Lesions Induced in the Nasal Turbinates of Neonatal Pigs Inoculated with *Pasteurella multocida* and/or *Bordetella bronchiseptica*

Toshifumi OYAMADA, Takashi YOSHIKAWA, Hiroyasu YOSHIKAWA, Makiko SHIMIZU, Toyotsugu NAKAI,¹⁾ and Katsumi KUME²⁾

Department of Veterinary Pathology, School of Veterinary Medicine and Animal Sciences, Kitasato University, Towada, Aomori 034, ¹⁾Research Center for Veterinary Science of The Kitasato Institute, Kashiwa, Chiba 277, and ²⁾The Kitasato Institute, 5-9-1, Shirogane, Minato, Tokyo 108, Japan

(Received 23 July 1985/Accepted 18 January 1986)

ABSTRACT. Specific-pathogen-free (SPF) neonatal pigs were intranasally inoculated with serotype D strains of *Pasteurella multocida* and/or strain L3 of *Bordetella bronchiseptica*, and their lesions were studied. Marked inflammatory changes were observed only after the infection with *B. bronchiseptica*. Both organisms were constantly recovered from the nasal cavities of the pigs during the experimental period; *B. bronchiseptica* was recovered in higher numbers than *P. multocida*. Degeneration and resorption of trabeculae in varying grades were observed in all the inoculated pigs. The turbinate bone lesions induced by *P. multocida* were characterized by the following changes: slight resorption of trabeculae by osteocytic osteolysis, active osteoid synthesis near trabeculae and periosteum due to increase of osteoblasts, and enhanced new bone formation. In many areas of the trabeculae, the trabeculae had been replaced by the proliferative osteoblasts and osteoids, thus, none of the pigs showed gross signs of swine atrophic rhinitis (AR). In contrast, the lesions induced by *B. bronchiseptica* were severe resorption of trabeculae by osteoclastic osteolysis, perforating resorption, increase of fibrous tissue near trabeculae, and impaired osteogenesis due to the damage to the osteoblasts. Thus, all the pigs showed severe gross signs of AR. Hence, quality and severity of the lesions observed between the 2 species of the bacteria were apparently different. Co-infection of the pigs with the 2 species of the bacteria apparently enhanced the turbinate lesion formation. Our observations are consistent with the hypothesis that *B. bronchiseptica* alone is responsible for pathogenesis of swine AR. The different mechanisms in pathogenesis by the 2 species of the bacteria in relation to the production of characteristic turbinate lesions are discussed.—**KEY WORDS:** atrophic rhinitis, *Bordetella bronchiseptica*, *Pasteurella multocida*, swine nasal turbinate.

Jpn. J. Vet. Sci. 48(2): 377–387, 1986

Swine atrophic rhinitis (AR) is a disease characterized by severe necrosis of epithelia of the upper respiratory tract and by deformity and reduction both in volume and size of the nasal turbinates and snouts [5, 15, 25]. Fetter *et al.* [7, 8] reported that the primary lesion induced in the trabeculae of the neonatal pigs affected with the disease was osteoporosis due to decreased bone formation by the degenerative osteoblasts and osteocytes, and they considered that resorption of the bone adjacent to the degenerative bone cells may have contributed to the overall loss of bone. Similar observations were reported by others [12, 18], though some differences in the

formed bone lesions both in quality and severity were noted among different studies. There has been a substantial amount of evidence supporting that *Bordetella bronchiseptica* is a major cause of the disease [2, 5, 7, 8, 12, 14, 15, 25, 31, 33], although many bacteria and/or factors responsible for the occurrence of swine AR have been suggested. Some studies positively suggested that dermonecrotic toxin (DNT) of *B. bronchiseptica* may act as an active component to produce the turbinate atrophy in neonatal pigs [11] or in young mice [29].

Certain strains belonging to a capsular serotype [3] D or A of *Pasteurella multocida*

Table 1. Experimental design

Group	Inoculation			No. tested	Age in days at inoculation	PID ^{e)} necropsy (days)
	<i>P. multocida</i>		<i>B. bronchiseptica</i>			
	SP-72	47459	L3			
A	5 × 10 ⁷ ^{a)}	—	—	4	7	49
	—	5 × 10 ⁷	—	4	7	49
	—	15 × 10 ⁷	—	4 ^{b)}	7, 14, 21	63 ^{c)}
B	—	—	5 × 10 ⁴	4	7	49
C	5 × 10 ⁷	—	5 × 10 ⁴	4	7	63
	—	15 × 10 ⁷	5 × 10 ⁴	4	7	63
D (Control)	—	—	—	2	—	(70) ^{d)}

a) Total viable cell numbers (i.n.) (CFU).

