PN-ABG-425
68867

RECEIVED
JUL 30 1987
STINFO

FINAL REPORT ON

GRANT NO.: DAN-5053-G-SS-5041

U.S. AGENCY FOR INTERNATIONAL DEVELOPMENT

WASHINGTON, D.C.

IMMUNOSUPPRESSION TO BACTERIAL VACCINES IN SCHISTOSOMIASIS

Principal Investigator:

OLAKUNLE O. KASSIM, Ph.D., MPH
Associate Professor
Department of Microbiology
Howard University College of Medicine
520 W Street, N.W.
Washington, D.C. 20059

SUMMARY

Both the vesicular and hepatosplenic forms of schistosomiasis are associated with the development of immunosuppression. But there is as yet no information if *Schistosoma mansoni* infection may result in immunological unresponsiveness to selected bacterial vaccines. Since the pathogenesis of *S. mansoni* is mice is similar to the clinical course of the disease in humans, we used the murine model to study the effects of an underlying schistosome infection to cellular and humoral immune responses to whole-cell vaccines of *Salmonella typhimurium*, *Bordetella pertussis*, *Streptococcus pneumoniae* and tetanus toxoid, all of which contribute to childhood morbidity and mortality in developing countries. Humoral immune responses were monitored with the measurement of specific IgG and IgM antibodies to PHA, *S. mansoni* SEA and antigenic extracts from the four bacterial vaccines. Cellular immune responses were monitored by spleen lymphocyte proliferation. The results indicate that the presence of an underlying *S. mansoni* infection did not impair the immunological capacity of mice to mount antibody responses to the various bacterial vaccines, except for *B. pertussis*. However, the results conclusively demonstrate that spleen lymphocytes from *S. mansoni*-infected mice were totally unresponsive to stimulation by pertussis antigen and tetanus toxoid. The findings therefore suggest the importance of considering the prevalence and incidence of a parasitic infection, such as schistosomiasis, malaria, onchocerciasis in the successful implementation of an immunization program that is designed for the control of whooping cough and tetanus.

INTRODUCTION

Schistosomiasis, malaria, whooping cough, bacterial meningitis and diarrheal diseases are associated with high rates of infant mortality and adult morbidity in many developing countries. A recent survey undertaken by the World Health Organization (WHO) estimated that over 500 million people are exposed worldwide to the three species of human schistosomes: Schistosoma mansoni, S. haematobium, and S. japonicum (1). Both the vesicular and hepatosplenic forms of schistosomiasis are chronic debilitating diseases which may result in nephrotic syndrome, bladder calcification, liver fibrosis and hepatosplenomegaly (2,3). Whooping cough, due to Bordetella pertussis, is an acute respiratory disease that is characterized by violent, asphyxiative cough and meningitis (4). Approximately 80% of all unimmunized children contract the disease, with a case fatility of 1-3% (5). Salmonella typhimurium, on the other hand, is associated with gastroenteritis and typhoid fever. The death rate from the disease varies from 2 to 10 percent. In addition to being associated with pneumonia, Streptococcus pneumoniae also causes meningitis, with case-fatality rates of 40-60% in many developing countries (5). Although diseases due to these bacteria have been largely controlled in industrialized countries, they still represent significant causes of mortality and morbidity in developing countries. While much research effort has been devoted to understanding the pathogenesis and immunology of these diseases, they have been investigated as single disease entities, thus ignoring their potential interaction in affected hosts.

A number of reports have shown that *in vitro* proliferative responses of spleen cells to mitogens and parasite antigens were depressed in human and murine schistosomiasis. Rocklin *et. al* (7) reported a suppression of lymphocyte proliferation in patients with *S. mansoni* infection. Ottesen (8) followed some patients from the acute to the chronic stage of schistosomiasis mansoni. He found that adherent suppressor cells, which were absent early in the infection, were present in the peripheral mononuclear cell population by the 20th month of infection. Keyes and Colley (9) also demonstrated a similar development of suppressor cell activity in spleen cells of mice with *S. mansoni* infection. Additional evidence of immunosuppression in parasitic diseases has come from the studies of Prost *et. al*. (10) who reported that only 7.1% of patients with onchocerciasis became immunized after an administration of tetanus toxoid vaccine, in contrast to 44.5% in the control group. Humoral and cellular immunosuppression to tuberculin skin tests and BCG vaccination has also been reported in individuals with onchocerciasis (11).

The present study was designed to determine the specificity of humoral and cellular immunosuppression resulting from experimental *S. manson* infection in mice. In order to elucidate this phenomenon, immune responses to selected bacterial vaccines were examined in bacteria-immunized and *S. mansoni* infected mice. Patterns of lympho-

cyte proliferative responses to the respective bacterial agents were investigated, including possible changes over a 21-day time period. Specific anti-bacteria IgG and IgM production was also examined, along with the kinetics of the humoral responses to each bacterial vaccine.

