INFECTION AND IMMUNITY, Jan. 1988, p. 234–240
0019-9567/88/010234-07$02.00/0
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Vol. 56, No. 1

Adherence of *Pasteurella multocida* or *Bordetella bronchiseptica* to the Swine Nasal Epithelial Cell In Vitro

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Received 19 May 1987/Accepted 29 September 1987

The interaction of *Bordetella bronchiseptica* or *Pasteurella multocida* with swine nasal epithelial cells was studied in vitro. The mean number of *B. bronchiseptica* organisms adhered per cell was about three times as high as that of *P. multocida* ($P < 0.01$), and the adherence was specifically inhibited by the homologous antiserum prepared with the whole-cell antigen of each bacterium. The poor affinity of *P. multocida* to the swine nasal mucosa as compared with that of *B. bronchiseptica* was also demonstrated in the cultured fragments of the nasal mucosa. When observed with a scanning electron microscope, *B. bronchiseptica* organisms colonized the fragments, whereas few *P. multocida* organisms adhered. Morphologically, the *P. multocida*-infected fragments had an essentially normal structure, whereas marked degeneration and marked desquamation of the epithelial cells and severe inflammatory reactions were observed in many areas of the *B. bronchiseptica*-infected fragments. These morphological observations were consistent with those for the nasal mucosa of *P. multocida*- or *B. bronchiseptica*-infected neonatal pigs (T. Nakai, K. Kume, H. Yoshikawa, T. Oyamada, and T. Yoshikawa, Jpn. J. Vet. Sci. 48:693–701, 1986; T. Oyamada, T. Yoshikawa, H. Yoshikawa, M. Shimizu, T. Nakai, and K. Kume, Jpn. J. Vet. Sci. 48:377–387, 1986). Cultured swine nasal fragments, however, were equally injured when they were incubated in a medium containing purified dermonecrotic toxin (DNT) preparations of *B. bronchiseptica* or *P. multocida*. Therefore, these DNT preparations can induce morphological damage closely resembling that induced in vivo. Hence, colonization of *B. bronchiseptica* and production of its DNT on the swine nasal mucosa appear to result in the production of mucosal damage. On the other hand, *P. multocida* seems to lack the ability to colonize normal swine nasal mucosa, thus resulting in no production or the slight production of DNT to such an extent as to produce mucosal damage. The present data support our previous hypothesis (Nakai et al.; Oyamada et al.) that *B. bronchiseptica* induces swine atrophic rhinitis, whereas *P. multocida* does not.

Swine atrophic rhinitis (AR) is a disease characterized by severe necrosis in the epithelia of the upper respiratory tract and by deformity and reduction in both the volume and size of the nasal turbinates and snouts (2, 4, 10, 11, 13, 23, 29). Phase I of *Bordetella bronchiseptica* causes a marked loss of cilia accompanied by the characteristic morphological changes of the nasal mucosa when the organisms are intranasally inoculated into gnotobiotic or specific-pathogen-free (SPF) neonatal pigs (2, 4, 10, 11, 13, 18, 23, 29, 30). Injection of pure cultures of *B. bronchiseptica* results in severe nasal turbinate atrophy in both gnotobiotic and SPF pigs; therefore, *B. bronchiseptica* has been considered as a primary causative agent for swine AR. A heat-labile toxin (dermonecrotic toxin [DNT]) extracted from the phase I organisms of *B. bronchiseptica* has been suggested as one of the virulence factors responsible for the production of turbinate atrophy in neonatal pigs, rats, and young mice (7, 8, 27).

The involvement of *Pasteurella multocida* having dermonecrotic activity in AR was first reported by de Jong et al. (M. F. de Jong, H. L. Oei, and G. J. Tetenburg, Int. Pig Vet. Soc. Proc., p. 228, 1980). Since then, other studies have implicated toxigenic strains of *P. multocida* and its DNT in a pathogenic role in swine AR or in the production of turbinate atrophy (5, 21, 22, 24–26). However, our previous data (14, 20) failed to confirm the results reported by Pedersen and Elling (22) and were consistent with the hypothesis that *B. bronchiseptica* alone is the causative agent of swine AR.

The adherence of pathogenic microorganisms to the mucosal surface is well recognized as an important first step in colonization and infection (12, 13, 28). Since *B. bronchiseptica* is known to preferentially multiply on the swine nasal mucosa (14, 20, 30), the adherence of this organism to the mucous membranes may play an important role in its pathogenicity. However, there is little information on the virulence characteristics of *P. multocida* in the upper respiratory tract or on the process by which the organism colonizes the swine nasal mucosa (6, 13, 26). We have previously demonstrated that toxigenic strains of *P. multocida* do not colonize normal swine nasal mucosa (14, 20). *P. multocida* colonizes the nasal mucosa of swine in a different manner from that of *B. bronchiseptica*, and possibly the difference may explain the differences in virulence between the two species of bacteria.