b) Inoculated 3 times at 1-week-intervals.

c) Days after the primary inoculation.

d) Days of age at necropsy.

e) Postinoculation days.

are also capable of producing DNT [4, 9, 30]. Difference was not observed between the *P. multocida* DNT and *B. bronchiseptica* DNT in their biologic and toxic properties [17]. A close correlation was demonstrated by de Jong *et al.* [4] between the pathogenicity of *P. multocida* in swine AR and the ability of the organisms to produce DNT. Additional studies have implicated the toxigenic strains of *P. multocida* as a causative agent for swine AR [13, 22, 23, 26, 27]. In some studies, however, in which the turbinate lesions were induced in neonatal pigs, inoculations of toxigenic strains of *P. multocida* were preceded application of acetic acid or inoculation of *B. bronchiseptica* [22, 23, 26, 27].

The purpose of the present investigation was to compare the lesions induced in the nasal turbinates of pigs by the toxigenic serotype D strains of *P. multocida* with those induced by *B. bronchiseptica*.

MATERIALS AND METHODS

Bacteria and culture media: *Pasteurella multocida* capsular serotype D strains SP-72 [17] and 47459 (provided by Dr. de Jong, Central Veterinary Institute, Rotterdam, The

Netherlands) and *Bordetella bronchiseptica* strain L3 [11] of pig origins were used. These strains produced DNT and were preserved as lyophilized cultures. Yeast extract-proteose peptone-cystine (YPC) agar medium [21] and Bordet-Gengou's (BG) agar medium [1] supplemented with 20% horse blood were used for propagation of *P. multocida* and *B. bronchiseptica*, respectively, throughout the study.

Neonatal pigs: Specific-pathogen-free (SPF) neonatal pigs, seven days of age, derived from 4 sows were used. These pigs were removed immediately at the birth from their dams and housed in a separate room in each infection group (Table 1). They were fed only sterile milk (Nippon Formula Feed Manufacturing Corp., Tokyo, Japan) without any antibiotics during the experimental period. Prior to the experiment, serum and nasal swab samples of each pig were collected, and these samples were checked serologically and bacteriologically for the presence of *P. multocida* and *B. bronchiseptica* infections according to the methods and the criteria described previously [24, 30, 32].

Preparation of inocula: *Pasteurella multocida* organisms grown on the YPC agar

plates for 6 hr at 37°C were harvested and suspended in 0.02 M phosphate-buffered solution, pH 7.0, containing 0.85% NaCl (PBS). Viable cells of the suspension were adjusted to approximately 5×10^7 colony-forming units (CFU)/0.5 ml by spectrophotometry (10^{10} cells/ml=optical density at 650 nm of 0.386) with a model 6/20 spectrophotometer (Coleman Instruments). Phase I of *B. bronchiseptica* [20] grown on the BG agar plates for 18 hr at 37°C was collected and suspended in PBS. Viable cells of the suspension were adjusted to approximately 5×10^4 CFU/0.5 ml by spectrophotometry. Inoculation was done within 30 min after the inocula was prepared.

Experimental infection: Experimental design is summarized in Table 1. Inoculation of *P. multocida* and/or *B. bronchiseptica* in neonatal pigs was performed via the nasal route by using syringe. These pigs were divided into 4 groups randomly distributed in regard to the origin: A, inoculation of *P. multocida* (12 pigs from 4 sows); B, inoculation of *B. bronchiseptica* (4 pigs from 4 sows); C, co-inoculation of the 2 species of the bacteria (8 pigs from 4 sows); and D, control (2 pigs from 2 sows). Among the 12 pigs in group A, 4 pigs were inoculated 3 times with the same dose of the 47459 inoculum at 1 week intervals. All the pigs were bled and sacrificed postinoculation days (PID) 49 or 63.

Clinical observation: Clinical signs of individual neonatal pigs, such as sneezing and coughing, were recorded daily during the experimental period. Gross lesions of AR characterized by shortening and deviation of the snouts in these pigs were graded at necropsy on a basis of 0 to 3 (0=normal, 1=mild, 2=moderate, and 3=severe). The pigs with the scores of 2 and 3 were considered as positive clinical AR.