MATERIALS AND METHODS

Parasite and Bacterial Antigens

The Puerto Rican strain of *Schistosoma mansoni* was routinely passaged in albino *Biomphalaria glabrata* snails and Swiss albino white mice. Six week-old female BALB/c mice were used in all experiments. The mice were infected intraperitoneally (ib) with about 100 *S. mansoni* cercariae. Stock cultures of *Bordetella pertussis*, *Salmonella typhimurium* and *Streptococcus pneumoniae* were obtained from the American Type Culture Collection (Rockville, MD.). *Salmonella* Vi antigen and tetanus toxoid were purchased from Difco Laboratories (Detroit, Michigan) and Wyett Laboratories (PA) respectively. The SEA used in the study was obtained through the auspices of the World Health Organization in Geneva, Switzerland. The bacterial cell cultures were grown in brain heart infusion at 37°C for 24 hours. The cells were then harvested by centrifugation. Whole cell vaccines and antigen extracts of the bacteria were prepared as follows: the cells were suspensed in PBS to a density of $1X10^{12}$ cells/ml, and the cell suspension was divided into two separate flasks. Cells in one flask were killed by treatment with 0.1% formaldehyde at 4°C for 48 hours and then resuspended in PBS to $1X10^{10}$ cells/ml. Prior to being used, these whole cell vaccines were heated at 56°C for 30 minutes to destroy any heat-labile toxicity. Cells in the second flask were sonicated for 5 minutes in an ice-cooled cell. After centrifugation at 100,000g for 30 min at 4°C, the supernatant was retained as the antigen.

Immunization

A total of 450 BALB/c mice were infected intraperitoneally with about 100 S. mansoni cercariae/mouse. At 10 weeks post-infection, the mice were randomly divided into five equal groups. Group I mice received 0.5ml/mouse ($1X10^9$ cells/ml) of whole-cell vaccine of B. pertussis, while mice in groups II and III received similar dosages of whole-cell vaccines of S. typhimurium and S. pneumoniae respectively. Mice in group IV received 0.5ml of appropriate dilution of tetanus toxoid. Group V mice received 0.5ml of phosphate buffered saline (PBS, pH 7.2). An equal number of uninfected mice (same age) were divided into five groups and immunized with similar dosages of the bacterial vaccines. An uninfected control group of mice also received 0.5ml of PBS. At 7, 14 and 21 days post-bacterial immunization, 20 to 25 mice from each infected and immunized group were bled for serum at the retro-orbital plexus and thereafter anaesthesized by ip injection of sodium pentobarbital. The spleen were aseptically removed from the mice and livers from those infected with S. mansoni checked for the presence of egg granulomas as a manifestation of infection. Spleen lymphocyte cell suspensions were prepared for the lymphocyte proliferation assays.

Lymphocyte proliferation assays

Responses of spleen lymphocytes from control, as well as uninfected immunized, S. mansoni infected and S. mansoni infected-immunized mice to phytohemagglutin (PHA), S. mansoni SEA, B. pertussis, Salmonella Vi, tetanus toxoid and Strep. pneumoniae were determined. For these experiments, $5X10^5$ viable spleen cells from the sacrificed mice were cultured in triplicate in RPMI 1640 medium in flat-bottom microtiter wells, supplemented with 100 units/ml of penicillin, 100 units/ml of streptomycin, 300 µg glutamine and 10% inactivated fetal calf serum. Also added to the appropriate cultures were 1 µg/ml SEA and 10 µg/ml of the bacterial antigen extracts. Control cultures without any stimulating antigens or mitogens were also included. The cultures were incubated in 5% CO_2-95% air at 37^oC for 3 days. Six hours prior to harvesting, each culture received 1 µCi/ml of triated thymidine (^{3}H-Tdr), (specific activity 2 Ci/mM). After harvesting, the cells were washed and processed in a solubilizing scintillation fluid and analysed for the amount of ^{3}H-Tdr incorporated in a spectrometer. The degree of lymphocyte response is expressed as net counts per minute (cpm) of stimulated cultures minus the cpm of unstimulated control cultures.

Assay of antibacterial and anti-SEA antibodies

Sera obtained at 7, 14 and 21 days post-bacterial vaccination from all groups of mice were analysed for specific IgG and IgM antibodies against the SEA and the bacterial antigens. A modification of the

enzyme-linked immunosorbent assay (ELISA) of Voller et al. (12) was employed in the quantitation of the antibodies. Briefly, 100 µl of the antigen extract in sodium bicarbonate coating buffer (pH 9.6) was used to sensitize the wells of polyvinyl microtiter plates and kept overnight at 4°C. After washing the plates with phosphate buffered saline (PBS) with Tween 20 (pH 7.4), duplicrte 100 µl serum samples (diluted 50X) were added to test wells. The plates were incubated at 20°C for 2 hours to permit the antibody from the serum samples to bind to the antigen. The plates were again washed and 100 µl of a 1/500 alkaline phosphatase conjugated rabbit-anti-mouse IgG or igM (Kirkgird Laboratories) in PBS-Tween was added to the wells and permitted to incubate at room temperature for another 2 hours. After a final wash, 100 µl of para-nitrophenyl phosphate (1 mg/ml) in diethanolamine buffer (pH 9.8) was added to each well. The reaction was stopped after 30 min. with 50 µl of 1N NaOH. The absorbance was read at [illegible] nm in an ELISA Reader (Dynatech Labs, VA). The intensity of the color reaction (OD) is directly proportional to the degree of antibody binding, and was thus a measure of anti-schistosomal or anti-bacterial IgG/IgM antibody.

All of the above experiments were duplicated for consistency and reliability.

Statistical analysis

Differences between data were analysed by either the Student's t-test or by one-way analysis of variance for statistical significance.

