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Studies with Double Cytokine-Deficient Mice Reveal That Highly Polarized Th1- and Th2-Type Cytokine and Antibody Responses Contribute Equally to Vaccine-Induced Immunity to Schistosoma mansoni

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A fundamental obstacle to vaccine development in schistosomiasis mansoni is a lack of understanding of what type of an immune response should be invoked. We have addressed this central issue by using the radiation-attenuated cercariae vaccine in mice genetically engineered to exhibit highly polarized type 1 (IL-10/IL-4-deficient) or type 2 (IL-10/IL-12-deficient) cytokine and Ab phenotypes. Our data show that while significant differences in immunity exist after a single vaccination with irradiated cercariae in double cytokine-deficient vs wild-type mice, these differences disappear after two vaccinations. The most important finding of these studies, however, was revealed in vaccinated IL-10-deficient mice. These mice developed a mixed and elevated type 1- and type 2-associated immune response and developed anti-schistosome immunity at levels equal to or better than those in wild-type mice. This immunity in IL-10-deficient mice correlated with higher parasite-specific Ab titers, greater proliferative capacity of lymphocytes, increased frequency of IFN-γ- and IL-4-secreting cells, elevated perivascular/peribronchial inflammatory responses in the lung, and greater in vitro schistosomulacidal capacity of parasite Ag-elicited cells. These results suggest that optimal vaccine-induced immunity against schistosomes is linked not to the development of a highly polarized response, but, rather, to the induction of both type 1- and type 2-associated immune responses. The Journal of Immunology, 1999, 163: 927–938.

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were harvested at 72 h and assayed for cytokine activity. IFN-γ, IL-5, and IL-10 were measured by specific two-site ELISA as previously described (24). IL-4 levels were determined by proliferation of CT245 cells. Cytokine levels were calculated using standard curves constructed using recombinant murine cytokines.

Measurement of SWAP-specific Ab responses

For assessment of serum Ig during protection experiments, sera were collected at 3 wk after secondary immunization and 3 wk after challenge infection. Immune 4 (Dynatech, Chantilly, VA) microritser plates were coated overnight at 4°C with SWAP (2 μg in 50 μl/well) diluted in PBS. Plates were blocked with 200 μl of 5% nonfat dry milk/PBS for 2 h at 37°C. The blocking solution was aspirated, and the wells were washed six times with PBS/0.05% Tween-20 (PBS/0.05% Tween). Specific SWAP-binding was determined by dilution 1/100 to 1/102,500 in 1% BSA/PBS, and 50 μl was added to appropriate wells. Plates were incubated at 37°C for 90 min and then washed six times with PBS/0.05% Tween-20. Fifty microliters of isotype specific HRP-conjugated rabbit anti-mouse Abs in 1% BSA/PBS diluted at 1/1,000 (measurement of IgG1 and IgG2b; Zymed, San Francisco, CA) were added to the wells and incubated at 37°C for 2 h. Wells were again washed six times with PBS/0.05% Tween-20, 100 μl of 2.2'-azino-di(3-ethylbenzthiazoline sulfonate) (ABTS: H₂O₂ substrate, Kirkegaard & Perry, Gaithersburg, MD) was added, and the reactions were developed in the dark at room temperature for 20–30 min. Absorbance at 405 nm was determined using a Vmax Microplate Reader (Molecular Devices, Palo Alto, CA). Specific SWAP isotype titers were calculated by the product of absorbance and the reciprocal of the sera dilution from an average of two points in the linear portion of the dilution curve.

Measurement of total SWAP-specific IgG/Ab was performed from the sera of vaccinated IL-10-deficient and C57BL/10 mice on days 0, 12, and 21 postchallenge. This ELISA was performed in essentially the same way as the isotype-specific Ab ELISAs described above using a Zymed HRP-conjugated rabbit anti-mouse IgG/Ab (diluted 1/1000 in 1% BSA/PBS).

Total serum IgE Abs were quantitated by ELISA using a protocol provided by Pharmingen (San Diego, CA). Briefly, plates were coated with anti-mouse IgE capture Ab from clone R35-72 in 0.1 M NaHCO₃, pH 8.2, overnight at 4°C. The secondary mAb was a biotinylated anti-mouse IgE from clone R35-92, and the streptavidin-peroxidase reagent was diluted 1/1000 in 1% BSA/PBS. A purified mouse IgE from clone IgE-2 (Pharmingen) was used as the control standard.

Ag-specific cellular proliferation

TALN and LALN cells from vaccinated IL-10-deficient and C57BL/10 mice were aseptically removed on days 0, 12, and 21 after challenge. Single-cell suspensions were made, cultured in 96-well tissue culture plates (Costar, Cambridge, MA) (5 × 10⁶ cells/well), and stimulated to proliferate by addition of 50 μg/ml SWAP or 1 μg/ml Con A. One microliter of [³H]thymidine was added to each well at 48 h, and the cells were incubated for an additional 18–24 h before harvesting. Between 66 and 72 h the cells were harvested, and the amount of Ag-specific proliferation was assessed by incorporation of thymidine. Each sample was assayed in duplicate.