Bacterial isolation: Isolation of *B. bronchiseptica* and *P. multocida* was attempted at PID 7, 21, 49, or 63 from the nasal cavity of

each neonatal pig as follows: Two cotton swab samples were obtained from each pig. One sample (about 0.1 g/sample) was directly streaked both on the YPC and BG agar plates. The other sample was suspended in 1 ml of PBS. Two fold serial dilutions were made in each 1 ml volume of PBS, and then 0.1 ml portions of each dilution were streaked both on YPC and BG agar plate. The viable cell numbers (CFU) were counted after the incubation for 10 (YPC) or 18 (BG) hr at 37°C. Numbers of the organisms (CFU/g of sample) were averaged in each infection group, and the result was expressed on a basis of - to +++ (-=no recovery, += $<10^2$ CFU, ++= $<10^6$ CFU, and +++= $>10^6$ CFU). Identification of the recovered organisms was done by the criteria described previously [24, 32]. The DNT-producing ability of the recovered *P. multocida* or *B. bronchiseptica* was investigated in guinea pigs, according to the procedure described in a previous report [17].

Serologic examination: Serum samples collected from individual neonatal pigs at necropsy were investigated by the rapid plate agglutination (RPA) [19] test with strain L3 of *B. bronchiseptica* and the indirect hemagglutination (IHA) [3] test with strain SP-72 of *P. multocida*.

Macroscopic observation of the nasal cross section: The nasal cross sections cut at a middle level between the canine and the first premolar teeth were obtained from the pigs within one hr after euthanasia. The nasal turbinate atrophy and distortion of the nasal septum were macroscopically evaluated and scored on a basis of 0 to 4 by using these cross sections, according to the criteria described by Maeda *et al.* [12]. The scores above 2 were considered as "positive" atrophy.

Histologic examination: The examination was carried out on the transverse sections of the snout mentioned above. Tissues were fixed in 10% neutral formalin solution, decalcified in 15% ethylenediaminetetraacetic acid

Table 2. Gross lesions of neonatal pigs inoculated intranasally with *P. multocida* or *B. bronchiseptica* or both

Group ^{a)}	No. tested	Clinical AR		Nasal turbinate atrophy	
		Score range	No. positive	Score range	No. of positive
A	12	0 (0.0) ^{b)}	0	0 (0.0) ^{c)}	0
B	4	2-3 (2.25)	4	2-4 (3.0)	4
C	8	3 (3.0) ^{c)}	8	3-4 (3.5)	8
D	2	0 (0.0)	0	0 (0.0)	0

a) See Table 1.

b) Shortening and deviation of the snout was grossly evaluated (0=normal, 1=mild, 2=moderate, and 3=severe). Scores 2 and 3 were considered as positive clinical AR. Mean in parenthesis.

c) Macroscopically evaluated and scored, according to the criteria described by Maeda *et al.* [12]. Scores above 2 were considered as positive.Table 3. Recovery of the inoculated organisms from the nasal cavities of the neonatal pigs that were given *P. multocida* or *B. bronchiseptica* or both intranasally

Group ^{a)}	No. tested	<i>P. multocida</i>					<i>B. bronchiseptica</i>				
		Pre	PID 7	21	49	63	Pre	PID 7	21	49	63
A	12	0 ^{b)(-)} ^{c)}	12 (+)	12 (+)	12 (+)	4 (+)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
B	4	0	0 (-)	0 (-)	0 (-)		0 (-)	4 (++)	4 (+++)	4 (+++)	
C	8	0	8 (+)	8 (++)	8 (++)	8 (++)	0 (-)	8 (++)	8 (+++)	8 (+++)	8 (+++)
D	2	0	2 (-)	2 (-)	2 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)

a) See Table 1.

b) No. cases with positive recovery.

c) Grade of bacterial recovery. See in the text.

solution, pH 7.3, at 37°C for 96 hr, dehydrated in alcohol, and embedded in paraffin. They were cut into thin sections approximately 6 μ m thick, which were stained with hematoxylin and eosin (H & E), Masson's trichrome, or phosphotungstic acid hematoxylin (PTAH).

RESULTS

Clinical findings: None of the pigs in group A that were given *P. multocida* alone (Table 1) showed any clinical signs, and their snouts appeared normal during the experimental period, irrespective of the strains and the dosage of the inocula employed (Table 2). In contrast, occasional sneezing and coughing were noted in the pigs in groups B and C that were given *B. bronchiseptica*

alone (group B) or those that were given both *P. multocida* and *B. bronchiseptica* (group C), respectively. These pigs showed moderate to severe lesions of AR at necropsy. All the controls in group D remained clinically normal during the period.

