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*Vet Pathol* 1991 28: 533

DOI: 10.1177/030098589102800611

The online version of this article can be found at:

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BRIEF COMMUNICATIONS and CASE REPORTS

Bronchiolar Metaplasia and Ulex europaeus agglutinin I (UEA-I) Affinity in Mycoplasma hyopneumoniae-infected Lungs of Six Pigs

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Key words: Bronchioles; lectins; pneumonia; swine.

Mycoplasma hyopneumoniae adheres to the cilia and apical plasma membrane of cells that line the trachea, bronchi, and bronchioles. Colonization results in ciliostasis and peribronchial and peribronchiolar nodular aggregates of lymphocytes and plasma cells.2,3 Although adhesion-receptor interactions between the organism and respiratory epithelial cells have been examined, little attention has been given to the morphologic and phenotypic changes that may occur in cells lining bronchioles during infection. In this study, we describe morphologic changes and differences in lectin staining patterns in the bronchioles from lungs of six pigs necropsied at sequential stages of infection with M. hyopneumoniae.

Nine, 10-week-old Yorkshire pigs of both sexes were obtained from a barrier maintained, closed herd at the animal resource station of Iowa State University (Ames, Iowa). The herd was originally established from Caesarian-derived, isolation-raised animals. Animals removed from the herd are routinely cultured for Mycoplasma hyopneumoniae, M. hyorhinis, and M. flocculare and other respiratory pathogens by nasal swabs pre- and post-inoculation and at necropsy. The pigs were fed a 16% protein swine growth ration that lacked growth promoters or antimicrobial compounds. The pigs were randomly distributed on the basis of litter and sex into two groups and housed in separate isolation rooms. Six pigs were inoculated intratracheally with 5 cc of 10% pneumatic lung suspension (pH 7.4) containing M. hyopneumoniae, strain 194, a pig passage inoculum free of detectable viruses and other bacteria.8 The lung homogenate contained 1 x 10^4 color changing units (CCU)/ml. Three control pigs were similarly inoculated with 5 cc of 10% pneumatic lung suspension (pH 7.4). Two infected pigs and one control were necropsied at 2, 4, and 6 weeks post-infection; 0.5 x 0.5 cm sections of lung from the left and right cranial lobes, along with trachea, were fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin. Sections were cut at 5 to 7 µm, and stained with hematoxylin and eosin. Peribronchial and peribronchiolar infiltrates of lymphocytes and plasma cells were subjectively judged in severity by a pre-determined scale: Minimal = rare infiltrates of cells; Mild = 0–2 layers of cells; Moderate = 2 to 10 layers of cells and small nodular aggregates; Marked = 8 to 12 layers of cells and nodular aggregates; and Severe = >10 layers of cells with extensive nodular aggregates. Additional sections were cut for lectin histochemical examination and attached to slides with a 0.1% poly-L-lysine solution. These sections were stained with a peroxidase conjugated lectin, Ulex europaeus agglutinin I (UEA-I), using previously described techniques with minor variations that included at TRIS buffer diluent with 0.1 mM Ca^{2+}, Mg^{2+}, and Mn^{2+}. The lowest concentration of lectin that resulted in specific staining was used. Lectin binding specificity was tested by the following: a) mixing the lectin with a 0.1 M solution of its inhibitory sugar (L-fucose) for 20 minutes before application; and b) treating sections with 1% sodium periodate prior to labeling for 10 minutes. Both techniques prevented staining. Additionally, unstained sections of formalin-fixed, paraffin-embedded lung tissue from three pigs submitted to the Iowa State University Diagnostic Laboratory (Ames, IA) that stained for swine influenza viral antigens by immunofluorescence and two pigs with experimental Actinobacillus (Hemophilus) pleuropneumoniae infection were stained with the lectin. Lungs from SIV-infected pigs were characterized histologically by a severe chronic-active bronchopneumonia; lungs from A. pleuropneumoniae-infected pigs were characterized by severe, multifocal, necrotizing, and fibrosing, chronic pneumonia. In lung tissue from control pigs, UEA-I stained the apical surface or entire cytoplasm of pseudostratified cells and submucosal gland cells of trachea and bronchi (Figs. 1, 2). These areas stained heavily in all pigs, control and infected, in this study. In contrast with the bronchial epithelial staining, bronchiolar epithelium in the control pigs had only 0–3 epithelial cells/bronchiole that stained lightly with UEA-I (Fig. 3). Rare bronchioles (approximately <5%) had scant to mild adventitial infiltrates of lymphocytes.

In M. hyopneumoniae-infected lungs 2 and 4 weeks post-inoculation, UEA-I stained serofibrinous and fibrinopurulent exudate present in bronchi, bronchioles, and alveoli. The lectin most likely stained the inflammatory proteaceous material (fibrin); however, the lectin also stained submucosal glands. In addition, UEA-I stained the cytoplasm of alveolar macrophages that contained ingested exudate. At 4 weeks post-inoculation, most lung sections had small amounts of intra-airway exudate and small areas of pseudostratified bronchiolar epithelium. The pseudostratified areas stained with UEA-I and were surrounded by moderate infiltrates of lymphocytes.

At 6 weeks post-inoculation, roughly 75 to 90% of bronchioles were lined by pseudostratified epithelium in a segmental to diffuse pattern. These airways also had marked
Fig. 1. Trachea; *Ulex europaeus I* (UEA-I) peroxidase stain, control pig necropsied 4 weeks post-inoculation. The epithelium and submucosal glands are stained by UEA-I. UEA-I peroxidase stain, Harris' hematoxylin counterstain.