In the present investigation, the interaction of toxigenic *P. multocida* or *B. bronchiseptica* with the swine nasal epithelium was studied in an in vitro system to evaluate quantitative bacterial adherence to nasal epithelial cells. To gain more insight into the mechanisms of surface infection by *P. multocida* or *B. bronchiseptica*, we also used a scanning electron microscope (SEM) to examine the morphological interaction between the two species of bacteria or their purified DNT preparations (9, 17) and the swine nasal mucosa.

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MATERIALS AND METHODS

Bacterial strains, culture media, and preparation of inocula. Capsular serotype D *P. multocida* SP-72 and 47459 (de Jong et al., Int. Pig Vet. Soc. Proc., p. 228, 1980) and *B. bronchiseptica* L3 and 0009 of pig origin were used (3, 7, 16). These strains were preserved as lyophilized cultures. *P. multocida* organisms or phase I organisms (18) of *B. bronchiseptica* were grown on yeast extract-proteose peptone (Difco Laboratories, Detroit, Mich.)-cystine agar medium (19) at 37°C for 6 h or on Bordet-Gengou agar medium (1) supplemented with 20% horse blood at 37°C for 18 h, harvested, and suspended in Eagle minimal essential medium (MEM; Nissui Seiyaku Co., Tokyo, Japan) supplemented with 5% fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.) to an optical density at 650 nm of 0.386 (equal to 10^{10} bacteria per ml) by use of a model 6/20 spectrophotometer (Coleman Instruments Div., The Perkin-Elmer Corp., Norwalk, Conn.). These bacterial suspensions were used as inocula for the adherence assay or for the inoculation test of cultured swine nasal tissue fragments.

Preparation of purified DNT. Purified *P. multocida* DNT and *B. bronchiseptica* DNT were prepared by previously described procedures from sonic extracts of the organisms (9, 17). Each DNT preparation used in the present investigation formed one precipitation line with homologous rabbit anti-DNT antiserum in Ouchterlony plates and migrated as a single protein band during sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The DNT preparations of *P. multocida* and *B. bronchiseptica* had ca. 1.0×10^6 and ca. 5.0×10^5 toxic units per milligram of protein for guinea pigs, respectively (9, 17).

Preparation of rabbit antisera. *P. multocida* SP-72 grown on yeast extract-proteose peptone-cystine agar plates at 37°C for 6 h or phase I organisms of *B. bronchiseptica* L3 grown on Bordet-Gengou agar plates supplemented with 20% horse blood at 37°C for 18 h were harvested and suspended to a concentration of 10^9 cells per ml (determined by spectrophotometry). Anti-*P. multocida* or anti-*B. bronchiseptica* antisera were prepared by injecting New Zealand White rabbits weighing about 2 kg with each cell suspension by a previously described procedure (17). Sera with indirect hemagglutination antibody titers of 1:2,560 against strain SP-72 (1) or those with agglutinin antibody titers of 1:10,240 against strain L3 (18) were pooled and stored at −20°C. Anti-*P. multocida* DNT or anti-*B. bronchiseptica* DNT antisera were also prepared by injecting rabbits with purified (100 μg of protein per ml) DNT preparations by a previously described procedure (17). Sera with anti-DNT (neutralization) antibody titers of 64 against the homologous DNT preparation (9, 17) were pooled and stored at −20°C. All the antisera were used for an adherence inhibition test.

Preparation of the swine nasal epithelial cell suspension. The swine nasal epithelial cell suspension was prepared by the method of Yokomizo and Shimizu (30). The nasal mucosae were obtained from the ventral turbinates of 1-day-old SPF neonatal pigs. The nasal epithelial cells were collected by scraping the nasal mucosa with a steel spoon. The cells were suspended in MEM, dispersed by vigorous pipetting, and kept at room temperature for 30 min. The contaminated mucus and dead cells remained in the supernatant fraction. The resulting sedimented fraction, consisting mostly of aggregated living cells, was washed three times and suspended to a concentration of 4×10^6 cells per ml in MEM.

