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Tracheal and bronchial polymeric immunoglobulin secretory immune system (PISIS) development in a porcine model

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ABSTRACT

Polymeric immunoglobulins (pIgs) mucosal secretion is mediated by the pIg secretory immune system (PISIS), which is composed of J-chain (JC) and antibody (IgM/IgA) producing cells (JC-AbPC), pIg receptor (pIgR) epithelial cell expression and the efficient release of secretory Igs (SIgs) to the mucosal lumen. A poor development or disturbances in this system may cause higher infection susceptibility, as observed in young and elderly people. In spite of this system's importance, few detailed studies regarding its development have been described in the lower respiratory tract of humans. Because the porcine model has been reported as an option for translational medicine to humans, we studied the tracheal and bronchial PISIS development in healthy, non-vaccinated, SPF, miniature Vietnamese pigs from birth to adulthood using immunohistochemistry and ELISAs. Our results demonstrated that pIgR was present at birth, and its expression increased with age. In contrast, JC-AbPC were low in neonatal pigs; however, colostrum was a source of IgM, SIgA, total IgA and IgG in respiratory secretions (trachea and bronchoalveolar lavages, nasal secretion and saliva) in piglets. JC-AbPC steadily increased in post-weaned, young and adult pigs, correlating with considerable increases in secretory and total Igs in the trachea and bronchi. These data suggest a compensatory role of maternal Igs at the respiratory mucosa in the absence of a structured PISIS before weaning. Furthermore, monomeric Igs (IgG and IgA) may also play an important role in respiratory protection and deserves a more thorough study.

1. Introduction

The respiratory tract is a mucosal site with higher antigenic exposition. A mechanism that controls the access of antigens (Ags) to the organism is the mucosal polymeric immunoglobulin (pIg) secretion, which is mediated by the polymeric immunoglobulin secretory immune system (PISIS). The PISIS elements include: pIg producing cells (synthetizing also the J-chain, JC); pIg receptor (pIgR) expression on the mucosal epithelial cell basolateral surface, which transport pIgs from the lamina propria (LP) to the mucosal surface and; pIg-pIgR complex liberation to the mucosal lumen, which is composed of the pIgR extracellular domain (i.e., the secretory component, SC) and the attached pIg (Brandtzaeg and Johansen, 2005; Reynolds, 1991; Strugnell and Wijburg, 2010). The absence of any of these elements could impair mucosal homeostasis (Holt et al., 2008; Karlsson et al., 2010), producing inflammatory conditions, such as asthma and allergies (Arnaboldi et al., 2005), chronic obstructive pulmonary disease (Gosman et al., 2006; Pilette et al., 2001) or enhance infection susceptibility (Arulanandam et al., 2001), which is observed in children (Renz et al., 2011) and elderly people (Blomberg and Frasca, 2011). Thus, PISIS development studies will help to better understand the respiratory immune system and also to determine the proper age for early mucosal vaccination.

The respiratory immune system comprises immune cells, including B and T lymphocytes, antigen presenting cells (macrophages and dendritic cells), monomeric and polymeric Igs, cytokine and chemokine production, receptors and extracellular matrix elements, whose presence and interactions orchestrate local protection (Holt et al., 2008). There are immune differences between the upper and the lower respiratory tracts (Holt et al., 2008; Reynolds, 1991); however, few reports have detailed these differences in humans because of the difficulties in obtaining adequate mucosal samples (Burnett et al., 1987; Heier et al., 2011; Soutar, 1976; Tschernig et al., 2001). In contrast, the development of nasal, larynx and bronchus associated lymphoid tissues (NALT, LALT

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and BALT, respectively) has been studied in animal models, such as mice (Rangel-Moreno et al., 2011), rats (Sosa et al., 2009), guinea pigs (Li et al., 2001), rabbits and others (Karol, 1994); however, their anatomic, physiologic and genetic differences with humans make the extrapolation of the results difficult. Recently, the use of the pig has been proposed as a model of human translational research (Judge et al., 2014). The immune advantages have been revised (Butler et al., 2009a) and experimental conditions have supported its use (Meurens et al., 2012). Developmental studies regarding the PISIS in the respiratory tract are limited (Bals et al., 1998; Bradley et al., 1976; Huang et al., 1990; Krejci et al., 2013; Morgan et al., 1980); therefore, we studied its development with age in a porcine model.

2. Materials and methods

2.1. Animals

Thirty-four, non-vaccinated, specific pathogen-free (SPF), Vietnamese potbellied minipigs from multiparous sows were used. These animals were obtained from the Animal Production and Experimentation Unit (UPEAL) of the Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), Mexico. The animals were divided into the following groups: Pigs who were weaned at birth (0DP, n = 6); suckled pigs at 2 days of age (2DP, n = 8); 6 week-old pigs, who were weaned 2 weeks earlier (6WP, n = 8); 3-month-old pigs (3 MP, n = 7) and; 6-month-old pigs (6 MP, n = 5). All of the animals were kept, handled and humanely euthanized following institutional guidelines (NOM-062-ZOO-1999), which were approved by the Institutional Use and Handling of Experimental Animals Committee (CICUAL).

2.2. Serum, nasal secretion and saliva

Prior to euthanasia, serum, nasal secretion and saliva samples were collected as described elsewhere (Guzman-Bautista et al., 2014).