Results

Spleen lymphocytes from bacteria-immunized, *S. mansoni*-infected, concurrent bacteria-immunized/*S. mansoni*-infected, unimmunized and uninfected control mice were separately exposed to PHA, *S. mansoni*, SEA and four bacterial antigens. The lymphocyte responses to the various antigenic stimulations were determined by the rates of ^{3}H-thymidine incorporation into DNA synthesis. For the spleen lymphocytes obtained from mice at 7 days post-bacteria immunization, the results presented in Tables 1 to 4 show that prior immunization of mice with tetanus toxoid, pertussis and pneumococcal vaccine extracts resulted in the suppression of lymphocyte proliferation to the respective bacterial antigens. Prior immunization of mice with the same bacterial vaccines also resulted in the inhibition of lymphocyte proliferation to SEA and *Salmonella* Vi antigen. Interestingly spleen lymphocytes from *Salmonella*-immunized, pertussis-immunized, *S. mansoni*-infected and *S. mansoni*-infected/pertussis immunized mice underwent considerable proliferation in the presence of PHA and pneumococcal vaccine (Tables 1 & 2). The results also indicate that the presence of *S. mansoni* infection in mice suppressed the proliferation of their spleen lymphocytes to pertussis and tetanus antigens, but not to *Salmonella* Vi and pneumococcal antigens.

The responses of spleen lymphocytes obtained from mice at 15 days post-bacteria immunization are presented in Tables 5 to 8. The data indicate that the suppression of lymphocyte proliferation to PHA, SEA and three of the bacterial antigens continued in mice immunized with the pneumococcal vaccine. Prior immunization with the Salmonella vaccine only suppressed lymphocyte proliferation to SEA and Salmonella Vi antigen. On the other hand, prior immunization with Salmonella vaccine increased the in vitro lymphocyte proliferation to pertussis and tetanus antigens. These observations therefore suggest a cross reactivity in lymphocyte responsiveness to Salmonella Vi antigen and S. mansoni SEA.

Significant differences were, however, observed in responses of lymphocytes from immunized and infected mice at 21 days-post bacterial vaccination. At this time, only lymphocytes from pneumococcal-immunized were totally unresponsive to all the various antigens. As in earlier time periods, lymphocytes from S. mansoni-infected mice did not respond to pertussis- and tetanus-antigen stimulation. But while spleen lymphocytes from pertussis-and tetanus-immunized mice were not responsive to their homologous-derived antigens, they showed significant proliferative responses to PHA, SEA and Salmonella Vi antigen.

Humoral immune responses

The enzyme-linked immunosorbent assay (ELISA) was used in measuring class-specific antibodies to the shistosome and bacterial antigens. As indicated in Table 13, *S. mansoni* infection did not have any noticeable effects on the antibody production to *Salmonella* Vi antigen. Similar levels of antibodies were raised to the antigen in both Salmonella-immunized mice and Salmonella immunized-*S. mansoni* infected mice. There was no evidence of cross-reactive antibodies between the *Salmonella* Vi antigen and SEA, as both IgG and IgM antibodies to SEA were comparatively low in *Salmonella* immunized mice and vice versa in *S. mansoni*-infected mice. The IgM antibody levels to SEA remained relatively high in *S. mansoni*-infected mice during experimental period. As shown in Tables 14, 15 and 16, presence of *S. mansoni* infection did not seem to impair IgG and IgM production to pertussis, pneumococcal antigens and tetanus toxoid, and there was no evidence of cross-reactive antibodies to the bacterial antigens as was also noted with the *Salmonella* Vi. However, IgM specific antibodies to pertussis and pneumococcal vaccines were significantly relatively higher than the IgG antibodies in pertussis-immunized and pneumococcal-immunized mice, even in the presence of a concurrent *S. mansoni*-infection. These IgM antibody levels were relatively lower than the IgM levels produced against the other bacterial antigens.

DISCUSSION

The development of hepatosplenomegaly, fibrosis, portal hypertension and immunological unresponsiveness in murine schistosomiasis mansoni resembles the clinical course of the disease in humans. Daily production of large numbers of eggs by the adult worm pairs represents a major source of antigenic stimulation, which initially is manifested by lymphocyte proliferation, followed by subsequent suppression. The capacity of T lymphocytes to respond to stimulation by S. mansoni SEA, PHA and Con A mitogens has been used as an index of the resulting immunosuppression (9, 14, 14). The decline in lymphocyte responsiveness has been shown to be due to increases in the numbers of suppressor T lymphocytes (8,9). It has also been shown that the decline in cell-mediated immunity in schistosomiasis is accompanied by increases in antibody titers to SEA. However, no study has hitherto been undertaken to determine the specificity of the associated immunosuppression to other antigens besides mitogens. Several bacterial diseases are endemic in geographical areas with a high prevalence of schistosome infection. We therefore used the murine model to study the cellular and humoral immune responses not only to SEA and PHA, but also to whole-cell vaccines of Salm. typhimurium, B. pertussis, S. pneumoniae and tetanus toxoid over a 21-day period after bacterial immunization. The S. mansoni infection in mice was 10-weeks old at the time of bacterial immunization. The 10-week infection time point was chosen

because this represents a period of maximum immune response in experimental schistosomias. A 21-day post-bacterial immunization period was also chosen so as to determine the kinetics of cellular and humoral immune responses to the bacterial vaccines.

At 7 days post-bacteria immunization, the results show a significant decline in blastogenic response to PHA in S. mansoni-infected mice, relative to controls. On the other hand, the results show significant increases in lymphocyte proliferation to PHA in S. typhimurium -, B. pertussis- and tetanus toxoid-immunized mice (Tables 1-3). There was no blastogenic response to PHA in pneumococcus-immunized mice. S. mansoni-infected mice showed maximum lymphocyte proliferation to SEA, Salmonella Vi and pneumococcal antigens, but were relatively unresponsive to pertussis and tetanus-antigen stimulation.