Frequency of cytokine-producing cells

The frequency of IFN-γ and IL-4-producing cells was determined by ELISPOT analysis as previously described (25). Briefly, Immulon 4 microtiter plates (Dynatech) were coated with rat anti-mouse IL-4 (Endogen, Boston, MA) or rat anti-mouse IFN-γ (BioSource, Camarillo, CA) in 50 μl of PBS overnight at 4°C. The plates were subsequently blocked by addition of 200 μl of 5% BSA/PBS overnight at 4°C. The blocking solution was aspirated, and the wells were washed six times with PBS/0.05% Tween-20. TALN and LALN cells from vaccinated IL-10-deficient and C57BL/10 mice were plated at 2 × 10⁵ cells/well, and at 2-fold dilutions up to 5 × 10⁶ cells/well. Cultures were stimulated by addition of 50 μg/ml SWAP. After a 24-h incubation at 37°C in 5% CO₂, the plates were washed and incubated for 2 h at room temperature with 50 μl/well of biotinylated secondary Abs (rat anti-mouse IL-4, no. 18112D; rat anti-mouse IFN-γ, no. 18112D). plates were washed, and incubated for 2 h at room temperature with 50 μl of a 1/5000 dilution (5% FCS/PBS/0.05% Tween-20) of streptavidin–alkaline phosphatase (PharMingen). Finally, the plates were washed, and 175 μl of a 4:l solution of 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) phosphate substrate (Sigma, St. Louis, MO) and low EEO agarsone (Sigma) was added at 56°C. The blue spots were allowed to develop overnight, and individual cytokine-secreting cells were counted using a ×10 objective on a Zeiss inverted microscope (Zeiss, New York, NY).
Histopathology

The degree of cellular inflammation associated with vascular and bronchial structures of the lung was examined in Giemsa-stained tissue sections. Essentially, two lung lobes were inflated with and fixed in Bouin-Holland solution, and histologic sections were processed and stained with Giemsa (Histo-Path of America, Clinton, MD). The degree of inflammation was estimated using an arbitrary scale, in which 1 represents minimal inflammation, and 6 represents maximal inflammation.

Peritoneal lavage and in vitro larvicidal assay

Peritoneal lavage cells were isolated in DMEM from immunized IL-10-deficient and C57Bl/10 mice injected 18–20 h previously with 250 μg of SWAP in 0.5 ml of PBS. Three-hour mechanically transformed schistosomula were prepared as previously described (20). Lavage cells were incubated at 37°C with schistosomula at a cell:target ratio of 10^5:1 in DMEM containing 4.5 mg/ml glucose, 10% FCS, and antibiotics. Schistosomula viability was assessed at 40 h by the criteria of motility and internal substructure granularity (20). NO production by lavage cells was assayed by the Greiss reaction from 100 μl of supernatant collected after 40 h of culture.

Statistics

Values for worm burdens, secreted cytokine proteins, serum ELISA data, perivascular inflammation, proliferation assays, and ELISPOTs were compared using Student’s two-tailed t test. p < 0.05 was regarded as significant. A minimum of two separate experiments was performed for all data.

Results

IL-4/IL-10- and IL-12/IL-10-deficient mice develop highly polarized type 1 and type 2 cellular and humoral immune responses, respectively, following vaccination with attenuated cercariae of S. mansoni

