Effect of Ovalbumin Aerosol Exposure on Colonization of the Porcine Upper Airway by Pasteurella multocida and Effect of Colonization on Subsequent Immune Function

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Seventy-three piglets were weaned at 1 week of age, randomly assigned to 10 groups (A to J), accommodated in stainless steel exposure chambers, and exposed continuously to a controlled environment containing aerosolized ovalbumin. The concentrations of ovalbumin dust were as follows (milligrams per cubic meter): A and F, 16.6; B and G, 8.4; C and H, 4.2; D and I, 2.1; E and J, 0. At weekly intervals, the pigs were bled via venipuncture and anesthetized for nasal lavage and tonsilar biopsies performed for subsequent bacteriologic analysis. At 2 weeks of age, the pigs in groups A to E were challenged with toxigenic Pasteurella multocida (10⁸ CFU pig⁻¹), and at 6 weeks of age, the pigs were euthanatized. At postmortem, the extent of turbinate atrophy was assessed on the snout sections by using a morphometric index. Exposure to aerial ovalbumin resulted in a dose-dependent increase in serum antiovalbumin immunoglobulin G (IgG; \( P < 0.001 \)) and serum antiovalbumin IgA (\( P < 0.001 \)). Exposure also caused a significant increase in the numbers of \( P. \) multocida organisms isolated from the upper respiratory tract (\( P < 0.001 \)) and a corresponding increase in turbinate atrophy, as judged by the morphometric index (\( P < 0.001 \)). Concurrent challenge with \( P. \) multocida and ovalbumin resulted in a significant decrease in both the IgG and IgA responses to ovalbumin (\( P < 0.001 \)). These results show that ovalbumin exposure increases pig susceptibility to \( P. \) multocida colonization and that toxigenic \( P. \) multocida modifies the serum IgG and IgA responses to ovalbumin in the pig. Both of these effects may enhance the virulence of this respiratory pathogen and so influence the pathogenesis of atrophic rhinitis in pigs.

Atrophic rhinitis is an upper respiratory tract disease of pigs characterized clinically by atrophy and degeneration of the bony and cartilaginous structures of the snout, in particular, the nasal turbinate and septum. In severe cases, this leads to visible shortening or twisting of the snout (12). In addition to these local effects, the disease is also associated with a decreased rate of weight gain, inefficient feed conversion, and increased time to market (6). The etiology and pathogenesis of atrophic rhinitis predisposes young pigs to pneumonia (9, 37). Experimentally, purified \( P. \) multocida toxin induces turbinate atrophy when aerosolized into the nasal cavity or injected into the subcutis, muscle, or peritoneum (6). The more severe, naturally occurring form of the disease is known as progressive atrophic rhinitis and is attributed specifically to colonization of the pig nasal cavity by toxigenic strains of \( P. \) multocida (25, 28, 31). However, the failure to reproduce the clinical disease consistently in experimental studies without depriving pigs of passive immunity, i.e., withholding colostrum (24), or pretreating the pigs’ nasal cavities prior to challenge with a chemical irritant such as acetic acid (26) supports the clinical impression that external factors, in particular, atmospheric pollutants, are necessary to the pathogenesis of progressive atrophic rhinitis as seen in commercial pig houses. We have previously established a clear synergistic role for gaseous ammonia in the etiology of \( P. \) multocida-induced atrophic rhinitis (18). We have also demonstrated that aerosolized ovalbumin predisposes young pigs to \( P. \) multocida colonization, which results in lesions typical of progressive atrophic rhinitis (16, 17).

The epidemiological study of Baekbo (2) supports this observation and shows that relatively small aerial dust concentration changes can significantly increase the incidence and severity of atrophic rhinitis.

The seminal observation that infection with microorganisms may decrease the immune response to a different pathogen was made in 1908 by von Pirquet, who showed that the tuberculin reaction, a typical expression of cell-mediated immunity, was depressed in patients with measles (35). This depressed reaction also applies to other clinical infections such as pertussis (4), typhus (38), scarlet fever (22), influenza (3), and brucellosis (1).

There are some data that suggest that toxin derived from \( P. \) multocida modulates the humoral immune response to atrophic rhinitis vaccine containing \( P. \) multocida toxoid (34). The objective of the present study was to explore the role of organic dusts in the etiology and pathogenesis of progressive atrophic rhinitis by observing (i) the effect of exposing pigs to an organic dust, ovalbumin, on their susceptibility to \( P. \) multocida colonization and (ii) whether the humoral immune response to aerial ovalbumin exposure was compromised by \( P. \) multocida colonization of the upper respiratory tract.

