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Actinobacillus pleuropneumoniae-Induced Thymic Lesions in Mice and Pigs†

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Actinobacillus pleuropneumoniae produces several hemolysins/cytotoxins that may be important in the pathogenesis of acute lesions. Little is known, however, about the role of these virulence factors in chronic disease or the carrier state. We investigated the effects of live bacterial infection and transthoracic injection of a sterile culture supernatant on primary lymphoid organs and lymphocyte populations. Transthoracic inoculation of mice or intranasal inoculation of pigs with virulent *A. pleuropneumoniae* serotypes 1 and 7 induced thymic cortical lymphoid necrosis. These lesions were reproduced in mice by transthoracic injection of a concentrated sterile culture supernatant. The cytotoxic effect of this culture supernatant was also demonstrated *in vitro* by using a tetrazolium dye reduction assay. Both porcine and murine thymic lymphocytes as well as splenic T lymphocytes were susceptible to the toxin. Porcine convalescent serum, but not preimmune serum, prevented thymic lesions and neutralized the *in vitro* cytotoxic effect of the culture supernatant on murine thymic lymphocytes. Thymic lesions also were reproduced in mice by using purified lipopolysaccharide (LPS) from *Escherichia coli* O111:B4; however, LPS had no *in vitro* cytotoxic effect on either porcine or murine thymic lymphocytes. These results suggest that secreted *A. pleuropneumoniae* toxin(s) is capable of affecting host T-lymphocyte populations and may affect host immune function.

Actinobacillus pleuropneumoniae is the etiologic agent of porcine pleuropneumonia, a disease which costs pork producers millions of dollars each year from death of animals, reduced feed efficiency, prophylactic medication, and vaccination costs (13). The disease is characterized by lesions which include severe necrohemorrhagic pneumonia, pleuritis, and suppurative lymphadenitis. Animals which survive acute infections often become chronic carriers, having poor growth characteristics and increased susceptibility to secondary infections (13, 15). The organism possesses many virulence factors (capsule, lipopolysaccharide [LPS], hemolysins, cytotoxins, and proteases) thought to be important in the pathogenesis of acute disease (1, 5, 6, 9, 14). A recent report has suggested that vaccination with preparations containing secreted factors, such as hemolysin/cytotoxin, provides enhanced protection from acute disease (4). Little is known, however, about the role of these virulence factors in the production of chronic disease and the carrier state. Therefore, we investigated the effects of live bacterial infection or sterile culture supernatants from two *A. pleuropneumoniae* serotype 1 and 7 reference strains on the lymphoid organs and lymphocyte populations of mice and pigs. If the organism or its putative virulence factors could affect the primary and secondary lymphoid organs as well as lymphocyte populations, then infection by the organism may inhibit the immunologic response of animals and subsequently contribute to chronic disease and the carrier state.

MATERIALS AND METHODS

Bacteria. *A. pleuropneumoniae* serotype 1 and 7 reference strains were obtained from Brad Fenwick, College of Veterinary Medicine, Kansas State University, Manhattan. Bacteria were grown and maintained as described previously (4).

To prepare inocula, bacteria were grown overnight in a shaker bath at 37°C in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) containing 0.01% (wt/vol) NAD (Sigma Chemical Co., St. Louis, Mo.). The following morning, 0.5 ml of the culture was added to 50 ml of fresh medium, and the culture was reincubated for an additional 3 h. The bacteria were harvested by centrifugation at 10,000 × g for 15 min and then resuspended in phosphate-buffered saline (PBS; pH 7.4) to an optical density at 600 nm (OD₆₀₀) of 0.05 to 0.075 (approximately 4 × 10⁷ CFU/ml). Additional dilutions of this suspension were made in PBS to prepare suspensions for intranasal inoculation of pigs and transthoracic injection of mice.

Animals and inoculation methods. Pigs used in this study were obtained from the University of Nebraska closed specific-pathogen-free herd, which is periodically monitored for the presence of respiratory pathogens and has had no previous clinical history of pleuropneumonia. BALB/c mice were obtained from a University of Nebraska breeding colony. All animals were housed and maintained in isolation facilities in accordance with our institutional animal care and use committee guidelines.

Acute pleuropneumonia was induced in pigs by using pressurized aerosolization through a nasal cannula as described previously (4). The inoculum consisted of 2 × 10⁶ CFU of serotype 1 or 4 × 10⁶ CFU of serotype 7 organisms suspended in 5 ml of PBS. Mice were injected transthoracically with 0.1 ml of a PBS suspension containing 2 × 10⁷ CFU of serotype 1 or 2 × 10⁷ CFU of serotype 7 organisms per ml. In certain murine experiments, the bacterial inoculum was replaced with 0.1 ml of a 10-fold concentrate from a sterile cytotoxic culture supernatant described below or 0.1 ml of PBS containing 1.0 mg of purified LPS per ml from *Escherichia coli* O111:B4 (Difco). Mice injected with 0.1 ml of 10-fold-concentrated uninoculated media served as a negative control. All mice and pigs were killed by euthanasia 24 h after inoculation, and tissues were immediately removed and fixed in 10% buffered formalin. Following fixa-