To test the hypothesis that polarization of the immune response toward the type 1 or type 2 direction may lead to differences in vaccine-induced immunity against S. mansoni, mouse strains displaying unique cytokine-producing profiles were vaccinated twice with 500 attenuated cercariae. To confirm that immune polarization occurred, vaccinated mice were analyzed for their in vitro parasite Ag-stimulated (SWAP) cytokine responses on day 18 after the second immunization (Fig. 1, A and B). Measurement of their SWAP-specific isotype profiles before and after challenge with unattenuated parasites was also performed to confirm immune polarization (Fig. 2, A and B). As described previously (22), vaccinated wild-type (wt) mice exhibited a mixed Th0-like profile of cytokine production in their gluteal (TALN; Fig. 1A) and LALN (Fig. 1B), although the IL-4 and IFN-γ responses were of greater magnitude in the TALN cultures. IL-10-deficient animals displayed a similar mixed IFN-γ/IL-4 SWAP-specific profile, although the response in general, particularly for IFN-γ, was significantly increased compared with that in wt animals. IL-4 production was more variable in the IL-10-deficient animals, showing an average increased level in the LALN cultures (Fig. 1B) and a similar or slightly decreased response in TALN cultures (Fig. 1A). Nevertheless, the overall mixed cytokine response in vaccinated IL-10-deficient mice was identical with the pattern previously reported in the spleens and mesenteric lymph nodes of 8-wk-infected IL-10 KO mice (26). IL-12-deficient animals showed very little change in their type 2-like cytokine profile compared with wt animals, while IFN-γ production was decreased in both the TALN (Fig. 1A) and LALN (Fig. 1B) cultures. IL-4-deficient mice, by contrast, showed an increased IFN-γ response. Nevertheless, the greatest increase in Ag-specific IFN-γ production was consistently observed in double IL-4/IL-10-deficient animals (Fig. 1, A and B), which when compared with wt, IL-10-deficient, or IL-4-deficient animals also showed the greatest defect in Th2-like cytokine production. Indeed, RT-PCR analysis confirmed no detectable IL-5 mRNA response in the lungs of IL-4/IL-10-deficient animals while wt, IL-10-deficient, and, to a lesser degree, IL-4-deficient animals, all developed significant IL-5 mRNA responses (wt-137, IL-10 KO-90, IL-4 KO-15, IL-10/IL-4 KO-0 arbitrary densitometric units; data not shown). The double IL-12/IL-10-deficient animals compared with wt, IL-10-deficient, or IL-12-deficient mice showed a similar and significant increase in their IL-4 responses (Fig. 1, A and B). Similar cytokine profiles were detected in splenocyte cultures from these animals, and RT-PCR analysis of cytokine mRNA expression in the lungs further confirmed that mice deficient in both IL-4 and IL-10 showed the greatest increase in IFN-γ mRNA expression (and a low Th2-type profile), while IL-12/IL-10-deficient animals developed a maximal IL-4/IL-5 mRNA response (and low IFN-γ message; data not shown). Furthermore, inclusion of anti-CD4 mAbs in SWAP-stimulated cultures confirmed that cytokine expression in vitro was largely attributable to a CD4+ T cell response (Fig. 1, A and B, far right panels).

The parasite-specific Ab response (Fig. 2) in large part reflected the cytokine-producing phenotype of the various cytokine-deficient animals. Thus, wt animals, as reported previously (20), produced Abs associated with type 1-associated (IgG2b) as well as type 2-associated (IgG1) immune responses. In accordance with their elevated and mixed cytokine profiles, the IL-10-deficient animals displayed elevated IgG2b and IgG1 titers at the postchallenge time point (Fig. 2B) and elevated IgG1 and IgE titers at the prechallenge time point (Fig. 2A). In some cases, these differences were not statistically different from those of wt mice. Not surprisingly, the double IL-12/IL-10-deficient animals developed a decreased IgG2b but similar IgE/IgG1 response compared with the IL-10-deficient mice (Fig. 2B), confirming that a more polarized type 2 immune response had developed in these animals. Single-IL-12-deficient animals showed a similarly skewed profile. The IL-4/IL-10-deficient double-deficient animals, in contrast, routinely showed the highest IgG2b titers both before (Fig. 2A) and after (Fig. 2B) challenge. These mice also displayed little or no IgG1 or IgE Abs, confirming that a highly polarized type 1/IFN-γ-dominant cytokine response was driving Ab production. IL-4-deficient animals displayed an Ab phenotype similar to but of lesser magnitude than the IL-4/IL-10 double-deficient animals, particularly at the prechallenge time point. Indeed, compared with the other mice in this study, the highest ratio of SWAP-specific IFN-γ/IgG2b and IgG2b/IgG1 was observed in mice deficient in both IL-4 and IL-10. In contrast, the IL-12/IL-10 double-deficient mice exhibited the highest ratio between their Ag-specific IL-4/IFN-γ and IgG1/IgG2b responses. Interestingly, the cytokine phenotypes were also extremely stable among the various cytokine-deficient groups. This was determined at the termination of the vaccine experiments (all challenged mice were perfused at 6 wk postchallenge), when the cytokine-producing profile of SWAP-stimulated spleen and LALN cell cultures reflected the profiles at earlier time points (data not shown).

Regardless of their cytokine/Ab production profile, mice vaccinated twice with irradiated cercariae develop highly significant, but similar, levels of immunity to a challenge infection

When the twice vaccinated mice were challenged with S. mansoni parasites and perfused 6 wk later, no significant difference in worm burdens or overall percentage of protection was observed between the type 1 polarized (IL-4/IL-10-deficient, 83.2%), type 2 polarized (IL-12/IL-10-deficient, 75.9%), or wt (84.2%) mice (Fig. 3). Indeed, all groups of vaccinated wt and cytokine-deficient animals
showed highly significant levels of immunity compared with their respective nonvaccinated control group. Worm burdens were reduced between 75.9–91.7% in the various cytokine-deficient animals, and only the IL-10-deficient group showed a significantly increased level of immunity compared with the vaccinated wt animals ($p<0.05$). The results obtained with IL-4-deficient animals confirmed the results reported by King et al., who also reported no difference between multiply vaccinated IL-4-deficient and wt mice (27). More importantly, however, they extend these observations and demonstrate that a similar level of protection can develop in multiply vaccinated animals regardless of the nature of the type 1 and/or type 2 cytokine or the Ab phenotype that dominates the response.

