INFECTION AND IMMUNITY, Jan. 1997, p. 219–226 Vol. 65, No. 1
0019-9567/97/$04.00+0

Developmental Differences Determine Larval Susceptibility to Nitric Oxide-Mediated Killing in a Murine Model of Vaccination against *Schistosoma mansoni*

S. FOUAD AHMED,[1]† I. P. OSWALD,[1]‡ P. CASPAR,[1] S. HIENY,[1] L. KEEFER,[2] A. SHER,[1] AND S. L. JAMES[1]*

Immunobiology Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892,[1] and Chemistry Section, Laboratory of Comparative Carcinogenesis, National Cancer Institute, National Institutes of Health, Frederick, Maryland 21702[2]

Received 25 July 1996/Returned for modification 23 September 1996/Accepted 18 October 1996

A persistent paradox in our understanding of protective immunity against *Schistosoma mansoni* infection in animals vaccinated with attenuated parasites has been that attrition of challenge parasites occurs during migration through the lungs in vivo, although parasites recovered from the lungs appear to be relatively resistant to cytotoxic effector mechanisms in vitro. We have compared the susceptibilities of different stages of larvae to killing by nitric oxide (NO), which was previously shown to be involved in the larvicidal function of cytokine-activated cytotoxic effector cells. Lung-stage larvae obtained 1 week after infection were not killed in vitro by NO generated either by a chemical NO donor or by activated cells. In contrast, parasites obtained from the portal system of control mice or from the lungs of vaccinated mice 2.5 weeks following challenge infection were killed by NO. As previously shown for mammalian cell targets, the effects of NO in susceptible larval stages may involve enzymes required for aerobic energy metabolism, since similar cytotoxicity was demonstrated by chemical inhibitors of the citric acid cycle or mitochondrial respiration. Taken together with previous observations of enhanced Th1 activity and expression of NO synthase in the lungs of vaccinated mice at 2.5 weeks after challenge infection, these observations elucidate the immune mechanism of vaccine-induced resistance to *S. mansoni* infection. Moreover, they suggest that conversion to a less metabolically active state may allow pathogens to escape the effects of the important effector molecule NO.

Schistosomiasis is one of the major tropical diseases targeted for control by the World Health Organization (34), and vaccine development has received much attention as a method for achieving such long-term control (3). Research with animal models indicates that vaccine development is feasible. High levels of protective immunity in mice are achieved by vaccination with radiation-attenuated parasites, and partially protective defined antigens are being identified (3). It has been reasoned that definition of the immune mechanisms operating in experimental vaccine models will facilitate the design of effective human vaccines.

Schistosome parasites undergo a series of complex transformations within the mammalian host, with newly transformed larvae (schistosomula) in the skin migrating via the bloodstream to the lungs and then on to the liver and hepatic portal system over a period of weeks (3). Previous studies with a murine model of a radiation-attenuated vaccine have shown that most larvae from a challenge infection are eliminated as they migrate through the lungs of the immunized host (8, 16). Parasite elimination in the lungs of vaccinated mice appears to be a protracted process, lasting several weeks. Histopathologic studies indicate that larvae transiting through the lungs become trapped in the pulmonary microvasculature or alveolar spaces (13). Inflammatory reactions are observed around larvae in both sites (13), and mononuclear cells are prominent in these reactions (32). Cell-mediated (Th1-type) immunity plays a major role in the resistance raised by a single vaccination, while antibody-dependent mechanisms become more prominent in multiply vaccinated mice (12). In mice exposed to a single immunization with attenuated parasites, depletion of either gamma interferon (IFN-γ) or nitrogen oxides results in substantially lower levels of resistance to challenge infection (25, 35). These in vivo observations, together with in vitro studies showing that macrophages and endothelial cells activated by cytokines, especially IFN-γ, are able to kill newly transformed schistosomula through an arginine-dependent NO-mediated mechanism (10, 19), have helped to define the importance of Th1-type immune responses in protective immunity. Furthermore, expression of mRNA for Th1 cytokines and NO synthase is elevated in the lungs of vaccinated mice after challenge infection with *Schistosoma mansoni* (35), an observation consistent with a role for these responses in protective immunity at the primary site of attrition in vivo. Previous findings that lung-stage larvae (recovered at approximately 1 week after infection) are remarkably resistant to killing by cytokine-activated macrophages in vitro (20, 21) would seem to challenge this hypothesis, however. Since these older parasites are also resistant to cytotoxic T lymphocytes and antibody-dependent killing (22), some have postulated that elimination of challenge parasites in this model is not directly mediated by an immune response but rather results from an unsuccessful attempt at migration by trapped larvae which ends in their escape into the alveoli, from which they are eventually expectorated (7). If true, this could have serious ramifications for

* Corresponding author. Mailing address: Parasitology and International Programs Branch, Solar Building, Room 3A-10, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892-7630. Phone: (301) 496-2544. Fax: (301) 402-0659.

† Present address: Cairo University, Guiza, Cairo, Egypt.

‡ Present address: INRA, Ecole Nationale Veterinaire de Toulouse, 31076 Toulouse Cedex, France.

any attempt to develop a human vaccine based on findings in this model.

We have previously shown that older larvae recovered from the portal system of infected mice exhibit a transient reexpression of susceptibility to killing by cytokine-activated effector cells (20). In this paper, we examine the physiologic basis of this surprising reversion of older larvae to a state of vulnerability to immune attack and relate it to the fate of challenge parasites trapped in the lungs of vaccinated mice.

MATERIALS AND METHODS

Animals and parasites. Female C57BL/6 mice were obtained from the Division of Cancer Treatment, National Cancer Institute (Frederick, Md.). Cercariae of *S. mansoni* (NMRI strain) were obtained from infected *Biomphalaria glabratra* snails (Biomedical Research Institute, Rockville, Md.). For vaccination, mice were exposed percutaneously to 500 50-krad-irradiated cercariae, as previously described (35). In this strain, vaccination by this method results in >60% protection against challenge infection (8, 10, 12).