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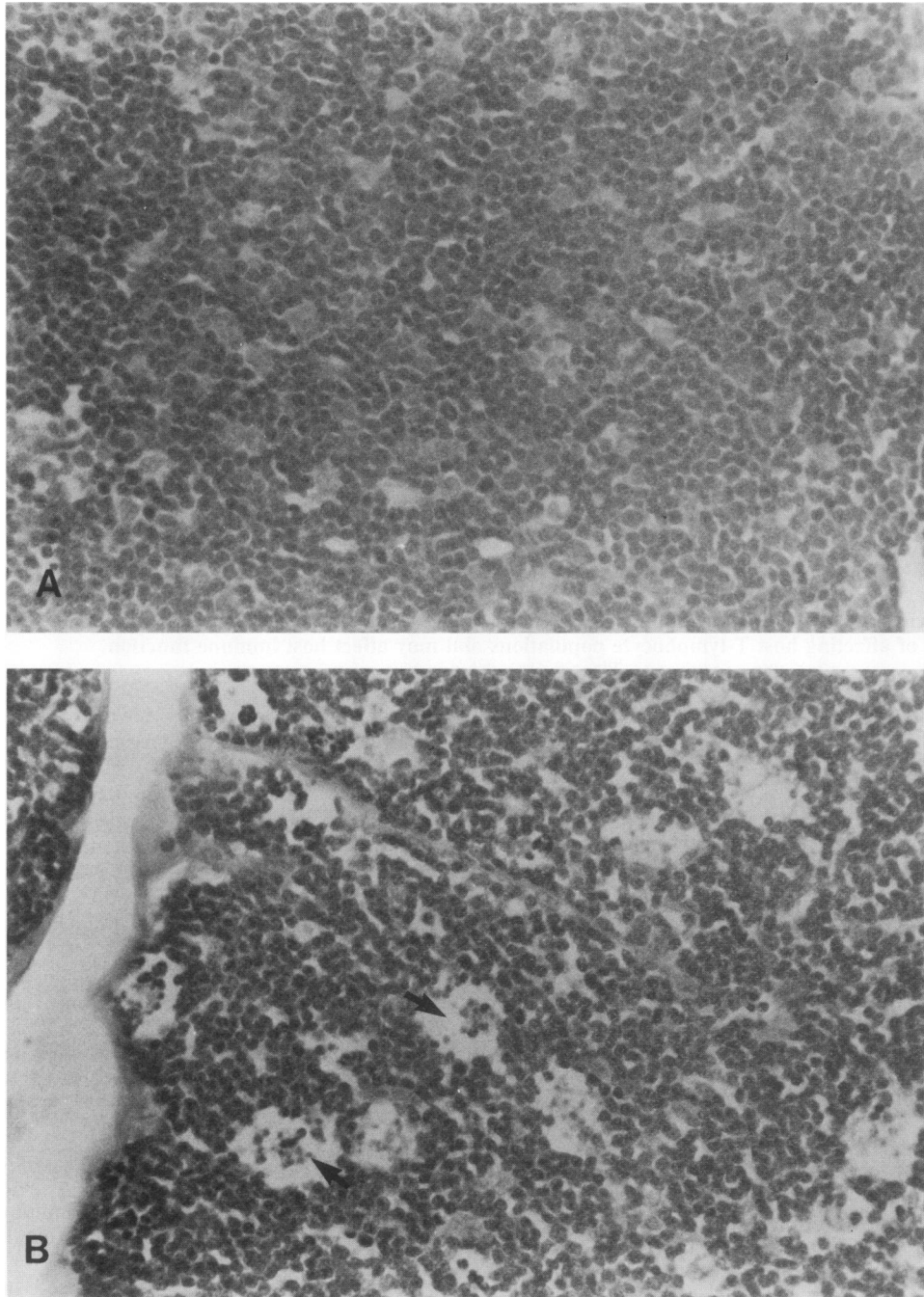


FIG. 1. (A) Normal pig thymic cortex. Magnification, $\times 25$. (B) Thymic cortex from a pig 24 h postinfection with *A. pleuropneumoniae* serotype 7 organisms. Arrows indicate areas of cortical lymphocyte necrosis and dropout. Magnification, $\times 25$. (C) Normal mouse thymus. Medulla (a); cortex (b). Magnification, $\times 25$. (D) Thymic cortex from a mouse transthoracically injected with serotype 1 10-fold-concentrated culture supernatant. Cortical lesion is characterized by severe diffuse lymphoid necrosis and dropout. (E) Thymic cortex from a mouse inoculated with serotype 1 10-fold-concentrated culture supernatant preincubated in homologous porcine convalescent serum. Thymic cortex was protected from lesion development.

tion, tissues were processed and stained with hematoxylin and eosin by routine methods for histologic examination.

