INFECTION AND IMMUNITY, Mar. 1985, p. 587–591
0019-9567/85/030587-05$02.00/0

Vol. 47, No. 3

Resistance to Adenovirus Infection After Administration of *Bordetella pertussis* Vaccine in Mice

ALVIN L. WINTERS,[1]* DAVID W. BAGGETT,[1] WILLIAM R. BENJAMIN,[2]† H. KEITH BROWN,[3] AND THOMAS W. KLEIN[2]

Department of Microbiology, University of Alabama, University, Alabama 35486,[1] and Departments of Medical Microbiology and Immunology[2] and Anatomy,[3] University of South Florida, Tampa, Florida 33612

Received 27 August 1984/Accepted 13 November 1984

Treatment of mice with *Bordetella pertussis* vaccine rendered mice resistant to mouse adenovirus infection. The resistant state took at least 5 days to develop, and susceptibility returned to a portion of the test population 35 days after treatment. Transient resistance developed in congenitally athymic mice also. Treatment with a dose of 25 μg (dry weight) of *B. pertussis* vaccine protected approximately 50% of the test population. Vaccines prepared from several different strains of *B. pertussis* were capable of inducing resistance, and the induction of resistance was not dependent on the mouse strain used for testing. Cross-reacting antibodies capable of neutralizing the virus or protecting against a challenging infection were not induced by treatment with *B. pertussis* vaccine.

A variety of immunomodulatory activities have been associated with *Bordetella pertussis* vaccine (BPV) (14, 17, 19, 20). Acellular components extracted from the *B. pertussis* cell can mimic some of these immunomodulatory activities (3, 4, 7, 8, 18, 22, 23). Treatment of mice with BPV or components extracted from *B. pertussis* can modify the pathogenesis of virus infections. Increased susceptibility to intranasal influenza virus challenge was observed 5 to 7 days after intraperitoneal (i.p.) injection of BPV (21). Increased resistance to intracranial rabies virus challenge was observed when an extract of *B. pertussis* was administered by the subcutaneous, intravenous, or i.p. route at the same time as the virus (19). Increased resistance to i.p. herpes simplex virus challenge was observed 7 days after i.p. injection of BPV; however, resistance was not observed when BPV was administered 3 days before virus challenge (13). Several acellular fractions of *B. pertussis*, namely, lipopolysaccharide, glycolipid, lipid A, or lipid X, induce a state of resistance to an i.p. challenge of encephalomyocarditis virus or a subcutaneous challenge of Semliki Forest virus when the *B. pertussis*-derived fractions were administered i.p. 24 h previously (1, 3). Discussions with the late Charles W. Fishel concerning the immunomodulatory activities of *B. pertussis* and the reports of antiviral activity of BPV suggested that BPV might modulate mouse adenovirus infection by a plaque-type variant of mouse adenovirus strain FL. The plaque-type variant strain, designated MAd1*pt*4, induces an interstitial pneumonia and death (25). Increased resistance to an i.p. challenge of mouse adenovirus was observed when BPV was injected i.p. 7 days before virus challenge (14). This report extends the characterization of BPV-induced resistance to mouse adenovirus infection.

MATERIALS AND METHODS

Animals. Female mice, strain BDF_1/Cox (C57Bl/6 Cox × DBA/2 Cox) were obtained from Laboratory Supply Co., Indianapolis, Ind. A colony of C3H/HeN (mammary tumor virus positive [MTV^+]) mice with the nude gene mutation was obtained from Carl Hansen, National Institutes of Health, Bethesda, Md., and bred and maintained at the University of South Florida, Tampa, Fla., as described previously (24). Male mice heterozygous for the nude gene and female mice homozygous for the nude gene were used in this study. A colony of C3H/HeN (MTV^-) mice was obtained from Carl Hansen and bred and maintained at the University of Alabama, University. Both male and female mice were used in this study.

