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Review

Response of the ruminant respiratory tract to *Mannheimia (Pasteurella) haemolytica*

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ABSTRACT – Pneumonia is a leading cause of loss to the sheep and cattle industry throughout the world. *Mannheimia (Pasteurella) haemolytica* is one of the most important respiratory pathogens of domestic ruminants and causes serious outbreaks of acute pneumonia in neonatal, weaned and growing lambs, calves, and goats. *M. haemolytica* is also an important cause of pneumonia in adult animals. Transportation, viral infections with agents such as infectious bovine rhinotracheitis virus, parainfluenza-3 virus or bovine respiratory syncytial virus, overcrowding, housing of neonates and weaned animals together and other stressful conditions predispose animals to *M. haemolytica* infection [1, 2]. This review assimilates some of the findings key to cellular and molecular responses of the lung from a pathologist’s perspective. It includes some of what is known and underscores areas that are not fully understood. © 2000 Éditions scientifiques et médicales Elsevier SAS

antimicrobial peptides / inflammation / innate immunity / pneumonia / pulmonary pasteurellosis

1. Innate immunity

*Mannheimia haemolytica* colonizes the mucosa of the upper respiratory tract and nasopharynx. By mechanisms poorly understood, *M. haemolytica* breeches the innate mucosal defense, including the mucociliary apparatus and antimicrobial factors to establish infection in the lung. Acute pulmonary infections caused by *M. haemolytica* are characterized by a peracute to acute fibrinosuppurative and necrotizing inflammatory response. Within hours after infection, the lung bronchi, bronchioles and alveoli contain dense infiltrates of neutrophils, fibrin, seroproteinaeous fluid and blood (figure 1). The exudate is associated with extensive parenchymal necrosis that is caused by *M. haemolytica* products such as leukotoxin, lipopolysaccharide and polysaccharide and also inflammatory factors released by neutrophils and other cells of the acute inflammatory process. The contribution of the neutrophil to parenchymal damage in bovine pulmonary pasteurellosis was demonstrated by a study in which calves depleted of neutrophils and inoculated with *M. haemolytica* had reduced parenchymal damage when compared with calves with normal levels of neutrophils [3]. Neutrophil constituents that potentially contribute to the tissue dam-

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Respiratory epithelial cell production of at least two antimicrobial peptides is increased during *M. haemolytica* pneumonia in cattle. These are the $\beta$-defensins termed tracheal antimicrobial peptide (TAP) [6] and lingual antimicrobial peptide (LAP) [7]. Both TAP and LAP have potent antibacterial activity; TAP also incites the degranulation of mast cells. The inducible form of nitric oxide synthase is produced at a high level in non-infamed bronchial and bronchiolar epithelial cells, but the level of its production by these cells decreases shortly after *M. haemolytica* infection [8]. There are a number of other epithelial cells in lung that likely also have a role in *M. haemolytica* pneumonia. Goblet cells and submucosal gland cells contribute secretion products to mucociliary layer and the inflammatory response. Epithelial cells (Clara cells) expressing mixed function oxidases for the detoxification of noxious compounds may initiate localized areas of inflammation; however, little is known of the responses of such specialized cells during *M. haemolytica* pneumonia.

The ruminant lung has anatomic features which limit its ability to resolve pneumatic episodes. Lungs of ruminants have relatively few pores of Kohn between alveolar units, which reduces the degree of collateral ventilation. This prevents the amount of air that can be delivered from alveolar units with adequate ventilation to those that are partially occluded by exudate or edema fluid. The ruminant lung has little interdependence due to extensive interlobular septa, which also reduces alveolar expansion. These features make it difficult for alveolar inflation during pneumatic episodes and reduce the capacity to expel alveolar exudate. Thus, a viable innate immune system consisting of an intact airway epithelium and functional muciliary apparatus is essential for preventing initial infection of the lower respiratory tract.

