Presence of N-acetylgalactosamine/galactose residues on bronchioalveolar cells during rat postnatal development

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Abstract

In mammals, the alveolarization process develops predominantly after birth. Airway cells display a complex assemblage of glycans on their surface. These glycans, particularly terminal glycan extensions, are important effective carriers of information that change during the differentiation process. Nevertheless, few systematic data are reported about the cell surface sugar residue content during postnatal lung development.

In the present work, we aimed to identify and semi-quantify N-acetylgalactosamine (GalNAc)/galactose (Gal) residues on the bronchioalveolar cell surface in rat lung sections from 1-, 4-, 8- day-old and adult animals and link these data with the lung glycocalyx composition. Horseradish peroxidase-conjugated lectin from Glycine max (soybean agglutinin, SBA) was used, and light microscopy methodologies were performed. SBA labelling intensity was studied before and after sialidase pre-treatment, in 1-, 4-, and 8-day-old animals and adult animals. For semi-quantitative evaluation of SBA binding intensity, two investigators performed the analysis independently, blinded to the type of experiment. Reactivity of the lectin was assessed in bronchiolar and respiratory portion/alveolar epithelial cell surfaces. We evidenced a stronger positive reaction when lung sections were pre-treated with neuraminidase before incubation with the lectin in 1- and 4-day-old animals and adult animals. These results were not so manifest in 8- day-old animals. This binding pattern, generally points towards the presence of terminal but mainly sub-terminal GalNAc/Gal residues probably capped by sialic acids on the rat bronchiolar/respiratory tract epithelial cells. As this glycan extension is common in O- and N-glycans, our results suggest that these glycan classes can be present in bronchioalveolar cells immediately after birth and exist during the postnatal period. The results observed in eight-day-old rat lung sections may be due to the dramatic lung morphologic changes and the possible underlying biological mechanisms that occur during this age-moment.

Introduction

The mammalian respiratory system is a complex branching structure that arises from the ventral foregut endoderm, with endoderm mesoderm interactions indispensable for the characteristic branching morphogenesis.1-3 During the embryonic period, the fetal period and the postnatal period, the lung develops from an outpouching of the foregut to a tree-like system and ultimately to a gas exchange area.4,5 Over the years, postnatal lung development has been extensively investigated in experimental animals and in humans, and it is now well known that at birth, the mammalian lung gas exchange apparatus undergoes very important structural modifications, being the formation of highly septated and alveolarized structures a key event.

During this phase, new septa are formed by a subdivision of the terminal air spaces, “sacculi”, followed by microvasculature maturation with the onset of a single-layered capillary network and still more alveoli generation.6-10 Unlike humans, rats and mice are born during the saccluar stage, but except for the phase of development at birth, rat lung postnatal development comprises the same developmental steps as human lung in the alveolarization process.7,10,11 With the advent of new imaging tools, it is now accepted that mammalian lung development continues throughout adulthood.12,13 Intrapulmonary epithelial cells, including ciliated cells, club cells, type II and type I cells, share a common lineage and undergo profound biochemical and morphofunctional changes during pre- and postnatal lung maturation.5,14-16

A complex and intricate array of monosaccharides or oligosaccharides, generically named glycans, surrounds the living cells of every organism and is required for critical cellular functions. The numerous physiological functions of glycans include organizational, modulatory, protective, interactive, and recognition roles and can be placed into three categories: structural, recognition and molecular mimicry of host glycans.12-15 Glycans located at the cell surface that define the molecular frontier of cells are closely implicated in cell-cell, cell-matrix and cell-molecule interactions and are involved in many biological events during development, such as differentiation and organogenesis.16-21 Carbohydrate chains of cell surface glycans can have spatial and temporal patterns of expression during development, and many glycan extensions are regulated not only during embryonic and fetal periods but also during postnatal development.22-24 Since the early 1950s, with the description of a pulmonary extracellular lining layer by Low23 and Macklin24, different histochemical methodologies, including those using lectins, have been applied in lung glycan analysis. Different studies have shown the presence of several glycoconjugate sugar residues during lung development using diverse lectins, and different morphological lung growth stages.23,24 However, to the best of our knowledge, systematic information on the composition of mammalian postnatal lung glycocalyx is scarce.

