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Plasma Cell Distribution in the Respiratory System of Pigs

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Abstract: The present study provided quantitative information on the plasma cell in respiratory system of pig without respiratory diseases. Plasma Cells (PC) were counted in the tonsils, epiglottis, trachea and 11 additional anatomical regions of the 2 lungs of adult pigs. Five clinically healthy animals were studied, of approximately 100 kg, serologically negative for *Mycoplasma hyopneumoniae*, PRRS, Aujesky and *Actinobacillus pleuropneumoniae*. Samples were processed by paraffin inclusion and stained with green methyl-pyronin. The tonsil (9.42±7.13 ***p>0.001) and right cranial lung lobe (apex) (3.24±4.74) were found to contain the highest amount of PC, while, the lowest amounts were detected in trachea (0.26±0.42) and left cranial lung lobe (apex) (0.6±0.44). These data, obtained from pigs clinically healthy, will enable comparisons to be made in future studies of pigs with infectious respiratory diseases.

Key words: Pig, respiratory system, plasmatic cells, distribution, respiratory diseases

INTRODUCTION

Studies on airway mucosal tissue immunity are relevant to examine the behavior of the immune system cell population and allow comparison between normal and abnormal parameters in respiratory processes (Kyd *et al*., 2001).

Research has focused on the study of mucosal immunity of the respiratory system in humans (Bienenstock, 1984) and the rat (Power *et al*., 1994; Holt, 2000) and different cell types in the lung of species such as calves (Pringle *et al*., 1988); however, few studies have been directed to other species such as the pig (Sinkora, 2002).

Curtis (2005) described the importance of the adaptive immune response of the lung and the interaction of 3 cell groups: T lymphocytes, Natural Killer cells (NK) and dendritic cells in infectious processes caused by virus, mycobacteria and fungi.

The localization and percentage of distribution of lymphoid tissue associated to respiratory mucosal tissue has been described in normal pigs; 8.38% was found in bronchi, 81.63% in bronchioles and 9.98% in respiratory bronchioles (Huang *et al*., 1990).

The function of neutrophils and alveolar macrophages has been examined along the different stages of production; however, no significant differences have been found between ages, or stages (Dumanoir *et al*., 2002).

Plasma Cells (PC) are effectors that participate in humoral immunity and are responsible for the synthesis and secretion of antigen-specific immunoglobulins (Shapiro-Shelef *et al*., 2003; Hargreaves *et al*., 2001; Iwakoshi *et al*., 2003; El-Nefiawy *et al*., 2003). Plasma cells arise as a response to antigens, both thymus-independent and dependent, the 1st week after exposure, at the extrafollicular focus of secondary lymphoid organs (Shapiro-Shelef *et al*., 2003).

In pigs, B cells are abundant in Bronchi Associated Lymphoid Tissue (BALT). Most of these cells produce IgA, a predominant immunoglobulin of crucial importance in the respiratory system. In the lower respiratory system, IgG is predominant in secretions (Tizard, 2002) and IgE is occasionally produced as a response to parasites, e.g.,

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against the larval phase of *Metastrongylus* sp. (Stevenson, 1998). The main surface markers of pig B lymphocyes are CD1, CD21, CD45 and SWC7 (Sinkora *et al*., 2002).

In pig lung infection by *Pasteurella multocida*, the detected surface markers are Class II MHC, SWC1, SWC3a, CD2a, CD4a, CD8b (Bernd and Muller, 1995). In *M. hyopneumoniae* infection, detected markers include CD3, CD79α, Mac 387, anti-CD45RA (3C3/9)-SLA-II-DQ (BL2H5) (Chianini, 2003). In addition, a significant increase in $CD8^+$ and CD16 (FCRγ III) was found in bronchial lymph nodes by cellular response flow cytometry, which could be related to observed lung lesions (Dayalu and Ross, 1990; Bhogal *et al*., 1992). *M. hyopneumoniae* also, induces an early and transitory rise of CD4 cells, followed by an increase in CD8 and mast cells in the lung parenchyma (Cruz *et al*., 2008). Sarradell *et al*. (2003) detected cells with the markers CD3, GASw/IgA, CD4 (74-12-4), CD8 (MCA 1223), SLA-II, lysozyme and S-100 in bronchial lymph nodes of slaughter-house pigs infected with the mycoplasm.

The pig respiratory complex is a multifactorial syndrome of known etiologic agents, interactions and clinical manifestations of the animals (Choi *et al*., 2006).

A marked humoral and cellular immune response is present in natural respiratory infections. For example, in the case of *Mycoplasma hyopneumoniae* infection (Sarradell *et al*., 2003), cells containing immunoglobulins specific against this agent have been found in lymphoid and lung tissue, 3-4 weeks after infection. The proportion of IgA:IgG-containing cells is 1:3 in nasal mucosa and retropharyngeal lymph nodes and this proportion rises to 1:15 in bronchial lymph nodes. Thus, the number of cells that produce IgM and IgG during the first phases of the infection is high, while IgA-producing cells increase progressively and the presence of antibodies coincides significantly with the appearance of lesions (Suter *et al*., 1985).