**Modest, but significant, differences in immunity are observed in singly vaccinated cytokine-deficient mice**

The preceding results were surprising, since earlier reports have clearly shown a requirement for IFN-γ (3, 16) and IL-12 (28) in the protective response induced by vaccination with attenuated parasites. Nevertheless, these previously published studies only examined immunity in mice vaccinated a single time with attenuated parasites in which cellular mechanisms are generally thought to dominate the protective response. Therefore, in subsequent experiments we examined the once vaccinated model to again determine whether mice exhibiting type 1 or type 2 skewed immune responses would display different patterns of immunity. In these

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**FIGURE 1.** SWAP-stimulated in vitro production of IFN-γ and IL-4 demonstrate that cytokine polarization occurred in response to the irradiated cercaria vaccine. Mice were vaccinated by two exposures to 500 irradiated cercaria, and tissues were harvested at 18 days after second exposure. Each bar represents the pooled cytokine response ($n=5$ mice/group) secreted from lymph nodes draining the tail (A) and draining the lung (B) after stimulation with medium, SWAP, or SWAP and CD4$^+$ T cell blockade (mean ± SE). The supernatants from these cultures were collected 72 h after stimulation and used in capture ELISA as described in Materials and Methods.
experiments, mice were vaccinated with 500 attenuated cercariae and then challenged with 120 unattenuated parasites 4–5 wk later. At 6 wk postinfection the mice were perfused to assess their worm burdens, and spleens were examined for their Ag-specific cytokine production profiles. As expected, all groups again showed the predicted cytokine response (data not shown). Nevertheless, in contrast to the twice vaccinated studies, significant differences in protection were observed between wt animals and several of the cytokine-deficient groups. Indeed, all vaccinated groups except the IL-10-deficient mice showed statistically significant differences in the number of recovered adult worms compared with the wt animals (Fig. 4). IL-10/IL-12-, IL-12-, IL-4-, and IL-10/IL-4-deficient mice all demonstrated slightly reduced resistance compared with wt vaccinated mice, whereas IL-10-deficient mice again demonstrated a slightly increased protective response (not significant). Regardless of these differences, however, all groups displayed a relatively high immunity, varying between 53.6–83.9%. These findings in part agree with the recent results of Anderson et al., who also showed that once vaccinated IL-12-deficient mice were partially, but not completely, defective in their protective response (28).

**Vaccinated IL-10-deficient mice, in contrast to their wt counterparts, develop a higher parasite-specific Ab titer and with accelerated kinetics**

Because of the surprising findings with the IL-10-deficient mice, in subsequent studies we examined their immune phenotype in more detail to determine the mechanisms of their protective response. In the following experiments, wt and IL-10-deficient mice were vaccinated with radiation-attenuated cercariae and then exposed 4 wk later to a second dose of attenuated parasites. This approach mimicked the procedures used in the two-vaccine experiments described above and assured that the immune response generated against the parasites in both groups of mice was not influenced by differences in Ag exposure, which can occur when mice are challenged with unattenuated parasites. At all time points (Fig. 5) IL-10-deficient mice exhibited significantly higher titers of SWAP-specific Abs than the wt control group.

**Vaccinated IL-10-deficient mice develop an enhanced cellular immune response: evidence for a marked expansion of parasite-specific lymphocytes producing IFN-γ or IL-4**

ELISPOT studies were performed to determine whether the altered IFN-γ and IL-4 responses previously observed in IL-10-deficient mice (Fig. 1) were due to increased cytokine production or were the result of changes in the frequencies of cytokine-producing cells. TALN and LALN cells from twice vaccinated mice were used in ELISPOT assays to determine the frequencies of IFN-γ- and IL-4-producing cells on days 0, 12, and 21 after the second exposure. As shown in Fig. 6, TALN and LALN cell cultures from IL-10-deficient mice consistently displayed a marked increase in IFN-γ-secreting cells compared with wt mice. This was evident at
all three time points examined. Interestingly, a higher frequency of IL-4-secreting cells was also observed in both TALN and LALN cultures from IL-10-deficient mice on days 0 and 21. IL-4 frequencies were not significantly different on day 12, and in agreement with the IFN-γ results, day 12 appeared to be the peak time point.

