NASAL INFECTION OF *ALCALIGENES BRONCHISEPTICUS* (BORDETELLA BRONCHISEPTICA) AND LESIONS IN NEWBORN RABBITS

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The mode of experimental infection with *Alcaligenes bronchisepticus* (Bordetella bronchiseptica) of pig and rabbit origin, and lesions in the respiratory organs were examined in three groups, A, B and C, of newborn rabbits. Groups A and C were free from the organism and agglutinating antibody and inoculated with the organisms of pig and rabbit origin, respectively. Group B had maternal antibody and was inoculated with the organism of pig origin. Establishment and persistence of infection with the organism were certified in the nasal cavities, trachea, or lungs of groups A and C 3 days after inoculation or later. Late establishment of infection occurred in trachea and lungs of group B. Agglutinating antibody was detected in groups A and B mostly 14 days after inoculation or later. Ventral turbinate atrophy occurred in groups A and B mostly 10 days after inoculation or later. Histologically, it was hypo-osteogenesis caused by degeneration of osteoblasts and proliferation of fibroblast-like cells in the osseous tissue. Catarrhal inflammation in the nasal and tracheal mucous membranes, and bronchopneumonia with peribronchiolitis developed commonly in all the groups. The fluorescent antibody technique revealed antigen of the organism of pig origin on the nasal mucosa, mostly of the dorsal and ventral meatus, and on the tracheal and bronchiolar mucosa in groups A and B.

Nasal infection of piglets with *Alcaligenes bronchisepticus* (Bordetella bronchiseptica) has been reported to cause nasal turbinate atrophy by many workers. Similarity on the development of lesions of the nasal turbinate has been observed in both experimental and natural atrophic rhinitis (AR). The inhibitory effect of immune antibody against *Alc. bronchisepticus* infection and its lesions, however, were not completely elucidated as yet. For basic investigation on these points, it may be necessary to find some experimental animals fit for AR research.

In rabbit herds an extensive outbreak of interstitial pneumonia in connection with *B. bronchiseptica* infection was reported by Oldenburg et al. Gwatkin et al. reported on rhinitis with the destroyed nasal turbinate in young rabbits inoculated with nasal material and *Pasteurella multocida* collected from diseased pigs. Genov observed rhinitis with turbinate atrophy in rabbits inoculated with *B. bronchiseptica* isolated from a diseased pig and performed passage intermediately in a guinea pig. Maeda recognized turbinate atrophy in newborn rabbits inoculated with...
Alc. bronchisepticus originated directly from a diseased pig. No detailed studies, however, have been conducted as yet on the mode of infection or lesion with Alc. bronchisepticus of pig origin in rabbits. This paper deals with the mode of infection of newborn rabbits with Alc. bronchisepticus originated from both pig and rabbit and respiratory lesions of the infection.

MATERIALS AND METHODS

Animals used: Seventy-four newborn white rabbits of 14 litters were divided into four groups, A to D. Group A consisted of 16 animals of three litters, group B of 20 animals of another five litters, group C of 23 animals of another four litters, and group D of 15 animals of the other two litters. The newborn of groups A, C, and D were proved to be free from Alc. bronchisepticus and agglutinin against the organism before inoculation. The newborn of group B were found to have maternal antibody in agglutinating titers from 1:40 to 1:80. All the newborn were nursed by their mothers in productive cages until 3 weeks of age, and raised in individual cages after that.

Inoculation: Two strains of Alc. bronchisepticus were used in the stage of phase I. They were the A-19 strain originated from a naturally diseased pig, and the F-36 strain isolated from a healthy rabbit. Group A was inoculated intranasally with 1.0 or 6.0 x 10^6 cells of the A-19 strain at 5 or 6 days of age. Group B was exposed to 8.0 x 10^5 cells of the A-19 strain some time between 1 and 10 days of age. Group C was instilled with 1.0 or 5.0 x 10^6 cells of the F-36 strain at 2, 3, or 6 days of age. Group D served as a control.

Pathological examination: All the animals were autopsied some time between 1 and 56 days after inoculation, as shown in Tables 1 and 2. Histological examination was performed on two transverse nasal sections at a level where the ventral turbinates and ethmoids showed a maximum development (Fig. 1). Specimens were also collected from the cervical trachea, each pulmonary lobe, and some other principal organs. They were fixed in 10% formalin, embedded in paraffin, and cut into thin sections, which were stained with hematoxylin and eosin. Specimens from the snout were decalcified before embedding.