Vaccines. BPV was provided by Connaught Laboratories, Swiftwater, Pa., and was adjusted to approximately 4.0 mg (dry weight) per ml in saline-thimerosal diluent (0.15 M NaCl in 0.02% thimerosal). Vaccines were made also from *B. pertussis* strains 18323 (James L. Cowell, Food and Drug Administration, Bethesda, Md.) and Tohama I (Stanley Falkow, Stanford University, Stanford, Calif.). The organisms were maintained on BG agar base (Difco Laboratories, Detroit, Mich.) supplemented with 17% defibrinated sheep blood. Vaccines were made by harvesting 4-day growth from Cohen-Wheeler agar in phosphate-buffered saline (pH 7.2) and inactivating the cells by heating (56°C for 30 min) in the presence of 0.02% thimerosal. The vaccines were adjusted to 4.0 mg (dry weight) in saline-thimerosal diluent and stored at 4°C.

Virus. MAd1*pt*4 was propagated in L cells (NCTC clone 929; American Type Culture Collection, Rockville, Md.) The virus titer of infecting stocks was determined by a plaque assay (24).

Leukocytosis. Mice were bled from the retroorbital plexus with heparinized capillary tubes. Samples (10 μl) of peripheral blood from each mouse were placed into tubes containing 490 μl of Turk solution (9), mixed, and counted with a hemocytometer.

Electron microscopy. Cells obtained by peritoneal lavage were sedimented by centrifugation at 450 × *g* for 10 min. The cell pellet was covered with fixative (2% paraformaldehyde–3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3) and postfixed with osmium tetroxide. After the cell pellet was embedded in epon, ultrathin sections (60 to 70 nm) were stained as previously described (24).

* Corresponding author.
† Present address: Hoffmann-LaRoche, Inc., Nutley, NJ 07110.

TABLE 1. Induction of resistance to MAd1*pt*4 challenge by BPV administration

Mouse strain	Treatment[a]	Virus dose (PFU)	Mortality (deaths/ total)
C3H/HeN (+/*nu*) (MTV^+)	Diluent	1.0×10^8	25/25
C3H/HeN (+/*nu*) (MTV^+)	BPV (Connaught)	1.0×10^8	0/20
BDF_1	Diluent	1.0×10^8	20/20
BDF_1	BPV (Connaught)	1.0×10^8	0/20
C3H/HeN (MTV^-)	Diluent	2.2×10^7	21/21
C3H/HeN (MTV^-)	BPV (Connaught)	2.2×10^7	0/21
C3H/HeN (MTV^-)	Diluent	2.8×10^7	10/10
C3H/HeN (MTV^-)	BPV (18323)	2.8×10^7	0/10
C3H/HeN (MTV^-)	Diluent	2.8×10^7	5/5
C3H/HeN (MTV^-)	BPV (Tohama I)	2.8×10^7	0/5

[a] Animals were injected i.p. with either saline-thimerosal diluent or 250 μg (dry weight) of BPV 7 days before i.p. virus challenge.

TABLE 3. Kinetics of BPV-induced resistance to MAd1*pt*4

Expt	Mouse strain	Time of treatment[a] (days)	Virus dose (PFU)	Mortality (deaths/ total)
1	BDF_1	0.02	1.0×10^8	5/5
	BDF_1	7	1.0×10^8	0/10
	BDF_1	14	1.0×10^8	0/10
	BDF_1	21	1.0×10^8	0/10
	BDF_1	35	1.0×10^8	4/10
2	C3H/HeN (MTV^-)	1	2.1×10^7	5/5
	C3H/HeN (MTV^-)	2	2.1×10^7	5/5
	C3H/HeN (MTV^-)	3	2.1×10^7	5/5
	C3H/HeN (MTV^-)	4	2.1×10^7	5/5
	C3H/HeN (MTV^-)	5	2.1×10^7	0/5
	C3H/HeN (MTV^-)	6	2.1×10^7	0/5
	C3H/HeN (MTV^-)	7	2.1×10^7	0/5

[a] Mice were injected i.p. with 250 μg (dry weight) of BPV (Connaught Laboratories) and challenged with virus at the designated times of treatment. Both experiments included diluent injected groups of mice as controls; the control groups exhibited 100% mortality.