A striking observation during these studies, however, was the markedly increased size of the draining lymph nodes in IL-10-deficient vs wt mice. The change in size (TALN and LALN) appeared to be a result of parasite migration, since mesenteric and inguinal lymph node size was not significantly affected at any time point postvaccination (data not shown). Indeed, when the frequencies of IFN-γ- and IL-4-producing cells were adjusted to take into account differences in total cell number, the frequencies of IFN-γ- and IL-4-producing cells per lymphoid organ were significantly increased at all time points in the IL-10-deficient mice (Fig. 7).

We also compared the proliferative capacity of lymphocytes from wt and IL-10-deficient animals. At all three time points examined, at sites draining the lung (LALN, Fig. 8A) and tail (TALN, Fig. 8B), cells from IL-10-deficient vs wt mice displayed greater proliferation in response to SWAP. Similar results were obtained with cultures stimulated with mitogen (Con A; data not shown). The proliferative capacities of TALN and LALN cultures in general appeared similar in magnitude at most time points; however, the response on day 21 was stronger in the LALN and remained elevated in the IL-10-deficient vs wt mice. This pattern

FIGURE 3. Polarized cytokine responses fail to significantly modify protection in twice vaccinated mice. Mice were immunized two times with 500 gamma-irradiated cercariae (4-wk interval between vaccinations) and challenged 4 wk later with 120 unattenuated parasites. Bars from bottom to top show the 10th, 25th, 50th, 75th, and 90th percentiles, respectively, of the tested samples. Single outliers are indicated as circles. The percent protection is indicated above each of the groups and was calculated according to the formula: (control − vaccinated/control) × 100. Statistically significant differences in worm recovery (p < 0.05) between wt mice and cytokine-deficient mice as determined by Student’s t test are indicated by an asterisk. All vaccinated mice were significantly protected compared with the control counterparts. Data were obtained from groups of mice containing 20–30 mice. These data are combined results from two or three separate experiments. Open boxes are worm burdens from control mice, whereas filled boxes are worm burdens from vaccinated mice.

FIGURE 4. Cytokine deficiencies significantly modify the levels of vaccine-induced immunity in once vaccinated mice. Mice were immunized one time with 500 gamma-irradiated cercaria and challenged 4 wk later with 120 unattenuated parasites. Bars from bottom to top show the 10th, 25th, 50th, 75th, and 90th percentiles, respectively, of the tested samples. Single outliers are indicated as circles. The percent protection is indicated above each of the groups. Statistically significant differences in worm recovery (p < 0.05) between wt mice and cytokine-deficient mice as determined by Student’s t test are indicated by an asterisk. All vaccinated mice were significantly protected compared with the control counterparts. Data were obtained from groups of mice containing between 7 and 30 mice. These data are combined results from two or three separate experiments. Open boxes are worm burdens from control mice, whereas filled boxes are worm burdens from vaccinated mice.

FIGURE 5. Serum Ab titers are increased in vaccinated IL-10-deficient mice. wt and IL-10-deficient mice were vaccinated with 500 irradiated parasites and challenged 4 wk later with 500 irradiated parasites. On days 0, 12, and 21 postchallenge, sera were obtained, and parasite-specific IgG/A/M was measured. Each circle represents the Ab titer from an individual mouse, and the bars indicate the SEM of each group (n = 5–6 mice/group). At each time point examined, IL-10-deficient mice showed a significantly higher parasite-specific Ab titer (Student’s t test) compared with wt mice. These data are representative of two separate experiments.
might be expected, since day 21 corresponds to the time when most of the attenuated schistosomula have reached the lung and are beginning to die (29). These results may explain why the number of cytokine-producing cells was increased in the vaccinated IL-10-deficient animals. In addition, an augmented effector T cell response may, in turn, provide a mechanistic explanation for why serum Ab titers were also elevated in the KO mice. Interestingly, no noticeable difference was observed in the expression of several cell surface markers between IL-10-deficient and wt mice when FACS analysis was employed (data not shown) on cells collected on days 0, 12, and 21 postchallenge, lymphocytes were collected from the LALN and TALN and stimulated with medium or SWAP, and IFN-γ-specific (A) and IL-4-specific (B) cells were enumerated by ELISPOT 24 h later. Bars represent the average number of cytokine-specific cells counted from at least two dilutions ± SEM (performed in duplicate). These data are representative of two separate experiments.
from the TALN and LALN. Cell surface markers examined included Mac-1, CD40, CD40 ligand, CD28, B7.2, MHC II, B220, and CD11c.

