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Experimentally Induced Pneumonia in *scid/beige* Mice, Using a Bovine Isolate of *Pasteurella haemolytica*

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Background and Purpose: **Intranasal challenge of immunocompetent mice with *Pasteurella haemolytica* results in little or no pulmonary inflammation. The study reported here was designed to investigate the inflammatory response in the lungs of immunodeficient scid/beige mice after similar challenge.**

Methods: **Fifty-five *scid/beige* mice were challenged intranasally with saline or one of three doses (2.8×10^6, 3.4×10^9, or 3.3×10^{11} colony-forming units [CFU]/ml) of *P. haemolytica*. The lungs were examined for changes in weight, bacterial count, and presence of gross and microscopic lesions at 24, 48, or 96 hours after challenge.**

Results: **Intranasal challenge with concentrations $\geq 3.4 \times 10^9$ CFU/ml of P. haemolytica induced significantly heavier lung weight, with severe pulmonary lesions, and development of suppurative and fibrinous bronchopneumonia in dose- and time-dependent manner 48 hours after challenge. *Pasteurella haemolytica* was consistently isolated from the lungs at 24 hours after challenge.**

Conclusions: **Bronchopneumonia was induced by *P. haemolytica* in mice without manipulation of the mouse or the bacteria. The lesions were similar to those that develop in the lungs of cattle infected with *P. haemolytica* and indicate potential use of the model for the study of this host/bacterial interaction.**

Pneumonic pasteurellosis in cattle represents a major source of economic loss in the cattle feedlot industry in North America (1). Although a variety of infective organisms have been associated with bovine respiratory tract disease, *Pasteurella haemolytica* biotype A serotype 1 is the principal microorganism associated with bovine pneumonic pasteurellosis (2). Pneumonic pasteurellosis induces fibrinous bronchopneumonia in affected cattle. This important and costly disease has been studied extensively in the laboratory and in the field; however, controlled experiments in cattle are limited due to financial constraints and the wide genetic variability among animals (3).

A number of attempts have been made to reproduce the disease in laboratory animals. Experimental models of *P. haemolytica* infection in mice have been described, but their use has yielded variable results. Outbred mice clear infection with *P. haemolytica* after intranasal, oral, or intraperitoneal exposure (4–8). This may be partly due to the fact that, despite its virulence for ruminants, *P. haemolytica* has little or no pathogenicity for clinically normal rodents (9). Disease in mice has been induced by manipulation of the bacteria through enhancement of virulence (4, 6, 9–11) or by physical intervention with the mouse (12).

Severe combined immune deficiency (SCID) is a rare congenital syndrome characterized by a lack of T and B cell-generated immunity. In 1983, a spontaneous mutation in an inbred line of BALB/c congenic C.B-17 mice yielded the *scid/scid* genotype *(scid)* and development of the SCID condition (13). Introduction of the beige gene (*bg*) into the *scid* mouse produced the double mutant *scid/scid.bg/bg* (*scid/beige*) mouse (14), which has reduced splenic natural killer cell function (15), as well as reduced T- and B-cell function. Compared with the *scid* mouse, the *scid/beige* mouse is more susceptible to opportunistic bacterial and viral pathogens. Many experimentally induced and naturally acquired infections of mice can be fatal in *scid/beige* colonies (16).

Immunodeficient *scid* and *scid/beige* mice have proved to be invaluable models for the study of disease pathogenesis of human pathogens, such as human immunodeficiency virus (17–19), Epstein-Barr virus, human cytomegalovirus (20), and bacterial respiratory tract pathogens, such as *Pneumocystis carnii* (21) and *Branhamella catarrhalis* (22). Studies of *scid* and *scid/beige* mice have documented susceptibility to many infective agents that are major animal pathogens, such as *Mycobacterium paratuberculosis* (23), bovine leukemia virus (24), feline immunodeficiency virus (25), simian immunodeficiency virus (26), *Rhodococcus equi* (27), *Theileria sp*. (28), *Cryptococcus neoformans* (29), and *P. haemolytica* (30, 31). In the study reported here, we describe development of fibrinous and suppurative bronchopneumonia in *scid/beige* mice after intranasal challenge with *P. haemolytica*.