### 1.1. Antimicrobial factors and peptides

Free radicals such as superoxide and hydroxy radical are produced by activated macrophages and neutrophils during *M. haemolytica* infection and cause oxidative damage of phospholipid membranes (conjugated diene formation), DNA, RNA, glycoconjugates, and proteins of parenchymal cells. The free radicals are also active against extracellular and internalized *M. haemolytica*. Eukaryotic cells protect their own membranes, cytosol and organelles from free radical damage by nonenzymatic (vitamin A, E, C) and enzymatic (superoxide dismutase) mechanisms. *M. haemolytica* organisms may be able to resist at least a certain level of free radical damage, since serotypes A1 and A2 produce superoxide dismutase [2, 9].

Recent work increasingly indicates that respiratory mucosal epithelia and leukocytes of ruminants produce antimicrobial peptides, including $\beta$-defensins, anionic peptides, and cathelicidins, all of which have potent microcidal activity [4, 6, 7, 10, 11, 12, 13]. In the ruminant lung, $\beta$-defensins of cattle include TAP and LAP and of sheep...
include sheep β-defensin 1 (SBD-1; SBD-2 is expressed in ovine ileum). Anionic peptide is also produced by epithelial cells of the lung of both cattle and sheep. TAP, LAP and probably SBD-1 expression can be enhanced by inflammatory stimuli; however anionic peptide appears to be constitutively expressed. Although cathelicidin production occurs in ruminant leukocytes and in human lung and skin, its production by respiratory mucosa in sheep and cattle has not yet been determined. The surfactant-associated proteins A and D are members of a family of collageneous host defense lectins, designated collectins. The collectins bind to carbohydrate and lipid moieties expressed by microorganisms and organic particles, and may modulate leukocyte activity [14]. The production of antimicrobial peptides and collectins in utero, by the neonate, or in the adult, remain poorly understood in cattle, sheep and other ruminants.

2. Inflammatory process initiated by M. haemolytica

Although it is known that M. haemolytica colonizes the upper respiratory tract of otherwise healthy ruminants, in some animals M. haemolytica causes severe pneumonia and death. The understanding of the mechanisms by which the bacteria colonize and eventually cause pneumonia is limited. M. haemolytica is a Gram-negative organism with a prominent polysaccharide capsule and resides in the mucus and in the secretion products of the ventral nasal meatus, sinuses and in tonsil. M. haemolytica contains a capsule composed of →3)-B-N-acetyllaminomannuronic-(1→4)-B-N-acetylmannosamine-(1→. This capsular polysaccharide may be involved in an adhesin-receptor interaction, with surfactant mediating the attaching of M. haemolytica in the alveolus (figure 2). Alveolar epithelial cells contain carbohydrate on the surface, which binds a lectin in the pulmonary surfactant. This lectin, in human and canine pulmonary surfactants, is a calcium-dependent, mannone-binding protein of 28 000 to 36 000 Da. M. haemolytica in the alveolus may attach to this surfactant layer by the lectin reaction of its capsular polysaccharide. The affinity of some lectins to the glycoalkyl of M. haemolytica can change when the bacteria are grown in chambers or exposed to serum [15], which suggests that the bacteria may alter glycoconjugate expression in vivo. These changes may affect colonization and invasiveness through bacterial glycoconjugates binding to receptors expressed by mucosal cells. Glycoconjugate/receptor binding may be further enhanced through the enzymatic activity of neuraminidase, which is produced by M. haemolytica in increased amounts during times of stress in the host [1, 16]. The increased neuraminidase is accompanied by increased replication of M. haemolytica [1, 16].

