stimulation to elicit activated macrophages to a particular tissue site has also been demonstrated in both BCG-immune (26) and *S. mansoni*-infected (10) mice and probably reflects the localized and transient nature of activation events (6, 27). That macrophages are the active cells in these populations is indicated by the observation that adherent cell monolayers, depleted of the majority of granulocyte contaminants, are superior to total cell populations or nonadherent cells. Indeed, previous studies have shown that the presence of contaminating cell types, such as eosinophils (14) or erythrocytes (personal observation), can inhibit the larvicidal activity of macrophages in suspension cultures. Thus, in vivo and in vitro observations suggest a role for activated macrophages in the effector mechanism of vaccine-induced resistance.

There is reason to believe that effector cell function is required for protective immunity in vaccinated mice. Experiments involving vaccination in μ-suppressed mice indicated a role for antibody in protective immunity. However, depletion of complement by treatment with cobra venom factor or use of congenitally C5-deficient animals had no effect on acquisition of vaccine-induced resistance, suggesting that neither the antibody-dependent classical pathway nor the alternate complement pathway is involved in the effector mechanism of immunity. An alternative function for antibody would be to direct or enhance the activity of effector cells against challenge parasites. Indeed, in the current study, pretreatment of schistosomula with antibody-containing immune serum enhanced the in vitro larvicidal activity of activated macrophages from vaccinated mice. In agreement with previous observations that the titer of anti-larval antibodies in vaccine serum is lower than that of chronic immune serum (8), serum from chronically infected mice was somewhat more effective than vaccine serum at enhancing macrophage larvicidal activity. Studies investigating the in vitro larvicidal activity of PC from *S. mansoni*-infected mice also support the concept of synergistic interaction between activated macrophages and antibody in parasite destruction (14). It is important to remember, however, that activation of the macrophages, either in vitro by LK or in vivo as a result of vaccination, is an absolute prerequisite for the expression of larvicidal capacity even in the presence of antibody; control macrophages have no activity in this system.

The killing of schistosomula by activated macrophages and antibody is the first potential effector mechanism of resistance to be described in the vaccine model. Studies are now underway to examine the activation of larvicidal macrophages and the induction of other antischistosome immune responses in strains of inbred mice that fail to become resistant as a result of vaccination, in an attempt to determine a causal relationship.

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