Materials and Methods

Animals: Fifty-five 8- to 9-week-old *scid/beige* mice (FOX CHASE C.B-17 SCID- BEIGE; 35 female and 20 male) were obtained from Taconic Farms (Germantown, NY). All mice were housed and handled in accordance with the guidelines of the Canadian Council on Animal Care and the Animal Care Committee of the University of Guelph. The experimental protocol was approved by the Animal Care Committee of the University of Guelph. Mice were housed under specific-pathogen-free conditions in microisolator cages, as described (32), were given unlimited access to autoclaved food and water, and were allowed to acclimatize for a period of at least 72 hours before experimentation. Sentinel *scid/beige* mice were consistently seronegative

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for antibodies to Sendai virus, pneumonia virus of mice, minute virus of mice, mouse parvovirus, murine polioencephalomyelitis virus, reovirus 3, *Mycoplasma pulmonis*, lymphocytic choriomeningitis virus, mouse adenovirus, ectromelia virus, K virus, and polyoma virus (Assessment Profile, Charles River Laboratories, Wilmington, MA). Results of routine histologic examination of sentinels were negative for *Pneumocystis carinii*.

Bacteria: *Pasteurella haemolytica* biotype A serotype 1 (A1) (American Type Culture Collection 43270), harvested in the mid-logarithmic phase of growth, was used for inoculation of the mice. A pure subculture from the stock was used to inoculate 500 ml of brain heart infusion broth warmed to 37°C, which was subsequently incubated on a shaking platform for 3 to 4 hours. The bacterial culture was harvested at optical densities of 0.600 and 0.800 at a wavelength of 525 nm because it was shown in a preliminary experiment, that this represented mid-logarithmic growth phase. The culture was centrifuged at 6,300 x g for 15 minutes at 4°C, then was resuspended in 2 ml of sterile phosphate-buffered saline (PBS). This concentration yielded approximately 10^{11} colony-forming units (CFU) of *P. haemolytica*/ml. Further dilutions were carried out in PBS when concentrations of approximately 10^9 and 10^6 CFU/ml were required. The actual CFU were determined by plating 100 μl of serial 10-fold dilutions onto blood agar plates and incubating them for 18 to 24 hours at 37°C and 5% CO_2.

Animal inoculation and tissue collection: Mice were randomly distributed to control or challenge groups (Table 1), then were anesthetized by placement into a 50-ml conical tube that had a small hole cut in the bottom. This tube was connected to another tube containing a cotton ball soaked with 1 ml of inhalant methoxyflurane (Janssen Pharmaceutica, Mississauga, Ontario, Canada). After being anesthetized, the mouse was removed from the tube and was held in an upright position while 0.05 ml of either physiologic saline (control) or *P. haemolytica* inoculum was slowly dropped into the nostrils by use of a tuberculin syringe. The challenge inoculum contained 3.3 x 10^{11}, 3.4 x 10^9, or 2.8 x 10^6, CFU of *P. haemolytica*/ml. To avoid a volume-induced pulmonary change (33), the volume of inoculum was determined by the smallest amount in which the 10^{11} CFU/ml challenge was no longer tenacious. Mice were returned to their microisolater cages and allowed to recover from anesthesia, which was complete in 3 to 4 minutes, with no apparent side effects. Mice were monitored every 6 to 8 hours for signs of clinical illness.

Mice were euthanized at 24, 48, or 96 hours after challenge by intraperitoneal administration of pentobarbital sodium (MTC Pharmaceuticals, Cambridge, Ontario, Canada). The lungs, liver, spleen, and kidneys were aseptically removed. The lungs were weighed and assessed for lesions and percentage of pulmonary tissue affected. The liver, kidneys and spleen were assessed subjectively. Macroscopic lesions were evaluated as group means. The left lung lobe, a kidney and half of the liver and spleen were placed in buffered 10% formalin for subsequent histologic evaluation. The lobes of the right lung and the remaining halves of liver and spleen and the remaining kidney were placed in sterile vials and stored at -20°C for subsequent bacterial isolation and enumeration.