Toxin production. Cytotoxin was prepared from the serotype 1 and 7 *A. pleuropneumoniae* reference strains by a modification of a method described previously (3). Briefly, bacteria were grown to log phase in the brain heart infusion-

NAD medium described above and harvested by centrifugation ($10,000 \times g$ for 15 min). The bacterial pellet was resuspended in twice the original volume of RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) containing 1.05 g of bovine serum albumin (BSA; Sigma) per liter. The culture was incubated in a shaker bath at 37°C for

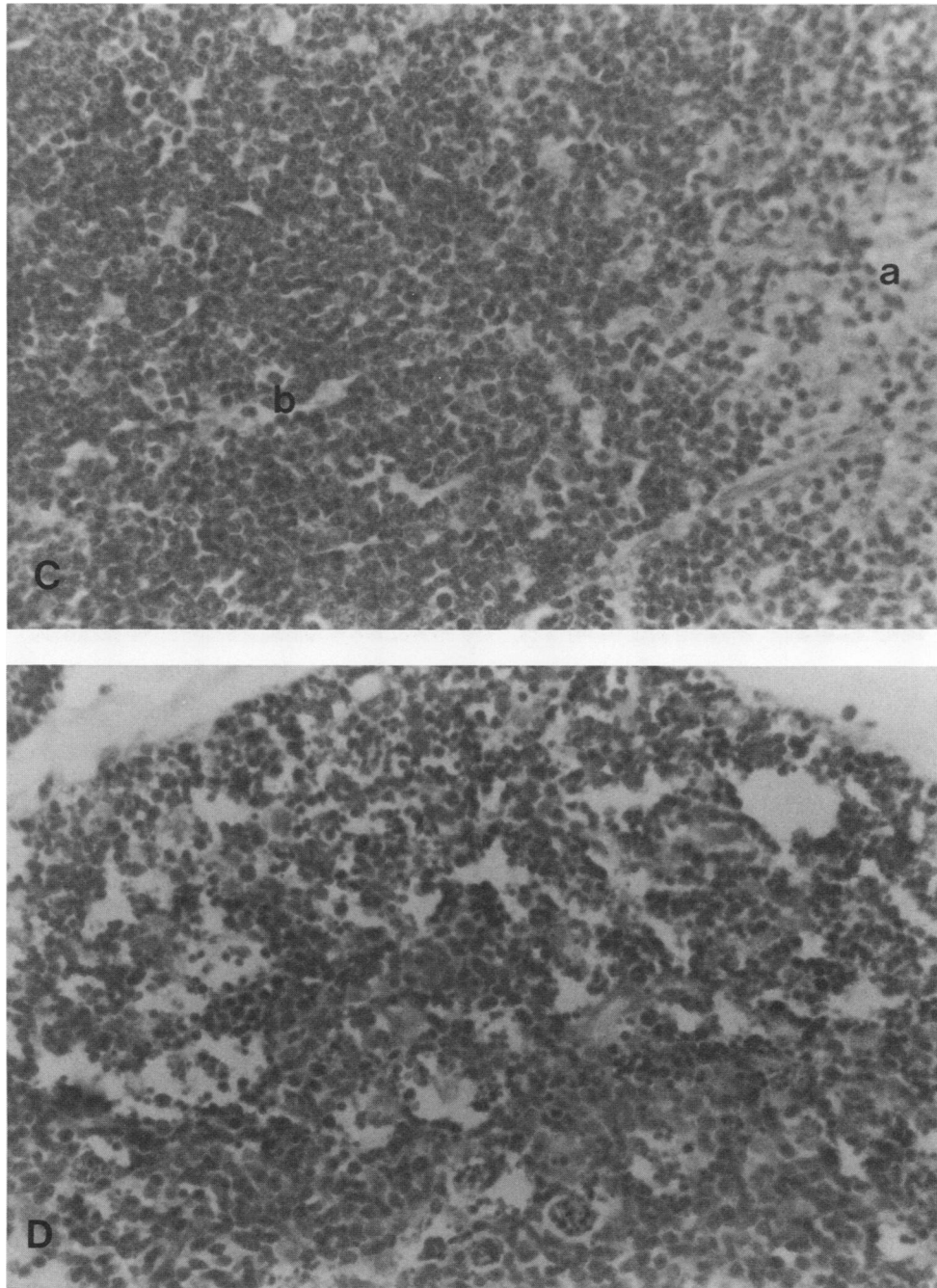


FIG. 1—Continued.

an additional 1.5 h. Bacteria were then removed from the culture by centrifugation as described above, and the culture supernatant was filter sterilized, divided into aliquots, and frozen at -20°C . For certain experiments, aliquots of the culture supernatants were heated for 30 min at 56°C to determine heat lability of the toxin or concentrated 10-fold in an Amicon stir cell (Diaflo; Amicon Div., Danvers, Mass.), using a 10-kDa retention membrane for use in Western blot (immunoblot) analysis. Other aliquots of the supernatants were concentrated in a stir cell, using a 100-kDa retention membrane. This retentate was reconstituted to its original volume with RPMI medium containing 0.25% BSA (RPMI-