Adherence assay. A 0.5-ml portion of the bacterial suspension of *P. multocida* or *B. bronchiseptica* (4×10^8 bacteria per ml) was added to an equal volume of the swine nasal epithelial cell suspension (4×10^6 cells per ml), and the bacterium-cell mixture was incubated at 37°C for 15 min with gentle shaking (30). The bacterium/cell ratio of the mixture was ca. 100:1. After incubation, bacteria remaining in the medium were removed by repeated (at least three times) washing and centrifugation at $100 \times g$ for 10 min. The washed epithelial cells were finally centrifuged at $400 \times g$ for 15 min and then smeared on a glass slide. The smears were fixed with phosphate-buffered saline (pH 7.0) containing 1% (vol/vol) Formalin (Wako Pure Chemical Co., Tokyo, Japan) for 2 h, washed with distilled water, stained for 1 h with 1% (vol/vol) Giemsa stain (Sigma Chemical Co., St. Louis, Mo.), and examined with a light microscope. The number of adherent bacteria per cell was counted by examining 60 cells on each smear. All experiments were done in triplicate; a total of 180 cells were examined in each sample. The mean numbers (± standard errors of the means) of bacteria attached per cell were calculated, and the statistical significance of the differences was determined by the goodness-of-fit test.

Adherence inhibition assay. Anti-*P. multocida*, anti-*B. bronchiseptica*, anti-*P. multocida* DNT, and anti-*B. bronchiseptica* DNT antisera were tested for their abilities to inhibit *P. multocida* or *B. bronchiseptica* adherence to swine nasal epithelial cells. Each antiserum (0.5 ml) was added to an equal volume of the *P. multocida* or *B. bronchiseptica* suspension (8×10^8 bacteria per ml) and incubated at 37°C for 30 min, followed by washing of the antiserum-bacterium mixture twice with MEM as described above and resuspension of the mixture in MEM to a bacterial concentration of 4×10^8/ml. A 0.5-ml portion of each mixture was added to an equal volume of the epithelial cell suspension (4×10^6 cells per ml) and incubated at 37°C for 30 min, and the number of adherent bacteria per cell was determined as described above. Normal rabbit sera lacking any detectable antibodies against *P. multocida* or *B. bronchiseptica* were collected, and a normal serum-bacterium mixture was used as a control for the adherence inhibition assay.

Preparation of swine nasal tissue fragments. The nasal mucosae were collected from the ventral turbinates of SPF neonatal pigs which had been euthanatized at 1 day of age. The nasal mucosa was removed from the turbinate cartilage and washed at least four times with MEM in a sterilized petri dish. The mucosa was then cut into tissue fragments consisting of an epithelial surface measuring about 5 by 5 mm. Each tissue fragment was placed in a test tube (1 by 10 cm) with a rubber stopper, and 1 ml of MEM containing 2×10^9 CFU of *P. multocida* or *B. bronchiseptica* per ml or containing 100 ng of *P. multocida* DNT or *B. bronchiseptica* DNT protein per ml was added to each tube. The infected or inoculated tissue fragments were incubated at 37°C for 15 min, 1 h, or 24 h. These cultured tissue fragments were washed at least four times with phosphate-buffered saline by vigorous shaking to remove nonadherent bacteria and to stop the effect of the DNT preparation on the mucosal surfaces of the fragments and were subjected to morphological examinations with a SEM and a light microscope.

SEM observations. Each sample of cultured swine nasal fragments was cut into two pieces; one portion was used for SEM observations, and the other was used for light microscope observations. Samples were fixed with 0.5% (vol/vol) glutaraldehyde (Eastman Kodak Co., Rochester, N.Y.). These prefixed samples were cut into small pieces in the usual manner, and portions of the samples were fixed in 1%