Histologic evaluation: Formalin-fixed tissues were embedded in paraffin and sectioned at 6-μm thickness. Sections of lung, liver, spleen, and kidney from each mouse were stained with hematoxylin and eosin. A subjective assessment of microscopic lesions was performed for sections of liver, spleen, and kidney, whereas lung sections were graded on the basis of airway or alveolar changes. Criteria for evaluation of the pulmonary lesions were based on modifications of those reported elsewhere (32). The lesions in the bronchi and bronchioles were graded as follows: 1 = histologically normal; 2 = a few inflammatory cells (neutrophils) present in lumen; 3 = lumen partially full of neutrophils; 4 = most lumens completely full of neutrophils; and 5 = most or all lumens full of neutrophils and obliteration of bronchiolar wall, with or without necrosis. The following criteria were used for the assessment of lesions found in the alveolar septa or lumen: 1 = histologically normal; 2 = mild focal neutrophil infiltrate; 3 = mildly diffuse or multifocal neutrophil infiltrate with some mild necrosis; 4 = moderately diffuse or multifocal neutrophil infiltrate with necrosis; and 5 = marked diffuse or coalescing neutrophil infiltrate with necrosis and fibrin deposition. Each lung was assessed in the cranial, central and caudal lobes, and given one numerical score for bronchiolar changes and one for alveolar changes in each area. The six individual numbers were summed and reported as a total lung score (range, 6 to 30). Sections were evaluated by one of the authors (CET) in blinded manner. Representative sections of lung from each group also were stained with silver for reticulin and with phosphotungstic acid hematoxylin (PTAH) for fibrin.

Bacterial isolation from murine tissues: Portions of spleen, liver, and kidney that were assessed for bacterial isolation were surface sterilized by immersion in 100% ethanol, followed by passage through a flame to remove the ethanol. The tissues were aseptically cut in half, and the exposed surface was smeared on blood and MacConkey agar plates, which were incubated for 18 to 24 hours at 37°C and 5% CO_2. Bacterial isolates were identified, using conventional methods (34).

The number of bacteria in the right lung was determined by homogenization of the cranial, central, caudal and postcaval lobes in 4.5 ml of sterile PBS in a Ten Broeck tissue grinder. A 100-μl aliquot of the homogenate was plated onto blood agar plates and incubated as previously described. Bacterial isolates were identified, using conventional methods (34). Colony counts were determined and reported as the mean of each group of mice. Bacterial growth was considered mild (+) if there were 0 to 500 CFU/ml, and moderate (++) if there were > 500 CFU/ml of lung homogenate.

Statistical analysis: All statistical analyses were performed, using the computer-based statistical program PC-SAS version 6.12 for Windows (SAS Institute Inc., Cary, NC). Analysis of variance was performed by use of a general linear models procedure followed by a Student-Newman-Keuls test for identification of significance. A P value < 0.05 was considered significant.

Results

Clinical assessment: At 24 hours after challenge, all mice appeared to be unaffected, with well groomed coats and normal activity. From 48 to 96 hours after challenge, mice that received sterile saline or 2.8 x 10^6 CFU of *P. haemolytica*/ml (group C) continued to appear normal whereas those of groups A and B had developed mildly scruffy coat. Activity was not affected. Respiratory difficulty was not seen in any of the mice.

Gross assessment of lungs, spleen, liver, and kidneys: At 24 hours after challenge, the lungs of all infected mice were

Table 1. Pulmonary changes and bacterial isolation from *scid*/*beige* mice after intranasal challenge with *Pasteurella haemolytica*

Group (challenge dose[a])	No. of mice, Time after challenge (h)			Gross pulmonary lesions[b], Time after challenge (h)			Lung weight (mg) mean ± SEM, Time after challenge (h)			Isolation[c] of *P. haemolytica*, Time after challenge (h)		
	24	48	96	24	48	96	24	48	96	24	48	96
A (3.3×10^{11})	5	5	5	-	++	+++	194 ± 11	188 ± 20	241 ± 61	++	-	-
B (3.4×10^{9})	5	5	5	-	+	++	167 ± 8	190 ± 14	175 ± 25	+	-	-
C (2.8×10^{6})	5	5	5	-	+	+	151 ± 13	136 ± 5	135 ± 15	+	-	-
D (saline)	3	3	4	-	-	-	127 ± 26	129 ± 8	146 ± 18	-	-	-

[a]Challenge dose is indicated in colony forming units (CFU) of *P. haemolytica*/ml.
[b]Gross pulmonary lesions were assessed in the left lung and were categorized as no lesions (-), < a quarter of the lung affected (+), a quarter to a half of the lung affected (++), and > a half of the lung affected (+++).
[c]No growth is indicated as (-), 1 to 500 CFU/ml of homogenate as (+) and > 500 CFU/ml of homogenate as (++).

grossly similar to those from mice of the non-infected saline group, having normal consistency and color. Gross changes were evident in the lungs of infected mice 48 and 96 hours after exposure to *P. haemolytica*, but were most extensive in mice 96 hours after challenge with 3.3×10^{11} CFU of bacteria/ml (Table 1). The extent of pulmonary injury was time dependent at the two highest challenge doses.