Recovery of the organisms: The inoculated organisms were recovered from the nasal cavities of all the infected pigs (Table 3). From all the pigs in group A, the toxigenic *P. multocida* was constantly recovered in small numbers during the experimental period. *B. bronchiseptica*, phase I, was recovered constantly in abundant numbers from all the pigs in group B throughout the period. Both *B. bronchiseptica* and *P. multocida* were recovered constantly in abundant numbers from all the pigs in group C during the period. Bacterial isolations from control animals (group D)

Fig. 1. Ventral turbinate core of a neonatal pig in group A that was given *P. multocida* alone intranasally, at PID 49. Resorption of trabeculae by osteolysis and osteoid synthesis are apparent. Proliferative osteoblasts are noted near the osseous core and periosteum (arrows). PTAH stain; $\times 350$.

Fig. 2. Ventral turbinate core of a neonatal pig in group A given *P. multocida* alone, at PID 43. Degeneration and resorption of trabeculae are observed. Marked proliferation of osteoblasts and osteoid synthesis are noted (small arrows). Osteoclasts are degenerative (large arrows). H & E stain; $\times 350$.

were negative for the 2 species of the bacteria during the period.

Determination of antibodies: All the pigs used were serologically negative for both *B. bronchiseptica* and *P. multocida* prior to the inoculation. Antibodies against *P. multocida* were not detected by the IHA test in all the pigs at necropsy. Antibodies against *B. bronchiseptica* in varying degrees were detected by the RPA test in all the pigs in group B and C. Controls were seronegative for the both species of the bacteria.

Macroscopic findings: No gross lesion was found in the nasal cross sections of all the pigs in group A (Table 2). In contrast, moderate to severe nasal turbinate atrophies were induced in all the pigs in groups B and C. The lesions induced in the group C were generally more severe than those induced in the pigs in group B. The nasal turbinates of the controls were normal.

Histologic lesions induced by *P. multocida*:

Nasal mucosa: The infected nasal mucosa in group A showed essentially normal structure, and most of the epithelial cells appeared intact with cilia. **Turbinate bones:** Slight reduction of the trabeculae both in volume and size was observed in all cases, and the lesions seemed to be induced by degeneration and resorption of trabeculae due to osteocytic osteolysis (Fig. 1). No increase in number of osteoclasts was noted as compared with that of controls. Osteoclastic osteolysis was rarely observed, however, most of the osteoclasts appeared to be degenerative. Marked proliferation of osteoblasts and osteoid synthesis in varying stages near trabeculae and periosteum resulted in further new bone formation (Fig. 2), and the trabeculae were replaced by proliferating osteoblasts and osteoids. In many areas, such osteoid tissues were surrounded by a mass of osteoblasts. Increase of fibrous tissue near the periosteum was apparent. The turbinate le

Fig. 3. Ventral turbinate core of a pig in group B that was given *B. bronchiseptica* alone intranasally, at PID 49. Many osteoclasts are noted in contact with the segmented bony trabeculae (small arrows). Proliferation of fibroblasts is apparent beneath periosteum. Most of the osteoblasts in osseous core are necrotic (large arrow). PTAH stain; $\times 175$.

sions induced in all the 12 pigs in group A were closely resembled.

Histologic lesions induced by *B. bronchiseptica*: Nasal mucosa: Marked degeneration and degenerative detachment of the epithelial cells were noted in all the pigs in group B, and cilia were lost from most of the epithelial cells. Marked hyperplasia of the epithelial cells was also observed in many parts of the epithelium, and a moderate number of lymphocytes and neutrophils infiltrated in the epithelium. Turbinate bones: The volume and size of the trabeculae were markedly reduced compared with those of controls, and these lesions seemed to be induced by degeneration and resorption of trabeculae mainly due to perforating resorption or osteoclastic osteolysis (Fig. 3). Osteoclastic cells

Fig. 4. Ventral turbinate core of a pig in group B given *B. bronchiseptica* alone, at PID 49. The trabeculae replaced by the proliferating fibrous tissue (arrow). H & E stain; $\times 350$.

were markedly increased in numbers in all the cases. Most of the cells in trabeculae were necrotic, and trabeculae were replaced by the proliferating fibrous tissues (Fig. 4). Slight increase of osteoblasts was found near trabeculae, however, most of them were undergoing severe necrotic changes (Fig. 5). Slight osteoid synthesis was also observed near trabeculae. Bone formation in their trabeculae was scarcely observed due to the damage to their osteogenesis, probably by impairing the osteoblasts. The turbinate lesions induced in all the 4 pigs in group B were closely resembled.