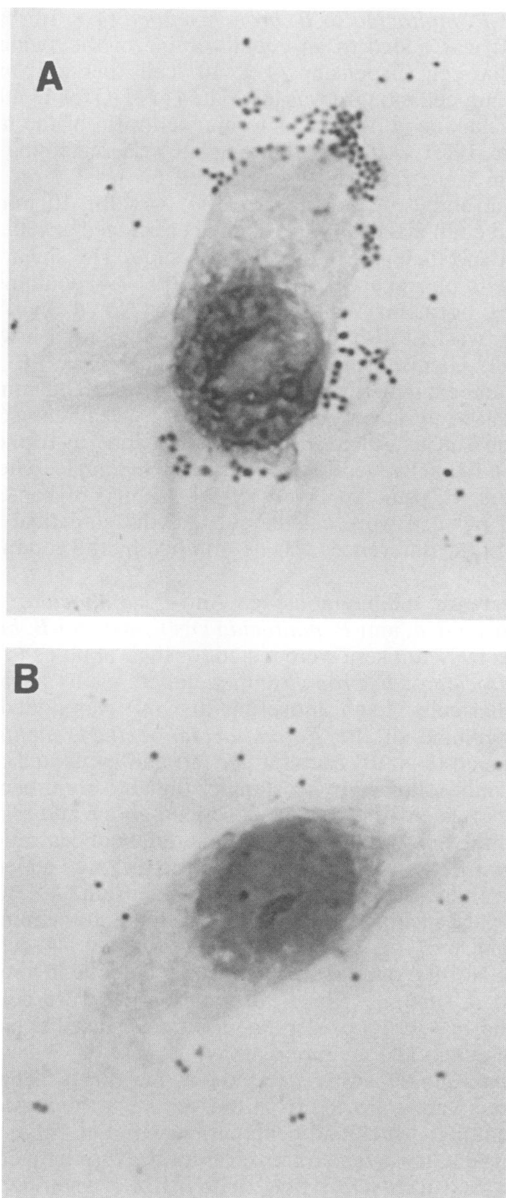

FIG. 1. Adherence of *B. bronchiseptica* L3 (A) or *P. multocida* 47459 (B) organisms to swine nasal epithelial cells cultured in vitro after 15 min of incubation at 37°C. The adherence assay was performed as described in the text. Giemsa stain; magnification ×1,200.

(vol/vol) OsO_4 solution for 2 h, dehydrated in a graded series of alcohol and isoamyl acetate, and dried by the CO_2 critical point method. The dried samples were cracked, processed for platinum shadow casting, and examined with a SEM (S-45; Hitachi Ltd., Tokyo, Japan) (14).

Light microscope observations. Samples were fixed in 10% (vol/vol) neutral Formalin, dehydrated in alcohol, and embedded in paraffin. They were cut into thin sections approximately 6 μm thick, and the sections were stained with hematoxylin and eosin.

RESULTS

Adherence of the two species of bacteria to swine nasal epithelial cells. *B. bronchiseptica* adhered well to swine nasal epithelial cells cultured in vitro during the 15-min incubation period (Fig. 1A), but few *P. multocida* organisms attached to the cells (Fig. 1B). Prolonged incubation did not significantly enhance the attachment of the two species of bacteria (Table 1). The mean number of *B. bronchiseptica* organisms (strains L3 and 0009) adhering per cell was about three times as high as that of *P. multocida* organisms (strains SP-72 and 47459) ($P < 0.01$).

Effect of antisera on adherence. The effect of various antisera on the adherence of *P. multocida* or *B. bronchiseptica* organisms to cultured swine nasal epithelial cells was investigated by an adherence inhibition assay (Table 2). Anti-*P. multocida* and anti-*B. bronchiseptica* antisera inhibited the adherence of homologous bacteria by 78.7 to 79.2% and by 88.3 to 89.9%, respectively, as compared with controls. The adherence of the two species of bacteria was not affected significantly by heterologous antisera or by anti-*P. multocida* DNT or anti-*B. bronchiseptica* DNT antisera (percentage of inhibition, <6.4%).

Morphological changes induced in *P. multocida*-infected or *B. bronchiseptica*-infected swine nasal fragments. Nasal fragments were cultured in MEM containing living *P. multocida* or *B. bronchiseptica* organisms. All the *P. multocida*-infected fragments had essentially normal structures, even after 24 h of incubation at 37°C, as observed by SEM. Deposition of a mucopurulent substance was rarely observed on the infected mucosa, and most of the epithelial cells appeared to be intact and had well-developed cilia (Fig. 2A). Small numbers of *P. multocida* organisms were infrequently observed on the mucosa by SEM (data not shown). Histologically, slight infiltrations of lymphocytes and neutrophils were observed in the lamina propria and submucosa (data not shown).

B. bronchiseptica become well attached to the swine nasal mucosa during the 1-h incubation period. Observation by SEM showed slight tissue damage on the mucosal surface (data not shown). Marked degeneration and desquamation of the epithelial cells were observed in all of the infected fragments after 24 h of incubation at 37°C (Fig. 2B). Cilia were lost from most of the epithelium, and deposition of a mucopurulent substance on many parts of the mucosa was apparent. Numerous organisms (small arrows) were observed near the remaining cilia or on the surface of the epithelium (Fig. 2C). In all of these samples, marked hyperplasia was observed in many parts of the epithelium, and marked infiltrations of lymphocytes and neutrophils were apparent in the lamina propria and submucosa (data not shown). All the cultured fragments of controls had essen-

TABLE 1. Adherence of *P. multocida* or *B. bronchiseptica* organisms to swine nasal epithelial cells cultured in vitro

Strain	No. of adherent bacteria/cell[a] after incubation for:	
	15 min	30 min
P. multocida		
SP-72	12.6 ± 3.2[b]	13.7 ± 2.1[b]
47459	13.1 ± 4.1[b]	15.9 ± 3.6[b]
B. bronchiseptica		
L3	44.7 ± 5.1	47.2 ± 1.3
0009	47.5 ± 4.4	49.6 ± 2.8

[a] Each value represents the mean ± standard error of the mean for 180 cells. The adherence assay was performed as described in the text.
[b] These values were significantly different from those for *B. bronchiseptica* ($P < 0.01$).