The inflammatory response that occurs during M. haemolytica pneumonia can be initiated by live M. haemolytica, heat-killed M. haemolytica, M. haemolytica lipopolysaccharide (LPS), M. haemolytica capsular polysaccharide, and M. haemolytica leukotoxin (LKT) [1, 2, 17, 18]. During infection with live, whole cell M. haemolytica, LPS, polysaccharide and LKT are released into the intraalveolar exudate [18, 19, 20]. LPS is also taken up by neutrophils, and polysaccharide localizes in the alveolus and alveolar macrophages [18, 19, 20]. The inflammatory response is mediated by several cell types, including the vascular endothelium, pulmonary alveolar and intravascular macrophages (PAMs and PIMs), neutrophils, mast cells, nerve fibers, lymphocytes, and airway epithelial cells (table I). M. haemolytica infection induces release of IL-1, TNF-α and IL-8 by lung tissue [21, 22] and initiates a profound procoagulant state resulting in extensive fibrin polymerization [23]. Other inflammatory mediators released by bovine lung in response to M. haemolytica products (LPS and LKT) include leukotriene B4, histamine and prostaglandin E2 [24, 25]. A wide-spreading cascade of other cytokines and inflammatory mediators are likely released during acute M. haemolytica pneumonia and would likely include platelet-activating factor; however, only a small subset of these factors has been characterized in experimental conditions. Recent work suggests that there may be a specialized pathway of LPS binding to the LPS receptor, CD14, in the lung [26]. The study demonstrated that human surfactant protein A binds rough LPS of Escherichia coli and mediates adherence of the rough LPS to both the membrane and soluble forms of CD14 [26]. Another study has shown that several hours (8 to 30 hours) after mice have been exposed to LPS a ‘late mediator,’ [27] HMG-1, is released. Increased levels of HMG-1 were associated with lethality. Activities of mediators such as human surfactant protein A and HMG-1 have not been investigated in M. haemolytica pneumonia; however, M. haemolytica LPS clearly has a prominent role in initiating the inflammatory response in ruminants. There is also likely a transition from acute pro-inflammatory cytokines to regulatory cytokines such as IL-12 and transforming growth factor beta (TGF-β) that occurs some time after the

Figure 2. Electron micrograph of an M. haemolytica bacterium closely associated to a pulmonary epithelial cell (type I) that lines the alveolar wall. The epithelial cell has an undulated membrane in the region of the bacteria. The bacteria is labeled with colloidal gold particles linked to an anti-M. haemolytica antibody. Lead citrate and uranyl acetate stain, × 28 000.
Table 1. Cells of the respiratory tract and key roles of these cells in responsiveness to M. haemolytica infection in ruminant lung.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Location</th>
<th>Function</th>
<th>Molecular expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cells</td>
<td>Tonsil, upper respiratory tract</td>
<td>Resist colonization</td>
<td>Surface glyconjugulates</td>
</tr>
<tr>
<td>Ciliated epithelial cells</td>
<td>Upper respiratory tract</td>
<td>Mucoiliary activity, resist colonization, adhesion molecule activity</td>
<td>ICAM-1, AMP*</td>
</tr>
<tr>
<td>Submucosal gland cells</td>
<td>Upper respiratory tract, bronchi</td>
<td>Mucinous and serous secretions</td>
<td>Possible AMP activity</td>
</tr>
<tr>
<td>Type II cells</td>
<td>Bronchi/bronchioles</td>
<td>Surfactant, surfactant proteins, reepithelization after type I cell injury</td>
<td>SP A-D [collectins] (opsonization, anti-microbial activity, LPS binding), AMP Mixed function oxidases</td>
</tr>
<tr>
<td>Clara cells</td>
<td>Bronchioles</td>
<td>Detoxification of toxins</td>
<td>Surfactant</td>
</tr>
<tr>
<td>Type I cells</td>
<td>Alveoli, pleura, interlobular septa</td>
<td>Air-blood barrier (gaseous exchange)</td>
<td>Collagen, tenasin, vitronectin, fibronectin</td>
</tr>
<tr>
<td>Extracellular matrix</td>
<td></td>
<td>Structural support, migration of leukocytes</td>
<td>Substance P</td>
</tr>
<tr>
<td>Nerve fibers</td>
<td>Interstitial tissue</td>
<td>Tone of vessels, airways; regulation of mast cell activity</td>
<td>Histamine, protease</td>
</tr>
<tr>
<td>Mast cells</td>
<td>Interstitial tissue</td>
<td>Acute inflammation, construction of vessels, airways</td>
<td>Adhesion molecules, tissue factor, cytokines, von willebrand factor for platelet adherence</td>
</tr>
<tr>
<td>Blood vessels/lymph</td>
<td>Interstitial area, alveolar septa</td>
<td>Gaseous exchange, inflammation, coagulation</td>
<td>Oxidative radicals, enzymes, AMP</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Circulation</td>
<td>Acute inflammatory response</td>
<td>Platelet activating factor, fibrin cross-links</td>
</tr>
<tr>
<td>Platelets</td>
<td>Circulation</td>
<td>Hemostasis</td>
<td>Cytokines, MHC II + pro-inflammatory mediators</td>
</tr>
<tr>
<td>Alveolar macrophage</td>
<td>Alveolus</td>
<td>Phagocytosis of debris, regulation of acute and chronic inflammation, antigen presentation</td>
<td>Cytokines, pro-inflammatory mediators</td>
</tr>
<tr>
<td>Intravascular macrophage</td>
<td>Alveolar capillaries</td>
<td>Internalization of hematogenous substances</td>
<td></td>
</tr>
</tbody>
</table>