Histologic lesions induced by co-infection with the 2 species of the bacteria: The changes induced in all the 8 pigs in group C (Fig. 6) were closely resembled to those induced by *B. bronchiseptica* (Figs. 3–5). However, the changes induced by the mixed infections were somewhat more severe than

Fig. 5. Ventral turbinate core of a pig in group B given *B. bronchiseptica* alone, at PID 49. Many pyknotic osteoblasts are observed around the rarefied trabeculae (small arrows). Some osteoclasts are phagocytizing the degenerative osteoblasts (large arrow). H & E stain; $\times 350$.

Fig. 6. Ventral turbinate core of a pig in group C that was given both *B. bronchiseptica* and *P. multocida* intranasally, at PID 63. Many osteoclasts are noted in contact with the rarefied bony trabeculae (small arrows). Marked proliferation of osteoblasts and fibroblasts is observed beneath the periosteum. Some osteoblasts are pyknotic (large arrows). H & E stain; $\times 350$.

Fig. 7. Ventral turbinate core of a control pig in group D euthanatized at 70 days of age. Showing normal structure. H & E stain; $\times 87.5$.

those induced by *B. bronchiseptica* alone.

Histologic findings in controls: The nasal mucosa of all the pigs in group D appeared normal (Fig. 7). Trabeculae were significantly larger in volume and size than those of the pigs in groups A, B, and C. In some parts of trabeculae, slight osteocytic osteolysis and resorption of trabeculae by osteoclasts were noted. Active osteoid synthesis by the osteoblasts, a normal osteogenesis in the growing neonatal pigs, was observed in many parts of trabeculae.

DISCUSSION

Pasteurella multocida was first associated with swine AR by Gwatkin and Dzenis [10], but its pathogenic role has never been definitely established; experimental infections of the pigs with the organism have frequently failed to reproduce the disease. Since de Jong *et al.* [4] suggested the presence of the DNT-producing strains among the *P. multocida* isolated from AR cases, the toxigenic strains and its DNT have been considered by some European researchers as a major causative agent and a virulent factor, respectively, in swine AR [6, 13, 22, 23, 26, 27]. *P. multocida* does not colonize itself on the nasal mucosa under natural and experimental conditions [9, 27], therefore, these workers employed a chemical irritation [6, 23] or *B. bronchiseptica* infection [13, 22, 26, 27] prior to the exposure to the toxigenic strains of *P. multocida*. The first aim of the present investigation was characterization of the histologic lesions induced in the nasal turbinates of the pigs that were given *P. multocida* or *B. bronchiseptica* alone to clarify the aetiological roles of the 2 species of the bacteria in the development of swine AR. The quality and severity of lesions observed between the 2 species of the bacteria were apparently different, and the present observations are consistent with the hypothesis that *B. bronchiseptica* is responsible for pathogenesis of swine AR.

Recently, Pedersen and Elling reported that the toxigenic serotype D [23] or serotype A [6] *P. multocida* strains induced swine AR which was characterized by the initial bone resorption by osteoclastic osteolysis accompanied by impaired osteogenesis in an early stage of the infection (within PID 14). The turbinate lesions in their experiments were similar to those of naturally occurring AR, hence, they concluded that the strains are able to induce the nasal turbinate atrophy in neonatal pigs. In contrast to their observation,

the present investigation showed that the intranasal inoculation of *P. multocida* alone (infection group A) resulted in the characteristic turbinate lesions at PID 49 or 63 including the following changes; slight resorption of trabeculae by osteocytic osteolysis, active osteoid synthesis beneath the osseous cores and periosteum due to increase of osteoblasts, and enhanced new bone formation. Many areas of the trabeculae have been replaced by thus proliferating osteoblasts and osteoids. Hence, macroscopically the nasal turbinates of the pigs were essentially normal, and none of these pigs showed clinical AR. The difference in quality of the formed turbinate lesions observed in the 2 studies can not be explained by the different experimental conditions and factors involved in the 2 tests, such as observation period, pre-treatment with acetic acid, strains employed, preparation of inocula, and proliferation of the organisms on the nasal mucosa. Further confirmation of the present results by an independent study is needed.