Fluorescent antibody technique (FAT): This technique was applied in the same manner as used in the preceding study. Immune globulin precipitated from hyper-immunized rabbit serum against the A-19 strain with ammonium sulfate was purified by Sephadex G-25 filtration and diethylaminoethyl (DEAE) cellulose chromatography after conjugation with fluorescein isothiocyanate. The purified product of conjugation was absorbed with rabbit liver powder. It showed a staining titer of 1:16. Thin sections cut from specimens were stained with four units of the product of conjugation at 4°C for 16 hours. A rabbit serum showing agglutinating titer of 1:2,560 blocked the antigenicity in the thin sections.

Recovery of the organism: Direct reisolation of the organism was tried from the nasal cavities, trachea, and lungs at autopsy. Colonies grown on 24-hour cultures on MacConkey agar enriched with 1% glucose and on DHL agar were identified by the slide agglutination with rabbit antiserum against the A-19 strain.

Agglutination test: The method by Shimizu et al. was applied to the sera of all the animals at autopsy, using formalin-killed antigen of pig origin purchased from the Kitasato Laboratories, Ltd. To the sera of the rabbits of group C inoculated with the organism of rabbit origin, live cells of this organism grown in an 18-hour culture of trypticase soy broth were used as antigen. A serum showing clear agglutination at 1:10 or higher dilutions was regarded as one containing agglutinating antibody.

### RESULTS

**1. Evidence of infection after inoculation**

Nasal establishment of the inoculum was confirmed by reisolation of the organism in both groups A and C 3 days after inoculation (hereinafter expressed as day 3), and in group B on day 1. Nasal infection was persistent until the end of each experimental period. Tracheal infection started in groups A and C on day 3 and in group B mostly on day 7. It lasted in each group until the

#### Table 1. Infection and lesions in newborn rabbits inoculated with the organism of pig origin (Groups A and B)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Days after inoculation</th>
<th>Recovery of organism</th>
<th>Agglutinating antibody</th>
<th>Nasal turbinate atrophy</th>
<th>Tracheitis</th>
<th>Pneumonia</th>
<th>Linear lesion in lungs</th>
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* The numerator indicates the number of animals from which the organism, antibody, and lesions were detected and the demoninator the number of animals tested. NT: Not tested

#### Table 2. Infection and lesions in newborn rabbits inoculated with the organism of rabbit origin (Group C)

<table>
<thead>
<tr>
<th>Days after inoculation</th>
<th>Recovery of organism</th>
<th>Agglutinating antibody</th>
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<th>Tracheitis</th>
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* See the remarks of Table 1.
end of the experimental period. Pulmonary infection was observed in groups A and C over a period from day 3 to the end of the experimental period, and in group B over a period between days 7 and 29. Late establishment of the organism was recognizable in the trachea and lungs of animals with maternal antibody. Early disappearance of the organism from the lungs was also detected in these animals. Agglutinating antibody of a titer ranging from 1:10 to 1:20 was detected from one of three rabbits examined on day 14 and from all the three rabbits examined later in group A. In group B, the antibody of a titer ranging from 1:40 to 1:80 was found in five of seven rabbits examined between days 1 and 8, and that of the titers ranging from 1:40 to 1:640 in all four rabbits examined between days 29 and 56. On the other hand, the antibody was not noticed in group C even when both antigens of pig and rabbit origin were used (Tables 1 and 2).

In group A nasal turbinate atrophy accompanied by mucous exudation was recognized in one of four rabbits examined on day 7, in one of two rabbits on day 10, and in all six rabbits on day 14 or later. Severe atrophy (Fig. 2) occurred in two rabbits on day 14 in this group. In group B, the atrophy was mild in 7 of 13 rabbits between days 7 and 56, and moderate in one rabbit on day 11. Only one rabbit exhibited mild atrophy in group C on day 14. In group A, 8 of 15 rabbits were affected bilaterally with lobular pneumonia (Fig. 3) of the apical and cardiac lobes between days 3 and 21. The pneumonia also occurred in six of nine rabbits of group B between days 7 and 11, and in 9 of 15 rabbits of group C between days 3 and 21. In addition to the pneumonia, gray linear pulmonary lesions were noticed along the bronchus and bronchioles mainly in the apical and cardiac lobes in these three groups. The lobular pneumonia was inclined to occur mostly in the middle stage of the experimental period, and the linear lesions appeared frequently in the late stage in common in the three groups. The organism or any lesion was not detected from group D.