**IL-10-deficient mice develop accentuated peribronchial/perivascular inflammatory reactions in response to migrating lung-stage schistosomula**

Because it is believed that the primary site of parasite elimination in vaccinated mice is in the lungs (30), by mechanisms still incompletely understood, we examined the morphological changes in the lungs of vaccinated wt and IL-10-deficient mice following a challenge infection with attenuated parasites. At the three time points examined, there was significantly greater peribronchial/perivascular inflammation in the lungs of IL-10-deficient vs wt mice (Fig. 9A). The inflammatory reactions were characterized by a larger accumulation of cells surrounding bronchial and vascular structures (Fig. 9B). There were also fewer infiltrating eosinophils associated with the lung tissue of IL-10-deficient compared with wt mice (data not shown), which may be explained by the increased IFN-γ response in the KO animals (31). Since it has been proposed that immature parasites may become trapped in pulmonary inflammatory foci and thus are prevented from completing their onward migration and maturation (32), the accentuated peribronchial inflammatory reactions may provide an additional mechanistic explanation for the excellent protective response of the IL-10-deficient animals.

**Parasite Ag-elicited cells from vaccinated IL-10-deficient mice display an enhanced ability to kill schistosomula ex vivo and produce greater amounts of the schistosomulacidal mediator, NO**

To determine whether cells from vaccinated IL-10-deficient mice could kill immature schistosomula, we performed an in vitro schistosomulacidal assay. In these experiments once vaccinated wt and IL-10-deficient mice were injected with SWAP i.p., and 18 h later parasites Ag-elicited cells were harvested and placed in culture with mechanically transformed schistosomula (Fig. 10). Surprisingly, SWAP-elicited cells from IL-10-deficient mice killed schistosomula ex vivo without the need for additional activation by IFN-γ (Fig. 9A). Indeed, the degree of killing by nonstimulated (medium)

**Discussion**

The discovery of age-dependent resistance (33), the identification of possible praziquantel-resistant parasites (34), and the data obtained from mathematical models predicting vaccine efficacy (35) all have insured that the search for an anti-schistosome vaccine will be a continuous and active process. Nevertheless, while many potential vaccine candidates have been identified (36), the specific type of immune response that a vaccine should evoke to confer maximal protection has not been defined. Toward this end, we used a well-studied experimental anti-schistosome vaccine and directly compared the protective responses in mice that preferentially develop either type 1 (IL-4/IL-10-deficient) or type 2 (IL-12/IL-10-deficient) cellular and humoral immune responses. Surprisingly, while differences in protection were observed in wt vs cytokine-deficient mice following a single vaccination with irradiated cercariae, differences were no longer evident when mice received a second immunization. Indeed, IL-10-deficient mice developed the most nonpolarized phenotype of all the cytokine-deficient mice and consistently showed high levels of protection in response to both one and two vaccinations. Together, these data demonstrate that high levels of vaccine-induced immunity can develop in immunized mice regardless of the cytokine-producing
phenotype of the responding parasite specific CD4⁺ T cell population.

According to the classic Th1/Th2 paradigm, Th1-type cytokines activate macrophages and induce strong cell-mediated immune responses, while Th2-type responses preferentially elicit nonphagocytic humoral defense mechanisms (9). Because the relative contributions of cellular and humoral anti-parasite effector mechanisms have been under debate in schistosomiasis, the double cytokine-deficient mice described here provided an excellent system to formally compare these two pathways in mice that are otherwise genetically matched. In agreement with previous pulmonary egg granuloma studies (22), mice vaccinated with attenuated parasites developed Ag-specific cytokine production profiles predicted from their cytokine deficiencies (Fig. 1). Thus, IL-4/IL-10-deficient animals produced abundant IFN-γ and no IL-4, while IL-12/IL-10 double-deficient mice produced the highest amount of IL-4 but little or no IFN-γ. These contrasting cytokine patterns were accompanied by markedly different Ab isotype profiles (Fig. 2), consistent with the accepted Th1/Th2 paradigm where IFN-γ triggers high IgG2 and low IgG1/IgE, and IL-4 induces high IgG1/IgE and low IgG2 Ab titers (37). Nevertheless, despite highly divergent cytokine and Ab profiles, the KO mice showed no significant difference in vaccine-induced immunity, particularly in the two-vaccination experiments in which a high level of immunity was achieved in all groups of mice. These findings suggest that while distinct anti-parasite effector mechanisms may be operating in type 1 vs type 2-polarized mice, both mechanisms are equally capable of evoking extremely high levels of protection. Alternatively, a similar protective mechanism may function on both poles of the immune response that is not significantly influenced by the phenotype of the responding CD4⁺ T cell population or the relative contribution of cellular vs humoral effector mechanisms.