At 15 days post-bacteria immunization, the results also show significant declines in the rates of lymphocyte proliferation to PHA, SEA, Salmonella and pneumoccal antigens in S. mansoni-infected mice. These results are consistent with the findings of previous investigators on the development of immunological unresponsiveness in chronic schistosomiasis (7-9). It is especially significant that spleen lymphocytes from S. mansoni-infected mice were relatively unresponsive to pertussis and tetanus antigens throughout the 21-day post-bacteria immunization period. It is also of interest to note that IgM antibody

levels to pertussis were comparatively low. Since it has been shown that the pathogenesis of S. mansoni in mice is similar to the clinical course of the disease in humans, it would be safe to assume the same pattern of immunological unresponsiveness to pertussis and tetanus vaccines in humans infected with S. mansoni. The interpretation of these experimental findings is that individuals with S. mansoni may not readily prime the pertussis and tetanus vaccines, and may thus represent a potential for failure of an immunization campaign program against pertussis and tetanus outbreaks in geographical areas that are endemic for S. mansoni, S. haematobium or S. japonicum. Indeed, it has been shown by Prost et al (10) that only 7.1% of individuals with onchocerciasis were immunized after an administration of tetanus toxoid vaccine. Other investigators have also demonstrated immunosuppression to bacterial vaccines in human acute malaria (15).

Data presented in Tables 13 to 16 indicate that the existence of an underlying S. mansoni infection did not impair the immunological capacity of mice to mount antibody responses to the various bacterial vaccines, although is is possible that most of the antibodies may not necessarily be protective. The two-to three-fold increase in lymphocyte proliferation to PHA in B. pertussis-immunized mice, irrespective of a concurrent S. mansoni infection, and the total nonresponsiveness of S. mansoni-infected mice to

pertussis and tetanus antigens indicate the development of a complex pattern of selective and specific immunosuppression to various bacterial antigens. It is therefore necessary to develop a good understanding of the specificity of immunosuppression, induced by S. mansoni or any other endemic parasitic infection, to pathogenic bacterial and viral agents that are associated with childhood morbidity and mortality. As has been demonstrated by the results of this study, understanding the nature of immunosuppression induced by a parasitic disease may be crucial to the success of an immunization program that is intended for the control or eradication of an immunizable disease in an endemic area.

REFERENCES

1. Iarotski, L.S. and Davis, A. 1981. The schistosomiasis problem in the world: results of a WHO questionnaire survey. Bull. WHO, 37:115-127.

2. Pugh, R.N.H., Jakubowski, A.W. and Gilles, H.M. 1979. Malumfashi Endemic Diseases Research Project, VI. Urinary schistosomiasis: abnormal urograms in infected males from the Malumfashi study area of northern Nigeria. Ann. Trop. Med. Parasit. 73:37-44.

3. Warren, K.S. 1973. The pathology of schistosome infections. Helminth. Abstr. Series A. 42:59-633.

4. Mahieu, J.M., Muller, A.S., Voorhoeve, A.M. and Dicken, H. 1978. Pertussis in rural area of Kenya: epidemiology and a preliminary report on a vaccine trial. Bull. WHO, 56:773.

5. Baird, D.R., Whittle, H.C. and Greenwood, B.M. 1976. Mortality from pneumococcal meningitis. Lancet ii: 1344-1346.

6. Todd, C.W., Goodgame, R.W. and Colley, D.G. 1979. Immune responses during human schistosomiasis mansoni. V. Suppression of schistosome antigen-specific lymphocyte blastogenesis by adherent/phagocytic cells. J. Immunol. 122:1440-1446.

7. Rocklin, R.E., Tracy, J.W. and Kholy, A.E. 1981. Activation of antigen-specific suppressor cells in human schistosomiasis mansoni by fractions of soluble eff antigens nonadherent to Con A sepharose J. Immunol. 127:2314-2318.

8. Ottesen, E.A. 1979. Modulation of the host response in human schistosomiasis. I. Adherent suppressor cells that inhibit lymphocyte proliferative responses to parasite antigens. 123:1639-1644.

9. Keyes, G.S. and Colley, D.G. 1979. Immunoregulation in experimental schistosomiasis: In vitro induction and assay of spleen cell suppressor activity. J. Immunol. 122:2340-2344.

10. Prost, a., Schlumberger, M. and Fayet, M.T. 1983. Response to tetanus immunization in onchocerciasis patients. Ann. Trop. Med. Parasit. 77:83-85.

11. Rougemont, A., Boisson-Pontal, M.E., Gridel, P.G. and Sangare, S. 1977. Tuberculin skin tests and BCG vaccination in a hyperendemic area of onchocerciasis. Lancet, ii: 309.

12. Voller, A. Bartlett, A. and Bidwell, D.E. 1976. Enzyme immunoassays for parasitic diseases. Trans. Roy. Soc. Trop. Med. Hyg. 70:98-106.

13. Pelley, R.P., Ruffier, J.J. and Warren, K. 1976. Supressive effect of a chronic helminth infection, schistosomiasis mansoni, on the in vitro responses of spleen and lymph node cells to the T-cell mitogens phytohemagglutinin and concanavalin A. Infect. Immun. 13:1176-83.

14. Oberlin, U-P and Weiss, N. Schistosomiasis mansoni in the hamster: cellular and humoral immune responses to soluble egg antigen (SEA). Am. J. Trop. Med. Hyg. 26:1178-1182.

15. Williamson, W.A. and Greenwood, B.M. 1978. Impairment of the immune response to vaccination after acute malaria. Lancet, 1:1328-1329.