In the present study, we continued previously published studies and sought to identify and compare the presence of N-acetylgalactosamine (GalNAc)/galactose (Gal) residues on rat bronchioalveolar epithelial cell surfaces using lectin histochemical light microscopy (LM) method-
ologies. The binding pattern of lectin from Glycine max (common name soybean agglutinin, SBA) with specificity to GalNac/Gal residues, before and after neuraminidase treatment, was systematically studied and compared in rat lung sections from 1-, 4-, 8-day-old and adult animals, and possible modifications of presence of these saccharides in all the age moments studied was surveyed.

**Materials and Methods**

**Animals and tissue collection**

Wistar rats, including adult rats, animals older than a week and pregnant females, were supplied by CIFRA, S.A. (Barcelona, Spain).

An intraperitoneal anaesthesia administration was performed using sodium pentobarbital (25 mg/kg), and the lungs were collected from 1-, 4-, and 8-day-old rat pups and from nine-week-old adult animals, for a total of 20 sacrificed animals.

After the thorax and abdomen were opened and an incision in the abdominal aorta was performed, the anaesthetized animals were submitted to lung perfusion via the right ventricle with a 0.1 M phosphate-buffered saline (PBS, pH 7.4) solution at a flow rate of 1 mL/min to 6 mL/min for 90 sec, according to the age. The fixation process followed this perfusion and began with a 5-min perfusion with 4% formaldehyde in PBS (pH 7.4) containing 0.5% glutaraldehyde. Collapsed lungs were excised and immersed in the same fixative solution for 16 h at 4°C. Samples from different pulmonary lobes were studied in each age group. Lung pieces were rinsed in 0.5 M ammonium chloride (NH4Cl) in PBS for 1 h at room temperature (RT) to block free aldehyde groups, then washed in PBS for one hour at RT, and finally processed for embedding in paraffin wax; 5 μm sections were made.

All the procedures were performed under the protocols established by the Ethics Committee at the Faculty of Medicine in Coimbra, Portugal.

**Lectin histochemistry**

Endogenous peroxidase was inhibited by treating sections after being routinely dewaxed with methanol containing 0.6% hydrogen peroxide (H2O2) for 30 min at room temperature (RT). Subsequently, tissue sections were incubated for one hour in a moist chamber with horseradish peroxidase-conjugated SBA (Sigma; Saint Louis, MO, USA, L-2650) diluted in 0.05 M Tris-buffered saline (TBS, pH 7.5) at the appropriate concentration, as shown in Table 1. Different lectin concentrations were studied to check reproducibility and reach the final concentration. After lectin incubation, the slides were rinsed in TBS and developed for 4% ammonium chloride (NH4Cl) in PBS for 10 min at RT (Sigma; D-5905; 48H8202). After rinsing the sections in distilled water, the nuclei were counterstained with haematoxylin, cleared in xylene and mounted.

The stained sections were observed under a microscope (Nikon Eclipse Ci-L, Konan, Minato-ku, Tokyo, Japan) equipped with a XFCA1M1080PHB/PHD CMOS digital camera (Toup Tek, Zhejiang, China) and programme ToupView. Digitalized images were captured under identical conditions.

**Sialidase digestion**

It is well known that sialic acids mainly occur as terminal components of cell surface glycans, acting as “caps” of underlying glycans. Bearing that in mind, neuraminidase digestion was performed in some experiments to remove terminal sialic acid residues prior to lectin staining. LM sections were incubated for 18 h at 37°C in a 0.1 M acetate buffer solution (pH 5.5) containing 0.15 M NaCl, 40 mM calcium chloride (CaCl2) and 0.8 U/ml neuraminidase Type X from Clostridium perfringens (Sigma; N-2133).

**Semi-quantitative evaluation of SBA binding intensity**

For semi-quantitative evaluation of SBA binding intensity, two investigators performed the analysis independently, blinded to the type of experiment (age-moment and pre-treated or non-pre-treated sections with sialidase). Reactivity of the lectin was assessed in bronchiolar and respiratory portion/alveolar epithelial cell surfaces. Binding intensity was evaluated using a semi-quantitative scale as follows: – negative labelling; + faint; ++ positive; +++ strong.

**Control for lectin staining**

The lectin staining specificity was evaluated by pre-incubation of the horseradish peroxidase-conjugated SBA with the corresponding inhibitory sugar (0.2 M; Table 1) for 50 min before staining.

**Results**

The cytochemical reaction observed after incubating rat lung sections with horseradish peroxidase-conjugated SBA was present on the surface of bronchiolar and respiratory tract epithelial cells, but it was generally faint. However, when tissue sections were pre-incubated with neuraminidase before SBA staining, the intensity of the reaction markedly increased, in most cases.