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MATERIALS AND METHODS

Animals. Seventy-three minimal-disease pigs were derived from Large White sows obtained from the Institute of Animal Health, Compton Laboratory, Newbury, England. The pigs were weaned onto a dry commercial ration (given twice daily) at 1 week of age and randomly assigned to 10 experimental groups (A to J). Before the experiment commenced, all of the pigs were screened for the presence of *P. multocida* by nasal lavage with 10 ml of phosphate-buffered saline (PBS) as described by Chantey and Rutter (6). The recovered fluid was cultured overnight at 37°C on 5% horse blood agar plates with added antibiotics (29).

Exposure chambers. All groups of pigs were housed in 1.4-m³ stainless steel Rochester exposure chambers built to the design of Timbrell et al. (32). The chambers were run at 25°C, giving an air change rate of 60 air changes per hour. Each exposure chamber via a HEPA filter and, after traveling through the chamber, was vented via a HEPA filter to prevent the release of biological material. Within the chamber, the air was maintained at a temperature of 25 ± 1°C and a relative humidity of 50 ± 5%.

Groups A to D were inoculated at 2 weeks of age with 10⁸ CFU of *P. multocida* by nasal lavage and without inoculation with *L. fermentum*. Groups E to F were inoculated for 10 days with five different concentrations of inhaled ovalbumin dust with and without inoculation. Groups A to E were inoculated at 2 weeks of age and inoculated with *L. fermentum*. Groups F to J were inoculated at 2 weeks of age and randomly assigned to 10 experimental groups (A to J). Before the experiment commenced, all of the pigs were screened for the presence of *P. multocida* by nasal lavage with 10 ml of phosphate-buffered saline (PBS) as described by Chantey and Rutter (6). The recovered fluid was cultured overnight at 37°C on 5% horse blood agar plates with added antibiotics (29).

Groups A to E were inoculated at 2 weeks of age with 10⁸ CFU of *P. multocida* by nasal lavage and without inoculation with *L. fermentum*. Groups F to J were inoculated for 10 days with five different concentrations of inhaled ovalbumin dust with and without inoculation. Groups A to E were inoculated at 2 weeks of age and inoculated with *L. fermentum*. Groups F to J were inoculated at 2 weeks of age and randomly assigned to 10 experimental groups (A to J). Before the experiment commenced, all of the pigs were screened for the presence of *P. multocida* by nasal lavage with 10 ml of phosphate-buffered saline (PBS) as described by Chantey and Rutter (6). The recovered fluid was cultured overnight at 37°C on 5% horse blood agar plates with added antibiotics (29).

Antibody detection. The antigen used for both ELISAs was grade V ovalbumin (Sigma), which was incubated overnight at 37°C with告诉她 about osteoarthritis (g). Finally, the alkaline phosphate substrate (1 mg ml⁻¹) was added. At this stage, all plates were left to incubate for approximately 10 min and read with a Multiskan Bichromatic plate reader (Molecular Devices, Ltd.) at 405 and 495 nm. The data were analyzed by using a computerized ELISA analysis package (ELISANALYSIS, release 5.53, J. H. Peterman, Birmingham, Ala.), and each sample serum was expressed as ELISA units (EU) by reference to a standard curve.

A positive IgG control and standard was produced in a pig injected at 3 weeks of age with grade V ovalbumin (Fremont incomplete adjuvant) and at 5 weeks of age with grade V ovalbumin (Fremont complete adjuvant). The serum from the pig was collected at 10 weeks of age. A positive IgA standard was raised by intramuscular inoculation of a lactating sow. Milk from the unweaned sow was collected, clarified by ultracentrifugation, and stored at −20°C. The IgG and IgA responses to ovalbumin exposure were calculated by subtracting the EU value at week 0 from the EU value at week 6 of the experiment.

**RESULTS**

Clinical signs. During the experiments, all animals retained a normal appetite and exhibited behavior consistent with good health. Clinical signs of disease were restricted to sporadic sneezing by some pigs in the groups inoculated with toxigenic *P. multocida*.