Table 1. In vitro proliferative responses of spleen lymphocyte from *Salmonella typhimurium*-immunized and *Schistosoma mansoni* - infected mice to various antigens at 7 days post immunization

In vitro sensitizing antigen	Spleen lymphocyte proliferative responses (cpm $\bar{X}$±SE)*			
	Non-infected control mice	*Sal. typhimurium* immunized mice	*S. mansoni* infected mice	*S. mansoni* infected and *Sal. typhim*-immunized mice
PHA	48,354 (±7,102)	87,315 (±13,408)	19,148 (±2,861)	44,739 (±2,276)
SEA	237 (±60)	2,078 (±1,51)	57,745 (±3,889)	35,478 (±5,479)
Salmonella	514 (±117)	12,618 (±3,259)	34,455 (±1,434)	19,427 (±3,405)
Pertussis	454 (±121)	196 (±147)	1,001 (±817)	229 (±41)
Tetanus	492 (±203)	364 (±213)	7,853 (±1,504)	51 (±23)
Pneumococcus	377 (±71)	49,703 (±12,991)	43,200 (±4,308)	29,340 (±2,196)

*Each mean is calculated from the results of eight cultures from two consecutive experiments and represents the cpm of stimulated cultures minus the cpm of unstimulated control cultures.

Table 2. In vitro proliferative responses of spleen lymphocytes from *Bordetella pertussis* - immunized and *Schistosoma mansoni* infected mice to various antigens at 7 days post immunization

	Spleen lymphocyte proliferative responses (cpm $\bar{X}$±SE)*			
In vitro sensitizing antigen	Non-infected control mice	*Bord. pertussis* immunized mice	*S. mansoni* infected mice	*S. mansoni* infected and Pertussis-immunized mice
PHA	48,354 (±7,102)	46,816 (±4,831)	19,148 (±2,861)	20,393 (±2,361)
SEA	237 (±60)	798 (±1,115)	57,745 (±3,889)	22,811 (±2,380)
Salmonella	514 117	2,241 (±4,87)	34,455 (±1,434)	20,687 (±2,212)
Pertussis	454 (±121)	138 (±46)	1,001 (±817)	133 (±87)
Tetanus	492 (±203)	197 (±15)	7,853 (±1,504)	1,234 (±1,27)
Pneumococcus	377 (±71)	9,922 (±1,657)	43,200 (±4,308)	19,934 (±1,521)

*Each mean is calculated from the results of eight cultures from two consecutive experiments and represents the cpm of stimulated cultures minus the cpm of unstimulated control cultures.

Table 3. In vitro proliferative responses of spleen lymphocytes from pneumococcus immunized mice and *Schistosoma* mansoni-infected mice to various antigens at 7 days post immunization

In vitro Sensitizing Antigen	Spleen lymphocyte proliferative responses (cpm X±SE)*			
	Non-infected control mice	Pneumococcus immunized mice	S. mansoni infected mice	*S. mansoni* and *Pneumococcus* immunized mice
PHA	48,354 (±7,102)	140 (±34)	19,148 (±2,161)	597 (±51)
SEA	237 (±60)	256 (±53)	57,745 (±3889)	596 (±272)
Salmonella	514 (±117)	139 (±110)	34,455 (±1434)	566 (±182)
Pertussis	454 (±121)	274 (±72)	1,001 (±817)	361 (±195)
Tetanus	492 (±203)	162 (±60)	7,853 (±1504)	251 (±75)
Pneumococcus	377 (±71)	321 (±79)	43,200 (±4,308)	772 (±151)

*Each mean is calculated from the results of eight cultures from two consecutive experiments and represents the cpm of stimulated cultures minus the cpm of unstimulated control cultures.

Table 4. In vitro proliferative responses of spleen lymphocytes from tetanus-immunized mice and Schistosoma mansoni-infected mice to various antigens at 7 days post immunization

In-vitro sensitizing antigen	Spleen lymphocyte proliferative responses (cpm X±SE)			
	Non-infected control mice	Tetanus immunized mice	S. mansoni infected mice	S. mansoni included and Tetanus-immunized mice
PHA	48,354 (±7,102)	405 (±95)	19,148 (±2,861)	16,936 (±2,353)
SEA	237 (±60)	190 (±61)	57,745 (±3889)	66 (±40)
Salmonella	514 (±117)	532 (±180)	34,455 (±1434)	51 (±23)
Pertussis	454 (±121)	216 (±35)	1,001 (±817)	20 (±3)
Tetanus	492 (±203)	176 (±54)	7,853 (±1504)	431 (±11)
Pneumococcus	377 (±71)	117 (±73)	43,200 (±4,308)	11,379 (±3480)

*Each mean is calculated from the results of eight cultures from two consecutive experiments and represents the cpm of stimulatec cultures minus the cpm of unstimulated control cultures.

Table 5. In vitro proliferative responses of spleen lymphocytes from *Salmonella typhimurium* and *Schistosoma mansoni* infected mice to various antigens at 14 days post immunization

In vitro sensitizing antigen	Spleen lymphocyte proliferative responses (cpm $\bar{X}\pm SE$)*			
	Non-infected control mice	*Salmonella typhimirium* immunized mice	*S. mansoni* infected mice	*S. mansoni* infected and *Sal typh.* immunized mice
PHA	51,950 (±213)	54,924 (±1156)	25,601 (±930)	33,064 (±4,635)
SEA	1,380 (±320)	2,931 (±878)	5,260 (±1129)	654 (±124)
Salmonella	2,304 (±178)	2,742 (±600)	5,514 (±630)	2,932 (±1191)
Pertussis	611 (±119)	15,260 (±1380)	804 (±183)	1,852 (±132)
Tetanus	593 (±145)	59,935 (3567)	206 (±106)	1,673 (±67)
Pneumococcus	3,661 (±411)	6,874 (±505)	10,265 (±1633)	16,313 (±2463)

*Each mean is calculated from the results of eight cultures from two consecutive experiments and represents the cpm of stimulated cultures minus the cpm of unstimulated control cultures.