BSA). The original unconcentrated supernatant, reconstituted retentate, and the supernatant filtrate were tested for toxicity to thymocytes. *A. pleuropneumoniae* serotype 1 cytotoxic culture supernatant was biosynthetically labeled in the following manner. Bacterial cultures were grown and harvested as above. Bacteria were then resuspended in 10 ml of RPMI medium without methionine (RPMI Select Amine Kit; GIBCO) and incubated at 37°C for 30 min. Bacterial cells were then reharvested and resuspended in 20 ml of RPMI-BSA medium containing $500\ \mu\text{Ci}$ of ^{35}S -methionine (New England Nuclear, Boston, Mass.). The culture was then incubated at 37°C for an additional 90 min, and the

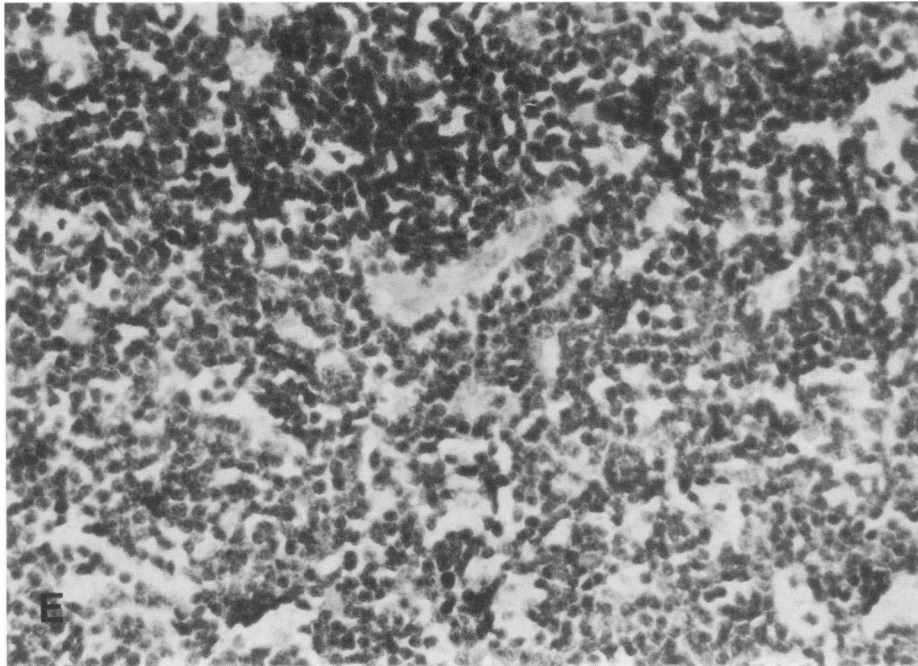


FIG. 1—Continued.

culture supernatant was harvested and sterilized as described above. Aliquots of the labeled supernatant were tested for cytotoxic activity on thymocytes.

Endotoxin determinations. Endotoxin present in the 10-fold-concentrated culture supernatants was measured by the *Limulus* amoebocyte lysate assay (Whittaker M.A. Bioproducts, Walkersville, Md.) per the manufacturer's instructions.

SDS-PAGE, Western blot, and autoradiographic analyses. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli (11) and immunoblot analysis procedures were performed as reported previously (4). Briefly, 10-fold concentrates of the culture supernatants were electrophoresed for approximately 2.5 h at a constant voltage of 75 V. Gels were stained with Coomassie brilliant blue dye for protein identification. The proteins in duplicate gels were transferred to nitrocellulose of 0.2- μ m pore size (Bio-Rad Laboratories, Richmond, Calif.). Successful transfer was determined by staining the nitrocellulose with 0.2% Ponceau S dye (Sigma), and molecular weight standards were marked with a felt-tip marker. The nitrocellulose was blocked overnight at 4°C in 3% fish gelatin (Sigma) and reacted for 1.5 h with 1:50 dilutions of preimmune, convalescent, or convalescent porcine sera adsorbed with homologous organisms (described below). The nitrocellulose blots were then washed and reacted with alkaline phosphatase-conjugated goat anti-porcine immunoglobulin G (Kirkegaard and Perry Laboratories, Gaithersburg, Md.). Color was developed by reaction with nitroblue tetrazolium-5-bromo-4-chloro-3-indolyl phosphate toluidinium substrate reagent (Kirkegaard and Perry) in accord with the manufacturer's instructions. Autoradiographs of the biosynthetically labeled serotype 1 toxin preparation were prepared by mixing a 100- μ l aliquot with an equal volume of 2 \times protein sample buffer without 2-mercaptoethanol. The sample was then heated at 100°C for 5 min and run in a 7% acrylamide gel as described above. Proteins in the gel were transferred to nitrocellulose and stained with Ponceau S to confirm protein

transfer. The nitrocellulose blot was then allowed to expose Kodak X-Omat film (Sigma) for 7 days at -80°C prior to development.