RESULTS

Induction of resistance. Treatment of mice with BPV induced resistance to an MAd1*pt*4 challenge (Table 1). Animals were injected i.p. with BPV; 7 days later, lethal doses of MAd1*pt*4 were administered. The animals were observed thereafter for clinical disease and death accompanied by hemorrhagic lungs at necropsy. Treatment of mice with BPV induced a resistant state to as much as 20 50% lethal doses of the virus. The treatment protected the mice also from overt clinical signs of disease, such as hunched back, ruffled fur, marked inactivity, and huddling. The long-term survival of mice after BPV treatment and MAd1*pt*4 infection was at least 65 days. The longest duration of disease and death observed after MAd1*pt*4 infection was 11 days; therefore, experiments were scored 21 days after infection. The 50% effective dose of BPV was approximately 25 μg (dry weight) or approximately 1.2 mg/kg of body weight (Table 2).

The kinetics of the induction of the resistant state suggest that BPV was not interacting directly with the virus. The resistant state did not develop immediately after administration of the vaccine, but was observed initially 5 days after BPV treatment (Table 3). The resistant state was transient, and susceptibility returned to a portion of the test population 35 days after BPV treatment.

A functioning thymus was not necessary for induction of the resistant state. Congenitally athymic mice were very susceptible to MAd1*pt*4 infection (Table 4). The kinetics of disease and death after high-dose virus infection was similar in both thymus-bearing and athymic animals. Infection with low doses of the virus produced no overt disease or death in thymus-bearing animals, whereas low-dose infection of athymic animals produced a chronic wasting disease and death with hemorrhagic lungs after an extended period of time. A transient BPV-induced resistant state was observed also in congenitally athymic mice. In contrast to the thymus-bearing animals, the athymic animals succumbed to high-dose infection and disease after the resistant state decayed (Table 4).

TABLE 2. Dose response of BPV-induced resistance to MAd1*pt*4 in BDF_1 mice

Treatment[a]	Mortality (deaths/ total)
Diluent	10/10
2.5 μg of BPV	10/10
12.5 μg of BPV	10/10
25 μg of BPV	19/47
125 μg of BPV	1/10
250 μg of BPV	0/10

[a] Mice were injected i.p. with the designated amounts (dry weight) of BPV (Connaught Laboratories) 7 days before i.p. virus challenge with 1.0×10^8 PFU.

TABLE 4. Dose response of MAd1*pt*4 in normal, athymic, and BPV-treated athymic mice

Mouse strain	Virus challenge (PFU)	Mortality (deaths/ total)	Time of death (days)
BDF_1	1.0×10^8	10/10	3, 3, 3, 3, 3, 3, 3, 3, 3, 3
BDF_1	1.0×10^7	10/10	3, 3, 3, 3, 3, 3, 3, 3, 4, 4
BDF_1	1.0×10^6	2/10	3, 3
C3H/HeN (+/*nu*)	1.0×10^8	15/15	3, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 5, 5, 5
C3H/HeN (+/*nu*)	1.0×10^7	8/10	6, 8, 8, 8, 8, 10, 10, 10
C3H/HeN (+/*nu*)	1.0×10^6	1/10	9
C3H/HeN (*nu*/*nu*)	1.0×10^8	5/5	5, 5, 5, 5, 6
C3H/HeN (*nu*/*nu*)	1.0×10^7	5/5	8, 10, 16, 21, 33
C3H/HeN (*nu*/*nu*)	1.0×10^6	5/5	18, 18, 22, 26, 31
C3H/HeN (*nu*/*nu*)	1.0×10^5	4/5[a]	35, 38, 38, 38
C3H/HeN (*nu*/*nu*)	1.0×10^4	5/5	26, 30, 33, 35, 39
C3H/HeN (*nu*/*nu*)	1.0×10^3	5/5	25, 29, 33, 35, 36
C3H/HeN (*nu*/*nu*)	1.0×10^2	5/5	33, 33, 36, 36, 45
BPV-treated C3H/HeN (*nu*/*nu*)[b]	1.0×10^8	15/15	7, 11, 37, 38, 39, 40, 43, 43, 46, 46, 48, 51, 54, 54, 63
Diluent-treated C3H/HeN (*nu*/*nu*)[b]	1.0×10^8	15/15	4, 4, 4, 4, 5, 5, 5, 5, 5, 5, 5, 5, 6, 9