Table 6. In vitro proliferative responses of spleen lymphocytes from *Bordetella pertussis*-immunized and *Schistosoma mansoni*-infected mice to various antigens at 14 days post immunization

In vitro sensitizing antigen	Spleen lymphocyte proliferative responses (cpm $\bar{X}$±SE)*			
	Non-infected control mice	*B. pertussis* immunized mice	*S. mansoni* infected mice	*S. mansoni* infected and *B. pertussis*-immunized mice
PHA	51,950 (±213)	112,733 (±1808)	25,601 (±930)	138,633 (±6413)
SEA	1,380 (±320)	418 (±193)	5,260 (±1129)	1,665 (±246)
Salmonella	2,304 (±198)	528 (±121)	5,514 (±630)	4,006 (±473)
Pertussis	611 (±119)	10,779 (±1677)	804 (±183)	20,017 (±2354)
Tetanus	593 (±145)	16,611 (±2349)	206 (±106)	17,889 (±2729)
Pneumococcus	3,661 (±111)	31,580 (±2856)	10,265 (±1633)	6,787 (±605)

*Each mean is calculated from the results of eight cultures from two consecutive experiments and represents the cpm of stimulated cultures minus the cpm of unstimulated control cultures.

Table 7. In vitro proliferative responses of spleen lymphocytes from pneumococcus - immunized and *Schistosoma mansoni*-infected mice to various antigens at 14 days post immunization

In vitro sensitizing antigens	Spleen lymphocyte proliferative responses (cpm X±SE)*			
	Non-infected Control mice	Pneumococcus immunized mice	*S. mansoni* infected mice	*S. mansoni* infected and pneumococcus immunized mice
PHA	51,950 (±213)	140 (±34)	25,601 (±930)	597 (±51)
SEA	1,380 (±320)	256 (±53)	5,260 (±1129)	596 (±272)
Salmonella	2,304 (±178)	139 (±110)	5,514 (±630)	566 (±182)
Pertussis	611 (±119)	74 (±12)	804 (±183)	361 (±195)
Tetanus	593 (±145)	162 (±60)	206 (±106)	251 (±75)
Pneumococcus	3,661 (±411)	32 (±11)	10,265 (±1,633)	772 (±151)

*Each mean is calculated from the results of eight cultures from two consecutive experiments and represents the cpm of stimulated cultures minus the cpm of unstimulated control cultures.

Table 8. In vitro proliferative responses of spleen lymphocytes from tetanus - immunized and *Schistosoma mansoni* - infected mice to various antigens at 14 days post immunization

In vitro sensitizing antigen	Spleen lymphocyte proliferative responses (cpm $\bar{X}$±SE)*			
	Non-infected control	Tetanus immunized mice	*S. mansoni* infected mice	immunized and *S. mansoni*-infected mice
PHA	51,950 (±213)	63,166 (±4846)	25,601 (±930)	55,532 (±4458)
SEA	1,380 (±320)	766 (±200)	5,260 (±1129)	214 (±28)
Salmonella	2,304 (±178)	738 (±263)	5,514 (±630)	126 (±75)
Pertussis	611 (±119)	39,528 (±4561)	804 (±183)	11,662 (±1155)
Tetanus	593 (±145)	43,123 (±2798)	206 (±106)	6,280 (±495)
Pneumococcus	3,661 (±111)	10,770 (±288)	10,265 (±1633)	33,430 (±2595)

*Each mean is calculated from the results of eight cultures from two consecutive experiments and represents the cpm of stimulated cultures minus the cpm of unstimulated control cultures.

Table 9. In vitro proliferative responses of spleen lymphocytes from *Salmonella typhimurium* -immunized and *Schistosoma mansoni*-infected mice to various antigens at 21 days post immunization

In vitro sensitizing antigen	Spleen lymphocyte proliferative responses (cmp $\bar{X}\pm SE$)*			
	Non-infected control mice mice	*Sal.* typhimurium immunized mice	*S. mansoni* infected mice	*S. mansoni*-infected and *Sal. typhim*-immunized mice
PHA	45,158 (±3542)	3,224 (±546)	28,254 (±4150)	73,934 (±1862)
SEA	719 (±226)	352 (1127)	40,040 (±1081)	33,383 (±5519)
Salmonella	979 (±190)	767 (±132)	27,509 (±1534)	34,560 (±2621)
Pertussis	659 (±80)	1,058 (±153)	95 (±37)	120 (±84)
Tetanus	566 (±204)	79 (±24)	212 (±70)	216 (±18)
Pneumococcus	1,214 (±442)	249 (±55)	31,858 (±2464)	34,176 (±1876)

*Each mean is calculated from the results of eight cultures from two consecutive experiments and represents the cpm of stimulated cultures minus the cpm of unstimulated control cultures.