Convalescent sera. Convalescent sera were produced by intranasal inoculation of one pig for each reference serotype as reported previously (4). Blood was collected before inoculation and at weekly intervals following infection. Sera were harvested and stored in small aliquots at -20°C. Sera from the blood collected from each animal on days 28, 35, and 42 postinoculation were pooled for use in Western blot analysis and passive protection experiments. A portion of each respective pooled convalescent serum sample was repeatedly adsorbed by incubation with approximately 10^{10} CFU of the homologous serotype 1 or 7 organisms per ml of convalescent serum at 37°C for 10 min followed by centrifugation at 15,000 \times *g* for 20 min to remove bacteria from the serum. Sera were heat inactivated (60°C for 40 min) prior to use in passive protection experiments.

Passive protection experiments. Passive protection experiments were performed with 5- to 8-week-old BALB/c mice (five animals per inoculation group). A 100- μ l aliquot of 10-fold-concentrated sterile culture supernatant was mixed with an equal volume of porcine convalescent serum (1:4 dilution in PBS) specific for the homologous bacterial serotype. The mixture (200- μ l total volume) was incubated at 37°C for 10 min and then transthoracically injected into mice. Mice inoculated with an identical aliquot of the supernatant mixed with 1:4-diluted homologous preimmune serum served as a positive control for evaluation of thymic lesions. Mice inoculated with 200 μ l of diluted convalescent serum served as a negative control. All mice were killed by euthanasia 24 h after inoculation, and tissues were collected and processed as described above.

T-cell enrichment. Both porcine and murine splenic T lymphocytes used in the *in vitro* cytotoxicity assays were prepared by a method described previously (8). Splenic lymphocytes were purified over Ficoll-Paque (Pharmacia,

Piscataway, N.J.), following the manufacturer's instructions. After cells were washed with sterile PBS, they were harvested and resuspended in RPMI-BSA medium at a cell concentration of 5×10^7 per ml. A total of 10^8 cells were then added to a syringe containing sterile nylon wool. The column was incubated at 37°C for 1 h. Nonadherent cells were harvested and resuspended in RPMI-BSA medium at a cell concentration of 10^7 per ml for use in cytotoxicity assays. Cell preparations typically contained >95% lymphocytes.

Cytotoxicity assay. Cytotoxic activity of the bacterial culture supernatants was determined by a modification of a method described previously (16). Briefly, twofold serial dilutions of unconcentrated culture supernatants or purified *E. coli* O111:B4 LPS (1 mg/ml) were made with RPMI-BSA in microtiter plates (100 μ l/well). Porcine or murine thymocytes (100 μ l of RPMI-BSA containing 10^7 viable cells) were then added to each well, and the plates were incubated at 37°C for 1 h. The plates were centrifuged at $500 \times g$ for 5 min, and the supernatants were removed from the cell pellets. The cells were resuspended in RPMI-BSA (120 μ l/well) containing 0.83 mg of a tetrazolium dye (MTT; Sigma) per ml. Plates were reincubated at 37°C for 3 h, followed by centrifugation and removal of the supernatants. Acid isopropanol (100 μ l/well) was added, and the plates were vibrated on a microtiter plate shaker to dissolve the dye precipitate within the cells. The OD₅₇₀ of each well was measured in a microtiter plate reader (Dynatech Laboratories Inc., Alexandria, Va.). The percent toxicity was determined as: $[1 - (\text{OD of toxin-treated cells}/\text{OD of untreated control cells})] \times 100$. Fifty percent toxicity end-point titers were defined as the reciprocal of the highest toxin dilution resulting in at least 50% toxicity to the thymocytes.

RESULTS

Pathology. Experimental inoculation of pigs with live *A. pleuropneumoniae* serotype 1 and 7 reference strains induced lesions typical of acute pleuropneumonia, including acute necrohemorrhagic pneumonia with pleuritis and severe suppurative bronchial lymphadenitis. An example of the thymic lesions induced in pigs by serotype 7 organisms is shown in Fig. 1B. These lesions were characterized by moderate multifocal cortical lymphocyte necrosis and drop-out, as evidenced by lymphocyte karyorrhexis and pyknosis with loss of cells in the regions of cortical dendrocytes. Pigs surviving acute bacterial infection had evidence of thymic atrophy and a decrease in the normal cortical/medullary ratio.

Transthioracic inoculation of mice with virulent *A. pleuropneumoniae* serotypes 1 and 7 induced lesions, including interstitial pneumonia, purulent pleuritis and lymphadenitis, lymphoid necrosis of splenic nodules and periarteriolar lymphatic sheaths, and diffuse acute thymic cortical lymphoid necrosis. The acute thymic lesions could be reproduced in mice following transthioracic injection of a 10-fold concentrate of bacterial culture supernatant (Fig. 1D) or by transthioracic injection of 0.1 mg of purified *E. coli* O111:B4 LPS (figure not shown). Less severe pyknotic nuclear changes were also observed in splenic lymphoid nodules in mice after injection of concentrated culture supernatants. Transthioracic injection of 10-fold-concentrated uninoculated medium had no pathologic effect on lymphoid tissues.