[a] The survivor was sacrificed by cervical dislocation 149 days after infection.

[b] Mice were injected with vaccine diluent or 250 μg (dry weight) of BPV (Connaught Laboratories) 7 days before virus challenge.

TABLE 5. Effect of heat treatment on the antiviral activity of BPV

Mouse strain	Treatment[a]	Mortality (deaths/total)
C3H/HeN (+/*nu*)	Diluent	15/15
C3H/HeN (+/*nu*)	BPV	0/10
C3H/HeN (+/*nu*)	Heated BPV[b]	0/15
BDF_1	Diluent	5/5
BDF_1	BPV	0/10
BDF_1	Heated BPV[b]	5/10

[a] Mice were injected i.p. with vaccine diluent or 250 μg (dry weight) of BPV (Connaught Laboratories) 7 days before virus challenge.
[b] BPV (Connaught Laboratories) was incubated at 80°C for 30 min before i.p. injection.

Treatment of BPV to inactivate pertussigen (80°C for 30 min) (19) did not affect resistance-inducing activity when assayed in C3H/HeN (+/*nu*) mice, but decreased activity when assayed in BDF_1 mice (Table 5). The heated vaccine failed to induce lymphocytosis in the peripheral blood of mice (Table 6).

Effects of treatment with BPV. High-dose MAd1*pt*4 infection did not effect a notable generalized immune response (splenomegaly) within 3 days of virus inoculation (Table 7). Treatment of mice with BPV induced a threefold increase in spleen size, and the splenomegaly was retained during 3 days of high-dose virus infection.

High-dose MAd1*pt*4 infection did not induce an observable increase in the number of peritoneal leukocytes 3 days after infection (Table 8). Similar to a previous study of Fishel and coworkers (5), BPV treatment effected a fivefold increase in peritoneal leukocyte number. The combination of BPV treatment and high-dose virus infection had a synergistic effect, and the peritoneal leukocyte number was increased approximately 10-fold. The activity of the BPV-induced peritoneal lavage cells increased markedly. Electron microscopy of diluent-treated peritoneal exudate cells 3 days after high-dose virus infection revealed numerous virus particles in the extracellular medium and in phagolysosomes (Fig. 1A). In contrast, BPV-treated peritoneal exudate 3 days after high-dose virus infection exhibited virtually no cells with virus particles (Fig. 1B).

Antibodies did not play a role in the development of the BPV-induced resistant state. Treatment of mice with BPV for 10 days did not induce MAd1*pt*4 neutralizing antibodies. Studies with monoclonal antibodies indicate that certain classes of antibodies do not neutralize, but will protect the test animal against a challenging virus infection (2, 15, 16). Treatment of mice with BPV for 21 days did not induce protective antibodies. Serum (0.5 ml) from the BPV-treated C3H/HeN (MTV^-) mice was injected i.p. into mice of the same strain and challenged with a high-dose virus inoculum 24 h later. Assuming a plasma volume of 1.1 ml (6), then potentially protective antibodies would have been diluted approximately threefold. A group of 10 mice treated in this manner succumbed to high-dose infection within 7 days.

TABLE 6. Effect of heat on lymphocytosis after BPV treatment of C3H/HeN (+/*nu*) mice

Treatment[a]	Peripheral blood leukocytes (cells/mm^3)	
	Mean	SD
Diluent	6,380	260
BPV	17,600	3,800
Heated BPV[b]	7,600	820

[a] Mice were injected i.p. with vaccine diluent or 250 μg (dry weight) of BPV (Connaught Laboratories) 4 days before bleeding.
[b] BPV (Connaught Laboratories) was incubated at 80°C for 30 min before i.p. injection.