Newly transformed (3-h) schistosomula were prepared by vortex shearing of cercarial tails followed by purification over a Percoll gradient (15). Mechanically transformed larvae were then incubated in Dulbecco's modified Eagle's medium (DMEM) with 4.5 g of glucose per liter (BioWhittaker, Rockville, Md.) supplemented with HEPES and antibiotics for 3 h at 37°C (13, 21). Lung-stage larvae were recovered from 1-week-infected (3,000 cercariae) mice, or from previously vaccinated mice at 1 or 2.5 weeks following exposure to 1,500 cercariae at 4 weeks after immunization, as previously described (20). Briefly, excised lungs were rinsed in DMEM containing 5% heat-inactivated fetal calf serum (FCS) (Hyclone, Logan, Utah), 2 mM glutamine, 100 U of penicillin, 100 μg of streptomycin, 10 mM HEPES, and 10 U of heparin per ml and incubated for at least 4 h at 37°C. Parasites were subsequently separated from mouse tissue through fine nylon. Older parasites (2.5 weeks) were perfused from the hepatic portal system (24) and washed free of contaminating blood cells in DMEM containing 5% FCS.

Reagents. The chemical donor molecule DETA/NO, the adduct of diethylenetriamine with NO {1-[*N*-(2-aminoethyl)-*N*-(2-ammonioethyl)amino]diazen-1-ium-1,2-diolate; Chemical Abstracts registry number 146724-94-9}, was used at a range of concentrations from 25 μM to 2 mM. The ability of DETA/NO to generate NO in aqueous solution has a half-life of 20 h (18). N^G-Monomethyl-L-arginine monoacetate (NMMA) (Calbiochem, La Jolla, Calif.) was used as a selective inhibitor of NO production by NO synthase. Inhibitors of enzymes involved in energy metabolism were used at concentrations bracketing those known to affect mammalian enzymes (6). These inhibitors included sodium fluoroacetate (Aldrich Chemical Company, Inc., Milwaukee, Wis.), an aconitase inhibitor; sodium azide, which inhibits electron transport between cytochromes a/a_3 and O_2; oligomycin B, an inhibitor of mitochondrial ATPase, preventing phosphoryl group transfer; antimycin A, an inhibitor of electron transfer between cytochromes *b* and *c*; 2,4-dinitrophenol, an uncoupler of oxidative phosphorylation; rotenone, an inhibitor of electron transport within the NADH-ubiquinone complex; and 2-deoxy-D-glucose, an inhibitor of glycolysis, utilized in glucose-free DMEM. All inhibitors except sodium fluoroacetate were purchased from Sigma Chemical Co. (St. Louis, Mo.). Recombinant murine IFN-γ and tumor necrosis factor alpha (TNF-α) were gifts from Genentech.

In vitro cytotoxicity assay. Inflammatory peritoneal exudative cells were harvested from C57BL/6 mice 4 days after injection with 3% thioglycolate broth (1.5 ml/mouse). Peritoneal exudative cells (>80% macrophages by differential staining) were activated with IFN-γ (100 U/ml) and used at a concentration of 10^6 cells/ml. A murine endothelial cell line (transformed brain endothelial cells) (19) was maintained in a mixture of 50% RPMI 1640 and 50% DMEM supplemented with 10% FCS. IFN-γ- and TNF-α (1,000 U/ml)-activated endothelial cells were cultured with newly transformed schistosomula or older parasites for 48 and 72 h, respectively, at effector/target ratios varying from 0.5×10^4:1 to 1×10^4:1 as previously described (19, 20). Larval killing was determined microscopically by the criteria of internal granularity and loss of mobility (11, 19–21).

Measurement of nitrite production. As appropriate, nitrite concentrations in the cell culture supernatants were monitored to ensure that observed differences in larval killing were not an artifact of differences in exposure to NO. Nitrite accumulation in culture supernatants was measured colorimetrically by a standard Griess reaction adapted to microplates (11, 19). Briefly, 100 μl of a 1:1 mixture of 1% sulfanilamide in 2.5% H_3PO_4 and 0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride in 2.5% H_3PO_4 was added to 100 μl of cell supernatants; after 10 min of reaction, the absorbance was read at 550 nm and NO_2^- was determined with reference to a standard curve with sodium nitrite at a concentration ranging from 250 to 1 μM in culture medium.

Lymphocyte cultures and cytokine assays. Mediastinal lymph node cells from infected mice were cultured at 37°C and 5% CO_2 in air, at a final concentration of 5×10^6/ml in 1 ml (final volume) of 50% RPMI–50% DMEM supplemented with 10% FCS, penicillin-streptomycin, nonessential amino acids, sodium pyruvate, and L-glutamine. Mechanically transformed 3-h schistosomula were frozen and thawed for use as the antigen for in vitro stimulations (5,000 larvae/ml) (31).