In vivo passive protection. Mice injected with the concentrated culture supernatant from serotype 1 organisms preincubated with diluted porcine homologous convalescent serum were passively protected from the development of

TABLE 1. Toxicity of culture supernatants

| Tested material | 50% Cytotoxic end-point titer for thymocytes ^a | |
|--|---|-----------------|
| | Murine | Porcine |
| Serotype 7 culture supernatant | 16 | 32 |
| Serotype 1 culture supernatant | 64 | 32 |
| Serotype 1 (heat inactivated) ^b | 8 | ND ^c |
| Serotype 1 culture retentate ^d | 32 | ND |
| Serotype 1 culture filtrate ^d | 0 | ND |
| Serotype 7 culture retentate ^d | 8 | ND |
| Serotype 7 culture filtrate ^d | 0 | ND |
| <i>E. coli</i> LPS | 0 | 0 |

^a Determined as the reciprocal of the highest toxin dilution causing 50% toxicity for 10^7 porcine or murine thymocytes.

^b Thawed aliquot of culture supernatant was warmed at 56°C for 30 min.

^c ND, activity not determined.

^d A thawed aliquot of culture supernatant was filtered through a membrane with 100-kDa retention. The filtrate was tested for activity, and the retentate was reconstituted to the original volume and then tested for activity.

thymic lesions (Fig. 1E). Porcine serum from preimmune animals had no protective capacity, whereas injection of convalescent serum alone had no toxic effect on thymic tissue (figures not shown).

In vitro cytotoxicity. Results from in vitro cytotoxicity assays and cytotoxic 50% end-point titers of *E. coli* LPS and various *A. pleuropneumoniae* culture supernatant preparations are summarized in Table 1. Unconcentrated culture supernatants from serotype 1 and 7 reference strains had a toxic effect on both porcine and murine thymocytes. Warming a thawed aliquot of the serotype 1 supernatant at 56°C for 30 min reduced the toxicity for murine thymocytes by eightfold. Cytotoxic analysis of serotype 1 and 7 culture supernatants filtered through a membrane with a 100,000-molecular-weight retention showed the retentates, but not the filtrates, to have cytotoxic activity for murine thymocytes. Concentrations of purified *E. coli* LPS capable of inducing acute thymic lesions in vivo were found to have no in vitro cytotoxic effect on either porcine or murine thymocytes (Table 1).

Serotype 1 culture supernatant was found to have 50% cytotoxic end-point titers of 8 and 16 for the respective murine and porcine splenic T-cell preparations, whereas the serotype 7 culture supernatant possessed a 50% cytotoxic end-point titer of 4 for each of the respective splenic T-cell preparations. Serotype 1 culture supernatant biosynthetically labeled with ³⁵S-methionine retained cytotoxic activity.

Endotoxin determination. The culture supernatant concentrates from serotype 1 and 7 reference strains contained 7.8×10^5 and 4.6×10^5 endotoxin units per ml, respectively, or approximately 7.8×10^{-3} and 4.6×10^{-3} mg of relative standard endotoxin per ml.

SDS-PAGE, Western blot, and autoradiographic analyses of toxin preparations. The protein profiles of the 10-fold concentrates from serotype 1 and 7 culture supernatants are shown in Fig. 2 (lanes B and C). Both culture supernatants contain a 105-kDa protein (arrows) which is absent from the uninoculated medium control. Western blot analysis of the concentrate from serotype 1 toxic culture supernatant blotted with various preimmune, convalescent, and adsorbed convalescent porcine sera is shown in Fig. 3. Convalescent sera from pigs infected with either the serotype 1 or 7 reference strain reacted with the 105-kDa protein. This reactivity was not removed by adsorption with *A. pleuropneumoniae* whole-cell suspensions of either the homo-

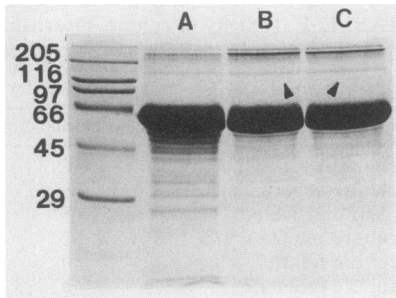


FIG. 2. SDS-PAGE of *A. pleuropneumoniae* 10-fold-concentrated toxic culture supernatants. Lane A, uninoculated RPMI-BSA medium control; lane B, serotype 1 culture supernatant; lane C, serotype 7 culture supernatant. Arrows indicate presence of protein band absent from medium control. Molecular weight markers (10^3) are indicated on the left.

gous or heterologous organisms. Serotype 1 convalescent serum also recognized additional high-molecular-weight bands in the homologous cytotoxic culture supernatant (lanes G and H). Serotype 7 convalescent serum failed to recognize these additional bands. Preimmune porcine sera failed to recognize the 105-kDa protein or other high-molecular-weight bands. The autoradiograph of the nonreduced ^{35}S -labeled toxin preparation is shown in Fig. 4. This preparation contained bands at 105, 120, and 210 kDa, as well as several lower-molecular-weight bands.