One mouse of group A (high dose) died approximately 35 hours after challenge, and at necropsy, marked edema and congestion were seen in all lung lobes. Mice of group A necropsied at 48 hours had visible lesions consistently in the left lung and right cranial lobe, whereas other lobes were affected to a lesser degree. The perihilar areas were a pale tan-gray, with consolidation of the tissue adjacent to the bronchi and larger airways. Ninety-six hours after challenge, the pulmonary lesions were similar to those at 48 hours, but were more extensive, often present in all lung lobes.

Mice of group B (middle dose) at 48 and 96 hours after challenge, had lung lesions similar to but not as extensive as those described for group-A mice. Only the left lung and right cranial lobes were affected.

In contrast, the lungs from mice that received the lowest dose of *P. haemolytica*, group C, had only mild gross changes. At 48 and 96 hours, consolidation of the perihilar region rarely involved the right cranial lobe and consistently affected < 25% of the left lung.

The spleen from one group-A mouse euthanized 48 hours after challenge was mildly enlarged but had no visible pathologic changes. The spleen was grossly normal in the rest of the bacteria-challenged mice. Gross changes were not observed in the lungs or spleen from any of the control mice. The liver and kidneys from all mice were grossly unaffected.

Bacterial isolation: Growth of *P. haemolytica* from a homogenate of the right lung lobes was quantitatively determined for every mouse. Low numbers of *P. haemolytica* were isolated from lungs of all the infected mice at 24 hours, but not at 48 or 96 hours after challenge (Table 1). There was no growth of *P. haemolytica* from the lungs of control animals. A mixed population of bacteria was isolated from the lungs of the mouse that died prematurely, but *P. haemolytica* was not recovered. The lungs of this mouse were not included in the data analysis. Qualitative analysis for *P. haemolytica* on the spleen, liver, and kidneys revealed no growth at any time.

Lung weight: At 24 hours after challenge, lung weight for group-A mice were significantly greater than that for mice of the other groups (Table 1). Lung weight for mice of groups B and C mice were similar, and both were significantly greater than that for control mice. At 48 hours after challenge, lung weight for mice of groups A and B was similar, but was significantly different from lung weight for mice of group C and mice of the control group. For mice euthanized 96 hours after infection, lung weight for group-A mice was significantly greater than that for mice of the other groups.

Histologic descriptions and pulmonary histologic scores: After intranasal inoculation with saline, the pulmonary histologic scores at 24, 48, or 96 hours after challenge were not significantly different from each other (Figure 1). Most lungs contained a few areas of mild congestion and atelectasis (Figure 2).

Among group-A mice, a time-dependent increase in lesion severity was observed (Figure 1). These differences were not statistically significant, likely due to the fact that the number of mice per group was inadequate to detect significance. Histologic examination revealed suppurative bronchopneumonia, edema, and fibrin deposition at 24, 48, and 96 hours after challenge with 10^{11} CFU of *P. haemolytica*/ml. At 24 hours after challenge, a moderate influx of neutrophils, with areas of necrosis, proteinaceous fluid, and fibrin deposition, was evident. More than half of the bronchioles contained a suppurative exudate. Forty-eight hours after challenge, there was a marked neutrophil infiltrate, patchy areas of necrosis, and fibrin and fluid accumulation in alveolar spaces. An expansive bronchiolar exudate with obliteration of the bronchiolar wall also was observed (Figure 3). Many areas of normal lung architecture were not discernible 96 hours after challenge due to marked suppurative inflammation and diffuse necrosis. When the sections stained with silver were examined, most of the alveolar basement membranes were disrupted. In addition, some of the bronchiolar walls had small disruptions in the basement membrane.

In group-B mice, the histologic scores were all significantly different from each other (17 ± 1.22, 19.8 ± 2.17, and 14.8 ± 0.44 at 24, 48, and 96 hours, respectively), with the pathologic changes being most severe at 48 hours and least severe at 96 hours after challenge. At 24 hours after challenge, there was a diffuse mild infiltrate of neutrophils in the alveolar spaces and in some of the bronchioles, with a few areas of necrosis. After 48 hours, the mice had developed multiple foci of suppurative bronchopneumonia. Neutrophils, macrophages, and variable amounts of proteinaceous fluid were evident in the bronchioles and adjacent alveolar spaces. Most of the bronchioles were partially or completely full of inflammatory exudate (Figure 4). The alveolar architecture appeared obscured on routine staining, although silver staining revealed minor disruptions in the basement membrane. A moderate amount of necrosis and fibrin deposition was observed. Less severely affected areas had increased numbers of neutrophils in the alveolar spaces, but lacked bronchiolar changes. At 96 hours, lesions were similar to those seen at 24 hours, but were less extensive.