*AMP = antimicrobial peptide, including TAP, LAP, anionic peptide, cathelicids, collectins.

acute inflammatory response subsides, but very little is known about the molecular aspects of the progressive nature of M. haemolytica pneumonia.

RTX toxins such as M. haemolytica LKT are not commonly produced by respiratory tract pathogens [28]. LKT, as its name indicates, can be toxic to ruminant leukocytes, and the dense infiltrates of neutrophils and other leukocytes which enter sites of lung infected with M. haemolytica are immediately vulnerable to the LKT activity. LKT binds the M. haemolytica RTX toxins such as M. haemolytica LKT are not completely eliminated by the host. Although LKT is toxic to leukocytes, including macrophages, subcytotoxic levels of LKT enhance alveolar macrophage production of NF-kappa beta, elevate calcium [36] and induce the production of TNF-α and IL-1β.

3. Acute and subacute inflammatory response

3.1. Vasculature

Although extensive intravascular coagulation does not occur in acute pasteurellosis, initiation of the coagulation cascade is evidenced by polymerization of fibrin admixed with pulmonary intravascular macrophages within capillaries and sub- and intraendothelial locations [19]. M. haemolytica LPS and IL-1 induce tissue factor production and procoagulant activity by endothelial cells [37, 38]. A monoclonal antibody to tissue factor prevents fibrin formation, platelet degeneration and severity of lung lesions in an experimental model in calves [38]. M. haemolytica infection also induces endothelial cell activation, since ICAM-1 mRNA is expressed in increased amounts as soon as six hours following experimental deposition of M. haemolytica in the lung [5]. This leads to increased leukocyte adherence and infiltration. LKT has no direct effect on vascular endothelial cells; however, LKT and LPS can
stimulate leukocyte activity, and the soluble products released by alveolar macrophages cause endothelial cell injury [39]. Little is known of the effects of *M. haemolytica* products on vascular tone, smooth muscle contractility, nitric oxide-induced vasodilation or endothelin-induced vasoconstriction.

The lymphatic vessels of the pulmonary interlobular septae become markedly distended and filled with polymerized fibrin and neutrophils during *M. haemolytica* pneumonia. Areas of necrosis often track along these regions and extend across interlobular septae into the adjacent lobules. Despite this characteristic and consistent finding, little is known of lymphatic drainage alterations or lymphatic endothelial cell activity during *M. haemolytica* pneumonia. It is a location where many aspects of the infectious process aggregate and this includes intracellular bacteria, neutrophils, macrophages, fibrin, and seroproteinaceous fluid. Enhanced delivery of antibiotics or other therapeutics to this location may be beneficial in improving the drainage of exudate from the lung.

### 3.2. Neutrophils and the acute inflammatory response

Neutrophil infiltration during *M. haemolytica* pneumonia is associated with alveolar epithelial cell damage and necrosis. The importance of neutrophil-mediated damage in the pathogenesis of *M. haemolytica* pneumonia is underscored by the fact that neutrophil depletion ameliorates the tissue damage and vascular leakage of protein [3]. Neutrophils mediate damage by release of enzymes such as elastase, oxidative radicals and cytokines, and these substances incite membrane damage, the formation of conjugated dienes such as malondialdehyde, and vascular leakage of protein. Affected cells respond by undergoing programmed cell death (apoptosis) or necrosis. Neutrophil-mediated damage to the lung mucosa removes an innate barrier and allows exposure of capillaries and small vessels to inflammatory mediators released in the lung and bacteria within the exudate (figure 3).