TABLE 7. Effect of BPV treatment and MAd1*pt*4 infection on spleen weight[a]

Treatment		Avg spleen wt on day 10 (g)
Day 0	Day 7	
Diluent	Medium	0.090
Diluent	1.0×10^8 PFU	0.130
BPV	Medium	0.352
BPV	1.0×10^8 PFU	0.338

[a] Groups of three C3H/HeN (+/*nu*) mice were injected i.p. with vaccine diluent or 250 μg (dry weight) of BPV. Seven days later, the mice were injected i.p. with cell culture medium or virus stock. After 3 days the mice were sacrificed by cervical dislocation, and the spleens were removed, blotted to remove excess fluid, and weighed.

DISCUSSION

Mouse adenovirus can be added to the list of virus infections that are modulated by treatment of the test animal with *B. pertussis* or materials derived from the microorganism. Development of the resistant state was not dependent on the strain of the microorganism. Although the *B. pertussis* strain from Connaught Laboratories, Inc., and strain 18323 (12) have a common lineage, strain Tohama I was derived from a completely different source (10). Development of the resistant state also was independent of the strain of mouse used.

The kinetics of development and decay of the resistant state indicated that a relatively long-term modification of the immunological mechanisms occurred. The modification probably did not involve thymus-derived lymphocytes in its mechanism of development, since congenitally athymic mice responded to BPV treatment with resistance. However, thymus involvement in the BPV-induced resistance in the thymus-bearing animal cannot be ruled out due to possible compensatory immune functions operating in congenitally athymic mice, such as increased numbers of natural killer cells. Thymus-derived cytolytic lymphocytes are necessary for final clearance of certain virus infections (11); consequently, the mechanism of BPV-induced resistance in thy-

TABLE 8. Effect of BPV treatment and MAd1*pt*4 infection on peritoneal leukocytes[a]

Treatment		Leukocytes in Peritoneal Lavage (cells × 10^{-6} per ml)			
		Day 7		Day 10	
Day 0	Day 7	Mean	SD	Mean	SD
Diluent		0.9	0.1	1.0	0.2
BPV		5.2	0.8	5.5	0.5
Diluent	1.0×10^8 PFU			1.1	0.2
BPV	1.0×10^8 PFU			10.0	1.0

[a] Groups of six C3H/HeN (+/*nu*) mice were treated by i.p. inoculation of vaccine diluent or 250 μg (dry weight) of BPV. Seven days later three mice from each group were sacrificed by cervical dislocation, and 4.0 ml of Dulbecco modified minimal Eagle medium was injected into the peritoneum. The peritoneal wall was massaged, 3.5 ml of lavage was withdrawn, and the cells were counted. The remaining three mice in each group were inoculated with virus and sacrificed for lavage 3 days later.