Pasteurella multocida DNT has been emphasized as a factor responsible for the pathogenesis of swine AR [4, 6, 13, 22, 23, 26, 27]. Elling and Pedersen [6] and Pedersen and Elling [23] stated that the *P. multocida* DNT may interfere with the physiological regulation of the development of the osseous core of the nasal turbinates in neonatal pigs. The nasal cross sections removed from neonatal pigs were cultured in a medium containing *P. multocida* or its purified DNT [18], and the lesions induced in these organ cultures were found very similar to those induced *in vivo* by *P. multocida* alone (unpublished data). Thus, it seems that *P. multocida* DNT is capable of producing the characteristic turbinate histologic lesions as mentioned above. Evidence is apparent that *P. multocida* DNT enhances osteoid synthesis both *in vivo* and *in vitro*. The mechanism of *P. multocida* DNT responsible for the osteoid synthesis is interesting to investigate. We concluded, therefore, that the toxigenic *P.*

multocida strains were responsible for the turbinate lesion formation characteristic to the bacterium, but probably not able to induce swine AR.

Pedersen and Barfod [22] and Rutter *et al.* [28] reported that the intranasal inoculation of neonatal pigs with *B. bronchiseptica* induced a transient atrophy of the nasal turbinates, but that the severe lesions observed in naturally occurring AR were not reproduced experimentally with *B. bronchiseptica* alone. In contrast, Elling and Pedersen [6] and Pedersen and Elling [23] demonstrated in SPF neonatal pigs that the toxigenic *P. multocida* strains are capable of inducing a disease comparable with naturally occurring swine AR and that the pathogenesis of the nasal turbinate atrophy involves both impaired osteoblastic bone formation and enhanced osteoclastic bone resorption. Interestingly, the turbinate bone lesions observed by them [6, 23] were very similar to those induced by *B. bronchiseptica* alone (infection group B) in our study. Inflammatory reaction was not observed in the *P. multocida* infected nasal mucosa [6, 23], whereas severe chronic catarrhal inflammatory changes were observed in the *B. bronchiseptica* infected ones. The turbinate lesions induced by *B. bronchiseptica* were very similar to those described previously in the nasal turbinates of the pigs that were given *B. bronchiseptica* [2, 8, 11, 12, 14, 15, 25, 31, 33]. In addition, the lesions induced in the nasal cross sections cultured in a medium containing *B. bronchiseptica* or its purified DNT were also similar to those induced *in vivo* by *B. bronchiseptica* alone (unpublished data). *B. bronchiseptica* DNT apparently impaired osteoblastic bone formation by damaging the osteoblasts resulting in enhancement of osteoclastic bone resorption. The present findings support the previous hypothesis [11] that *B. bronchiseptica* DNT is a major virulent factor of the organisms for pathogenesis of swine AR.

Infection with *B. bronchiseptica* followed by infection with the toxigenic *P. multocida* caused a progressive AR corresponding to the disease observed in the field [22, 27]. The observations were confirmed by the present study in infection group C. The phenomenon may be interpreted as follows: Both species of the bacteria colonize on the nasal mucosa, producing an abundant amount of DNT by autolysis of the cells [16]. The osteoclastic and osteolytic bone resorption enhanced by co-infection with the 2 species of the bacteria may be overcome by the osteoid synthesis promoted by *P. multocida* DNT. In addition, impaired osteoblastic bone formation observed in groups B and C may enhance the production of severe nasal turbinate atrophy in the pigs.

P. multocida DNT and *B. bronchiseptica* DNT located intracellularly in viable organisms [16]. The DNT was not secreted from the actively growing cells. A low amount of cell-free DNT was detected in the culture supernatants after autolysis of the cells. Experimental studies have shown that *B. bronchiseptica* well colonizes on the nasal mucosa and causes severe chronic catarrhal inflammatory reaction [33], whereas *P. multocida* establishes poorly on the normal nasal mucosa without causing much damage to the mucosa [9, 15, 27]. These findings were consistent with those of the present studies, suggesting the different ability of the 2 species of the bacteria in production of DNT *in vivo*. Our preliminary study showed that the nasal mucosa cultured *in vitro* in the presence of the purified DNT of *P. multocida* or *B. bronchiseptica* were equally injured by the toxin. These observations also supported our previous hypothesis that *P. multocida* is probably not able to induce clinical swine AR. In conclusion, *B. bronchiseptica* is a known causative agent for swine AR and its DNT is responsible for pathogenesis of the nasal turbinate atrophy.