**MI and bacteriological findings.** There was a direct linear correlation between the MI and the cumulative number of *P. multocida* organisms isolated per group per chamber (linear regression; *R*² = 0.965, *P* < 0.001) (Fig. 1). There was no significant difference in the cumulative total number of *P. multocida* organisms isolated from the nasal cavity between groups B, C, and D and groups E (the group that was inoculated with *P. multocida* but not exposed to ovalbumin dust) (GLM). However, Fig. 1 does suggest a tendency for both the cumulative numbers of *P. multocida* organisms and the MI to increase with the concentration of ovalbumin dust (from groups E to A) and fits a quadratic equation (Pearson correlation coefficient, *R*² = 0.921). This is further illustrated by Fig. 2. The increase in the cumulative numbers of *P. multocida* organisms isolated from the nasal cavity was significantly greater in group A than in groups B, C, and D (0.001). Furthermore, a significantly greater cumulative number of *P. multocida* organisms was isolated in group E from both the nasal cavity (linear regression; *R*² = 0.645 [P < 0.001]) and the tonsil (quadratic regression, *y* = 0.010*x*² – 0.065*x* + 0.665; *R*² = 0.963 [P < 0.001]) compared to the dust concentration. Groups F to J (pigs not challenged with *P. multocida*) had a mean MI of 48.4% (standard deviation [SD], ±4.43%), which was sig-
significantly lower than the MIs of all of the corresponding groups challenged with *P. multocida*, which ranged from 54.63% (SD, 6.2.43%) in group E to 74.23% (SD, 6.2.12%) in group A (*P*, 0.001 [GLM]).

**Serum antibody.** All pigs exposed to ovalbumin dust in the absence of *P. multocida* showed a significant increase in the level of serum IgG (*P*, 0.001 [GLM]) and IgA (*P*, 0.001 [GLM]) to ovalbumin at the end of the experiment (Fig. 3 and 4), and this response was dose dependent (*R*^2^ = 0.858, *P* < 0.001 [RA], and *R*^2^ = 0.539, *P* < 0.001 [RA], respectively). Pigs infected with *P. multocida* and concurrently exposed to ovalbumin showed significantly lower (effectively no) IgG and IgA responses to ovalbumin (*P* < 0.001 [GLM]) compared to exposure to ovalbumin alone (Fig. 3 and 4). Levels of antiovalbumin IgG and IgA in pigs exposed to *P. multocida* plus ovalbumin (groups A to D) were not significantly higher than in pigs exposed to neither (group E; GLM) (Fig. 3 and 4).

**DISCUSSION**

Pigs reared in intensive production systems are continuously exposed to dusts which are principally organic in nature and originate largely from the animals’ feed and integument. Epidemiological studies have demonstrated a link between dust
exposure and nasal turbinate scores (2, 27). Ovalbumin is an organic dust, novel to the pigs in this study, which can be mixed to a specific range of particle sizes; hence, it has been used in inhalation studies (15). The aerial dust concentrations of 2.1 to 16.6 mg m\(^{-3}\) used in this study cover the upper range of dust concentrations measured in commercial piggeries (5).

In this study, the pigs were presented with the antigen (ovalbumin) in the form of a respirable aerosol which will have come into contact with the upper respiratory tract, conjunctiva, and mucosa of the gastrointestinal tract. The upper respiratory tract has been shown to be an important site for the deposition of organic dust, which has a profound influence on the colonization kinetics of \(P.\) multocida (17). Ovalbumin was used in this study to replicate the predominately organic nature of dusts in swine confinement buildings. Furthermore, it is an organic food substance antigenically distinct from pig feed and available as a sterile powder which is easily milled to a size suitable for inhalation. The data presented in this study demonstrate that ovalbumin dust at the maximal exposure concentration (16.6 mg m\(^{-3}\)) increased the pigs’ susceptibility to \(P.\) multocida colonization of the upper respiratory tract at both the tonsil and nasal cavity \((P < 0.001)\) (Fig. 2). This concentration gave the highest yield of \(P.\) multocida from both sites in the upper respiratory tract and resulted in the severest turbinate damage (Fig. 1). This implies increased susceptibility to \(P.\) multocida colonization. Potentially, this could be achieved by one or more mechanisms, e.g., (i) modulation of the local immune system, lowering its effectiveness against the bacteria; (ii) disruption of the integrity of the host’s mucosal surfaces; (iii) alteration of the competing commensal flora; or (iv) a direct effect on the viability and/or growth of the pathogen.

The extent of colonization of the tonsil with \(P.\) multocida was proportional to the extent of turbinate damage \((R^2 = 0.968,\) Fig. 1). Hamilton et al. (18) observed a similar effect when studying interactions between \(P.\) multocida and inhaled ammon. There is a significant increase in the number of \(P.\) multocida organisms isolated from the nasal cavity \((R^2 = 0.921,\) Fig. 1), which has a quadratic relationship with the MI. However, because the numbers of \(P.\) multocida organisms isolated are expressed as CFU per millilitre of recovered fluid from the nasal lavage, it is not possible to calculate the concentration of \(P.\) multocida in the recovered mucus alone. Therefore, it is possible that the relationship between the number of \(P.\) multocida organisms isolated from the nasal lavage and the MI is influenced by the unmeasured proportion of mucus recovered in the PBS lavage. It is not clear which is the more important site (tonsil or nasal cavity) for toxin absorption, but previous studies suggest that local absorption of toxin may be indirectly mediated through unidentified products of immune cells in the upper respiratory tract mucosa (14, 26). Therefore, both sites may have equal importance in the absorption of the toxin but previous experimental observations suggest that the toxin is cytotoxic to lymphocytes, which renders the pig less able to initiate an effective response to foreign antigens, which may include invading bacteria on the mucosa of the respiratory tract.