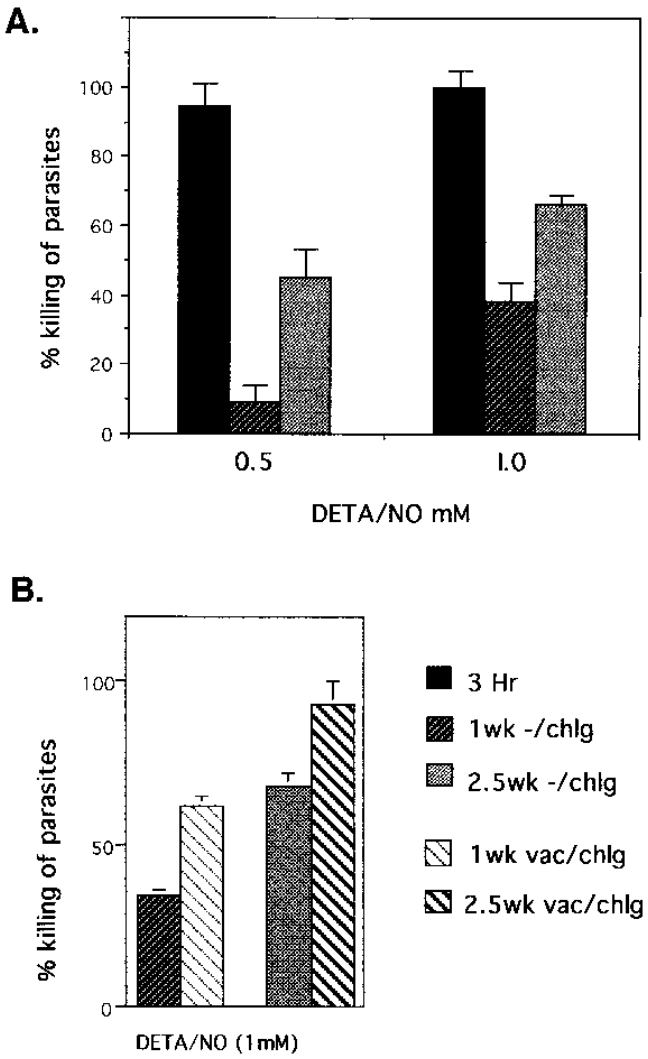

FIG. 1. Comparison of the susceptibilities of different parasite stages to NO produced by the chemical donor molecule DETA/NO. Parasites, prepared in vitro (3 h) or collected at the indicated times after infection of untreated control C57BL/6 mice (−/chlg) or mice vaccinated 4 weeks previously (vac/chlg), were incubated for 48 h in vitro with the chemical, which releases NO with a half-life of 20 h, and then mortality was evaluated microscopically. Results represent means ± standard errors of the means for five experiments. Larval mortality in the absence of DETA/NO was less than 15% at 48 h.

A soluble adult worm antigen preparation was prepared from frozen adult worms and was used at a concentration of 50 μg/ml for in vitro stimulation (31). For detection of IFN-γ and interleukin-10, 72-h culture supernatant fluids were used and two-site enzyme-linked immunosorbent assays were performed as previously described (31).

RESULTS

Susceptibility of larval schistosomes to NO. Because previous studies indicating a role for NO in cell-mediated killing of larval schistosomes were indirect, involving treatment of activated effector cell cultures with inhibitors of NO production (10, 19), it remained possible that some related form such as peroxynitrite anion functioned as the actual effector molecule. To determine whether NO plays a role in parasite killing in the absence of other products of activated effector cells, we examined the level of parasite mortality in the presence of the chemical NO donor DETA/NO. As shown in Fig. 1A, newly

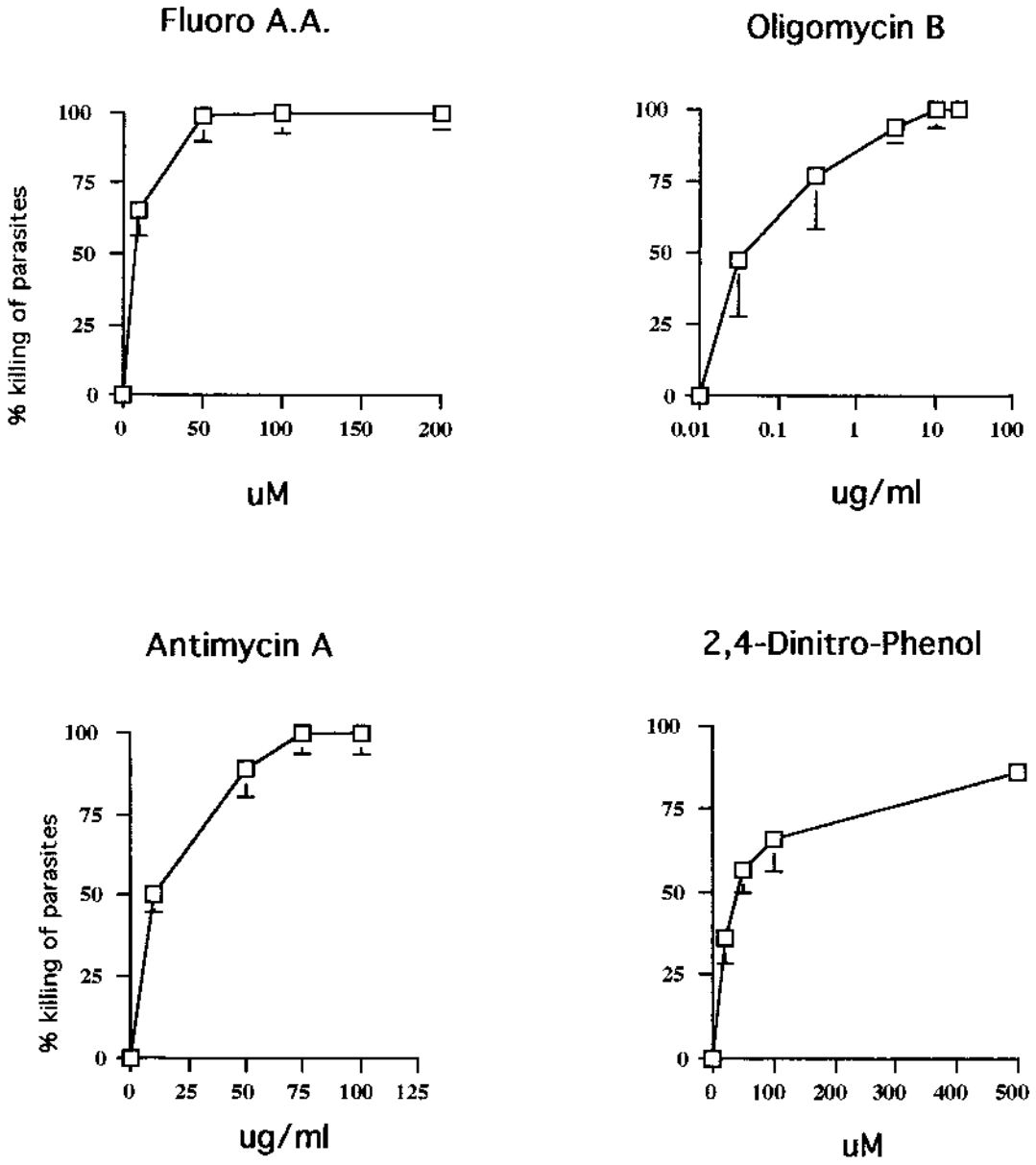

FIG. 2. Susceptibility of 3-h schistosomula to various inhibitors of aerobic metabolism. Parasites were incubated for 48 h in vitro with a range of concentrations of each inhibitor, and mortality was evaluated. Results represent the means for at least four separate experiments ± standard errors of the means. Fluoro A.A., fluoroacetate.

transformed (3-h) schistosomula were completely killed in vitro by concentrations of DETA/NO previously shown to inhibit proliferation of mammalian cells (18), indicating that the larvicidal action of NO does not require interaction with other effector cell products.