REFERENCES

1. Bordet, J., and Gengou, O. 1906. Le microbe de la Conqueluche. *Ann. Inst. Pasteur Paris* 20: 731-741.
2. Brassinne, M., Devaele, A., and Gouffanux, G. 1976. Intranasal infection with *Bordetella bronchiseptica* in gnotobiotic piglets. *Res. Vet. Sci.* 20: 162-166.
3. Carter, G. R. 1955. Studies on *Pasteurella multocida*. I. A hemagglutination test for the identification of serological types. *Am. J. Vet. Res.* 16: 481-484.
4. de Jong, M. F., Oei, H. L., and Tetenburg, G. J. 1980. AR-pathogenicity-tests for *Pasteurella multocida* isolates. *Proc. 5th Intern. Pig Vet. Soc.* (Copenhagen, Denmark), 211.
5. Duncan, J. R., Ross, R. F., Switzer, W. P., and Ramsey, F. K. 1966. Pathology of experimental *Bordetella bronchiseptica* infection in swine: atrophic rhinitis. *Am. J. Vet. Res.* 27: 457-466.
6. Elling, F., and Pedersen, K. B. 1983. Atrophic rhinitis in pigs induced by a dermonecrotic type A strain of *Pasteurella multocida*. pp. 1234-135. In: Atrophic rhinitis in pigs (Pedersen, K. B., and Nielsen, N. C., eds.), Office for Official Publications of the European Communities, Luxembourg.
7. Fetter, A. W., and Capen, C. C. 1971. The fine structure of bone cells in the nasal turbinates of pigs with naturally occurring atrophic rhinitis. *Am. J. Pathol.* 62: 265-282.
8. Fetter, A. W., Switzer, W. P., and Capen, C. C. 1975. Electron microscopic evaluation of bone cells in pigs with experimentally induced *Bordetella* rhinitis (turbinate osteoporosis). *Am. J. Vet. Res.* 36: 15-22.
9. Gois, M., Barnes, H. J., and Ross, R. F. 1983. Potentiation of turbinate atrophy in pigs by long-term nasal colonization with *Pasteurella multocida*. *Am. J. Vet. Res.* 44: 372-378.
10. Gwatkin, R., and Dzenis, L. 1953. Rhinitis of swine. VIII. Experiments with *Pasteurella multocida*. *Can. J. Com. Med. Vet. Sci.* 17: 454-464.
11. Hanada, M., Shimoda, K., Tomita, S., Nakasa, Y., and Nishiyama, Y. 1979. Production of lesions similar to naturally occurring swine atrophic rhinitis by cell-free sonicated extract of *Bordetella bronchiseptica*. *Jpn. J. Vet. Sci.* 41: 1-8.
12. Maeda, M., Inui, S., and Konno, S. 1969. Lesions of nasal turbinates in swine atrophic rhinitis. *Natl. Inst. Anim. Health Q.* (Jpn.). 9: 193-202.
13. Martineau, G. P., Broes, A., de Jong, M. F., and Doizé, B. M. 1982. Experimental reproduction of atrophic rhinitis with *Pasteurella multocida* on gnotobiotic and conventional piglets. *Proc. 6th Intern. Pig Vet. Soc.* (Mexico), 88.
14. Miniates, O. P., and Johnson, J. A. 1980. Experimental atrophic rhinitis in gnotobiotic pigs. *Can. J. Com. Med.* 44: 358-365.
15. Nakagawa, M., Shimizu, T., and Motoi, Y. 1974. Pathology of experimental atrophic rhinitis in swine infected with *Alcaligenes bronchiseptica* or *Pasteurella multocida*. *Natl. Inst. Anim. Health Q.* (Jpn.). 14: 61-71.
16. Nakai, T., Sawada, A., and Kume, K. 1985. Intracellular locations of dermonecrotic toxins in *Pasteurella multocida* and in *Bordetella bronchiseptica*. *Am. J. Vet. Res.* 46: 870-874.
17. Nakai, T., Sawata, A., Tsuji, M., and Kume, K. 1984. Characterization of dermonecrotic toxin produced by serotype D strains of *Pasteurella multocida*. *Am. J. Vet. Res.* 45: 2410-2413.
18. Nakai, T., Sawata, A., Tsuji, M., Samejima, Y., and Kume, K. 1984. Purification of dermonecrotic toxin from a sonic extract of *Pasteurella multocida* SP-72 serotype D. *Infect. Immun.* 46: 429-434.
19. Nakase, Y. 1957. Studies on *Haemophilus bronchisepticus*. I. The antigenic structures of *H. bronchisepticus* from guinea pigs. *Kitasato Arch. Exp. Med.* 30: 57-72.
20. Nakase, Y. 1957. Studies on *Haemophilus bronchisepticus*. II. Phase variation of *H. bronchisepticus*. *Kitasato Arch. Exp. Med.* 30: 73-78.
21. Namioka, S., and Murata, M. 1961. Serological studies on *Pasteurella multocida*. 1. A simplified method for capsular typing of the organisms. *Cornell Vet.* 51: 498-507.
22. Pedersen, K. B., and Barfod, K. 1981. The aetiological significance of *Bordetella bronchiseptica* and *Pasteurella multocida* in atrophic rhinitis of swine. *Nord. Vet. Med.* 33: 513-522.
23. Pedersen, K. B., and Elling, F. 1984. The pathogenicity of atrophic rhinitis in pigs induced by toxigenic *Pasteurella multocida*. *J. Comp. Pathol.* 94: 203-214.
24. Pittman, M. 1974. Genus *Bordetella* Moreno-López 1952 pp. 282-283. In: *Bergey's Manual of Determinative Bacteriology*, 8th ed. (Buchanan, R. E., and Gibbons, N. E. eds.). Williams & Wilkins, Baltimore.
25. Ross, R. F., Duncan, J. R., and Switzer, W. P. 1963. Turbinate atrophy in swine produced by pure cultures of *Bordetella bronchiseptica*. *Vet. Rec.* 58: 566-570.
26. Rutter, J. M. 1983. Virulence of *Pasteurella multocida* in atrophic rhinitis of gnotobiotic pigs infected with *Bordetella bronchiseptica*. *Res. Vet. Sci.* 34: 287-295.
27. Rutter, J. M., and Rojas, X. 1982. Atrophic rhinitis in gnotobiotic piglets: difference in the pathogenicity of *Pasteurella multocida* in combined infections with *Bordetella bronchiseptica*. *Vet. Rec.* 110: 531-535.

