Rabbit pasteurellosis: Respiratory and renal pathology of control and immunized rabbits after challenge with Pasteurella multocida

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Summary. Gross and microscopic lesions of pasteurellosis were studied in control and immunized pasteurella-free rabbits after challenge with virulent Pasteurella multocida. Pathologic responses were compared in rabbits immunized intravenously or mucosally with P. multocida or with J5, a cross protective core LPS mutant of E. coli. All rabbits were challenged conjunctivally with approximately 2xL50 of P. multocida. Rabbits were necropsied and examined for histopathology of the respiratory tract and kidneys. Lung lesions varied in severity depending on the duration of the disease, the route of vaccination, and the vaccine used. The most severe lung lesions occurred in rabbits vaccinated intravenously with P. multocida and challenged with the same strain. Some of these rabbits had purulent bronchopneumonia and pleuropneumonia. Lung lesions were absent or less severe in rabbits vaccinated by a mucosal (aerosol, conjunctival) route and in unvaccinated controls. In these animals there was no bronchopneumonia or pleuropneumonia, and bronchiolitis, if present, was less severe. Kidney lesions were found only in rabbits vaccinated intravenously. There was an interstitial nephritis, some collagen deposition, mononuclear cell infiltration, and a loss of tubular architecture in the cortex. Some glomeruli were affected.

These results indicate that intravenous immunization contributes to the formation of lesions whereas mucosal immunization prevented lesion formation to some degree.

Key words: Pasteurella multocida, Rabbit, Pneumonic Pasteurellosis, Immunization

Introduction

Respiratory diseases are common in domesticated rabbits. This may vary from a mild, chronic mucopurulent upper respiratory infection (snuffles) to more acute or subacute bronchopneumonia leading to high mortality (Hagen, 1958). Epizootics and enzootics of acute fatal pneumonia have been reported (Alexander et al., 1952; Hagen, 1959; Flatt and Dungworth, 1971).

It has been reported that Pasteurella multocida frequently causes either acute bronchopneumonia in the cranio-ventral portion of the lungs of rabbits dying of pneumonia or chronic purulent to fibrinopurulent bronchopneumonia (Weisbroth, 1979).

This study is part of a project to compare the effectiveness of vaccines administered intravenously, by aerosol, and in the conjunctival sac. This includes the gross and microscopic changes in lungs and kidneys of control and immunized rabbits after challenge with Pasteurella multocida.

Materials and methods

Rabbits

Ninety-six male Pasteurella free (Pf) rabbits (Dutchland Laboratories, Inc., Denver, PA), 8 to 9 weeks of age and weighing 1.5-2.5 kg, were used for vaccination and challenge as has been described (Al-Lebban et al., 1988). Briefly, 58 Pf rabbits were vaccinated intravenously with boiled cells (at 22% transmission) of P. multocida 1059, P. multocida 1062, E. coli J5, or sterile saline solution. The rabbits were given six I.V. injections, 1.0 ml/INjection, at 2-day intervals. Two routes were used for mucosal vaccination with P. multocida 1062, E. coli J5, or sterile saline. Aerosols were created by an ultrasonic nebulizer (Ultramist III, Macrosound Corp, Rahway, NJ) with approximately 3 ml of aerosolized vaccine exposure and given to 18 rabbits. Each rabbit was exposed for 15 minutes, 3 times,
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at 5-day intervals. Conjunctival vaccination (20 rabbits) was done by dropping 0.1 ml of the vaccine into both conjunctival sacs six times at 2-day intervals. Twenty-one days after the first vaccination, all rabbits were challenged by placing 0.1 ml of a suspension of *P. multocida* (containing approximately 2x the LD₅₀) into the conjunctival sac. Additional groups of four rabbits immunized intravenously and mucosally with *P. multocida*, *E. coli J5*, and saline were necropsied without being challenged.

Necropsy Examination

All rabbits were necropsied; sections of trachea, lungs, and kidney no thicker than 0.5 cm were taken and fixed in 10% buffered neutral formalin. The tissues were dehydrated through graded ethanols, cleared in xylene, and infiltrated with embedding paraffin in a routine automated processor cycle (Autotechnicon, Technicon Corporation, Chauncey, N.Y.), cut at 6 μm, mounted on glass slides, stained by hematoxylin and eosin (H & E) using an automated slide stainer (Histotech, Ames Company, Div. Miles Laboratories, Inc., Elkhart, IN.), and covered with glass coverslips. The periodic acid Schiff (PAS) stain on kidney tissues was done by hand (Luna, 1968).