Because previous studies have shown a differential susceptibility of older parasites to killing by cytokine-activated effector cells, the DETA/NO susceptibility of larvae obtained 1 week (lung stage) or 2.5 weeks (liver stage) following infection of naive mice was also examined (Fig. 1A). In agreement with results obtained with whole-cell populations (20, 21), lung-stage larvae were relatively resistant to NO effects while liver-stage parasites showed an intermediate level of susceptibility. That all cultures contained equivalent levels of nitrite was confirmed by the Griess reaction (data not shown).

Susceptibility of schistosomula to inhibitors of energy metabolism. One of the mechanisms by which NO has been shown to exert direct cytotoxic and cytostatic effects on mammalian cells is through inactivation of key metabolic enzymes (33). To determine whether similar metabolic inhibition would be toxic to schistosomula, and thus might account for the larvicidal activity of NO, 3-h parasites were treated with inhibitors of various enzymes or activities related to the tricarboxylic acid cycle or oxidative phosphorylation (Fig. 2). In each case, larval killing was observed at concentrations of these inhibitors approximately equivalent to those known to be toxic for mammalian cells (6). In three additional experiments, 10 μM rotenone, another inhibitor of mitochondrial respiration, was found to kill 79 ± 3% of 3-h larvae in culture, while 50 μM was 100% toxic.

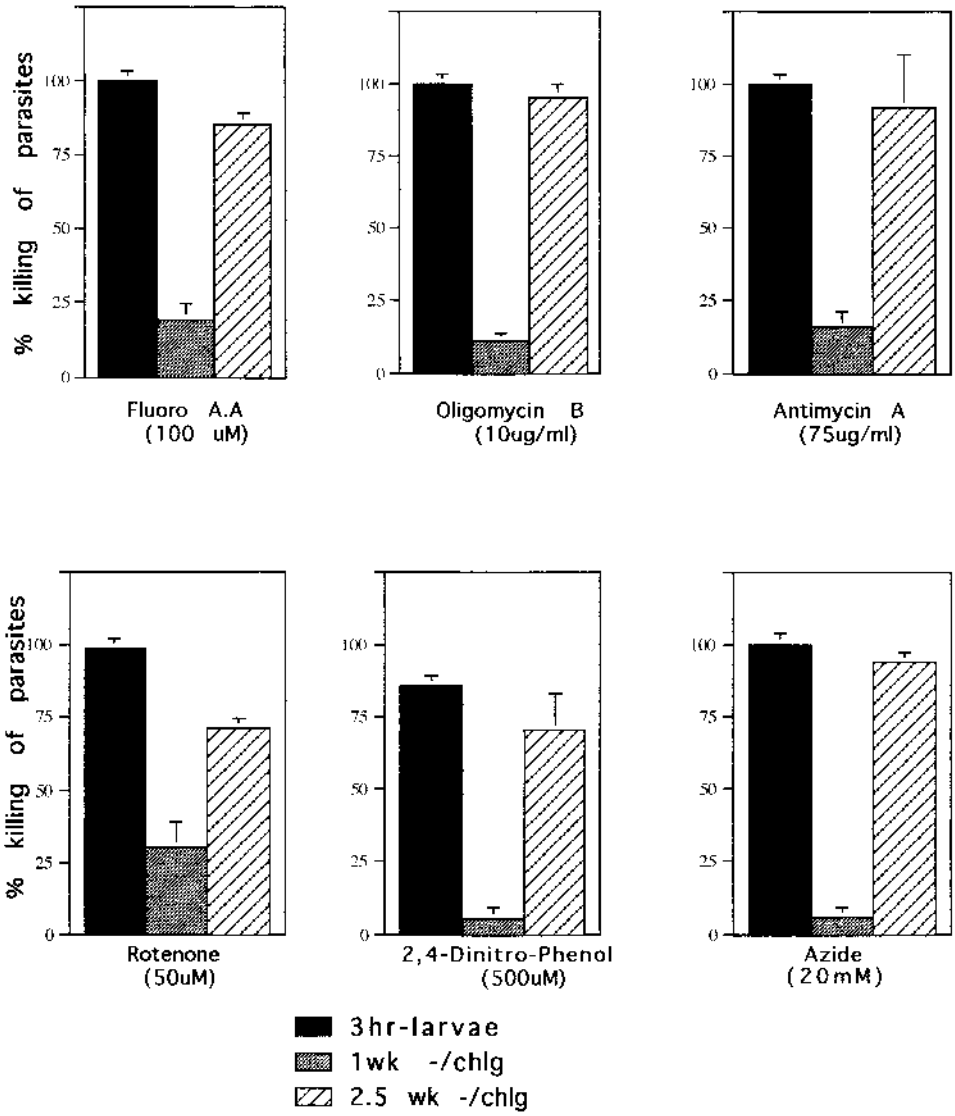

FIG. 3. Comparison of the susceptibilities of different parasite stages to metabolic inhibitors. Parasites, collected at the indicated times after infection of untreated control C57BL/6 mice, were incubated for 48 h in vitro with inhibitors, and then mortality was evaluated microscopically. Inhibitors were utilized at the minimum concentration that demonstrated the highest level of killing of 3-h larvae. Results represent means ± standard errors of the means for four experiments. Abbreviations are as described in the legends to Fig. 1 and 2.

Because it has been reported that while newly transformed schistosomula retain aerobic metabolism for a short time, older parasites are almost entirely dependent on anaerobic metabolism, the effects of these metabolic inhibitors on 1-week lung-stage and 2.5-week liver-stage worms were also tested (Fig. 3). At concentrations of inhibitor found to produce maximal killing of 3-h parasites, the same pattern was observed in these studies as was seen as described above with DETA/NO and previously with activated cells (20). That is, the 1-week larvae were resistant to killing, while the 2.5-week larvae reverted to susceptibility. These studies suggest that the observed biphasic pattern of larval susceptibility to NO killing may be due to differences in energy requirements at different stages of parasite development. In support of this conclusion, schistosomula were observed to lose motility at inhibitor concentrations two- to fivefold lower than those which were directly toxic (data not shown).

A similar comparison was performed with 2-deoxy-D-glucose, an inhibitor of glycolysis, which was substituted for glucose in the culture medium. In two experiments, means of 80% of 3-h worms, 78% of 1-week worms, and 97% of 2.5-week worms were killed under these conditions. These results verify that 1-week lung-stage parasites utilize anaerobic metabolism to acquire energy and that glycolysis is also prerequisite for energy metabolism by other stages.