In group-C mice that received the lowest challenge dose of *P. haemolytica*, the characteristic histologic feature was an in-

crease in cellularity in the cranial and middle regions of the left lung. At 24 hours, there were some areas of congestion and atelectasis, with a mild multifocal influx of neutrophils into the interstitium. After 48 hours, neutrophils were more numerous. There was diffuse thickening of the alveolar septa by infiltration of neutrophils and mononuclear cells, and plump alveolar epithelium (Figure 5). At 96 hours, the microscopic changes were similar to those seen at 48 hours, but the neutrophil influx was more extensive. The bronchi and bronchioles were not affected at this dose. The histologic scores at 24, 48, or 96 hours (10 ± 1.64, 9.8 ± 0.44, and 11.4 ± 1.67, respectively) were not significantly different from each other. When similar time points were evaluated among groups, the differences in histologic scores were found to be significantly dose dependent.

The spleen of all mice was similar and was typical of the morphology associated with the *scid* mouse genotype. The splenic follicles contained few lymphoid cells with extramedullary hematopoeisis and prominent stromal cells and macrophages. The liver and kidneys of the mice were unremarkable.

Discussion

Intranasal administration of 0.05 ml of *P. haemolytica* at a dose ≥10^9 CFU/ml to *scid*/*beige* mice induced gross and microscopic pulmonary lesions. The histologic findings were similar to those seen in cattle with pneumonic pasteurellosis, with evidence of suppurative bronchopneumonia, inflammation, proteinaceous fluid exudation, and fibrin deposition (35–37). To the authors' knowledge, this is the first report of suppurative and fibrinous bronchopneumonia being induced in mice by *P. haemolytica*, without physical manipulation the mice or enhancement of bacterial pathogenicity. The severity of the pulmonary lesions was dose dependent, and was affected by time at challenge concentrations of 10^9 and 10^{11} CFU/ml.

At a dose of 10^6 CFU of *P. haemolytica*/ml, the bronchi and bronchioles were not affected, but large numbers of neutrophils were seen in the interstitium and alveolar septae. Exposure to *P. haemolytica* has been documented to elicit rapid influx of neutrophils into the pulmonary tissue of calves and rats (5, 38–40), but with little damage to rat pulmonary epithelial cells (41). The microscopic changes in the lungs of mice that received saline intranasally were consistent with the effects of barbiturate anesthesia (42) and saline administration (41, 43).

Lesions were consistent in the cranial portion of the left lung, but were less consistent in the right cranial lobe. The inoculum was administered equally to left and right nostrils, and there was no effort to position the mice in left lateral recumbence for anesthesia recovery. The primary bronchus on the right side is situated more craniad than that on the left; the reason for the discrepancy in lung lesions is unclear.

Numerous mouse models for *P. haemolytica* infection are detailed in literature. Only two reports have outlined intranasal route of inoculation, both of which documented rapid clearance of the bacteria from the lungs, with minimal pulmonary injury (6, 8). Jian et al. (6) reported that moderate, but inconsistent, focal bronchopneumonia could be induced in young Swiss mice. The lungs of most mice were similar to those of mice exposed to PBS. A more severe and repeatable bronchopneumonia was induced only when *P. haemolytica* was delivered intranasally 30 minutes after previous inoculation with *Bordetella parapertussis*. In an earlier report, BALB/c mice were challenged intranasally with 10^8 CFU of *P. haemolytica*/ml under variable temperatures and relative humidity (8). At 24 hours after challenge, clearance of the bacteria from the lungs was rapid, but gross and microscopic pulmonary pathologic changes were not evaluated.