Results

Macroscopic Findings

Trachea: There were few abnormalities except for a diffusely hyperemic mucosa in rabbits that died 12 to 144 hours post-challenge. Survivors that were necropsied 180 hours post-challenge had no tracheal lesions or patches of hyperemic mucosa.

Lungs: The severity of lung lesions varied depending on how soon the rabbit died after challenge, the route of vaccination and the type of antigen used. Rabbits that were vaccinated but not challenged had no lung lesions. Rabbits that died 12-24 hours post-challenge generally had few lesions. The lungs from rabbits that died latter had either no lesions, various degrees of hyperemia, or multifocal to diffuse areas of consolidation of the apical and cardiac lobes. Survivors that were necropsied 180 hours post-challenge had more severe lung lesions. The lesions were most severe in rabbits vaccinated intravenously. Lesions were less severe in rabbits vaccinated by conjunctival and aerosol routes and in unvaccinated rabbits.

Severe lung lesions varied from bilateral diffuse fibrinous pleuritis to consolidated areas of lung that were firm and varied from red to grey or black. Cross-sections through such altered lobes were alternately dark red to greywhite. Some survivors had no changes, small red or grey areas, or slightly enlarged lungs.

Kidneys: Macroscopic changes in the kidney were found in challenged and unchallenged rabbits vaccinated by the intravenous route. There were scattered dark red foci on the outer surfaces of the kidney that varied in size and number. These lesions were the most frequent and numerous in rabbits vaccinated with *E. coli J5*.

Microscopic findings

Trachea: There were no marked differences in lesions of the trachea with respect to route of immunization, antigen used, or saline. Tracheal lesions in rabbits that died during the first 24 hours post-challenge varied from no microscopic change to a severely congested mucosa. Most rabbits that died later or were killed 180 hours post-challenge developed an inflammatory response that varied from a mild to a moderate catarrhal inflammation. In some, cuboidal epithelial cells had detached while the basal layer remained intact, and there was some accumulation of mucus and heterophils in the lumen. Tracheal blood vessels of rabbits that died after challenge were moderately to severely congested. Tracheal lesions varied in rabbits that survived one week post-challenge. In some, there were no marked changes, and in others, there was severe catarrhal inflammation with desquamation of the epithelial lining, and the lumen was filled with heterophils and mucus.

Fig. 1. Lung of a PF-rabbit vaccinated intravenously with *P. multocida* 1062 and sacrificed 7 days after challenge with *P. multocida* 1062. The alveoli are filled with heterophils, few mononuclear cells and fibrin. Hematoxylin-eosin stain. × 313
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Table 1. A summary of the histopathology of the respiratory tract of Pasteurella-free New Zealand white rabbits challenged with 2 x LD_{50} of two strains of Pasteurella multocida following vaccination with P. multocida or Escherichia coli J5.