FIGURE 9. Perivascular and peribronchial inflammation is increased in the lungs of vaccinated IL-10-deficient mice compared with wt mice. The wt and IL-10-deficient mice were vaccinated with 500 irradiated parasites and challenged 4 wk later with 500 irradiated parasites. Lungs were examined at days 0, 12, and 21 postchallenge. Perivascular/peribronchial inflammation was then assessed for each mouse. A, At each time point measured, lung inflammation was significantly greater in IL-10-deficient mice than in wt mice. Each circle represents an individual mouse, and the bars represent the SEM of each group (n = 5–6 mice/group). Statistical significance is given above each comparison (Student’s t test). B, A photomicrograph (day 12) showing inflammation in lung sections of IL-10-deficient- and wt mice (examined at ×40 magnification). Arrows indicate cellular inflammation surrounding bronchi (B). These data are representative of two separate experiments.
These findings were somewhat surprising, since numerous studies suggested a central role for CD4+ T cells (13, 15), IFN-γ (3, 16), and IL-12 (28) in the protection elicited by attenuated cercariae. Nevertheless, these conclusions were based almost entirely on results obtained with the one-vaccine model, where, in contrast to the two-vaccine model, cellular, not humoral, mechanisms are believed to play a major role in the protective response. Indeed, it has been suggested that polarizing the immune response in favor of Th1-dependent cell-based mechanisms might prove an effective strategy to achieve maximal levels of immunity in mice receiving one vaccine (38, 39). Th2-type cytokines are induced by one vaccine in mice (39) and have been hypothesized to antagonize the Th1-dependent effector mechanisms (40). In support of these conclusions, we observed a reduced level of protection in the IFN-γ (3) and IL-12 (28) in the protection elicited by attenuated cercariae. Nevertheless, in agreement with the recent published results of Anderson et al., we still observed quite high levels of protection in the IL-12-deficient animals (exceeding 50%) (28). Perhaps more importantly, however, by the second immunization, there appeared to be no significant requirement for IL-12 in the development of the protective response (Fig. 3). Indeed, mice that developed the most type 1 polarized phenotype (IL-10/IL-4 deficient) failed to show increased protection and, in fact, displayed reduced levels compared with wt or single IL-10-deficient mice (Fig. 4). This result along with the excellent protection data observed in the IL-10/IL-12-deficient mice (type 2 polarized) suggest that IFN-γ-mediated effector devices are not the sole immunological mechanisms driving immunity in the one-vaccine model. These findings suggest that the humoral arm of the immune response may play a more significant role in protection than was previously hypothesized. Indeed, recent studies in B cell-deficient mice confirmed an important role for Abs, even in the one-vaccine model (19).

Based on our findings from the KO mice (Fig. 2), we argue that the major Ab isotype contributing to protection is probably the IgG class. This is in agreement with serum transfer experiments of Mangold and Dean, who showed that the IgG class is the Ab isotype responsible for protection in immune mice (21). IgE is believed to play little or no role in immunity in murine schistosomiasis (2, 3), which is consistent with our observations, since highly divergent levels of IgE were detected between the two double cytokine-deficient groups (Fig. 2), yet they displayed similar levels of protection (Fig. 3). Our data suggest that as long as a significant anti-parasite IgG response is generated, the specific IgG subclass may not be an important determinant in generating high levels of immunity. While we have no data to support whether the IgG Abs on the type 1 pole (IgG2a) vs those on the type 2 pole (IgG1) would be more or less protective, purified IgG1 Abs have been used to successfully transfer protection to naïve recipients (41). We are currently planning passive transfer experiments to address this question. The double cytokine-deficient mouse provide an ideal model to perform such studies.

More importantly, however, these data suggest that polarizing the immune response in the context of an anti-schistosome vaccine may be a less important goal than was previously thought (12). In fact, our data demonstrate that generating an immune response intermediate between the Th1/Th2 poles may be even more advantageous. This was most clearly observed in the vaccinated IL-10-deficient mice that developed the most nonpolarized cytokine-producing phenotype. These mice developed very high levels of protection and displayed increases in both type 1 and type 2-associated cytokine responses. Because wt mice display a similar phenotype (Figs. 1 and 2), but of a lesser magnitude, a mixed or nonpolarized type immune response may be a more realistic goal for an anti-schistosome vaccine, as has recently been hypothesized (42).

Interestingly, the IL-10-deficient mice displayed several characteristics that would be considered ideal for a vaccine. Perhaps most importantly, the IL-10-deficient animals responded to the attenuated parasites with a more vigorous and rapid Ab response (Fig. 5). In addition, there was a highly significant expansion of the Ag-specific cytokine-producing effector cell population in the IL-10-deficient mice (Figs. 6 and 7). This expansion was characterized by an increased frequency of both type 1 and type 2 cytokine-producing cells, which probably explains the presence of both IgG2b and IgG1 Ab isotypes in these animals. The cellular expansion was also accompanied by an increased proliferative response to parasite Ags (Fig. 8), complementing the work of Sher et al. and King et al., who showed that IL-10 inhibits lymphocyte proliferation in murine and human schistosomiasis (43, 44). The enhanced proliferative response probably explains the markedly enhanced lymph node sizes of the vaccinated IL-10-deficient animals. These observations are potentially important for vaccine research, because they suggest a mechanism in which IL-10 antagonism might be used to significantly expand the CD4+ T cell memory population.