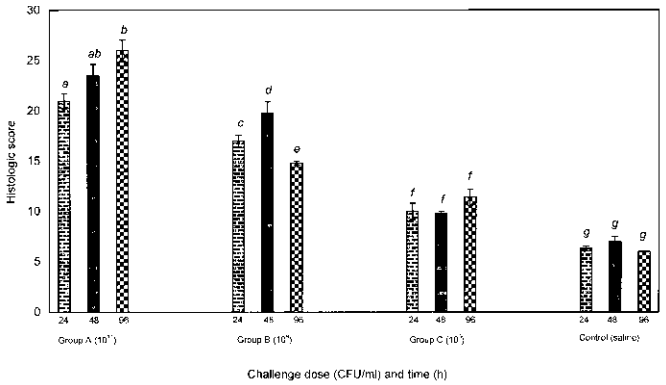

Figure 1. Mean ± SEM pulmonary histologic scores from *scid*/*beige* mice after intranasal challenge with *Pasteurella haemolytica*. Significant differences between histologic scores are indicated by different letters. Similar bar patterns represent comparison of histologic scores at similar times for each challenge dose. Significance was determined at $P < 0.05$.

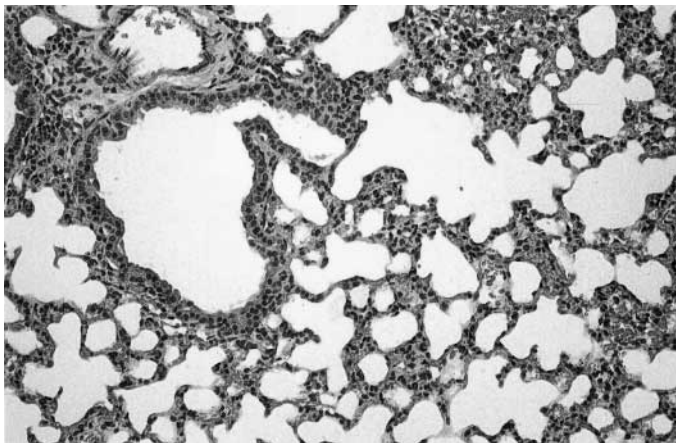

Figure 2. Photomicrograph of a section of left lung from a *scid*/*beige* mouse 48 hours after intranasal administration of saline. Notice few areas of focal congestion and atelectasis. H&E stain; magnification = 61x.

Most observations relating to experimentally induced infection of mice with *P. haemolytica* indicated challenge of the animals with aerosolized doses ≤ 10^8 CFU/ml. Aerosolization of *P. haemolytica* may render the organism fragile, and decrease its survivability through the effects of humidity and temperature (8, 44). Experimental evidence documenting histologic changes associated with challenge > 10^9 CFU/ml is sparse because most studies measured the rate of pulmonary clearance of *P. haemolytica* but did not evaluate pulmonary lesions (5, 11, 44, 45). Therefore, it was not clear whether higher concentrations of *P. haemolytica* would be sufficient to induce bronchopneumonia in immunocompetent mice. In pilot studies performed prior to the study reported here, BALB/C mice exposed to an intranasal dose of 0.05 ml of 10^{11} CFU of *P. haemolytica*/ml did not result in bronchopneumonia, whereas concentration ≥ 10^{10} CFU/ml

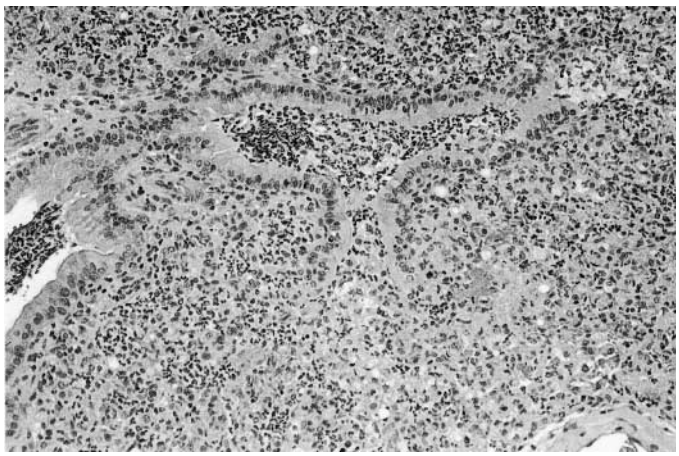

Figure 3. Photomicrograph of a section of left lung from a *scid*/*beige* mouse 48 hours after intranasal administration of 0.05 ml of a concentration of $3.3x10^{11}$ colony-forming units (CFU) of *P. haemolytica*/ml. Notice severe necrotizing bronchiolitis, bronchopneumonia, and fibrin deposition. H&E stain; magnification, 61x.