<table>
<thead>
<tr>
<th>Route of Vaccination</th>
<th>Antigen</th>
<th>No. of Rabbits</th>
<th>Number of Rabbits with lesions</th>
<th>Interstitial Pneumonitis</th>
<th>Broncho-Pneumonia</th>
<th>Pleuro-Pneumonia</th>
<th>Bronchiolitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M/M/\textsuperscript{a}</td>
<td>S/\textsuperscript{b}</td>
<td>M/M</td>
<td>S</td>
</tr>
<tr>
<td>Intravenous</td>
<td>P. multocida</td>
<td>2\textsuperscript{c}</td>
<td>0 0 0 0 1 0 0 0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
<td>1 0</td>
</tr>
<tr>
<td></td>
<td>homologous</td>
<td>13\textsuperscript{d}</td>
<td>4 2 4 1 2 3 5 6</td>
<td>4 0</td>
<td>0 0</td>
<td>5 0</td>
<td>0 0</td>
</tr>
<tr>
<td></td>
<td>heterologous</td>
<td>8\textsuperscript{e}</td>
<td>4 0 0 0 0 0 5 0</td>
<td>4 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
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<tr>
<td></td>
<td>E. coli</td>
<td>10\textsuperscript{a}</td>
<td>5 0 0 0 0 0 0 0</td>
<td>5 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>14\textsuperscript{b}</td>
<td>4 0 0 0 0 0 7 0</td>
<td>4 0</td>
<td>0 0</td>
<td>0 0</td>
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<tr>
<td></td>
<td></td>
<td>1\textsuperscript{f}</td>
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<td>0 0</td>
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<tr>
<td>Mucosal</td>
<td>P. multocida</td>
<td>8\textsuperscript{g}</td>
<td>7 0 0 0 0 0 4 0</td>
<td>6 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td></td>
<td>homologous</td>
<td>7\textsuperscript{h}</td>
<td>6 0 0 0 0 0 1 0</td>
<td>6 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td></td>
<td>E. coli J5</td>
<td>7\textsuperscript{i}</td>
<td>5 0 0 0 0 0 3 0</td>
<td>8 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>8\textsuperscript{j}</td>
<td>2 0 0 0 0 0 4 0</td>
<td>8 0</td>
<td>0 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
</tbody>
</table>

a. Moderate to marked
b. Slight
c. Death occurred < 96 hours post inoculation
d. Euthanatized 180 hours post inoculation.

Table 2. Incidence of renal pathology according to route of vaccination

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Route</th>
<th>Rabbits with renal pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive/Total</td>
<td>Percent Positive</td>
</tr>
<tr>
<td>None</td>
<td>0/23</td>
<td>0.00</td>
</tr>
<tr>
<td>Pasteurella multocida</td>
<td>11/28</td>
<td>39.3</td>
</tr>
<tr>
<td>Intravenous</td>
<td>0/15</td>
<td>0.00</td>
</tr>
<tr>
<td>Mucosal</td>
<td>8/15</td>
<td>53.3</td>
</tr>
<tr>
<td>E. coli J5</td>
<td>0/15</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Lungs: The major lung lesions were interstitial pneumonia, bronchopneumonia, pleuropneumonia, and bronchiolitis. The frequency and severity of these lesions in the various groups is shown in Table 1.

Rabbits that died during the first 24 hours post-challenge had few microscopic lesions in the epithelial lining of the bronchioles or terminal bronchioles. There was some mucus in the lumen. The alveolar wall was normal or slightly thickened and infiltrated with heterophils. Alveolar spaces were clear or filled with serous fluid, and the blood vessels were congested.

Lung lesions in rabbits killed 180 hours post-challenge were variable, and severity was correlated with the route of vaccination and the antigen used.

Rabbits vaccinated intravenously developed lung lesions of variable severity. The condition of the bronchioles ranged from relatively normal to severe epithelial desquamation that included the basal layer cells. The lumen of the bronchioles in these rabbits contained copious amounts of fibrin, heterophils, and noncellular debris. The terminal bronchioles generally had the same response as the bronchioles, but were more frequently affected. The alveolar walls were thickened in rabbits of most groups with a marked increase in severity.
Fig. 2. Lung of a Pr-rabbit vaccinated intravenously with *P. multocida* 1059. The alveoli contain many heterophils, red blood cells, fibrin and edema. Hematoxylin-eosin stain (A) x 112 and (B) x 313.

Fig. 3. Lung of a Pr-rabbit vaccinated intravenously with *P. multocida* 1059 and sacrificed 7 days after challenge with *P. multocida* 1059. The alveoli are filled with heterophils, proteinaceous material, fibrin, and edema. Hematoxylin-eosin stain (A) x 112 and (B) x 313.
Fig. 4. Fibrinous pleuropneumonia of a Pf-rabbit vaccinated intravenously with *P. multocida* 1059, and sacrificed 7 days after challenge with *P. multocida* 1059. The pleura is thickened and there is extensive margination of the heterophils. Hematoxylin-eosin stain. × 112

Fig. 5. The kidney of Pf-rabbit vaccinated intravenously with *P. multocida* 1062 and challenged with *P. multocida* 1062. There is a focal interstitial nephritis and mononuclear cell infiltration. Hematoxylin-eosin stain. × 112