![Figure 10](http://www.jimmunol.org/)

**FIGURE 10.** SWAP-elicited peritoneal cells from vaccinated IL-10-deficient mice display greater parasite killing and increased production of NO. IL-10- and wt-vaccinated mice were injected i.p. with 250 μg of SWAP 4 wk postimmunization. The SWAP-induced peritoneal cells were then assayed for their ability to kill 3-h mechanically transformed schistosomula and to secrete NO. Separate cultures were also treated with IFN-γ (100 U/ml), Nω-monomethyl-l-arginine monoacetate (l-NMMA; 1 mM), or a combination of IFN-γ and l-NMMA. The data are the mean ± SD of triplicate determinations and are representative of two experiments performed. Note the increased schistosomula killing and NO production from SWAP-elicited IL-10 peritoneal cells even in the absence of added IFN-γ.
The increased perivascular inflammatory response observed in IL-10-deficient mice might also contribute to the high levels of protection in these animals (Fig. 9). Similar findings were also recently reported in 8-wk-infected mice, where increased egg-induced granuloma formation was observed in IL-10-deficient animals (26). It has been suggested that the inflammatory reactions that surround the lung stage schistosomula might contribute to the trapping of parasites and thus prevent their onward migration and maturation (32, 45). Indeed, Th1-like responses were hypothesized to promote parasite attrition in vaccinated mice by allowing the formation of tight inflammatory foci, which effectively trap the lung stage parasites (46, 47). These tight inflammatory foci potentially increase the parasite’s exposure to the schistosomulacidal mediator NO, which is produced at maximal levels by IFN-γ-activated macrophages and endothelial cells (48). Our data show that parasite Ag–elicited cells from IL-10-deficient mice have an increased capacity to kill parasites, ex vivo, through an NO-dependent mechanism (Fig. 10). However, it is important to note that the inflammatory reactions that we observed in the IL-10-deficient mice were peribronchial and perivascular and were not in all cases directly associated with the lung stage parasites themselves. Nevertheless, these findings support the results of Gazinelli et al., who showed that IL-10 could inhibit the killing of schistosomula by parasite Ag-elicited cells (49).

Previously, our laboratory demonstrated that cellular and humoral immune responses were increased in vaccinated mice exposed to attenuated parasites in the presence of exogenous rIL-12 (18). Interestingly, we observed a similar situation in the vaccinated IL-10-deficient mice, since both humoral and cellular-dependent mechanisms were increased. However, the humoral response in IL-12-vaccinated mice was dominated by IgG2 Abs, an Ab isotype typically associated with polarized type 1 immune responses, while both type 1 and type 2 Abs were observed in the IL-10-deficient animals. This observation was surprising, since we have previously hypothesized that the presence of type 2-associated effector mechanisms might inhibit the protective mechanisms that operate on the type 1 pole (39, 50). The current data show that type 1 effector mechanisms are indeed functional in IL-10-deficient mice (Fig. 10), which supports the conclusions of Caulada-Benedetti et al., who also demonstrated highly effective cell-mediated anti-schistosome effector mechanisms in the midst of a significant type 2 response (20).

Our results suggest that a major consequence of schistosome-induced IL-10 production is to down-regulate the host’s immune response against the parasite. We recently demonstrated that IL-10 also plays an important role in down-regulating hepatic granuloma formation during the acute stage of infection (26). Thus, the induction of IL-10 by the parasite may be an important factor ensuring the survival of the parasite as well as the host, since it reduces several key anti-parasite effector mechanisms and simultaneously limits the extent of tissue pathology. Both effects would help insure a favorable host-parasite relationship. Thus, as has been suggested by Muraille and Leo (51), IL-10 appears to be a central dampening cytokine of the immune response. Moreover, these studies support the recent findings of Pearce et al., who have also suggested that generating a balance between Th1- and Th2-type cytokine expression may be a more beneficial goal for preventing severe disease in schistosomiasis (52). Additionally, results obtained from humans suggest that IL-10, in fact, may be a commonly used protective cytokine induced by many helminth parasites (44, 53, 54). Finally, these data revealed a potential strategy whereby short term IL-10 antagonism might be exploited to boost cellular and humoral protective mechanisms induced by vaccination. Nevertheless, whether IL-10 neutralization at the time of vaccination alone can duplicate the results obtained from the IL-10-deficient animals has yet to be determined. Studies with schistosome vaccine candidates have often yielded disappointing results (36); targeting IL-10 might prove an effective approach to increase the efficacy of some of the defined vaccine Ags and contribute to a highly effective vaccine for schistosomiasis.

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References