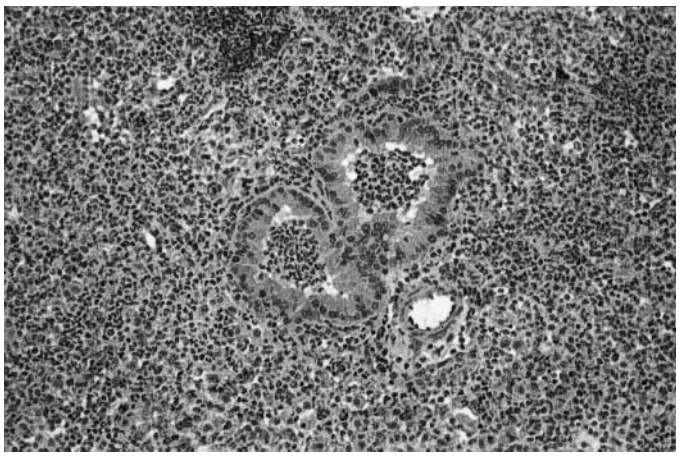

Figure 4. Photomicrograph of a section of left lung from a *scid*/*beige* mouse 48 hours after intranasal administration of 0.05 ml of a concentration of $3.4x10^{9}$ CFU of *P. haemolytica*/ml. Notice suppurative bronchopneumonia, as evidenced by many bronchioles containing an inflammatory exudate with numerous neutrophils in adjacent alveolar spaces. H&E stain; magnification, 61x.

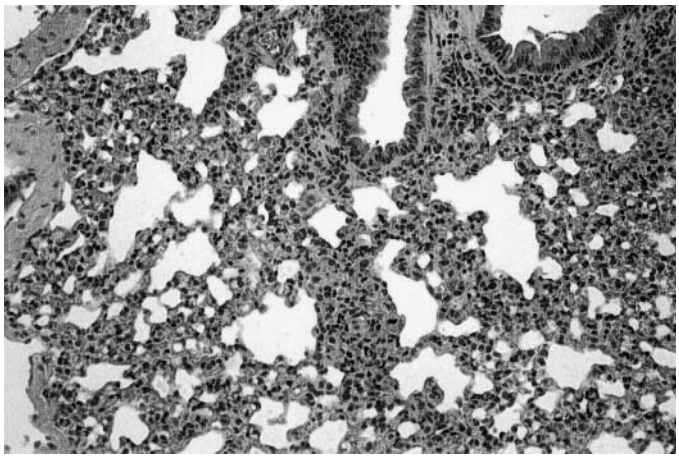

Figure 5. Photomicrograph of a section of left lung from a *scid*/*beige* mouse 48 hours after intranasal administration of 0.05 ml of a concentration of $2.8x10^{6}$ CFU of *P. haemolytica*/ml. Notice multifocal consolidation as indicated by collapse of alveolar walls and mild neutrophil influx. No bronchiolar changes were seen. H&E stain; magnification, 61x.

consistently induced bronchopneumonia in *scid*/*beige* mice. In the *scid*/*beige* mouse model, the lowest concentration that induced bronchopneumonia was 10^9 CFU/ml. Interestingly, in experimental models of *P. haemolytica* infection in the calf, the minimal challenge dose of *P. haemolytica* reported in literature that consistently results in bronchopneumonia is approximately 25 ml of a concentration of 10^9 CFU/ml (40, 46–49).

In the *scid*/*beige* mouse, *P. haemolytica* could be recovered consistently from the lungs 24 but not 48 or 96 hours after challenge. Pulmonary clearance of *P. haemolytica* from mouse lungs after following aerosol exposure has been documented to be efficient. Using adult white mice, Lillie and Thomson (7) observed that by 8 hours after aerosol challenge, 93% of the inhaled *P. haemolytica* had been cleared, and < 5% of the bacteria remained in the lung after 48 hours. Similar findings in mice have been reported by others (4, 5, 44, 45, 50).

Induction of bronchopneumonia in the Swiss Webster mouse was reported after intrabronchial challenge with *P. haemolytica* (12). Midcervical tracheostomy was performed, and the bacteria were delivered through an intratracheal catheter to the lung via a flexible plastic tube, similar to a method described in calves (51). However, in these mice, it was necessary to leave the surgical site open to prevent tracheal collapse until the mice were euthanized 24 hours after challenge. Bronchopneumonia developed in mice that received $\geq 5 \times 10^7$ CFU *P. haemolytica*/ml; however, ultrastructural studies revealed lack of cellular alterations in the neutrophils and macrophages of the affected mice. Development of bronchopneumonia may have been due to virulence factors other than those associated with host cell lysis or to secondary bacterial contamination.