Fig. 6. The kidney of a Pf-rabbit vaccinated intravenously with *E. coli* J5 and challenged with *P. multocida* 1059. There is a diffuse interstitial nephritis and infiltration of mononuclear cells. Periodic Acid Schiff Stain (A) × 112 and (B) × 313
in rabbits vaccinated intravenously and challenged with the autologous strain. The alveoli were filled with heterophils. Edema and fibrin were prominent (Fig. 1). The alveolar space of these rabbits was either clear or filled with heterophils, fibrin and noncellular debris (Fig. 2). Some rabbits developed hemorrhagic lesions in which the interstitial tissue and the alveolar spaces were filled with erythrocytes (Fig. 3). It was striking that bronchopneumonia occurred only in intravenously immunized animals.

The pleura varied in response to challenge. In some rabbits the pleura was relatively normal; in others the pleura was thickened and infiltrated with fibrin, heterophils, a few mononuclear cells, and bacterial colonies (Fig. 4). These severe changes were seen only in rabbits vaccinated intravenously with *P. multocida* and challenged with the autologous strain.

Rabbits vaccinated by the aerosol or conjunctival route developed an interstitial pneumonia of variable severity. None of the rabbits in these two experiments developed a fibrinous pneumonia, and the pleura was unaffected.

**Kidney.** Kidney lesions were restricted to challenged or unchallenged rabbits vaccinated intravenously. Other rabbits in this study that were vaccinated by aerosol and conjunctival routes including the controls did not develop kidney pathology (Table 2). Multifocal interstitial nephritis with mononuclear cell infiltration and fibroblast proliferation was frequently observed (Fig. 5). In some kidneys, diffuse interstitial nephritis with collagen deposition was present (Fig. 6). The lesions were prominent in the cortex with lesser lesions found in the medulla. In affected glomeruli, there was a loss of glomerular architecture, debris in Bowman’s space, and a mononuclear cell infiltrate. Heterophils were absent from most lesions. The epithelium of the proximal convoluted tubule had retracted from the basement membrane, and there was a loss of tubular architecture. In some there was a segmental loss of the brush border, and in others there was mononuclear cell infiltration. Lesions in the distal convoluted tubules were generally the same as those in the proximal tubules. The tubular epithelial nuclei were hyperchromatic. Two rabbits had areas of mononuclear and heterophil aggregation in the collecting tubules.

**Discussion**

It is clear from the results presented above that lesions were correlated with the route of immunization and the antigen as well as the time of death post challenge.

The most severe lung lesions were found in animals vaccinated intravenously with *P. multocida* and sacrificed one week following challenge, probably because there was time for lesions to develop. It was interesting that the rabbits protected by immunization were the ones that developed the most severe pneumonia. This points out the paradox of the positive and negative sides of the systemic immune response. Bacteremia and death were prevented in most of these animals (Al-Lebban et al., 1988). In that sense, the inflammatory response in the lung, presumably mediated by systemic immunity, was protective. But it was at the cost of considerable damage to the host in the form of moderate to severe pneumonia. This is comparable to Markham & Wilkie’s findings of more severe pneumonia in vaccinated calves than in controls (Markham and Wilkie, 1980a). They suggested that since immunized serum enhances phagocytosis of *P. hemolytica* in challenged animals (Markham and Wilkie, 1980b) and that since *P. hemolytica* is cytotoxic for alveolar macrophages, the destruction of macrophages and release of enzymes in immunized animals may account for the more severe pneumonia in this group (Kachler et al., 1980). Similar phenomena may be occurring in systemically immunized rabbits in our study. Immune complex deposition could be involved also as suggested by Wilkie et al. (1974). Physiologically, the predominance of IgG antibody at the alveolar level does not hold in the upper Airways, where IgA prevails (Bienenstock, 1985).

Most natural immune complex-mediated intra-alveolar injury is likely to be induced by IgG complexes or those of both IgG and IgA. Also, it is well established that IgG immune complex injury requires an intact complement system (Henson and Cochrane, 1971; Johnson and Ward, 1974; Larsen et al., 1981), and the role of complement appears to relate to the intrapulmonary activation of complement and subsequent immigration of heterophils into the site. Recent evidence suggests that IgG immune complex-induced lung injury is dependent on neutrophils and the production of toxic oxygen metabolites (Johnson and Ward, 1981; McCormick et al., 1981; Morganroth, 1986). This phenomenon may also occur in systemically immunized rabbits in our study: where their serum IgG titers are high, they have the most severe pneumonia, and the affected areas of the lungs are infiltrated almost entirely with heterophils.