At present, no data are available on the normal parameters of cell populations in the pig lung and this information would contribute to understand the immunopathogenesis of respiratory processes. For instance, little is known about the normal parameters and appearance of some cell groups, such as plasma cells, in the porcine respiratory system (Chianini *et al*., 2001). The aim of the present research, was to determine PC distribution in the tonsil, trachea, epiglottis and 11 additional anatomical sites of the pig lung by methyl green-pyronine staining of lung tissue samples.

MATERIALS AND METHODS

Animals: Five clinically healthy Yorkshire pigs of 100 kg weight, serologically negative to *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae, Pasteurella multocida*, PRSS and Aujesky were obtained from the Center for Teaching and Research on Pig Production (Centro de Ensenanza, Investigación y Extensión en Produccion Porcina), of the veterinary medicine and zootechnics faculty, national Autonomous University of Mexico (UNAM) (CEEIEPE, Jilotepec, Estado de Mexico, Mexico).

Necropsy and sample collection: Pigs were sedated, anesthetized and sacrificed by bleeding. The presence of pneumonic lesions was evaluated and lungs were aseptically dissected to obtain fragments of tonsil, epiglottis and trachea; from the right lung: cranial lobe (apex), medial lobe (dorsal border), medial lobe (acute border), caudal lobe (caudal end of the dorsal border), caudal lobe (central portion), accessory lobe (acute border of the medial portion); from the left lung: cranial lobe (apex), cranial lobe (caudal portion of the dorsal border), cranial lobe (acute border of the caudal portion), caudal lobe (caudal end of the dorsal border) and caudal lobe (central portion). Samples were fixed with (1:10 v v^{-1}) Carnoy to detect plasma cells. A total of 14 anatomical sites from each animal were sampled. Other fragments of the same sites were fixed in 10% buffered formalin for histopathology.

Histopathology: Samples preserved in 10% buffered formalin were sectioned and stained with eosin-hematoxylin to search for changes in microscopic structure.

Plasma cell detection: To determine PC, histologic sections were stained with methyl green-pyronine and washed with absolute methyl alcohol (Kiernan, 1990). Slides were cleared, mounted in resin and observed under light microscope at 40X. PC were identified by spotting cells with brilliant red-stained active RNA, differentiating them from other pyronine-stained structures. Lymphocytes showed the same characteristics in all organs.

Cell count and statistical analysis: Positive cells were counted in no <10 randomly selected non-confluent fields, using the Image Pro-Express program (Version 4.01 Media Cybernetics) at 400X. The mean and standard error was obtained for each animal. Results were analyzed by ANOVA with Tukey's multiple comparison test, using the graph pad prism program version 3.

RESULTS AND DISCUSSION

Little is known about the distribution of plasma cell populations in the pig. The present study analyzed plasma cell distribution in the pig respiratory system by taining specific regions of the lung with methyl green pyronine.

Neither macroscopic nor microscopic lesions were found in the sampled tissues. The mean number of CP were: tonsil 9.42±7.13, epiglottis 0.82±1.00, trachea 0.26±0.42. Right lung Cranial lobe (apex) 3.24±4.74, medial lobe (dorsal border) 1.48±0.86, medial lobe (acute border) 1.24±0.42, caudal lobe (caudal extreme of the dorsal border) 3.18±3.83, caudal lobe (central portion) 1.94±1.19, accessory lobe (acute border, medial portion) 2.16±3.03. Left lung: Cranial lobe (apex) 0.6±0.44, cranial lobe (caudal portion, dorsal border) 1.38±1.61, cranial lobe (caudal portion, acute border) 0.8±0.72, caudal lobe (caudal extreme of the dorsal border) 2.02±1.60, caudal lobe (central portion) 1.12±0.67. Figure 1 shows the mean number and standard error of plasma cells obtained in samples taken from various lung regions of 5 pigs.

As shown, the tonsil and the right cranial lung lobe (apex) displayed the highest amount of plasma cells: (9.42±7.13) and(3.24±4.74), respectively. Only the number of PC in tonsil was significantly above the mean $p<0.001$, while the trachea presented the lowest mean number of PC (0.26±0.42) followed by the left cranial lung lobe (apex) (0.6±0.44).