TABLE 2. Effect of antisera on the adherence of *P. multocida* or *B. bronchiseptica* organisms to swine nasal epithelial cells cultured in vitro

Strain	Mean no. of adherent bacteria/cell[a] (% inhibition)[b] after treatment with the following antiserum:				
	Anti-SP-72	Anti-L3	Anti-*P. multocida* DNT	Anti-*B. bronchiseptica* DNT	Normal rabbit serum (control)
P. multocida					
SP-72	4.3[c] (78.7)	18.9 (6.4)	20.3 (−0.5)	19.2 (4.9)	20.2 (0)
47459	3.8[c] (79.2)	17.3 (5.5)	18.9 (−3.3)	17.4 (5.5)	18.3 (0)
B. bronchiseptica					
L3	45.8 (−5.0)	5.1[c] (88.3)	44.2 (−1.4)	45.4 (−4.1)	43.6 (0)
0009	46.9 (2.3)	4.9[c] (89.8)	47.2 (1.7)	49.1 (−2.3)	48.0 (0)

[a] The adherence inhibition assay was performed as described in the text (incubation for 30 min).
[b] Percent inhibition was calculated as follows: (number of adherent bacteria on the test sample/number of adherent bacteria on the control sample) × 100.
[c] These values were significantly different from those obtained with normal serum ($P < 0.01$).

tially normal structures during the experimental period (Fig. 2D).

Morphological changes induced in swine nasal fragments after inoculation with DNT preparations from *P. multocida* or *B. bronchiseptica*. Swine nasal fragments were incubated in MEM containing purified preparations of *P. multocida* DNT or *B. bronchiseptica* DNT. Slight morphological changes were observed as early as 15 min after the start of incubation at 37°C with *P. multocida* DNT or *B. bronchiseptica* DNT. Morphological changes induced in *P. multocida* DNT-inoculated (Fig. 3A and C) or *B. bronchiseptica* DNT-inoculated (Fig. 3B and D) fragments after 24 h of incubation at 37°C were similar to those induced in *B. bronchiseptica*-infected fragments (Fig. 2B and C). A marked loss of cilia and deposition of a mucopurulent substance on the surface of the nasal mucosa were apparent, as observed by SEM (Fig. 3A and B). Histologically, marked hyperplasia of the epithelial cells and marked infiltrations of lymphocytes and neutrophils were apparent (Fig. 3C and D). Purified DNT preparations of *B. bronchiseptica* and *P. multocida* caused similar mucosal damage to swine nasal fragments. All the cultured fragments of controls had essentially normal morphology during the experimental period (Fig. 3E).

DISCUSSION

Previously, we (14, 20) demonstrated that *B. bronchiseptica*-infected nasal mucosae of SPF neonatal pigs showed extensive ultrastructural and histological changes, whereas *P. multocida*-infected nasal mucosae showed few changes. In addition, *B. bronchiseptica* was found in abundant numbers on the infected nasal mucosae, but *P. multocida* was scarce. Colonization of the swine nasal mucosa by *B. bronchiseptica* seems to be essential for the production of macroscopic and histological turbinate lesions and for the persistence of infection in situ. Thus, we (14, 20) concluded that *B. bronchiseptica* induces swine AR and that *P. multocida* is not a primary pathogen for swine AR. The aim of the present study was to clarify the etiological roles of *B. bronchiseptica* and *P. multocida* and their purified DNT preparations in the development of swine AR. To reach this goal, we used cultured swine nasal tissue fragments removed from SPF neonatal pigs and examined ultramicroscopic and histological lesions induced on the fragments by *B. bronchiseptica*, *P. multocida*, or their purified DNT preparations.