DISCUSSION

The purpose of this study was to determine whether infection by *A. pleuropneumoniae* and secretion of virulence

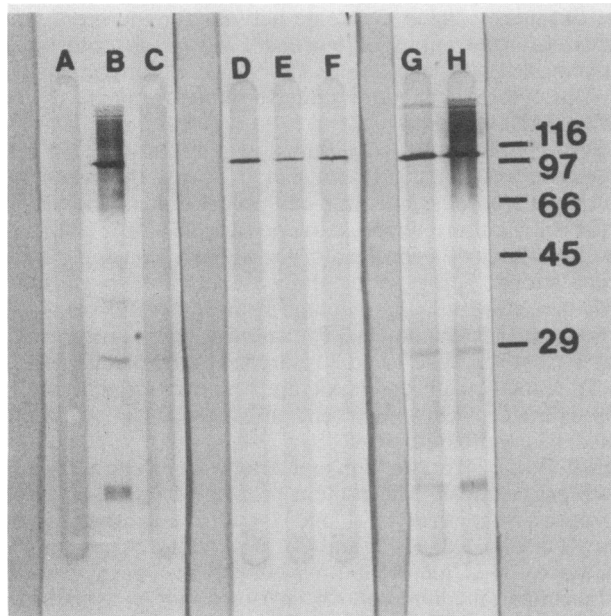


FIG. 3. Immunoblot of *A. pleuropneumoniae* serotype 1 (S1) $10\times$ toxic culture supernatant with the following porcine sera: S1 preimmune serum (lane A), S1 antiserum (lane B), serotype 7 (S7) preimmune serum (lane C), S7 antiserum (lane D), S7 antiserum adsorbed with S7 bacteria (lane E), S7 antiserum adsorbed with S1 bacteria (lane F), S1 antiserum adsorbed with S1 bacteria (lane G), and S1 antiserum adsorbed with S7 bacteria (lane H).

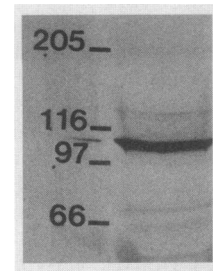


FIG. 4. Autoradiograph of a nitrocellulose blot from a nonreducing 7% acrylamide gel containing unconcentrated *A. pleuropneumoniae* serotype 1 culture supernatant. Approximate molecular weights (10^3) are marked on the left.

factors might adversely affect an animal's lymphoid tissue and thus impair its ability to mount an effective immune response. Such impairment could prevent clearance of the organism after infection and contribute to the establishment of chronic disease and the carrier state. Previous results have shown that various serotypes of this organism produce toxins that kill alveolar macrophages, monocytes, and neutrophils (1, 9, 14). Frey and Nicolet (6) have purified a 105-kDa protein from the serotype 1 reference strain and have demonstrated that it possesses hemolytic activity. Recently, a protein of similar molecular weight from the serotype 1 reference strain has been shown to form pores in phospholipid membrane bilayers (12).

Our results indicate that live bacterial infection by serotype 1 and 7 reference strains can induce thymic lesions in mice and pigs. Such lesions may contribute to organ atrophy in pigs surviving live bacterial infection. Similar lesions were reproduced in mice by transthoracic injection of 10-fold concentrates of sterile culture supernatants from these reference strains. Thymic lesions could be prevented by preincubation of the concentrated culture supernatants with homologous porcine convalescent serum. Convalescent sera from animals infected with either the serotype 1 or 7 reference strain reacted in immunoblot analysis with a 105-kDa protein in the concentrated culture supernatant from the serotype 1 organism. Adsorption of these sera with *A. pleuropneumoniae* whole cells of either reference serotype failed to remove reactivity to this protein, suggesting that it is a secreted product.

Unconcentrated culture supernatants from both reference strains were toxic for porcine and murine thymocytes in an in vitro dye reduction assay. This toxicity was heat labile and present in a fraction of the supernatant having a molecular weight of $>100,000$. These results suggest that a high-molecular-weight product secreted by *A. pleuropneumoniae* has cytotoxic activity for thymocytes. That convalescent sera neutralized lesions in vivo and reacted to a 105-kDa protein and several high-molecular-weight bands in immunoblot analysis suggests that one or several of these proteins may have a role in cytotoxic activity. In an effort to identify the macromolecules present in the high-molecular-weight fraction with in vitro cytotoxic activity, we biosynthetically labeled the cytotoxic preparation from *A. pleuropneumoniae* serotype 1. This fraction contained molecules of 105, 120, and 210 kDa. Whether any or all of these molecules are responsible for the cytotoxic activity is not clear; however, the 210-kDa protein may represent a dimer of the 105-kDa protein since a single monoclonal antibody recognizes both molecules (unpublished observation).

That *A. pleuropneumoniae* can induce thymic lesions in

swine is a new finding. The exact pathogenesis of these lesions, however, is still undetermined. The observation that purified *E. coli* endotoxin was capable of inducing thymic lesions in the mouse model suggests that at least some endotoxins can induce thymic lesions in this system and that the endotoxin of *A. pleuropneumoniae* may also play a significant role in the production of these in vivo lesions. In contrast, the heat lability of the in vitro toxic effect on murine thymocytes and the failure of purified *E. coli* LPS to produce a direct cytotoxic effect on either murine or porcine thymocytes suggest the presence of a proteinaceous toxin. It is also possible that *A. pleuropneumoniae* endotoxin may possess biological or species-specific activity different from that of *E. coli* endotoxin. The determination of the exact pathogenic mechanisms involved may require purification of the cytotoxic substance(s).