Fig. 2. Lung; control pig necropsied 2 weeks post-inoculation. The epithelium and submucosal glands of the bronchus are stained by *Ulex europaeus I* (UEA-I). UEA-I peroxidase stain, Harris' hematoxylin counterstain.

Fig. 3. Lung; control pig necropsied 6 weeks post-inoculation. The bronchiolar epithelium is not stained by *Ulex europaeus I* (UEA-I) lectin, and lacks adventitial infiltrates of lymphocytes. UEA-I peroxidase stain, Harris' hematoxylin counterstain.

Fig. 4. Lung; pig 6 weeks post-inoculation with *Mycoplasma hyopneumoniae*. The bronchiole has mild adventitial infiltrates of lymphocytes. The epithelium is pseudostratified and stains intensely with *Ulex europaeus I* (UEA-I). UEA-I peroxidase stain, Harris' hematoxylin counterstain.
infiltrates of lymphocytes, with occasional plasma cells and macrophages, diffusely scattered in the adventitial layer and also in dense nodular aggregates. Lesser numbers of lymphocytes extended into the lamina propria. The apical surface and the entire cytoplasm of bronchiolar lining cells stained with UEA-I in a strikingly strong staining pattern (Fig. 4). In many bronchioles, UEA-I staining of cuboidal and pseudostratified areas were associated with marked adventitial infiltrates of lymphocytes and few plasma cells (Figs. 5, 6). In less affected bronchioles, there was segmental staining of cuboidal epithelial cells in areas associated with mild to moderate infiltrates of lymphocytes in the subjacent adventitia. Fibrinopurulent exudate, which was prominent in the lungs of all pigs at 2 and 4 weeks post-inoculation, was present only in small amounts and was limited to rare alveolar lumina. This material and the cytoplasm of alveolar macrophages that contained ingested fibrinous exudate stained with UEA-I.

In pig lungs infected with swine influenza virus, bronchiolar lining cells did not stain with UEA-I, and the epithelium was cuboidal (non-pseudostratified). Occasional bronchioles in A. pleuropneumonia-infected lungs were surrounded by marked fibroplasia. All of the UEA-I stained bronchioles in these lungs were surrounded by the fibrous connective tissue and lined by a pseudostratified epithelial layer. The remaining bronchioles stained similarly to bronchioles of lungs from control pigs.

The staining pattern by UEA-I suggests that changes in differentiation of bronchiolar epithelium from a cuboidal cell layer to a pseudostratified-type layer is associated with alterations in glycoconjugate expression. Although the conditions in this study were experimental, these changes may influence adherence of Mycoplasma and play a role in persistent infection. The UEA-I lectin binds L-fucose residues and Mycoplasma lack the enzyme fucosidase.8 UEA-I staining of the cytoplasm of tracheal, bronchial, and pseudostratified bronchiolar cells indicates that fucose may be a terminal residue on glycoconjugates synthesized by these cells. Biochemical studies have shown that fucose is a common terminal sugar moiety of glycoconjugates in normal porcine tracheal mucus.3 These glycoconjugates are largely produced by submucosal glands in the trachea and bronchi. The strong affinity of UEA-I to the submucosal gland cells likely was due to the presence of fucose residues. We were able to subjectively use this affinity as an internal control for UEA-I specificity to L-fucose.

Differentiation and phenotypic changes in glycoconjugates that occur in bronchiolar cells may be induced by persistent lymphoplasmacytic infiltrates via the elaboration of cytokines that would influence bronchiolar cell growth. Segmental bronchiolar UEA-I staining patterns associated with subjacent lymphocytes implied such a relationship. Gamma interferon from activated T-cells and interleukin-1 from activated macrophages may initiate physiologic cascades, such as prostaglandin synthesis, and induce differentiation.47 Alternatively, the pseudostratification may merely reflect nonspecific changes that occur in chronic pneumonia as seen, to a much lesser degree, in the Actinobacillus-infected lungs. It could be argued that the UEA-I stained residual fibrinous exudate, which could accumulate on the bronchiolar epithelial cell surface of pigs at 4 and 6 weeks post-inoculation, but this is unlikely since a) this material was not present historically on the surface of cells of the bronchioles, bronchi, or in their lumens, and was present in the lumen of only rare alveoli; b) in addition to the apical surface, UEA-I also stained the cytoplasm of the bronchiolar cells; and c) long-term (6-weeks) accumulation of fibrinous exudate on the surface or cytoplasm of bronchiolar cells has not been a documented phenomenon.

Acknowledgement

The authors thank Dr. S. Messier for porcine lung tissues.

References


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Fig. 5. Lung; control pig necropsied 6 weeks post-inoculation. Bronchioles are not stained by Ulex europaeus I ± (UEA-I), and lack lymphocytic infiltrates. UEA-I peroxidase stain, Harris’ hematoxylin counterstain.

Fig. 6. Lung; pig 6 weeks post-inoculation with Mycoplasma hyopneumoniae. Several bronchioles are partially surrounded by nodular aggregates of lymphocytes and plasma cells. The epithelium lining these airways stains intensely with Ulex europaeus I (UEA-I). UEA-I peroxidase stain, Harris’ hematoxylin counterstain.