Plasma cells are well-known immunoglobulin producers, they are therefore expected to be present in low numbers in pathogen-free regions. In adult equines, jejunum biopsies of the lamina propria villi, mean PC number was 18±10.8 PC, while in lamina propria crypts the mean was 35±10.2 PC (Packer *et al*., 2004); in cats, the mean number of IgA-secreting PC in duodenum was 8.2±3.4, in jejunum, 7.0±2.8 and in ileon, 3.7±0.9 (Waly *et al*., 2001). In humans, jejunum sections contained a mean number of 22.9 IgA-secreting and 9.5 IgM-secreting PC (Kingston *et al*., 1981), while in the rectal mucosa the mean ranged from 1-2.1 PC (Leonard *et al*., 1982). In adult rat endometrium, mean PC number was 10.38±1.37, while, in myometrium, it was 1.67±0.30 (Kanter *et al*., 2003). According to these results, under normal conditions, the population of PC is scarce. This agrees with the results found herein for most sampled tissues; thus, an increase in PC would be expected when these tissues are exposed to pathogens. In this study, tonsil tissue showed the highest number of PC, mainly because it forms part of the immune system of the respiratory and digestive mucosae and because the tissue stemmed from adult pigs whose immune system had been previously stimulated by diverse antigens (Fig. 2). In contrast, trachea showed the lowest mean number of PC, which could be due to 2 reasons: the 1st, that trachea is an avascular tissue, being irrigated by peripheral blood vessels; the 2nd that it is not in direct contact with the environment, but protected by

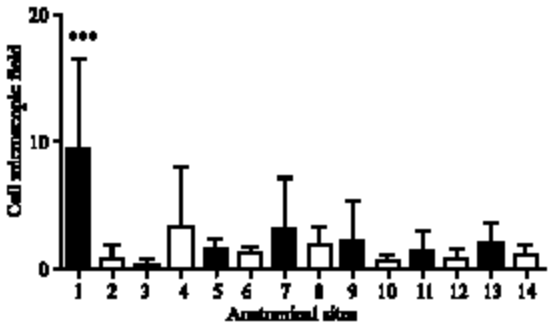

Fig. 1: Number of plasma cell in different anatomical regions in the pig respiratory tract (n = 5). Bars represent the mean and standard error of positive cells in a minimum of 10 microscopic field per animal, 1: Tonsil, 2: Epiglottis, 3: Trachea, 4: Right lung (RI): cranial lobe (apex), 5: R1 medial lobe (dorsal border), 6: R1 medial lobe (acute border), 7: R1 caudal lobe (caudal extreme of the dorsal border), 8: R1 caudal lobe (central portion), 9: R1 accessory lobe (acute border, medial portion), 10: Left lung (L1), cranial lobe (apex), 11: L1 cranial lobe (caudal portion, dorsal border), 12: L1 cranial lobe (caudal portion, acute border), 13: L1 caudal lobe (caudal extreme of the dorsal border), 14: L1 caudal lobe central portion. Statistically significant differences ***($p<0.001$)

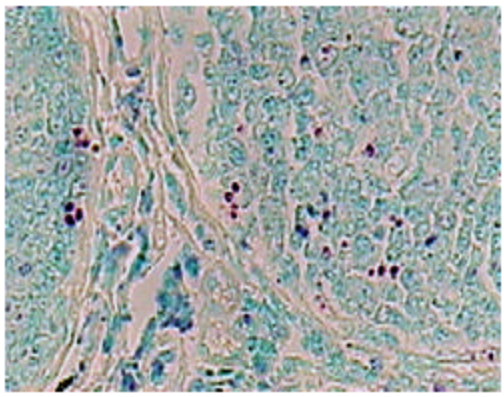

Fig. 2: Section of tonsil. Showing plasma cells (red cells). Methyl green-pyronine stain

physical barriers, such as the mucociliary system, where particles and pathogens are trapped, which would stimulate interaction with B cells and thus PC formation.

Peeters *et al*. (2005) quantified the number of PC in dog nasal cavity, tracheal carina, primary and secondary bronchi and terminal bronchioles by immunohistochemical staining. IgA, IgG and IgM were detected. The mean numbers of IgA-secreting (2.56±0.3) and IgM-secreting PC (1.02±0.14) in tracheal carina were significantly different to mean IgG-secreting PC (0.71±0.16). Also in dog, the primary, secondary and tertiary bronchi, showed IgA-secreting PC numbers which were significantly higher than those of other PC types. These data reveal that there are no great numerical differences between species.

In pigs, the highest mean was found in the tonsil and the right cranial lung lobe (apex). Anatomically, pigs display a bronchus that ventilates the right cranial lobe and directly originates in the trachea, thus allowing the entry into this portion of air, which has been previously filtered by the barriers where pathogens are trapped.

CONCLUSION

The present study provided quantitative information on the plasma cell in respiratory tract of pig clinically healthy. Plasma cells are dispersed and scarce in the lung tissue of clinically healthy pigs. PC showed ovoid morphology, with eccentric nucleus and dense chromatin. The highest number of PC was obtained in the tonsil and the lowest number in the trachea. Of the different pulmonary regions, the right cranial lung lobe (apex) revealed the highest number of PC. This will enable comparisons to be made in future studies of pigs suffering from infectious respiratory diseases.

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