A guinea pig model of pneumonic pasteurellosis has been reported (52). Intrabronchial inoculation with *P. haemolytica* induced fibrinous pleuropneumonia with prominent areas of necrosis, similar to the gross lesions seen in cattle. Microscopic examination of sections revealed infiltration of the pleura and alveoli by granulocytes, macrophages, and fibrin exudate within the alveoli. However, 61% of the guinea pigs developed septicemia.

Development of laboratory animal models of bovine pneumonic pasteurellosis has been hampered by the low pathogenicity of *P. haemolytica* for laboratory rodents (4, 9, 10). Intraperitoneal, aerogenic, and oral routes of *P. haemolytica* administration to mice have resulted in similar lack of pathogenic effect. Preferential pathogenicity for ruminants was documented by injection of *P. haemolytica* into the mammary glands of mice, rats, rabbits, sows, and cows; disease was induced exclusively in the cows (53). All clinical isolates of *P. haemolytica* secrete a soluble, heat-labile, protein leukotoxin during logarithmic growth phase (39, 54) that is specific for ruminant leukocytes (55, 56) and platelets (57). Intratracheal inoculation of *P. haemolytica* supernatant containing leukotoxin was likewise, not appreciably toxic to the rat lung, inducing inflammatory changes that were similar to those associated with saline inoculation (58). Virulence of *P. haemolytica* in murine models has been enhanced by use of gastric mucin, iron- chelating compounds, and dextran, or in combination with other pathogens (4,

6, 9–11). Despite lack of natural pathogenicity for the mouse, the murine model has been used in several studies to determine efficiency of *P. haemolytica* vaccines (8,10, 59).

The *bg* gene defect is selective for NK cell dysfunction, with no effect on the cytolytic function of granulocytes, macrophages, and T cells (60). Earlier reports suggested that *beige* mice were defective in the chemotactic and antimicrobial activities of granulocytes (61) and in T- cell cytolytic ability (62). In the *scid*/*beige* mouse, intranasal administration of *P. haemolytica* induced a strong chemotactic neutrophil response in the lungs. There was no evidence to suggest dysfunction in neutrophil chemotaxis in the *scid*/*beige* mouse model.

At 96 hours after challenge with 10^9 CFU/ml, the neutrophil influx and histologic changes were significantly less severe than those seen at 48 hours, suggesting that the lesions were starting to resolve. The influx of neutrophils into pulmonary tissues after exposure to *P. haemolytica* is thought to be responsible for efficient elimination of the bacteria from the lungs of mice (5). Resolution of bronchopneumonia requires an intact alveolar basement membrane, readily cleared exudate, and effective killing or clearance of the infective agent. Complete resolution is unlikely if there is necrosis of the alveolar wall, copious amounts of exudate, or persistence of the offending pathogen (35). The alveolar basement membrane was intact at 48 hours after challenge, and there was no recovery of *P. haemolytica* after culture of the lung homogenate. Clearance of bacteria from the lungs of the *scid*/*beige* mice appeared to be similar to that observed in immunocompetent mice, possibly suggesting that the neutrophils were functioning in effective manner. Mice of *scid* genotype are capable of producing cytokines via T lymphocyte-independent mechanisms (63), and similarly, NK cells from *beige* mice are capable of normal cytokine production (64). This suggests that cell-to-cell signaling and intercellular mediators are able to effect of neutrophil and macrophage chemotaxis in the lungs.

There are no reports of intranasal challenge of *P. haemolytica* by use of concentrations > 10^9 CFU/ml in immunocompetent mice indicating time- and dose-related effects on pulmonary lesions. In *scid*/*beige* mice, intranasal administration of *P. haemolytica* resulted in a dose- responsive fibrinous and suppurative bronchopneumonia histologically similar to that seen in affected cattle. Therefore, the *scid*/*beige* mouse model should prove useful for future studies of *P. haemolytica*-induced pneumonia. The potential exploitation of these mice for disease study includes xenogenic engraftment with lymphoid cells from immunocompetent animals. The *scid*/*beige* mouse provides a suitable biological environment in which the immune response may be manipulated by addition of various bovine cell types. These mice could prove useful for understanding the consequences resulting from the presence of various bovine cells in pulmonary infection with *P. haemolytica*.

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