### 3.3. Adhesion molecules of the acute inflammatory response

Adhesion molecules that mediate passage of neutrophils into inflammatory sites initially involve relatively unstable adherence between selectin molecules and their glycoconjugate receptors (fucosylated oligosaccharide molecules). L-selectin is expressed on neutrophils and mediates neutrophil binding to endothelial cells. E- and P-selectins are expressed mainly on endothelial cells and mediate binding to neutrophil selectin receptors. Cytok-
Microbes and Infection

ines released during pneumonia activate neutrophils which results in membrane surface expression of the $\beta_2$ integrins by neutrophils and immunoglobulin superfamily molecules such as ICAM-1 and PECAM-1 by endothelial cells (figure 3).

The $\beta_2$ integrins are glycoprotein heterodimers which consist of one of four $\alpha$ subunits (CD11a, b, c or d) and a constant $\beta_2$ subunit (CD18): CD11a/CD18 [LFA-1], CD11b/CD18 [Mac-1], CD11c/CD18 [p150,95], and CD11d/CD18. CD11b/CD18 is the predominant $\beta_2$ integrin on neutrophils. Neutrophil $\beta_2$ integrin binds ICAM-1 molecules expressed on activated endothelial cells and this mediates stable adherence between the neutrophils and endothelial cells. During endothelial cell activation, PECAM-1 production increases at the interendothelial cell junctions and PECAM-1 mediates transendothelial cell passage. In the extravascular stroma, fibroblasts can also express ICAM-1 and are thought to bridge passage of neutrophils from the vasculature to laminae in the basement membrane of pulmonary epithelia. Respiratory epithelial cells lining bronchi and bronchioles of cattle express ICAM-1 [5], which mediates the final leg of neutrophil infiltration into the airway space. In addition, recent work suggests that neutrophils can adhere to VCAM-1 produced by endothelial cells and extracellular matrix proteins such as tenascin-C via $\beta_1$ integrins ($\alpha_4 \beta_1$) [40]. Impaired expression of adhesion molecules can be lethal. For example, mutations resulting in minimal expression of the $\beta_2$ integrins has been reported in humans beings, holstein cattle and Irish setter dogs resulting in severe, debilitating infections of the respiratory and alimentary tracts [41, 42].

Calf models of M. haemolytica in which neutrophil adhesion molecules have been inhibited or eliminated have been used to identify new therapeutic regimens [43, 44, 45]. It is thought that inhibition of neutrophil-mediated pulmonary damage during early M. haemolytica may have synergistic effects with antibiotic therapy. Such therapeutic regimens would potentially lessen both the leukocyte- and microbial-induced aspects of pulmonary damage.

Experimentally, inhibition of $\beta_2$ integrin-mediated adherence during M. haemolytica infection results in reduced neutrophil infiltration into the pulmonary bronchi and bronchioles [44], but does not appear to reduce infiltration into the pulmonary alveoli [43]. This distribution of $\beta_2$ integrin-mediated adherence in the bronchi and bronchioles correlates with the distribution of the $\beta_2$ integrin receptor, ICAM-1, by bronchi and bronchicolar epithelial cells. Evidently, the epithelium of these airways and the extensive extravascular tissue matrix requires $\beta_2$ integrin-mediated adherence. In contrast, neutrophil infiltration into alveoli during M. haemolytica pneumonia does not require $\beta_2$ integrins and alveolar epithelial cells of cattle express relatively low amounts of the $\beta_2$ integrin receptor, ICAM-1 [5]. The reason for ability of neutrophils to pass the alveolar wall independently of $\beta_2$ integrins has not been determined, nor has the alternative set of adhesion molecules that mediate this passage. The $\beta_2$ integrin-independent passage is likely facilitated by the thin walls of alveolar epithelial cells, the small amount of extracellular matrix in alveolar septa, and fenestrations in the basement membranes of the vascular endothelial cells and alveolar epithelial cells.