2.3. Tracheal and bronchial samples

After euthanasia, tracheas were obtained and ligated below the larynx and above the right cranial lobe bronchus. Lungs were obtained and extensively washed with physiological saline through the pulmonary artery to remove the intravascular blood, as described by Balam-May et al. (2014). Tracheal and bronchoalveolar lavage (TL and BAL, respectively) samples were obtained with PBS. The volume used for TL/BAL samples was adapted according to the animal's age as follows: 0DP and 2DP (500 μL/10 mL), 6WP (2 mL/40 mL), and 3 MP and 6 MP (5 mL/50 mL). All of the mucosal samples were mixed with a protease inhibitor cocktail; TPCK 50 μg/mL, TLCK 25 μg/mL (Sigma, Switzerland), and PMSF 174 μg/mL (Sigma, China) at a 20:1 (sample:cocktail) ratio and were immediately stored at −20 °C until used.

The lower respiratory tract was examined for macro and micro inflammatory signs. Tissue samples were taken from the middle longitudinal tracheal region. The porcine tracheobronchial tree was obtained from a procedure that was standardized in our laboratory. Because of the complexity of the tissue, two clusters of bronchi were defined using the nomenclature of Judge et al. (2014), which depends of the site of ventilation and identification by bronchoscopy: the larger bronchi cluster was formed by the left cranial lobe bronchus (LB1), left caudal lobe bronchus (LB2), right cranial lobe bronchus (RB1), right middle lobe bronchus (RB2), right accessory lobe bronchus (RB3) and right caudal lobe bronchus (RB4). The smaller branches cluster comprises the smaller branches of the LB1, RB2 and RB3. Tissues were immediately fixed in 4% formaldehyde in PBS (Baker, Mexico) and finally embedded in paraffin (Paraplast®, Leica Richmond, USA).

2.4. Immunohistochemistry

The paraffin embedded tissues were cut in 5 μm thick serial sections and collected on (3-aminopropyl) trimethoxysilane (Sigma Aldrich, Germany) coated slides. Dewaxed tissue sections were stained for immunohistochemistry (IHC). For optimal staining, enzymatic-epitope retrieval was applied to the samples with trypsin 0.1% (Gibco, Canada) in a buffered solution, pH = 7.0, 200 mM Tris (Invitrogen, USA), 4 mM $CaCl_2$ (Sigma, Japan) for 15 min at 37 °C. For the JC producing cells, 6 M urea (Sigma, Germany) was used for 30 min at room temperature, as reported by Korsrud and Brandtzaeg (1980). To block the endogenous peroxidase activity, 6% hydrogen peroxide in methanol was used and non-specific Ig attachment was blocked with normal goat and porcine serum. Both were incubated for 60 min at room temperature. The utilized primary antibodies, with their dilutions, were as follows: Rabbit anti-human J chain, 1/2000 (InvivoGen, USA), goat anti-porcine IgM-HRP conjugated, 1/600 (Bethyl, USA), mouse anti-porcine IgA, clone F9, 1/25 (U. of Bristol, UK) and mouse anti-porcine secretory component, 1/400, clone K60 1F1 (Serotec, USA). Each antibody was incubated overnight at 4 °C in a humid chamber. Biotin-xx-goat anti-rabbit IgG (H + L), 1/300, cat: B2770 (Invitrogen, USA) or biotin-xx-goat anti-mouse IgG (H + L), 1/2000, cat: B2763 (Invitrogen, USA), were used for 60 min at room temperature, according to the primary antibody species. Where appropriate, streptavidin-HRP, 1/500 (Zymed, USA) was incubated for 60 min at room temperature, and 3,3-diaminobenzidine (Sigma, USA) was applied to develop the reaction. ChromPure rabbit IgG, 1/10000, cat: 011-000-003 (Jackson ImmunoResearch, USA) or a non-related mouse primary antibody, cat: 08-6599 (Invitrogen, USA) isotype control was run as primary Ab in every sample. Other sections were stained with H&E for histological evaluation.

2.5. Cell population quantification

Analysis of the stained slides was performed using a light microscope, Eclipse E4000 (Nikon, Japan) and the pictures were digitally recorded with Image-Pro Plus 7.0 (Media Cybernetics, USA) at 200× for quantification. Three zones for analysis were established in the trachea, the larger bronchi and the smaller branches according to Tschernig et al. (2006), which included the epithelium, lamina propria (LP; 100 μm from the basement membrane) and the submucosa (SM; 100–400 μm from the basement membrane). The cell density was recorded as the total number of positive cells per square millimeter (cells/mm^2), from 10 to 15 visual fields from each analyzed animal. Glands and intercartilage areas were excluded from the counts.

2.6. IgM, IgG, SIgA and total IgA ELISA quantification

IgM, IgG, SIgA and total IgA (IgA, pIgA and SIgA) were measured in the serum, TL, BAL, nasal secretion and saliva with quantitative ELISA tests. A kit was used to evaluate the porcine IgM, E100-100 (Bethyl, USA) and porcine IgG, E100-104 (Bethyl, USA), according to the manufacturer instructions. The samples were diluted threefold, starting with 1/1500 for serum and 1/5 for the mucosal secretions. Because the kit did not discriminate amongst IgA, pIgA and SIgA, the three were measured together as total IgA. To measure the SIgA, a sandwich ELISA test was standardized in our laboratory, as a modification of the Jaffar et al. (2009) procedure. Both determinations (SIgA and total IgA) were run simultaneously.