2. Development of lesions

Rhinitis and turbinate atrophy: Three stages of rhinitis were commonly observed in groups A, B, and C. They were acute catarrhal rhinitis (acute rhinitis), acute catarrhal rhinitis with the proliferation and desquamation of epithelial cells (subacute
Acute bronchiseptic infection and lesion in newborn rabbits

Acute rhinitis (Fig. 4) was represented by mild karyopyknosis and vacuolation of epithelial cells accompanied by a mild heterophil infiltration in the epithelial layer. Mild heterophil infiltration and hyperemia were also seen in the lamina propria. Acute rhinitis was extended all over the nasal mucous membrane in groups A and C between days 3 and 7, and was mainly located at the tip of the ventral turbinate and in the dorsal meatus in group B between days 1 and 14. Subacute rhinitis (Fig. 5) exhibited localized proliferation or desquamation of epithelial cells, together with a marked heterophil and lymphocytic infiltration in the lamina propria. The desquamation was more conspicuous in subacute rhinitis. The rhinitis was situated in common at the tips of the ventral and dorsal turbinates and in the dorsal meatus in the three groups. It was observed mostly in groups A and C between days 14 and 28, and in group B between days 7 and 29. Chronic rhinitis showed a marked focal lymphocytic infiltration with a mild desquamation of epithelial cells (Fig. 6), in addition to a mild lymphocytic and plasmacytic infiltration in the lamina propria. The rhinitis occurred to the tips of the turbinates and in the dorsal meatus in group B mostly on day 56 and in group C mainly on day 42. Catarrhal ethmoiditis (Fig. 7) was observed in all the newborn of these three groups.

In the osseous cores of the atrophic ventral turbinates, the bone trabeculae presented a decrease in number and rarefaction. And degeneration of osteoblasts and an increase in number of fibroblast-like cells (Fig. 8) were additionally shown in the medullary spaces, mainly at the tips of the ramifications.
of the ventral turbinates. The degenerative osteoblasts exhibited karyopyknosis or vacuolation. Such hypo-osteogenesis as this was conspicuous in the case of subacute rhinitis in groups A and B in the middle and late stages of the experimental period. In two rabbits of group B examined on day 56, the ventral turbinates showed an increase of collagenous fibers beneath the periosteum of the osseous tissue.

Tracheitis: Acute and chronic catarrhal tracheitis were observed. Acute tracheitis was manifested by a mild desquamation of epithelial cells and heterophil infiltration in the epithelial layer (Fig. 9), as well as by hyperemia in the lamina propria. Chronic tracheitis exhibited a lymphocytic and plasmacytic infiltration in the lamina propria. Acute tracheitis was detected in groups A and C some time between days 3 and 10, and in group B some time between days 3 and 11. Chronic tracheitis developed subsequently in each group.

Pneumonia and linear lesion: In each group, pneumonia was manifested histologically by catarrhal bronchopneumonia accompanied by a marked heterophil infiltration and desquamation of alveolar and bronchiolar epithelial cells. Histiocytic infiltration and proliferation of fibroblasts were also remarkable in the alveolar septa (Fig. 10) and around the bronchioles. The linear lesion corresponded to peribronchitis or peribronchiolitis with marked lymphocytic infiltration (Fig. 11). A mild heterophil infiltration was seen frequently in the mucosa of the bronchus and bronchioles.