Inhibition of selectin adherence by the oligosaccharide mimetic, TBC1269, appears to reduce the extent of neutrophil infiltration and vascular leakage of protein during M. haemolytica pneumonia in calves [45]. TBC1269 reduces adherence of all three (L, E, and P) selectins and recent work suggests that TBC1269 increases the level of apoptosis of infiltrating neutrophils [8]. The decrease in vascular leakage of protein and infiltration of neutrophils in combination with the increase in neutrophil apoptosis during TBC1269 treatment of calves would suggest that the compound may reduce damage of the pulmonary tissue. The degree of membrane damage as measured by conjugated diene formation is reduced by TBC1269 treatment; however, the reduction was not significant statistically. Additional studies using higher numbers of animals and older cattle may demonstrate a protective effect of at least this one selectin inhibitor in M. haemolytica pneumonia. A possible synergistic role between TBC1269 or other adhesion molecule inhibitors with antibiotics during M. haemolytica pneumonia may be the basis for new types of therapy.

3.4. Mast cells and substance P nerve fibers

Mast cells are located in the submucosa throughout the respiratory tract, from the rostral nasal cavity to deep in the pulmonary alveoli. The number of mast cells is limited in neonatal ruminants and increases markedly with age. In lungs of neonatal calves, there are 7.36 ± 0.8 mast cells/mm² [46]. These cells degranulate during M. haemolytica infection and the number of stainable mast cells is reduced to 2.8 ± 0.6 mast cells/mm² by six hours after M. haemolytica inoculation [46]. Sheep have a higher number of intrapulmonary mast cells; however, the number of cells also decreases during acute M. haemolytica infection. Interestingly, the degranulation process of mast cells occurs only in areas of active inflammation (associated with dense infiltrates of neutrophils). Evidently in areas of lung lacking neutrophilic infiltrates, mast cells are resistant or not exposed to degranulation stimuli. If mast cells were to degranulate throughout the entire lung tract during M. haemolytica pneumonia, it would likely result in severe edema, and broncho- and vasoconstriction in both infected and noninfected areas. The limitation of mast cell degranulation to the site of intense leukocyte infiltration during M. haemolytica pneumonia contrasts sharply with the mast cell activity in pulmonary hypersensitivity reactions and asthma.

In addition to neutrophil infiltration and IgE cross-linking, mast cells can be stimulated to degranulate through the release of substance P from autonomic nerve fibers. These fibers are most densely arranged in the rostral portion of the nasal septum, but are also present in the trachea and lung. They may be important in initiation of the acute inflammatory response during the very early phases of M. haemolytica infection.

3.5. Pulmonary alveolar macrophages

PAMs are regulatory cells that control inflammatory, immune and repair processes through the release of cytok-
ines and other regulatory substances such eicosinoids and platelet-activating factor. Circulating monocytes are the precursors of PAMs, PIMs and dendritic cells of the bronchus-associated lymphoid tissue. Monocyte adherence is mediated by a broad set of adhesion molecules including L, E, and P selectins, \( \beta_1 \) integrins (VLA-4), \( \beta_2 \) integrins, ICAM-1, PECAM-1, and VCAM-1. Because of their location in lung, PAMs are exposed to M. haemolytica or M. haemolytica constituents (such as LPS or LKT) almost immediately after entrance of the bacteria into lung alveoli.

M. haemolytica polysaccharide reduces internalization of M. haemolytica by PAMs [47] and M. haemolytica LPS incites mRNA expression and protein production of TNF-\( \alpha \), and IL-1\( \beta \) in PAMs [22]. M. haemolytica LPS also incites IL-8 mRNA expression in a biphasic fashion with peaks at 1 to 2 and 16 to 24 hours after incubation [48]. PAMs express tissue factor during M. haemolytica pneumonia, and procoagulant activity of macrophages recovered from pulmonary lung is increased 30-fold. The enhanced procoagulant activity is exacerbated by the fact that macrophage and neutrophil profibrinolytic activity is reduced 23-fold [23]. This imbalance results not only in extensive fibrin deposition, but also in retarded fibrin removal. As indicated, antibodies to tissue factor greatly reduce fibrin deposition [38].