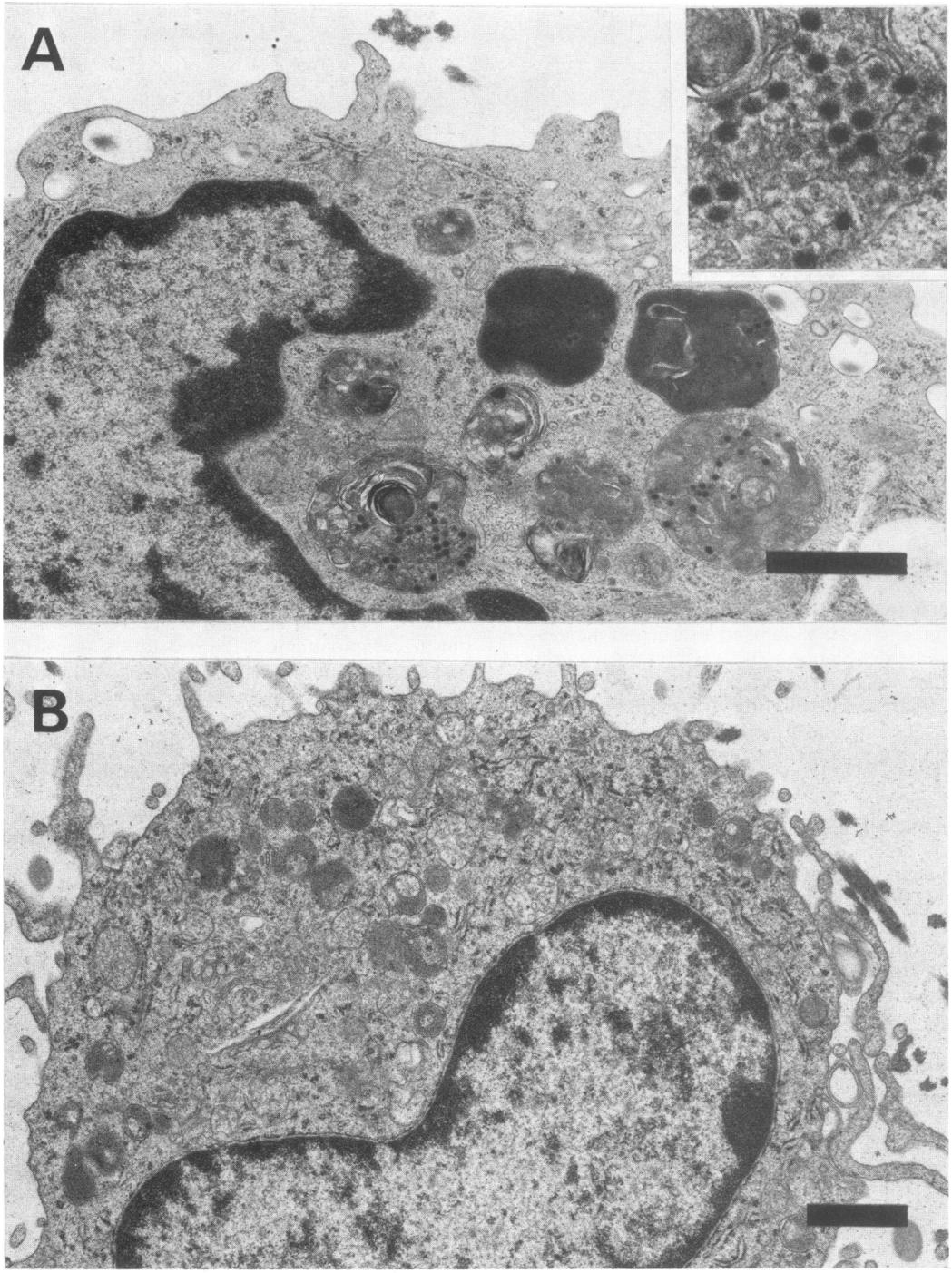

FIG. 1. Electron micrograph of ultrathin sections of peritoneal lavage cells from C3H/HeN (+/*nu*) mice. Bar, 1.0 μm. (A) Representative cell obtained after treatment with vaccine diluent (7 days) followed by MAd1*pt*4 infection (3 days). The inset shows an increased magnification of a phagolysosome containing virus particles. (B) Representative cell obtained after BPV treatment (7 days) followed by MAd1*pt*4 infection (3 days).

mus-bearing mice is probably an expression of retarded virus growth until the proper T-lymphocyte clones can develop. This conclusion is supported by the similarity of disease patterns in BPV-treated and low dose-infected athymic mice.

The immune response of mice appears to be overwhelmed by high-dose MAd1*pt*4 infection; death occurs before a systemic response in the form of splenomegaly, leukocytosis, and increase leukocytes in the peritoneum can develop. The treatment of mice with BPV could provide the mouse with a systemic response at the time of virus inoculation; however, one must consider that clinical signs of systemic response might not be involved in the resistant state. We have observed that acellular fractions of *B. pertussis* have decreased ability to induce splenomegaly and leukocytosis, yet retain resistance-inducing activity (R. S.