Whether or not the induction of thymic lymphoid necrosis and possible organ atrophy could lead to a generalized immunosuppression is yet to be determined. There is recent evidence, however, that animals removed from conventional herds via a medicated early weaning program and subsequently reared in isolation have larger mean thymic weights and enhanced growth characteristics when compared with animals reared in the conventional herd (7). These findings suggest possible relevance to the presence of thymic organ atrophy related to the overall health of the animal and its growth potential.

The observation that *A. pleuropneumoniae* produces a toxin that can affect T lymphocytes also suggests that the organism may induce a localized immunosuppression of mucosal immune responses due to the destruction of local T-cell populations. *A. pleuropneumoniae* is known to cause severe suppurative and necrotizing lymphadenitis of bronchial and mediastinal lymph nodes. Similar lesions have been observed in porcine tonsillar tissues, and the organism has been isolated from tonsillar crypts long after initial infection (2, 13). This destruction of secondary lymphoid tissues may provide a mechanism for persistence of the organism at mucosal surfaces and facilitate its spread to susceptible hosts.

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REFERENCES

1. Bendixon, P. H., P. E. Shewen, S. Rosendal, and B. N. Wilkie. 1981. Toxicity of *Haemophilus pleuropneumoniae* for porcine lung macrophages, peripheral blood monocytes, and testicular cells. *Infect. Immun.* **33**:673-676.
2. Briesch, S. 1990. M.S. thesis. University of Nebraska, Lincoln.
3. Clinkenbeard, K. D., D. A. Mosier, and A. W. Confer. 1989. Transmembrane pore size and role of cell swelling in cytotoxicity caused by *Pasteurella haemolytica* leukotoxin. *Infect. Immun.* **57**:420-425.
4. Fedorka-Cray, P. J., M. J. Huether, D. L. Stine, and G. A. Anderson. 1990. Efficacy of a cell extract from *Actinobacillus (Haemophilus) pleuropneumoniae* serotype 1 against disease in swine. *Infect. Immun.* **58**:358-365.
5. Fenwick, B. W., and B. I. Osburn. 1986. Immune responses to the lipopolysaccharides and capsular polysaccharides of *Haemophilus pleuropneumoniae* in convalescent and immunized pigs. *Infect. Immun.* **54**:575-582.
6. Frey, J., and J. Nicolet. 1988. Regulation of hemolysin expression in *Actinobacillus pleuropneumoniae* serotype 1 by Ca^{2+} . *Infect. Immun.* **56**:2570-2575.
7. Harris, D. L. Personal communication.
8. Henry, C., et al. 1980. Cell separation: adherence, p. 182. In B. B. Mishell and S. M. Shiigi (ed.), *Selected methods in cellular immunology*. W. H. Freeman and Co., San Francisco.
9. Inzana, T. J., J. Ma, T. Workman, R. P. Gogolewski, and P. Anderson. 1988. Virulence properties and protective efficacy of the capsular polymer of *Haemophilus (Actinobacillus) pleuropneumoniae* serotype 5. *Infect. Immun.* **56**:1880-1889.
10. Kamp, E. M., and L. A. M. G. van Leengoed. 1989. Serotype-related differences in production and type of heat-labile hemolysin and heat-labile cytotoxin of *Actinobacillus (Haemophilus) pleuropneumoniae*. *J. Clin. Microbiol.* **27**:1189-1191.
11. Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature (London)* **227**:680-685.
12. Lalonde, G., T. V. McDonald, P. Gardner, and P. D. O'Hanley. 1989. Identification of a hemolysin from *Actinobacillus pleuropneumoniae* and characterization of its channel properties in planar phospholipid bilayers. *J. Biol. Chem.* **264**:13559-13564.
13. Lynch, J. A., and S. Laiviere. 1988. *Actinobacillus (Haemophilus) pleuropneumoniae* symposium. *Can. Vet. J.* **29**:571-582.
14. Rosendal, S., J. Devenish, J. I. MacInnes, J. H. Lumsden, S. Watson, and H. Xun. 1988. Evaluation of heat-sensitive neutrophil-toxic, and hemolytic activity of *Haemophilus (Actinobacillus) pleuropneumoniae*. *Am. J. Vet. Res.* **49**:1053-1058.
15. Sebunya, T. N. K., and J. R. Saunders. 1983. *Haemophilus pleuropneumoniae* infection in swine: a review. *J. Am. Vet. Med. Assoc.* **182**:1331-1337.
16. Vega, M. V., S. K. Maheswaran, J. R. Leininger, and T. R. Ames. 1987. Adaptation of a colorimetric microtitration assay for the quantifying *Pasteurella haemolytica* A1 leukotoxin and antileukotoxin. *Am. J. Vet. Res.* **48**:1559-1564.