B. bronchiseptica caused clinical signs of AR and produced both macroscopic and histological lesions in the nasal turbinates of infected neonatal pigs (2, 4, 10, 11, 13, 14, 20, 23, 29, 30). Histologically, the mucosal epithelial cells lost their cilia, and lymphocytes and neutrophils infiltrated the submucosal areas, resulting in chronic catarrh (4, 10, 13, 14, 20). After intranasal inoculation, *B. bronchiseptica* colonized the infected nasal mucosa of SPF neonatal pigs in abundant numbers during the experimental period (14, 20). *B. bronchiseptica*-infected nasal mucosae (Fig. 2B and C) showed extensive ultrastructural and histological changes in vitro which were similar to those induced in vivo (14, 20). The organism was found in large numbers on infected swine nasal tissue fragments by SEM. The firm adherence of *B. bronchiseptica* to swine nasal epithelial cells cultured in vitro was also demonstrated (Tables 1 and 2). Thus, our present findings suggest that the ability of *B. bronchiseptica* to adhere to swine nasal epithelial cells may be one of the important factors responsible for virulence in upper respiratory tract infections caused by *B. bronchiseptica*. In addition, the present data indicate that colonization of the swine nasal mucosa by *B. bronchiseptica* is essential for the production of macroscopic and histological lesions and for the persistence of the infection caused by *B. bronchiseptica*, as previously suggested (14).

Cultured swine nasal tissue fragments infected with *P. multocida* appeared to be essentially normal in morphology (Fig. 2A). Colonization by the organisms was infrequently observed by SEM. The present findings agreed well with previous findings for the nasal mucosa of *P. multocida*-infected neonatal pigs (6, 13, 14, 20) or *P. multocida*-infected rats (8). The lower affinity of *P. multocida* than of *B. bronchiseptica* for swine nasal epithelial cells was also demonstrated (Tables 1 and 2). Therefore, the application of an irritant such as acetic acid or the inoculation of *B. bronchiseptica* was a prerequisite for colonization of the nasal mucosa of SPF or gnotobiotic neonatal pigs by toxigenic *P. multocida* (5, 20, 21, 22, 24). Thus, it is likely that *P. multocida* probably exists as a normal resident on the nasal mucosa of healthy pigs.

There is much evidence that *B. bronchiseptica* is a major cause of swine AR (2, 4, 10, 11, 13, 14, 20, 23, 29), although many bacteria and/or factors responsible for the occurrence of the disease have been suggested. Hanada et al. (7) suggested that *B. bronchiseptica* DNT may act as an active component to produce nasal turbinate atrophy in neonatal pigs. Toxigenic strains of *P. multocida* have also been considered as a major causative agent of swine AR, and their DNTs have been indicated as the cause of their virulence (5, 21, 22, 24, 26). Our previous data (14, 20), however, failed to support this indication, since we could not produce the nasal