Modulation of the immune system with \(P.\) multocida has also been observed in an epidemiological study which looked at the isolation of \(B.\) bronchiseptica and \(P.\) multocida from the nasal cavities of piglets (13). This study found that total immunoglobulin concentrations in serum (IgG, IgA, and IgM) decreased in proportion to an increase in the concentration of \(P.\) multocida isolated per piglet (CFU per piglet) over a 6-week period. The authors did not comment on this observation. It is, however, consistent with our own observations (Fig. 3 and 4).

Experimental studies on gastrotoxic \(P.\) multocida have shown that crude \(P.\) multocida toxin is a poor immunogen but that when the toxin is made inactive (toxoid), it becomes a potent vaccine (6). This implies that active immunity is seen in pigs when the toxin is given in its biologically inactive state (by intranasal vaccination) but not its biologically active state (by natural infection). A recent study (34) found that a commercially available \(P.\) multocida vaccine imparted protection against atrophic rhinitis with a subsequent rise in antibodies to \(P.\) multocida. However, when animals were vaccinated simultaneously with a \(P.\) multocida toxin challenge, no detectable systemic humoral or cellular response to \(P.\) multocida toxin was found. Challenge with active \(P.\) multocida toxin before vaccination appeared to slow down the immune responses initiated by the toxoid in the vaccine; when vaccination and challenge were simultaneous, the immune response was abolished.

The findings of the current study show that colonization of the upper respiratory tract with toxigenic \(P.\) multocida (and consequent exposure to \(P.\) multocida) immunologic pneumonia can occur as early as 2 to 8 weeks of age (19). This systemic modulation of the immune system may be an important (and as yet undocumented) virulence factor in this complex multifactorial disease. Indeed, this effect may have more far-reaching implications for other opportunistic bacterial infections in pigs and other farm animals intensively housed in polluted environments. Progressive atrophic rhinitis can severely impair growth rate and food conversion efficiency in commercial pig herds (6, 33). However, it is not clear why such a localized condition can have such a prolonged systemic effect. One possibility is that it exacerbates the effects of concurrent or subsequent infections, such as enzootic pneumonia. Atrophic rhinitis and enzootic pneumonia (mycoplasma-induced respiratory disease complex) are clearly quite distinct conditions, and the majority scientific opinion is that there is no mechanistic or epidemiological relationship between the two (30). However, Cowart et al. (9) found that in individual pigs, an increasing atrophic rhinitis score was related to an increasing pneumonia score at 8 weeks but not at 6 months of age. This finding is consistent with the study by Chanter and Rutter (6) of the colonization kinetics of toxigenic \(P.\) multocida following experimental inoculation. This showed that \(P.\) multocida colonized the nasal cavity in large numbers at 3 weeks of age but fell to below 10% at 2 months of age. Thus, \(P.\) multocida-induced immune modulation may occur early in life and wane when the pig ages. Clinical evidence indicates that enzootic pneumonia can occur as early as 2 to 8 weeks of age (19). This would coincide with the time of maximal immunosuppres-
sion due to *P. multocida* although not with the time the effects of turbinate atrophy are most apparent.

In conclusion, exposure of young pigs to ovalbumin predisposed the nasal cavity and the tonsils to colonization by *P. multocida* in a dose-dependent manner. Colonization of the upper respiratory tract with *P. multocida* was directly proportional to the severity of turbinate atrophy, as judged by the MI. Challenge with *P. multocida* profoundly suppressed the humoral immune response induced by chronic exposure to ovalbumin dust. On the basis of these observations, we propose a new hypothesis which may partly explain the roles of *P. multocida* and respirable dusts in the etiology and pathogenesis of ill thrift due to endemic respiratory diseases in housed pigs. Organic dusts predispose the upper respiratory tract to colonization with *P. multocida*. Colonization with *P. multocida* induces immunomodulation, which may not only increase the severity of atrophic rhinitis but also predispose the pig to enzootic pneumonia and similar, more chronically debilitating infections of the lower respiratory tract.

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