Susceptibility of larvae from vaccinated mice to NO-mediated killing. In order further to examine the potential role of cell-mediated NO production in vaccine-induced resistance, the susceptibility of older parasites recovered from challenge infection of vaccinated mice was compared to that of parasites developing from initial infection of naive animals (Fig. 1B). In the former case, however, because challenge parasites persist in the lungs of previously vaccinated animals rather than transitting on to the portal system as they do in normal mice (8, 16), both 1-week and 2.5-week old larvae were obtained from the lungs. A substantial percentage of parasites recovered 2.5

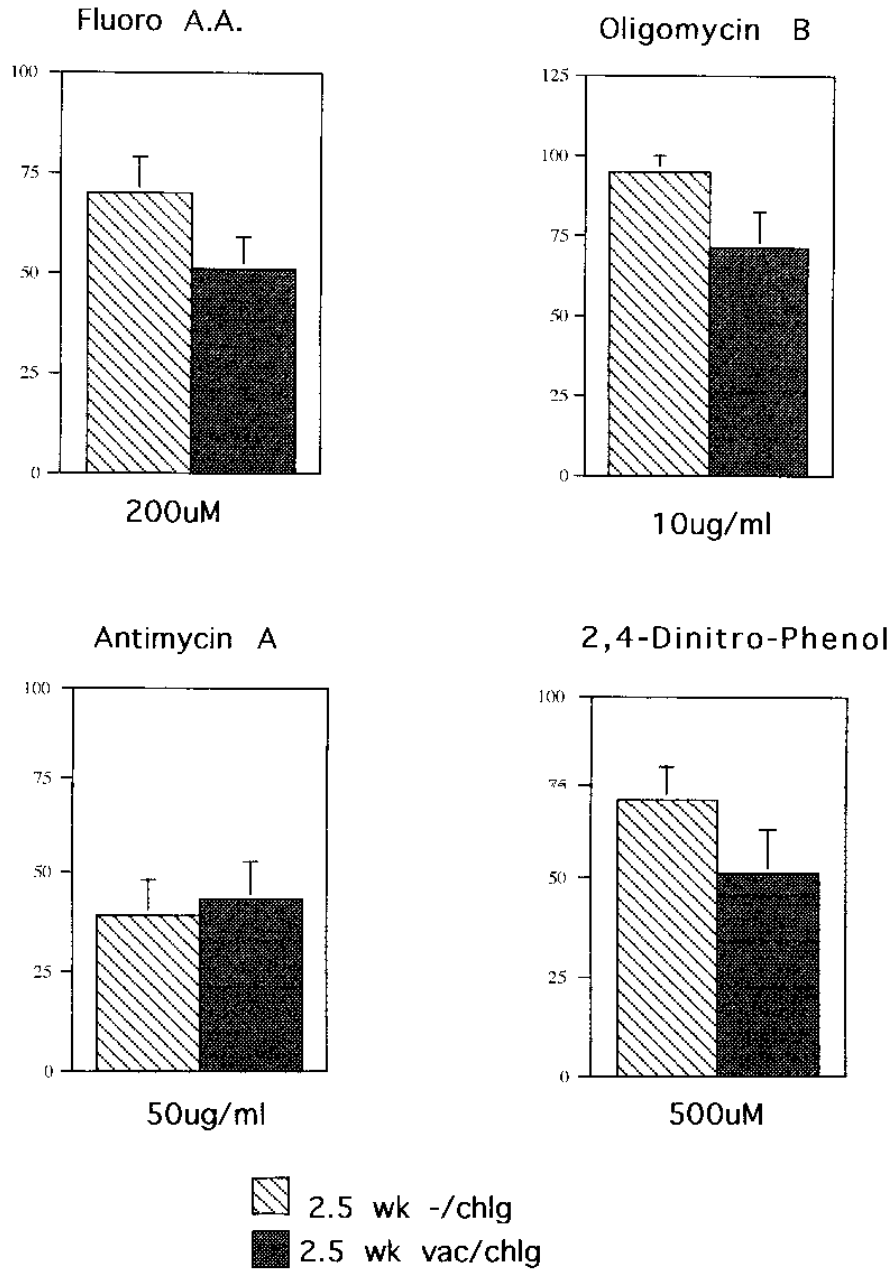

FIG. 4. Comparison of the susceptibilities to metabolic inhibitors of parasites collected from the livers of untreated control mice or the lungs of previously vaccinated mice 2.5 weeks after challenge infection. Results represent means ± standard errors of the means for four experiments. Abbreviations are as described in the legends to Fig. 1 and 2.

weeks after infection of either normal or vaccinated mice was found to be killed by DETA/NO, indicating that by this stage of development parasites have reverted to susceptibility regardless of the physiologic compartment which they occupy. It also appeared that 1-week lung-stage larvae from vaccinated mice were slightly more susceptible to NO-mediated killing than were 1-week larvae from normal mice, an observation that could reflect the early effects of protective immune mechanisms operating in vivo before the larvae were isolated.

A comparison of the susceptibilities of 2.5-week larvae recovered from infection of vaccinated mice and naive mice to several metabolic inhibitors revealed little difference (Fig. 4). Sufficient numbers of lung-stage parasites could not be recovered from 1-week-infected vaccinated mice to perform a similar survey.

Susceptibility of larvae from vaccinated mice to cell-mediated killing. To examine whether the results observed with DETA/NO might reflect the basis of the lung phase of resistance in vaccinated mice, the different larval stages were cultured in vitro with cytokine-activated murine macrophages or endothelial cells (Fig. 5). As previously shown (11, 19–21), 3-h larvae were killed by either cell type by a mechanism that is inhibitable by NMMA. Larvae obtained 1 week after infection of either normal or vaccinated mice showed little susceptibility to cell-mediated killing, although as observed with DETA/NO, the larvae from vaccinated animals appeared to be slightly