The picture seems quite different in mucosally immunized animals, however. Protection against lethal bacteremia with these very invasive strains of *P. multocida* was not as good as with homologous *P. multocida* systemic immunization. Even so, protected animals did not have severe or even moderate bronchopneumonia or fibrinous pneumonia. Bronchiolitis, if present, was relatively mild. This may be due to the higher IgA/IgG ratio produced in bronchoalveolar washes of the rabbits as compared with serum IgA/IgG ratio (Wilkie et al., 1980). Mucosal immunization would be expected to produce more IgA antibodies than would systemic immunization.

It has been suggested that IgA may serve to prevent antigen adherence to mucosal surfaces (Williams and Ribbons, 1972), is a more efficient agglutinator than IgG (Reynolds and Thompson, 1973), and is bactericidal in the presence of complement and lysozymes (Newhouse et al., 1976), and is much less phlogistic than the IgG and IgM classes, which are more profound complement activators (Lamm, 1976). This low phlogistic potential of the IgA class is an important factor for protection of mucous membranes, where antigen-antibody reactions along the lining of the respiratory tract are continuous occurrences. Any or all of these factors could have been
important in reducing the severity of the pathology in those groups vaccinated by aerosol or into the conjunctival sac. Protection may also be related to cell-mediated immunity and/or IgA initiation of adherence resulting in less colonization.

The lower incidence of pneumonia in animals systematically immunized with J5 than in those systemically immunized with P. multocida is of interest. It may be that lower antibody titers with cross reacting antigen produce less opsonization and fewer immune complexes.

As renal lesions were observed only in rabbits that were vaccinated intravenously, the pathology may have been caused by endotoxic damage or immunologic injury. Endotoxins administered intravenously in rabbits are known to produce severe disseminated intravascular coagulation (DIC) that culminates in bilateral renal cortical necrosis (Thomas and Good, 1952; Lee, 1963). Most rabbits die as a consequence of the severe kidney damage. In the typical Schwartzman reaction, there are widespread occlusions of the glomerular capillaries by dense fibrin deposits. Although some of the rabbits vaccinated intravenously did have abnormal glomeruli, the lesions were not seen throughout the cortex. Although we did not see severe generalized Schwartzman reactions, there may have been some «Schwartzman-like» damage after vaccination. Affected rabbits were necropsied 10 to 17 days following the last vaccination and 20 to 27 days following the second intravenous injection of bacterial antigen. Since Schwartzman reactions are usually demonstrated 24 hours after the second injection of endotoxin, the chronic inflammation we observed may represent an attempt to repair the initial damage. The intravenous injections of an antigen into an animal with a humoral antibody response can result in glomerulo-nephritis due to deposition of immune complexes in the glomeruli. Unanue and Dixon suggested that immune complexes may be involved in the pathogenesis of any nephritis induced by repeated injection of antigenic materials (Unanue and Dixon, 1963). The continuous formation of complexes between injected antigens and host antibody might lead to renal injury. As renal lesions were observed in both challenged and unchallenged rabbits vaccinated intravenously, it is possible that the initial damage was produced by immune complex deposition. This possibility needs to be confirmed by additional tests for the presence of immune-complexes.

In conclusion, it is clear that although intravenous immunization provided the highest serum antibody titers and the best protection against bacteremia, it also produced the greatest immunopathology in the lung and kidney. Protection by mucosal immunization is much less costly in terms of immune damages to the host.

Acknowledgements. The authors would like to thank Drs. Erby Wilkinson and Doris Gove for their editorial work; Mr. Kreis Weigel and Mr. Brian Rice for preparing the micrographs; and Ms. Sheila Manis and Ms. Betsy Cagle for their secretarial assistance.

This study was supported in part by Animal Health and Disease Formula Funds; contribution No. 82-513-J from Kansas Agricultural Experimental Station.

References


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Accepted August 12, 1988