3. Distribution of antigen detected by FAT

Nasal cavity: Two forms, granular and

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**Fig. 8.** Osseous tissue of ventral turbinate. Group A on day 14. Proliferation of fibroblast-like cells. HE staining, × 250

**Fig. 9.** Tracheal mucosa. Group A on day 3. Acute catarrhal tracheitis. HE staining, × 250

**Fig. 10.** Lung. Group A on day 14. Histiocytic and fibroblastic infiltration in alveolar septa. Alveolar epithelial cells are proliferative. HE staining, × 250

**Fig. 11.** Lung. Group B on day 56. Peribronchiolitis with lymphocytic infiltration. HE staining, × 100

*Maeda, M. and Shimizu, T.*

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laminated, of antigen were detected from epithelial cells and nasal exudate. No antigen was demonstrated in the lamina propria. Fine granular antigen was revealed mainly on the nasal epithelial cells of the dorsal meatus, and slightly on the ventral turbinate (Fig. 12) in groups A and B. On the nasal epithelial cells of the ventral meatus much laminated antigen (Fig. 13) was seen in these groups. In group C, much laminated antigen was detected on the ventral meatus and a little granular antigen on some other parts. These forms of antigen were demonstrated frequently in the case of acute and subacute rhinitis in groups A and C between days 7 and 28 and in group B between days 3 and 29.

Trachea and lung: A little granular antigen was detected on the tracheal epithelium (Fig. 14) in some animals of groups A and B examined between days 3 and 10, and on the tracheal mucosa in group C on day 28. In the lung there was granular antigen mainly on epithelial cells of the bronchiolar crypts (Fig. 15) and in bronchiolar exudate. Antigen was demonstrable in group A between days 7 and 21, and in group B between days 7 and 56. Alveolar exudate contained a homogeneously antigenic substance in an animal of group B on day 11.

DISCUSSION

No precise description has been made on the experimental infection of rabbits with
Alc. bronchiseptica of pig origin or on its lesions.

In the present study, newborn rabbits which were free from Alc. bronchiseptica before inoculation (free newborn) were infected intranasally with the organism of pig origin (group A) or rabbit origin (group C). Infection with the inoculated organism took place in the nose, trachea, and lungs, regardless of the origin of the organism. Catarhal rhinitis, catarhal tracheitis, bronchopneumonia, and peribronchitis developed in common after infection. Antigen was commonly detected also by FAT on epithelial cells of the nasal, tracheal, and bronchiolar mucosa. On the other hand, nasal turbinate atrophy, as well as agglutinin against the organism, appeared in group A alone. Antigen detectable by FAT was distributed mostly on the ventral and dorsal meatus in the case of both acute and subacute rhinitis in group A, while in group C it was mainly on the ventral meatus which showed no inflammatory changes. These differences between both groups might come from the different pathogenicity of both organisms to the newborn rabbit.

In experimental AR produced by nasal infection with Alc. bronchiseptica (B. bronchiseptica) of pig origin in piglets, nasal establishment of the organism has been reported to occur mostly 1 or 2 weeks after inoculation. Persistent infection was shown after the establishment in the nose, trachea, and lungs. Agglutinating antibody was detected in hysterectomy-produced, colostrum-deprived piglets on day 35 or later by Shimizu et al. and on day 15 or later by Kemeny. Nasal turbinate atrophy was recognized to initiate in piglets or weeks after inoculation. It was shown to develop in piglets as a result of hypo-osteogenesis in osseous tissue in relation to acute catarhal rhinitis exhibiting conspicuous proliferation and desquamation of epithelial cells. Following acute catarhal rhinitis, bronchopneumonia was observed together with an increase in fibroblasts and connective tissue in the alveolar septa and around the bronchioles. Antigen detectable by FAT was distributed on the nasal mucosa, mainly on the ventral turbinate and dorsal meatus, and also on the tracheal and bronchiolar mucosa.

In the present study of newborn rabbits inoculated with the organism of pig origin (groups A and B), establishment of infection with the organism was certified in the respiratory organs in these rabbits earlier (on day 3 in group A) than in piglets. Agglutinating antibody or high titers of agglutinin came out in the rabbits, as well as in piglets, mostly 2 or 4 weeks after inoculation. Nasal turbinate atrophy in this study became detectable on day 7. It was shown due to the same hypo-osteogenesis at the turbinate bones as in piglets. On the other hand, acute and subacute rhinitis on the nasal turbinate were more transient and localized in newborn rabbits. Antigen detected by FAT in the nasal cavity was distributed predominantly on the ventral and dorsal meatus in the newborn rabbit, while it was distributed excessively on the ventral turbinates in piglets. Generally, some similarities on the mode of infection and development of lesions seemed to be present between newborn rabbits and piglets both of which had been inoculated with the organism of pig origin.

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LITERATURE CITED