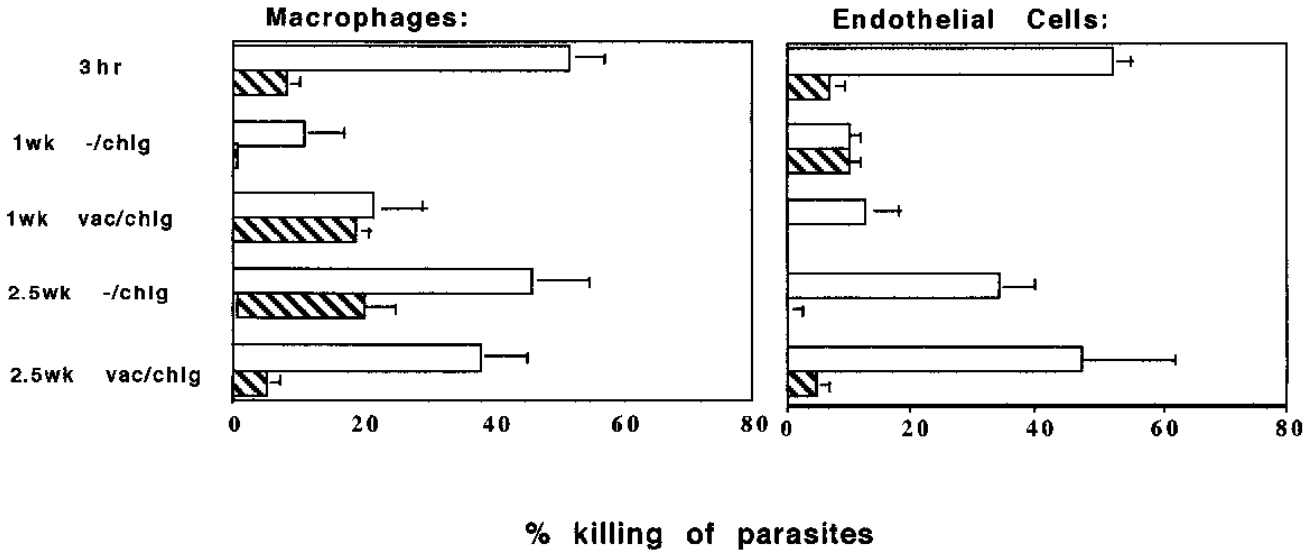

FIG. 5. Comparison of the susceptibilities of different parasite stages to killing by cytokine-activated macrophages or endothelial cells. Parasites collected as described were cultured for 48 h with IFN-γ-treated murine peritoneal macrophages or with IFN-γ-plus-TNF-α-treated murine endothelial cells in the presence (hatched bars) or absence (open bars) of the inhibitor of NO production NMMA. Results represent the means ± standard errors of the means for four experiments with macrophages and two experiments with endothelial cells. Abbreviations are as described in the legend to Fig. 1.

more susceptible to killing. The observation that NMMA did not inhibit the cytotoxic activity of activated macrophages suggests that these parasites may have suffered prior damage in vivo which was not reversible. It was apparent, however, that 2.5-week larvae obtained from infection of either vaccinated or normal mice were readily killed by cytokine-activated cells in a manner that was largely inhibited by NMMA.

When mediastinal lymph nodes were taken from mice used as lung donors for larval recovery and lymphoid cells were restimulated in vitro with parasite antigens, the cytokine pattern observed was consistent with conditions favoring cellular activation in vivo. Thus, cells from animals receiving prior vaccination produced high levels of the activating cytokine IFN-γ and lower levels of the inhibitory cytokine interleukin-10 (data not shown). These results confirmed, for these particular donor animals, the more extensive previous report of cytokine expression patterns within the lungs of vaccinated and challenged mice (35).

DISCUSSION

NO is known to be involved in multiple biologically important reactions, including those with transition metal ions, thiols, and redox forms of oxygen (26). It has been postulated that the toxic effects of NO on helminths are likely due to its reaction with superoxide anion to produce the highly toxic peroxynitrite ion (2, 5). Previous studies showing inhibition of cell-mediated killing in the presence of NMMA did not rule out this possibility, since the cytokine-activated effector cells might produce reactive oxygen as well as nitrogen intermediates. Observation of the same pattern of larval killing with NO generated directly by a chemical donor molecule (Fig. 1) demonstrates that production of superoxide by effector cells is not required in this system, a result consistent with the hypothesis that NO is acting directly on the parasite target. This interpretation is supported by earlier ultrastructural studies of newly transformed and 2.5-week larvae cultured with activated macrophages, in which it was observed that cytotoxicity was not directed against the parasite surface. Rather, progressive disintegration of internal structures, beginning with perturbation of the mitochondria within subtegumental muscle cells and culminating in widespread vacuolation, was observed (17, 20). Such observations are consistent with the principal effector role of NO proposed for mammalian cell targets, which involves inactivation of key enzymes (33). The aconitase enzyme of the Krebs cycle, NADPH-ubiquinone oxidoreductase and succinate:ubiquinone oxidoreductase of the electron transport chain, and the ribonucleotide reductase involved in DNA synthesis and cell proliferation have all been identified as potential enzymatic targets of NO inhibition in mammalian cells (33). The observation that macrophage-mediated killing of schistosomula is inhibited by addition of excess iron to the culture medium (11) also supports the interpretation that Fe-containing moieties might serve as the ultimate NO target molecules within the parasite. It must be noted, however, that the biochemistry of schistosomes is incompletely understood and that therefore these parasites may also contain as-yet-unknown enzymatic or other targets of NO inactivation.

It is widely believed that schistosomes rapidly convert from the aerobic free-living stage to a form primarily dependent on fermentative metabolism after infection of the mammalian host (27, 28, 30). Early experiments confirmed that this transition in energy metabolism correlated with decreased susceptibility to NO-mediated killing by activated macrophages (11, 20, 21). It was later observed, however, that the parasite underwent another transient period of susceptibility to macrophage killing between 2 and 4 weeks after infection (20). By 6 weeks after infection, adult worms were again resistant to macrophage cytotoxicity. The observation that parasites which have entered into a form that is invulnerable to virtually every immune effector mechanism (21, 22) subsequently revert to a susceptible form was inexplicable at the time.