28. Rutter, J. M., Francis, L. M. A., and Sansom, B. F. 1982. Virulence of *Bordetella bronchiseptica* from pigs with or without atrophic rhinitis. *J. Med. Microbiol.* 15: 105–116.
29. Sawata, A., and Kume, K. 1982. Nasal turbinates atrophy in young mice inoculated with *Bordetella bronchiseptica* of pig origin. *Am. J. Vet. Res.* 43: 1945–1947.
30. Sawata, A., Nakai, T., Tsuji, M., and Kume, K. 1984. Dermonecrotic activity of *Pasteurella multocida* strains isolated from pigs in Japanese field. *Jpn. J. Vet. Sci.* 46: 142–148.
31. Shimizu, T., Nakagawa, M., Shibata, S., and Suzuki, K. 1971. Atrophic rhinitis produced by intranasal inoculation of *Bordetella bronchiseptica* in hysterectomy produced colostrum-deprived pigs. *Cornell Vet.* 4: 699–705.
32. Smith, J. E. 1974. Genus *Pasteurella*. pp. 370–373. In: *Bergey's Manual of Determinative Bacteriology*, 8th ed. (Buchanan, R. E., and Gibbons, N. E., eds.), Williams & Wilkins, Baltimore.
33. Yokomizo, Y., and Shimizu, T. 1979. Adherence of *Bordetella bronchiseptica* to swine nasal epithelial cells and its possible role in virulence. *Res. Vet. Sci.* 27: 15–21.

要 約

Pasteurella multocida および *Bordetella bronchiseptica* の単独あるいは混合接種により形成された SPF 新生豚の鼻甲介病変：小山田敏文・吉川 堯・清水まき子・中井豊次¹⁾・久米勝己²⁾（北里大学獣医畜産学部獣医病理学教室，¹⁾北里研究所柏獣医学研究所，²⁾北里研究所）——*Pasteurella multocida* (Pm) SP-72 株および 47459 株と *Bordetella bronchiseptica* (Bb) L3 株を 1 週齢 SPF 豚の鼻腔内に単独あるいは混合接種し，7 あるいは 9 週後に病理学的観察を行った。両菌は実験期間中鼻腔より回収されたが，回収菌数は Bb がはるかに多く，炎症性変化も Bb 接種豚で明らかであった。Pm 接種豚の鼻甲介は肉眼変状を示さず，組織学的には骨細胞性骨融解による骨梁の吸収が存在するものの，活発に増生した骨芽細胞と類骨に置換していた。これに対し，肉眼的に明らかな萎縮が認められた Bb 接種豚の鼻甲介には破骨細胞性骨融解による重度の穿孔性骨梁吸収が認められ，同時に骨芽細胞障害による骨新生の減弱と骨梁周囲の線維増生を伴っていた。Pm, Bb を混合接種した場合の骨梁病変は Bb 接種のそれよりも重度であった。以上の成績から両菌の鼻甲介に対する病原性は明らかに異なり，AR 病変の形成は主に Bb で展開されることが強く示唆された。