Table 10. In vitro proliferative responses of spleen lymphocytes from *Bordetella pertussis* - immunized and *Schistosoma mansoni* - infected mice to various antigens at 21 days post immunization

In vitro sensitizing antigen	Spleen lymphocyte proliferative responses (cpm X±SE)			
	Non-infected control mice	*B. pertussis* immunized	*S. mansoni* infected mice	*B. pertussis* immunized *S. mansoni* infected mice
PHA	45,158 (±35421)	126,772 (±15,412)	28,254 (±4150)	12,638 (±2074)
SEA	719 (±226)	22,597 (±4117)	40,040 (±1081)	7,859 (±1610)
Salmonella	22,979 (±190)	23,423 (±2667)	27,509 (±1534)	6,728 (±1370)
Pertissus	659 (±80)	32 (±5)	95 (±37)	149 (±87)
Tetanus	566 (±204)	152 (±90)	212 (±70)	497 (±241)
Pneumococcus	1,214 (±442)	38,583 (±2,766)	31,858 (±2464)	6,038 (±1648)

*Each mean is calculated from the results of eight cultures from two consecutive experiments and represents the cpm of stimulated cultures minus the cpm of unstimulated control cultures.

Table 11. In vitro proliferative responses of spleen lymphocytes from *pneumococcus* - immunized and *Schistosoma mansoni* -infected mice to various antigens at 21 days post immunization.

In vitro sensitizing antigen	Spleen lymphocyte proliferative responses (cpm $\bar{X}$±SE)			
	Non-infected control mice	Pneumococcus immunized mice	*S. mansoni* infected mice	*S. mansoni* infected and pneumococcus immunized
PHA	45,458 (±35421)	31 (±15)	28,254 (±4150)	88,608 (±9093)
SEA	719 (±226)	294 (±207)	40,040 (±1081)	19,419 (±4056)
Salmonella	979 (±190)	94 (± 19)	27,509 (±1534)	9,540 (±2482)
Pertussis	659 (±80)	91 (±18	95 (±32)	408 (±216)
Tetanus	566 (±204)	528 (±33)	212 (±70)	1,453 (±312)
Pneumococcus	1,214 (±442)	49 (±8)	31,858 (±2464)	14,786 (±3775)

*Each mean is calculated from the results of eight cultures from two consecutive experiments and represents the cpm of stimulated cultures minus the cpm of unstimulated control cultures.

Table 12. In vitro proliferative responses of spleen lymphocytes from tetanus -immunized and *Schistosoma mansoni*-infected mice to various antigens at 21 days post immunization.

In vitro sensitizing antigen	Spleen lymphocyte proliferative responses (cpm $\bar{X}$±SE)			
	Non-infected control mice	Tetanus immunized mice	*S. mansoni* infected mice	*S. mansoni* infected and tetanus-immunized mice
PHA	45,158 (±3,5421)	49,821 (±5,104)	28,254 (±4,150)	32,918 (±8,923)
SEA	719 (226)	3,192 (±1,36)	40,040 (±1,081)	34,441 (±3,658)
Salmonella	979 (±1,904)	4,193 (±7,58)	27,509 (±1,534)	23,712 (±2,345)
Pertussis	659 (±80)	417 (±154)	95 (±37)	98 (±23)
Tetanus	566 (±204)	277 (±36)	212 (±70)	702 (±238)
Pneumococcus	1,214 (±4,42)	4,298 (±1,466)	31,858 (±2,464)	28,851 (±4,487)

*Each mean is calculated from the results of eight cultures from two consecutive experiments and represents the cpm of stimulated cultures minus the cpm of unstimulated control cultures.

Table 13. Comparison of serum antibody responses to Salmonella Vi antigen and soluble egg antigen of Schistosoma mansoni in immunized and infected mice at various time intervals

Immunization and infection status of mice (post-immunization in days)	Serum antibody responses by ELISA ($\bar{X}$±SE) to: Salmonella Vi antigen		S. mansoni egg antigen	
	IgG	IgM	IgG	IgM
S.m*. + Salm Vi (7 dy)	0.683 (±0.045)	0.483 (±0.349)	0.608 (±0.072)	1.370 (±0.117)
S.m. + Salm Vi (14 dy)	0.890 (±0.184)	0.510 (±0.265)	0.780 (±0.113)	1.640 (±0.300)
S.m. + Salm Vi (21 dy)	1.470 (±0.171)	0.800 (±0.156)	1.056 (±0.156)	1.571 (±0.108)
S.m. Control	0.198 (±0.110)	0.350 (±0.085)	0.730 (±0.155)	1.540 (±0.197)
Uninfected Control	0.180 (±0.035)	0.373 (±0.266)	0.112 (±0.021)	0.181 (±0.034)
Salmonella Vi (7 dy)	0.722 (±0.143)	0.532 (±0.268)	0.121 (±0.041)	0.255 (±0.068)
Salmonella Vi (14 dy)	0.844 (±0.204)	0.556 (±0.169)	0.105 (±0.081)	0.261 (±0.107)
Salmonella Vi (21 dy)	1.380 (±0.334)	0.486 (±0.156)	0.114 (±0.016)	0.328 (±0.108)

S.m*. refers to 10 week-old infections of S. mansoni, at which time infected and uninfected mice were immunized with Salmonella Vi antigen. Antibody (IgG and IgM) production to Salmonella Vi antigen and S. mansoni soluble egg antigen (SEA) was measured by ELISA as absorbance at 405nm. Each mean represents determinations from 30 to 40 mice from two consecutive experiments.