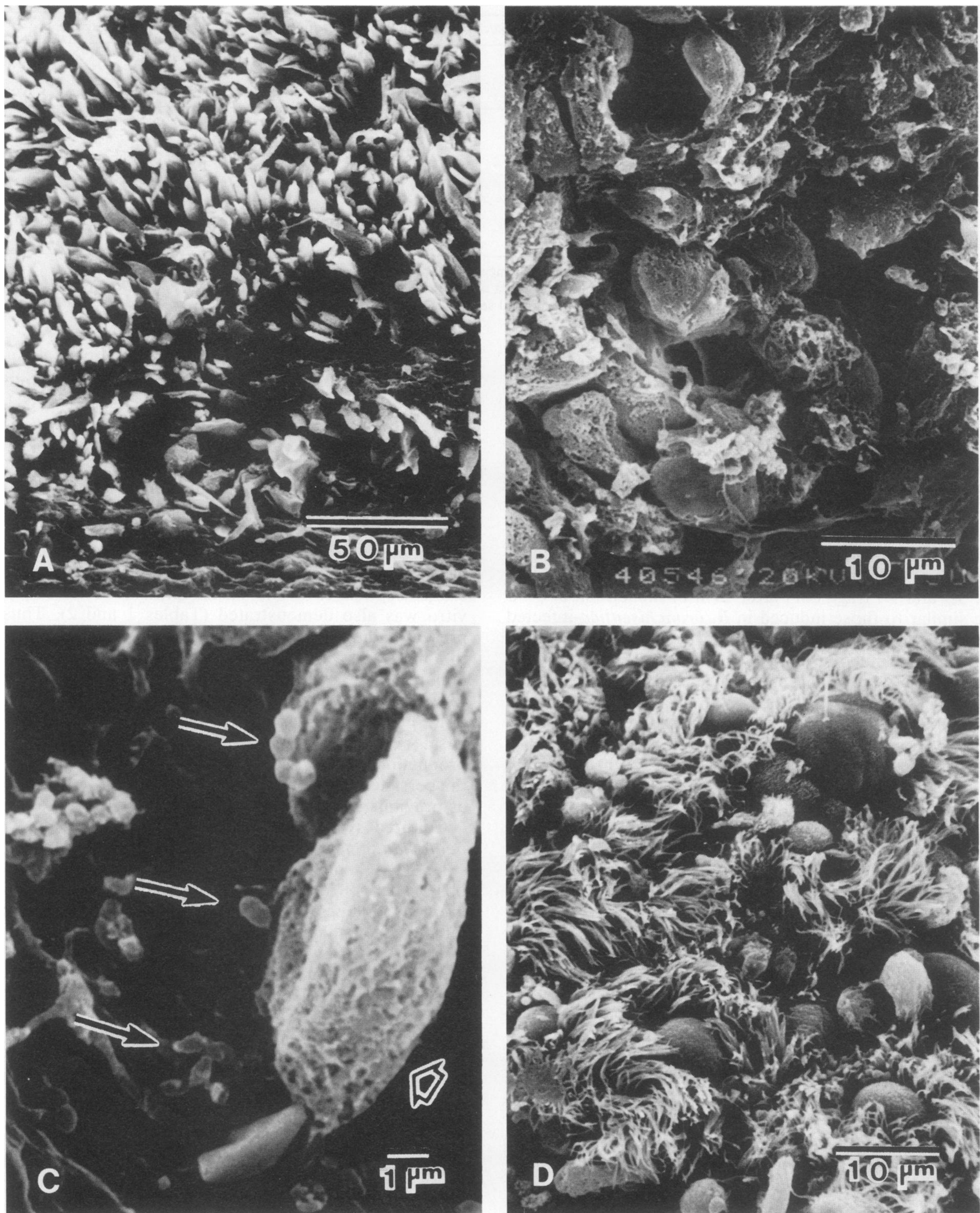

FIG. 2. Morphological changes (SEM observations) induced in the *P. multocida*-infected (A), *B. bronchiseptica*-infected (B and C), and uninfected control (D) swine nasal tissue fragments (5 by 5 mm) after 24 h of incubation at 37°C. The dose was adjusted to 2×10^9 bacteria per fragment. Fragments were prepared as described in the text. (A) *P. multocida* SP-72-infected nasal mucosa showing a normal structure. Slight deposition of a mucopurulent substance was observed in a limited area of the mucosa. (B) *B. bronchiseptica* L3-infected nasal mucosa. Marked degeneration and desquamination of the epithelial cells were apparent. (C) *B. bronchiseptica* L3-infected nasal mucosa. Marked loss of cilia and deposition of a mucopurulent substance (arrowhead) on the mucosa were apparent. Organisms (small arrows) were present near the remaining cilia or on the surface of the epithelium. (D) Uninfected control nasal mucosa showing an essentially normal structure.