Stinson, J. D. Lee, L. Williamson, and A. Winters, Abstr. 19th Natl. Meet. Reticuloendothel. Soc. 1982, abstr. no. 56, p. 70). The mechanism of BPV-induced resistance markedly decreased virus particles from the inoculum or subsequent multiplication in peritoneal lavage cells obtained 3 days after infection (or both).

Study of the molecules or complex of molecules in *B. pertussis* that are responsible for induction of the virus-resistant state, and investigation of their mechanism of immunomodulation might lead to new methods for prevention and treatment of virus infections.

ACKNOWLEDGMENTS

This work was initiated at the Department of Medical Microbiology and Immunology, University of South Florida, Tampa. Support was provided by University of Alabama Research Committee projects no. 1116 and 1143, Public Health Service Biomedical research support grants NIH 5 S07 RR05749-05 and PHS 2 S07 RR07151-06 from the National Institutes of Health, Public Health Service Grant AI 15108-01 from the National Institutes of Allergy and Infectious Disease, and Office of Naval Research contract N00014-83-K-0597.

LITERATURE CITED

1. **Ayme, G., M. Caroff, R. Chaby, N. Haeffner-Cavaillon, A. LeDur, M. Moreau, M. Muset, M.-C. Mynard, M. Roumiantzeff, D. Schulz, and L. Szabo.** 1980. Biological activities of fragments derived from *Bordetella pertussis* endotoxin: isolation of a nontoxic, Shwartzman-negative lipid A possessing high adjuvant properties. Infect. Immun. **27**:739–745.
2. **Balachandran, N., S. Bacchetti, and W. E. Rawls.** 1982. Protection against lethal challenge of BALB/c mice by passive transfer of monoclonal antibodies to five glycoproteins of herpes simplex virus type 2. Infect. Immun. **37**:1132–1137.
3. **Chaby, R., G. Ayme, M. Caroff, R. Donikian, N. Haeffner-Cavaillon, A. Le Dur, M. Moreau, M.-C. Mynard, M. Roumiantzeff, and L. Szabo.** 1979. Structural features and separation of some of the biological activities of the *Bordetella pertussis* endotoxin by chemical fractionation, p. 185–190. *In* C. R. Manclark and J. C. Hill (ed.), International symposium on pertussis. Department of Health, Education, and Welfare publication no. (NIH)79-1830. U.S. Government Printing Office, Washington, D.C.
4. **Fish, F., J. L. Cowell, and C. R. Manclark.** 1984. Proliferative response of immune mouse T-lymphocytes to the lymphocytosis-promoting factor of *Bordetella pertussis*. Infect. Immun. **44**:1–6.
5. **Fishel, C. W., D. G. Halkias, T. W. Klein, and A. Szentivanyi.** 1976. Characterization of cells present in peritoneal fluids of mice injected intraperitoneally with *Bordetella pertussis*. Infect. Immun. **13**:263–272.
6. **Friedman, J. J.** 1959. Circulating and tissue hematocrits of normal unanesthetized mice. Am. J. Physiol. **196**:420–422.
7. **Hewlett, E. L., C. O. Roberts, J. Wolff, and C. R. Manclark.** 1983. Biphasic effect of pertussis vaccine on serum insulin in mice. Infect. Immun. **41**:137–144.
8. **Ho, M.-K., A. S. Kong, and S. I. Morse.** 1980. The *in vitro* effects of *Bordetella pertussis* lymphocytosis-promoting factor on murine lymphocytes. V. Modulation of T cell proliferation by helper and suppressor lymphocytes. J. Immunol. **124**: 362–369.
9. **Jerne, N. K., C. Henry, A. A. Nordin, H. Fuji, A. M. C. Koros, and I. Lefkovits.** 1976. Antibody synthesis *in vitro*. Methods Immunol. Immunochem. **5**:303–374.