3.6. Pulmonary intravascular macrophages

PIMs adhere tightly to endothelial cells of pulmonary capillaries of several species including cattle, sheep, goats, pigs, llama, deer, reindeer, horses and cats. PIMs function to clear cellular and acellular material from the blood during pulmonary conditions and release cytokines and other regulatory factors similar to, but slightly different from, PAMs. PIMs are present in other species (rabbits, dogs, rats, mice, guinea pigs and human beings), but they are few in number and difficult to discriminate from adherent and activated monocytes. The numbers of PIMs in ruminants are limited in neonates but increase with age [49, 50, 51]. PIMs of neonatal calves appear to be more differentiated than those of neonatal pigs. PIMs occupy 3.65% of the total capillary volume in cats [51], 15.3% of the total capillary volume in sheep [50], and 6 and 25% of the total capillary volume in neonatal and 30-day-old pigs [49], respectively. PIMs are more active metabolically than PAMs when stimulated by the calcium ionophore, A23187 and produce eight arachidonic metabolites. The major cyclooxygenase metabolite of A23187-stimulated PIMs was hydroxyeptadecatrienoic acid and lipoxygename products including 5-HETE, 12-HETE, and LT\( \beta_2 \) [52]. PIMs take up LPS and produce cytokines IL-1, TNF, and PAF in addition to eicosinoids.

PIM adherence to pulmonary endothelial cells is mediated by cell junctions termed ICAPs that are approximately 12 to 20 nm of electron-dense material that, in man, has been demonstrated to consist of the \( \beta_2 \) integrin, LFA-1 [53]. ICAPs have similarities to belt desmosomes of epithelial cells (zona adherens, intermediate junctions), and the ICAP distinguishes PIMs from adherent monocytes and PAMs. Another characteristic ultrastructural feature of PIMs is the cytoplasmic lamellar structures termed micropinocytosis vermiciformes.

PIMs from sheep and goats stain for alpha-naphthyl butyrate esterase, secrete lysozyme, generate oxygen radicals, release TNF and express Fc receptors [51]. PIMs are highly phagocytic during M. haemolytica infection and internalize neutrophils, platelets, and fibrin [19]. In swine, PIM activity is reduced by viral infections (porcine reproductive and respiratory syndrome virus) and mycotoxins (Fusarium moniliforme) [54]. Little is known of the activity of PIMs of cattle or sheep during M. haemolytica pneumonia.

3.7. Lymphocytic inflammation

A detectable number of lymphocytes are sequestered in the alveolar capillaries of calves with acute M. haemolytica pneumonia [20]. However, most studies of lymphocyte activity in the field of M. haemolytica pneumonia are concerned with the responsiveness of these cells to vaccines. Live M. haemolytica vaccines, for example, induce IFN-\( \gamma \) production and strong proliferation of lymphocytes from mediastinal lymph node and, to a lesser degree, superficial cervical lymph node. On the other hand, M. haemolytica bacteria incite strongest proliferative responses in cells and IFN-\( \gamma \) from superficial cervical lymph node (near the vaccination site) [55].

4. Active immunity

Live M. haemolytica vaccines can enhance resistance to infection; however, these vaccines can be associated with local tissue reactions, transient anorexia, fever, and even septicemia [56, 57]. Experimental vaccines with partially purified M. haemolytica leukotoxin can induce an antibody titer [56], but the effectiveness in preventing pneumonia has not been fully determined. Despite some improvement in managerial practices, vaccines and clinical therapies, M. haemolytica pneumonia remains a widespread problem and strategies to enhance host resistance to colonization and pneumonia by M. haemolytica are needed.