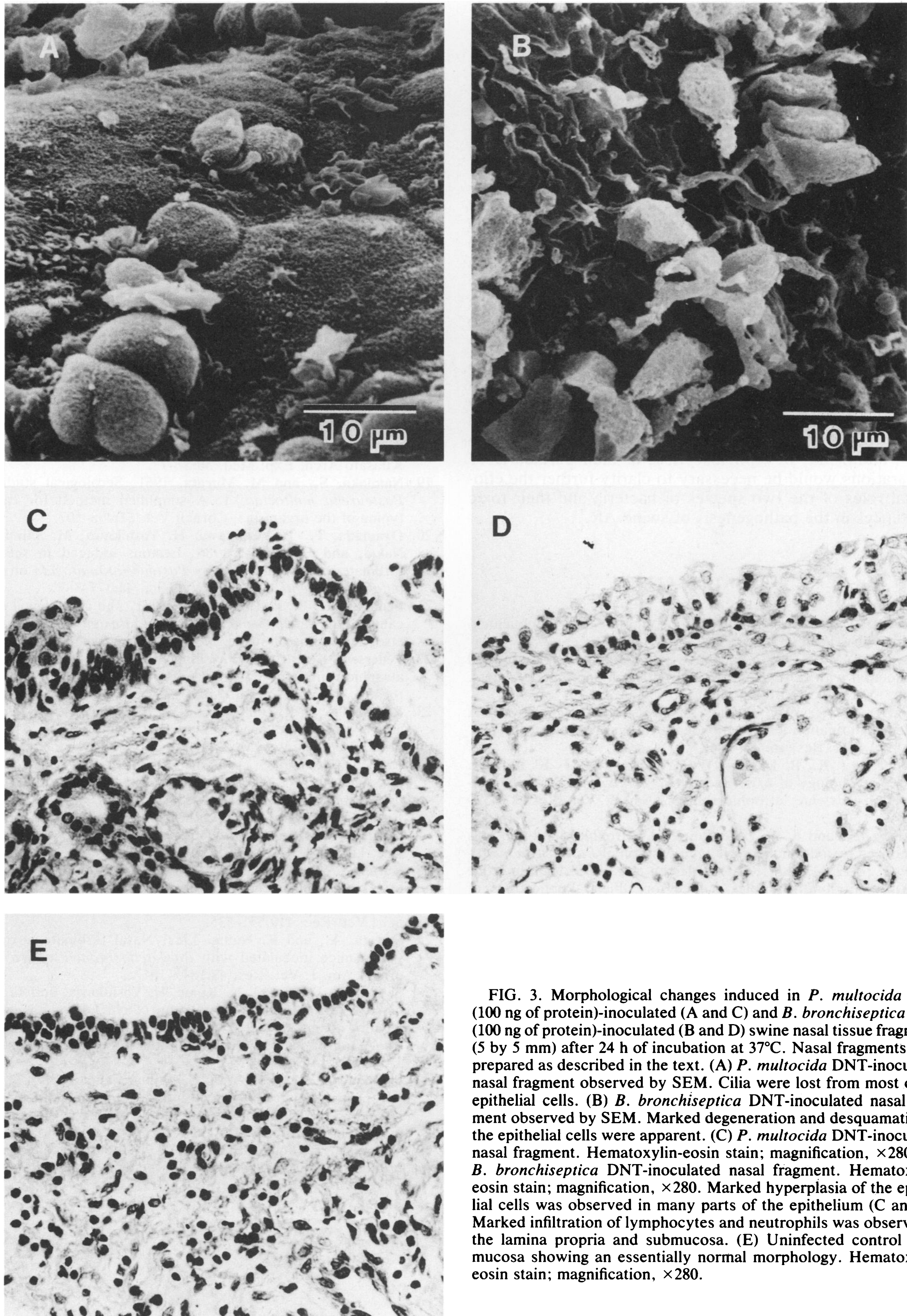

FIG. 3. Morphological changes induced in *P. multocida* DNT (100 ng of protein)-inoculated (A and C) and *B. bronchiseptica* DNT (100 ng of protein)-inoculated (B and D) swine nasal tissue fragments (5 by 5 mm) after 24 h of incubation at 37°C. Nasal fragments were prepared as described in the text. (A) *P. multocida* DNT-inoculated nasal fragment observed by SEM. Cilia were lost from most of the epithelial cells. (B) *B. bronchiseptica* DNT-inoculated nasal fragment observed by SEM. Marked degeneration and desquamation of the epithelial cells were apparent. (C) *P. multocida* DNT-inoculated nasal fragment. Hematoxylin-eosin stain; magnification, ×280. (D) *B. bronchiseptica* DNT-inoculated nasal fragment. Hematoxylin-eosin stain; magnification, ×280. Marked hyperplasia of the epithelial cells was observed in many parts of the epithelium (C and D). Marked infiltration of lymphocytes and neutrophils was observed in the lamina propria and submucosa. (E) Uninfected control nasal mucosa showing an essentially normal morphology. Hematoxylin-eosin stain; magnification, ×280.

turbinate atrophy characteristic of AR after intranasal inoculation of toxigenic *P. multocida* strains into SPF neonatal pigs. Purified preparations of both *P. multocida* DNT and *B. bronchiseptica* DNT were capable of producing the mucosal damage characteristic of AR on cultured swine nasal tissue fragments (Fig. 3A, B, C, and D). However, *P. multocida* did not colonize the nasal mucosa, and the mucosal surface of infected nasal fragments appeared to be normal in morphology (Fig. 2A). In general, *P. multocida* DNT and *B. bronchiseptica* DNT were not produced from actively growing cells, but low amounts of the DNTs were released from the cells by autolysis (15). Therefore, proliferation of the organisms is essential for the production of DNTs by the two species of bacteria. If DNT is responsible for the production of nasal turbinate atrophy in neonatal pigs, as reported by some researchers (5, 21, 22, 24, 26), then colonization of the nasal mucosa by *P. multocida* might be essential for the production of its DNT in amounts sufficient to cause histological changes in infected swine nasal mucosa. However, our previous (6, 13, 14, 20) and present data strongly suggest that *P. multocida* does not colonize to such an extent as to produce its DNT in normal swine nasal mucosa. Organ cultures of swine nasal turbinate bone in a medium containing *P. multocida*, *B. bronchiseptica*, or their purified DNT preparations would be necessary to clarify further the etiological roles of the two species of bacteria and their toxic substances in the pathogenesis of swine AR.

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