10. **Kasuga, T., Y. Nakase, K. Ukishima, and K. Takatsu.** 1954. Studies on *Haemophilus pertussis*. V. Relation between the phase of bacilli and the progress of the whooping-cough. Kitasato Arch. Exp. Med. **27**:57–62.
11. **Kees, U., and R. V. Blanden.** 1976. A single genetic element in *H-2K* affects mouse T-cell antiviral function in poxvirus infection. J. Exp. Med. **143**:450–455.
12. **Kendrick, P. L., G. Eldering, M. K. Dixon, and J. Misner.** 1947. Mouse protection tests in the study of pertussis vaccine: a comparative series using the intracerebral route for challenge. Am. J. Public Health **37**:803–810.
13. **Kirchner, H., M. T. Scott, H. M. Hirt, and K. Munk.** 1978. Protection of mice against viral infection by *Corynebacterium parvum* and *Bordetella pertussis*. J. Gen. Virol. **41**:97–104.
14. **Klein, T., W. Benjamin, A. Winters, and H. Friedman.** 1981. Pertussis induced immunomodulation, p. 275–292. *In* H. Friedman, T. W. Klein, and A. Szentivanyi (ed.), Immunomodulation by bacteria and their products. Plenum Publishing Corp., New York.
15. **LeFrancois, L.** 1984. Protection against lethal viral infection by neutralizing and nonneutralizing monoclonal antibodies: distinct mechanisms of action in vivo. J. Virol. **51**:208–214.
16. **Mathews, J. H., and J. T. Roehrig.** 1982. Determination of the protective epitopes on the glycoproteins of Venezuelan equine encephalomyelitis virus by passive transfer of monoclonal antibodies. J. Immunol. **129**:2763–2767.
17. **Morse, S. I.** 1976. Biologically active components and properties of *Bordetella pertussis*. Adv. Appl. Microbiol. **20**:2–25.
18. **Munoz, J. J., H. Arai, R. K. Bergman, and P. L. Sadowski.** 1981. Biological activities of crystalline pertussigen from *Bordetella pertussis*. Infect. Immun. **33**:820–826.
19. **Munoz, J. J., and R. K. Bergman.** 1977. *Bordetella pertussis*, immunological and other biological activities, vol. 4. Marcel Dekker, Inc., New York.
20. **Munoz, J. J., and R. K. Bergman.** 1979. Biological activities of *Bordetella pertussis*, p. 143–150. *In* C. R. Manclark and J. C. Hill (ed.), International symposium on pertussis. Department of Health, Education, and Welfare publication no. (NIH)79-1830. U.S. Government Printing Office, Washington, D.C.
21. **Parfentjev, I. A.** 1955. Bacterial allergy increases susceptibility to influenza virus in mice. Proc. Soc. Exp. Biol. Med. **90**:373–375.
22. **Sato, Y., J. L. Cowell, H. Sato, D. G. Burstyn, C. R. Manclark.** 1983. Separation and purification of the hemagglutinins from *Bordetella pertussis*. Infect. Immun. **41**:313–320.
23. **Sugimoto, M., Y. Nakanishi, M. Otokawa, N. Uchida, T. Yasuda, H. Sato, and Y. Sato.** 1983. Effect of *Bordetella pertussis* leukocytosis(lymphocytosis)-promoting factor (LPF) on the physical lymphoepithelial-cell association studied with the use of an *in vitro* model of mouse thymus. J. Immunol. **130**:2767–2774.
24. **Winters, A. L., and H. K. Brown.** 1980. Duodenal lesions associated with adenovirus infection in athymic "nude" mice. Proc. Soc. Exp. Biol. Med. **164**:280–286.
25. **Winters, A. L., H. K. Brown, and J. K. Carlson.** 1981. Interstitial pneumonia induced by a plaque-type variant of mouse adenovirus. Proc. Soc. Exp. Biol. Med. **167**:359–364.