The results presented here suggest that the early conversion to anaerobic metabolism is not permanent; rather, migrating schistosome larvae pass through stages at which they are more or less dependent upon enzymes associated with aerobic respiration for energy metabolism. Thus, the pattern of susceptibility to NO toxicity was found to correlate with that of sus-

ceptibility to chemical inhibitors of aconitase and mitochondrial respiration. The second phase of vulnerability was observed at around 2 to 2.5 weeks after infection in parasites recovered from the livers of unimmunized mice or from the lungs of mice vaccinated with attenuated cercariae, where they are trapped by host inflammatory response (20) (Fig. 1, 3, and 5). It is well known that parasitic helminths generally have the capacity to adapt to different environments by the use of different methods of energy generation (14, 27, 28) and that all parasitic helminths are capable of oxidative phosphorylation (1). It has been reported that even adult schistosomes utilize aerobic pathways to generate the energy needed for motility (9, 29). Adult parasites have also been shown to express mRNAs for two enzymes involved in oxidative metabolism, mitochondrial malate dehydrogenase and cytochrome oxidase subunit 1 (23), and to obtain approximately 30% of their total ATP production through aerobic mechanisms (30). In fact, most studies of the metabolic activity of schistosomes have involved only adult parasites, because they are relatively easy to obtain and are logical targets for development of chemotherapeutic agents. Similar metabolic or molecular studies of migrating larvae are hampered by the inability to obtain parasites free of host tissue. One such study monitoring the development of *S. mansoni* in hamsters reported an interesting dip in lactate formation at between 2 and 4 weeks after infection, with a coincident peak in CO_2 production (30), however, which could be interpreted to support our hypothesis. The concentrations at which inhibitors were found to be toxic to schistosomula were generally not very different from those found to be cytostatic for mammalian cells, an observation that may be surprising given that these helminth larvae are complex multicellular organisms. Moreover, while more difficult to quantify, it should be noted that in the experiments reported here, 2.5-week larval schistosomes completely lost their motility at inhibitor concentrations lower than those resulting in absolute larval mortality.

These results supply an important but hitherto missing piece in the puzzle of how this attenuated vaccine protects against challenge *S. mansoni* infection. Thus, it has previously been shown that immunologic conditions favorable to the activation of cytotoxic macrophages or endothelial cells exist within the lungs of vaccinated and challenged mice (35). Furthermore, levels of mRNA for inducible NO synthase are highly elevated in the lungs of these animals between 2 and 3 weeks after challenge infection, suggesting that toxic NO is being produced locally at this time (35). More than 60% of detectable parasites resulting from challenge infection of singly vaccinated mice are found in the lung after 3 weeks, and the majority of these, whether located in the blood vessels or alveoli, are surrounded by an inflammatory cellular infiltrate (13, 32). NO synthase expression has been demonstrated in the inflammatory foci surrounding challenge schistosomula in the lung (35). In the current study, it has now been shown that the parasite itself is extremely vulnerable to NO-mediated killing at exactly the time of peak NO expression. Previous studies (13) have argued that no evidence for larval damage in the lung is observed; given that NO-mediated killing leaves the parasite tegument intact (17, 20), however, it is unlikely that low-resolution histopathologic and autoradiographic examination would distinguish live from dying parasites. Certainly, the type of parasite paralysis observed at lower inhibitor concentrations could never be detected by these methods but might explain why parasites are unable to escape the trapping phenomenon.

Taken together, these results strongly suggest that protective immunity in this vaccine model is acting through a standard cytotoxic-cytostatic mechanism, likely involving the function of activated macrophages in the alveolar spaces as well as activated endothelial cells in the pulmonary vasculature. Such a mechanism should be reproducible by using defined vaccines that elicit strong Th1-type reactivity.

In addition, these results help to define conditions by which a human pathogen can escape the toxic effect of NO, a molecule which has recently been shown to kill a multitude of eukaryotic and prokaryotic organisms and against which no other defense mechanism has, to our knowledge, been reported. Recent experiments showing that conversion of *Toxoplasma gondii* to the relatively quiescent bradyzoite stage is induced upon treatment with exogenous NO or with inhibitors of mitochondrial respiration (4) suggest that other pathogens may be found to utilize this immune evasion mechanism as well.

ACKNOWLEDGMENTS

This work was supported in part by the UNDP/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases and the Schistosomiasis Research Project (a collaborative program between the Ministry of Health Egypt and the USAID).

We thank Tom Wynn, Dragana Jankovic, and Tanya Kersten for helpful discussions during the conduct of the research and preparation of the manuscript.

REFERENCES

1. **Barrett, J.** 1984. The anaerobic end-products of helminths. Parasitology **88:**179–198.
2. **Beckman, J. S., T. W. Beckman, J. Chen, P. A. Marshall, and B. A. Freeman.** 1990. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc. Natl. Acad. Sci. USA **87:**1620–1624.
3. **Bergquist, N. R., B. F. Hall, and S. James.** 1994. Schistosomiasis vaccine development: translating basic research into practical results. Immunologist **2:**131–134.
4. **Bohne, W., J. Heesemann, and U. Gross.** 1994. Reduced replication of *Toxoplasma gondii* is necessary for induction of bradyzoite-specific antigens: a possible role for nitric oxide in triggering stage conversion. Infect. Immun. **62:**1761–1767.
5. **Brophy, P. M., and D. I. Pritchard.** 1992. Immunity to helminths: ready to tip the biochemical balance? Parasitol. Today **8:**419–422.
6. **Dawson, R. M. C., D. C. Elliott, W. H. Elliott, and K. M. Jones.** 1986. Antimetabolites, antibacterial agents, and enzyme inhibitors, p. 255–335. *In* Data for biochemical research. Clarendon Press, Oxford, United Kingdom.
7. **Dean, D. A., and B. L. Mangold.** 1992. Evidence that both normal and immune elimination of *Schistosoma mansoni* take place at the lung stage of migration prior to parasite death. Am. J. Trop. Med. Hyg. **47:**238–248.
8. **Dean, D. A., B. L. Mangold, J. R. Georgi, and R. H. Jacobson.** 1984. Comparison of *Schistosoma mansoni* migration patterns in normal and irradiated cercaria-immunized mice by means of autoradiographic analysis. Am. J. Trop. Med. Hyg. **33:**89–96.
9. **Foster, L. A., G.-Z. Chen, E. A. VandeWaa, R. A. Pax, and J. L. Bennett.** 1989. Glutamine- vs glucose-supported motor activity in *Schistosoma mansoni*: physiological relevance of aerobic metabolism. Exp. Parasitol. **69:**44–53.
10. **James, S. L., and D. L. Boros.** 1994. Immune effector role of macrophages in experimental *Schistosomiasis mansoni*. Immunol. Ser. **60:**461–473.
11. **James, S. L., and J. Glaven.** 1989. Macrophage cytotoxicity against schistosomula of *Schistosoma mansoni* involves arginine-dependent production of reactive nitrogen intermediates. J. Immunol. **143:**4208–4212.
12. **James, S. L.** 1992. Experimental models of immunization against schistosomes: lessons for vaccine development. Immunol. Invest. **21:**477–493.
13. **Kassim, O. O., D. A. Dean, B. L. Mangold, and F. von Lichtenberg.** 1992. Combined microautoradiographic and histopathologic analysis of the fate of challenge *Schistosoma mansoni* schistosomula in mice immunized with irradiated cercariae. Am. J. Trop. Med. Hyg. **47:**231–237.
14. **Komuniecki, R., and P. R. Komuniecki.** 1995. Aerobic-anaerobic transitions in energy metabolism during the development of the parasitic nematode *Ascaris suum*, p. 109–121. *In* J. C. Boothroyd and R. Komuniecki (ed.), Molecular approaches to parasitology. Wiley-Liss, New York, N.Y.
15. **Lazdins, J. K., M. J. Stein, J. R. David, and A. Sher.** 1982. *Schistosoma mansoni*: rapid isolation and purification of schistosomula of different developmental stages by centrifugation on discontinuous density gradients of Percoll. Exp. Parasitol. **53:**39–44.
16. **Mastin, A. J., Q. D. Bickle, and R. A. Wilson.** 1983. *Schistosoma mansoni*: migration and attrition of irradiated and challenge schistosomula in the mouse. Parasitology **87:**87–102.
17. **McLaren, D. J., and S. L. James.** 1985. Ultrastructural studies of the killing