Non-vaccinated cattle infected with M. haemolytica develop serum IgM titers by five days postinfection and IgG titers by seven days [58]. Commercial vaccines in the United States induce a degree of protection in most trials. Vaccines are typically killed bacterins or modified live products with or without LKT. In one study, both a bacterin-toxoid (OneShot, Pfizer) and a modified live vaccine (Once PMH, Bayer Corp.) induced protection against challenge; however, the bacterin-toxoid provided the greatest degree of protection. The oil adjuvant in the bacterin-toxoid may have contributed to the difference between the two vaccines, since oil adjuvants are more effective than aluminum hydroxide for reducing lung lesions upon challenge [57] and for stimulating antibody response. Adjuvants such as muramyl dipeptide also enhance vaccine protection [59]. Non-living M. haemolytica vaccines (OneShot [Pfizer], Prespense HP/TK, and Septimune PHK) induce antibody titers to whole cell M. haemolytica between seven and 14 days after vaccination, but only two of the vaccines (OneShot and Prespense) induced antibody titers to LKT. Upon revaccination, cattle generated an anamnes-
tic response. Antibodies were increased for up to 84 days after vaccination or revaccination [60].

Delivery is an important factor of vaccination. Cattle receiving intranasal M. haemolytica vaccines develop antibodies in nasal secretions and bronchoalveolar lavage fluid. In contrast, cattle receiving vaccine at the tracheal bifurcation produce antibodies in the bronchoalveolar lavage fluid, but not nasal secretions [61].

Several laboratories have utilized LKT as an antigenic molecule for vaccination. Reductions in the activity or production of LKT would reduce the degree of LKT-induced leukocyte damage and apoptosis. LKT with an aluminum adjuvant stimulated a humoral response to LKT and only a low response to formalized M. haemolytica or to outer membrane proteins of M. haemolytica [56]. An LKT-IL-2 molecular chimera did not increase antibody production; however, it may increase lymphocyte proliferation [62].

Outer membrane proteins (OMPs) of M. haemolytica also have potential to be important antigens for acquired immunity. Antibodies to M. haemolytica OMPs are associated with resistance to M. haemolytica challenge in cattle. Many of the OMPs are exposed on the surface of M. haemolytica S1 strains and, thereby, are readily recognized by leukocytes and circulating antibodies [63]. Loss of three membrane lipoproteins in an M. haemolytica mutant resulted in enhanced susceptibility to complement-mediated killing [64]. One lipoprotein, PlpE, has sequence homology to OmlA of M. haemolytica [65]. An LKT-IL-2 molecular chimera did not increase antibody production; however, it may increase lymphocyte proliferation [62].

The lipid portion of these molecules may be responsible for the immunogenicity. One of the four, Plp4, is a 31-kDa lipoprotein with regions of homology with antigens from Haemophilus somnus, Neiseseria meningitidis, and the OmpA family.

4.1. Immune complex-mediated vascular change

Immune complexes may contribute to the pathology of M. haemolytica pneumonia. Immune complexes composed of IgG complexed with OMPs of M. haemolytica and complement fragments are present in the lungs of a mouse model [67]. Other work has demonstrated an Arthus-like reaction in rabbits first vaccinated with M. haemolytica bacterin, then receiving M. haemolytica LPS intratracheally [68]. The lungs of the rabbits had extensive vascular edema and neutrophil infiltration with features similar to those of M. haemolytica pneumonia.

5. Chronic pneumonia

The gross and microscopic changes that occur following the initial acute necrotizing pneumonic episode of M. haemolytica pneumonia have been recognized for many years but infrequently studied. Necrotic areas are infiltrated by macrophages and fibroblasts and become encased in capsules of fibrous trabeculae. In time, these regions become remodeled into a granulomatous area. In chronic lung injuries in human patients with bacterial pneumonia, mast cell numbers increase. The numbers of mast cells in chronic bovine pulmonary pasteurellosis have not been determined. Potentially, therapies aimed to remodel granulomas and fibrous connective tissue could be developed. These regions may then become sites for new bronchiola and alveolar units to form.

Although the number of mast cells is greatly reduced following acute M. haemolytica infection, there is a significant increase in mast cells at 15 days following experimental M. haemolytica infection in sheep (R. Ramirez-Romero, personal communication). This increase is present only in the areas of lung in which there was an intense acute inflammatory response. The increase in mast cells in these areas may predispose the lung to reinfection. Alternatively, the mast cells may contribute to the hypersensitivity type of reactions that can be seen with viruses such as respiratory syncytial virus or with allergic responses such as asthma.

References


Response of respiratory tract to Mannheimia haemolytica