Table 14. Comparison of serum antibody responses to whole-cell vaccine of Bordetella pertussis and soluble egg antigen of *Schistosoma mansoni* in Immunized and infected mice at various time intervals

Immunization and infected status of mice (post-immunization in days)	Serum antibody responses by ELISA ($\bar{X}$±SE) to: Pertussis antigen		*S. mansoni* egg antigen	
	IgG	IgM	IgG	IgM
S.m.* + Pertussis (7 dy)	0.299 (±0.040)	0.726 (±0.078)	0.876 (±0.210)	1.571 (±0.383)
S.m. + Pertussis (14 dy)	0.443 (±0.107)	0.736 (±0.151)	1.168 (±0.135)	1.744 (±0.101)
S.m. + Pertussis (21 dy)	0.656 (±0.133)	0.815 (±0.117)	0.962 (±0.226)	1.424 (±0.164)
S.m. Control	0.320 (±0.045)	0.800 (±0.155)	0.840 (±0.136)	1.720 (±0.215)
Uninfected Control	0.180 (±0.055)	0.223 (±0.087)	0.195 (±0.030)	0.060 (±0.042)
Pertussis (7 dy)	0.250 (±0.040	0.730 (±0.227)	0.060 (±0.025)	0.248 (±0.042)
Pertussis (14 dy)	0.387 (±0.133)	0.758 (±0.164)	0.206 (±0.045)	0.266 (±0.055)
Pertussis (21 dy)	0.496 (±0.149)	0.718 (±0.094)	0.180 (±0.106)	0.360 (±0.177)

S.m.* refers to 10 week-old infections of *S. mansoni*, at which time infected and uninfected mice were immunized with whole-cell vaccine of *B. pertussis*. Antibody (IgG and IgM) production to *B. pertussis* antigen and *S. mansoni* soluble egg antigen was measured by ELISA as absorbance at 405nm. Each mean represents determinations from 30 to 40 mice from two consecutive experiments.

Table 15. Comparison of serum antibody responses to whole-cell vaccine of *Streptococcus pneumoniae* and soluble egg antigen of *Schistosoma mansoni* in immunized and infected mice at various time intervals

Immunization and infection status of mice (post-immunization in days)	Serum antibody responses by ELISA ($\bar{X}$±SE) to: Pneumococcus antigen		*S. mansoni* antigen	
	IgG	IgM	IgG	IgM
S.m.* + Pneumo (7 dy)	0.454 (±0.195)	1.960 (±0.956)	0.464 (±0.089)	1.040 (±0.200)
S.m. + Pneumo (14 dy)	0.502 (±0.188)	1.482 (±0.543)	1.180 (±0.154)	1.700 (±0.194)
S.m. + Pneumo (21 dy)	0.586 (±0.165)	1.940 (±0.094)	1.07 (±0.142)	1.460 (±0.061)
S.m. Control	0.362 (±0.064)	0.485 (±0.071)	0.62 (±0.075)	1.140 (±0.086)
Uninfected Control	0.216 (±0.042)	0.184 (±0.055)	0.121 (±0.024)	0.148 (±0.055)
Pneumococcus (7 dy)	0.242 (±0.056)	1.195 (±0.462)	0.364 (±0.070)	0.285 (±0.081)
Pneumococcus (14 dy)	0.275 (±0.121)	1.300 (±0.089)	0.320 (±0.085)	0.048 (±0.260)
Pneumococcus (21 dy)	0.480 (±0.093)	1.934 (±0.082)	0.400 (±0.226)	0.210 (±0.044)

Sm* refers to 10 week-old infections of *S. mansoni*, at which time infected and uninfected mice were immunized with whole-cell vaccine of *Streptococcus pneumoniae*. Antibody (IgG and IgM) production to pneumococcal antigen and *S. mansoni* soluble egg antigen (SEA) was measured by ELISA absorbance at 405nm. Each mean represents determinations from 30 to 40 mice from two consecutive experiments.

Table 16. Comparison of serum antibody responses to tetanus toxoid and soluble egg antigen of *Schistosoma mansoni* in immunized and infected mice at various time intervals

Immunization and infection status of mice (post-immunization in days)	Serum antibody responses by ELISA ($\bar{X}$±SE) to: Tetanus toxoid		*S. mansoni* egg antigen	
	IgM	IgM	IgM	IgM
S.m.* + Tetanus (7 dy)	0.120 (±0.044)	0.164 (±0.062)	0.756 (±0.246)	0.946 (±0.155)
S.m. + Tetanus (14 dy)	0.562 (±0.199)	0.105 (±0.041)	0.996 (±0.171)	1.460 (±0.219)
S.m. + Tetanus (21 dy)	0.987 (±0.135)	0.230 (±0.056)	1.114 (±0.153)	1.613 (±0.136)
S.m. Control	0.140 (±0.028)	0.185 (±0.035)	0.730 (±0.155)	1.560 (±0.226)
Uninfected Control	0.095 (±0.012)	0.161 (±0.042)	0.188 (±0.063	0.330 (±0.093)
Tetanus (7 dy)	0.300 (±0.070)	0.046 (±0.033)	0.198 (±0.013)	0.153 (±0.090)
Tetanus (14 dy)	0.832 (±0.076)	0.130 (±0.063)	0.175 (±0.056)	0.137 (±0.065)
Tetanus (21 dy)	1.480 (±0.259)	0.073 (±0.017)	0.150 (±0.092)	0.147 (±0.058)

S.m.* refers to 10 week-old infections of *S. mansoni* at which time infected and uninfected mice were immunized with *Clostridium tetani toxoid*. Antibody (IgG and IgM) production to tetanus toxoid and *S. mansoni* soluble egg antigen was measured by ELISA as absorbance to 405nm. Each mean represents determinations from 30 to 40 mice from two consecutive experiments.