of schistosomula of *Schistosoma mansoni* by activated macrophages in vitro. Parasite Immunol. **7:**315–331.
18. **Mooradian, D. L., T. C. Hutsell, and L. K. Keefer.** 1995. Nitric oxide (NO) donor molecules: effect of NO release rate on vascular smooth muscle cell proliferation in vitro. J. Cardiovasc. Pharmacol. **25:**674–678.
19. **Oswald, I. P., I. Eltoum, T. A. Wynn, B. Schwartz, P. Caspar, D. Paulin, A. Sher, and S. L. James.** 1994. Endothelial cells are activated by cytokine treatment to kill an intravascular parasite, *Schistosoma mansoni*, through the production of nitric oxide. Proc. Natl. Acad. Sci. USA **91:**999–1003.
20. **Pearce, E. J., and S. L. James.** 1986. Post lung-stage schistosomula of *Schistosoma mansoni* exhibit transient susceptibility to macrophage-mediated cytotoxicity in vitro that may relate to late phase killing in vivo. Parasite Immunol. **8:**513–527.
21. **Sher, A., S. L. James, A. J. G. Simpson, J. K. Lazdins, and M. S. Meltzer.** 1982. Macrophages as effector cells of protective immunity in murine schistosomiasis. III. Loss of susceptibility to macrophage-mediated killing during maturation of *S. mansoni* schistosomula from the skin to the lung stage. J. Immunol. **128:**1876–1879.
22. **Sher, A., and G. Moser.** 1981. Immunologic properties of developing schistosomula. Am. J. Pathol. **102:**121–126.
23. **Skelly, P. J., L. D. Stein, and C. B. Shoemaker.** 1993. Expression of *Schistosoma mansoni* genes involved in anaerobic and oxidative glucose metabolism during the cercaria to adult transformation. Mol. Biochem. Parasitol. **60:**93–104.
24. **Smithers, S. R., and R. J. Terry.** 1965. The infection of laboratory hosts with cercariae of *Schistosoma mansoni* and the recovery of adult worms. Parasitology **55:**695–700.
25. **Smythies, L. E., P. S. Coulson, and R. A. Wilson.** 1992. Monoclonal antibody to IFN modifies pulmonary inflammatory responses and abrogates immunity to *Schistosoma mansoni* in mice vaccinated with attenuated cercariae. J. Immunol. **149:**3654–3658.
26. **Stamler, J. S., D. J. Singel, and J. Loscalzo.** 1992. Biochemistry of nitric oxide and its redox-activated forms. Science **258:**1898–1902.
27. **Thompson, D. P., D. D. Morrison, R. A. Pax, and J. L. Bennett.** 1984. Changes in glucose metabolism and cyanide sensitivity in *Schistosoma mansoni* during development. Mol. Biochem. Parasitol. **13:**39–51.
28. **Tielens, A. G. M.** 1994. Energy generation in parasitic helminths. Parasitol. Today **10:**346–352.
29. **Vanden Bossche, H.** 1985. How anthelmintics help us to understand helminths. Parasitology **90:**675–685.
30. **van Oordt, B. E. P., A. G. M. Tielens, and S. G. van den Bergh.** 1988. The energy metabolism of *Schistosoma mansoni* during its development in the hamster. Parasitol. Res. **75:**31–35.
31. **Williams, M. E., P. Caspar, I. Oswald, H. K. Sharma, O. Pankewycz, A. Sher, and S. L. James.** 1995. Vaccination routes that fail to elicit protective immunity against *Schistosoma mansoni* induce the production of TGF-β, which down-regulates macrophage antiparasitic activity. J. Immunol. **154:**4693–4700.
32. **Wilson, R. A., and P. S. Coulson.** 1989. Lung-phase immunity to schistosomes: a new perspective on an old problem. Parasitol. Today **5:**274–278.
33. **Woods, M. L., J. Mayer, T. G. Evans, and J. B. Hibbs Jr.** 1994. Antiparasitic effects of nitric oxide in an in vitro murine model of *Chlamydia trachomatis* infection and an in vivo murine model of *Leishmania major* infection. Immunol. Ser. **60:**179–195.
34. **World Health Organization.** 1993. The control of schistosomiasis. *In* Second Report of the WHO Expert Committee. World Health Organization, Geneva, Switzerland.
35. **Wynn, T. A., I. P. Oswald, I. A. Eltoum, P. Caspar, C. J. Lowenstein, F. A. Lewis, S. L. James, and A. Sher.** 1994. Elevated expression of Th1 cytokines and nitric oxide synthase in the lungs of vaccinated mice after challenge infection with *Schistosoma mansoni*. J. Immunol. **153:**5200–5209.

Editor: J. M. Mansfield