Therefore, we observed a weak reaction on the luminal surface of bronchiolar cells from 1-day-old rat lungs (Figure 1A). However, the apical cell surface of bronchioles and the respiratory tract from one-day-old rats are intensely stained when SBA was incubated after neuraminidase pre-treatment (Figure 1B and Table 2). When we compare the non-pre-treated sections with those for lung sections pre-treated with sialidase, the same labelling pattern was evident in 4-day-old (Figure 1 C,D; Table 2). Accordingly, in four-day-old rat lungs, we could also see an evident positive reaction on the luminal surface of bronchiolar cells and in the respiratory portion when the sections were pre-treated with the enzyme (Figure 1D). Comparatively, a weak reaction is seen in bronchiolar and respiratory cells when non-pre-treated lung sections were incubated with SBA (Figure 1C).

In 8-day-old rat lung sections, the difference in reaction intensity between non-pre-treated and pre-treated sections was not so evident (Figure 1E; Table 2).

In adult animals, SBA reactivity was detected on the luminal surface of ciliated and non-ciliated bronchiolar cells (Figure 2A). The alveolar epithelium of an adult animal was weakly stained by SBA, as shown in Figure 2C.

However, when the sections were pre-treated with neuraminidase, the staining

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**Table 1. Lectin characteristics.**

| Botanical name | Common name | Acronym | SBA concentration (g/mL) | Saccharide affinity | Inhibitory saccharide |
|----------------|-------------|---------|--------------------------|---------------------|----------------------|
| Glycine max    | Soybean     | SBA     | 20                       | GalNac>>Gal         | GalNac               |
intensity increased. Thus, the luminal surface of club and ciliated cells is heavily stained with SBA in enzyme pre-treated sections (Figure 2B). The alveoli epithelial cells also showed a stronger affinity for SBA in pre-treated than in non-pre-treated sections (Figure 2D,C).

The semi-quantitative binding evaluation (Table 2) reflects the evident increase in SBA binding when lung sections were pre-treated with sialidase, which can be observed in all age-moments studied except for the 8-day-old age.

The lectin staining was entirely absent when one-day-old rat lung sections were pre-incubated with SBA and the corresponding inhibitory sugar (Figure 3).

Discussion

The rat lung is not completely mature at birth, and the gas-exchange surface area expands during the postnatal period.9,36,37 The cytodifferentiation of the epithelial cells that line the bronchioalveolar airway is a continuous process occurring mostly after birth.38 A dynamic expression pattern of molecular mediators regulating cell differentiation was demonstrated in rat lung development during pre- and also postnatal periods.39-41

In addition to generating energy in the cell, carbohydrates act as signalling effectors and recognition markers and are key elements in post-translational modifications of proteins. It is now clear that the expression of certain glycans changes in different stages of development, which implies different roles for these glycoconjugates.19,42 It was proven that N-glycans are important for airway epithelium organization during development. On the other hand, animals lacking O-fucose glycans in the lung have deficits in pulmonary development, particularly due to a lack of secretory cells.43-45 Therefore, the study of the bronchioalveolar cell surface glycan composition during postnatal rat lung development can contribute to a better understanding of lung development.

Since the middle of the 20th century, lectin histochemistry is one of the major approaches to characterize glycoconjugates, and the binding patterns of different lectins have been studied in different organs of humans and experimental animals, including rats.34,46-49 Concerning lung histochemical investigations, authors generally used different lectins, pointing out the most obvious results for each lectin.50-53

Our LM lectin histochemistry data, although not covering the second phase of alveolarization, showed that SBA binds to bronchiolar and respiratory tract epithelial cells in rat lung sections from 1-, 4-, 8-day-old and adult animals. However, although present, the binding sites are generally much more evident after neuraminidase treatment. These results are in accordance with Iwatsuki et al.,31 who revealed “a small number of SBA binding sites” on adult rat alveolar cells. Shimizu et al.54 also reported that only a small percentage of rat tracheobronchial cells reacted with SBA as well as with other lectins studied. Castells et al.,35

![Figure 1. An increase in SBA binding is evident in sialidase pre-treated sections (arrows in B and D) when compared with non-pre-treated sections (arrowheads in A and C) from 1-day-old (A and B) and from 4-day-old (B and C). This increase is not so evident in sections from 8-day-old rat tissue sections (arrowheads in E and arrows in F).](image)

**Table 2. Semi-quantitative binding intensity of SBA to bronchiolar and respiratory portion/alveolar cell surfaces in four different postnatal age moments.**

| AGE MOMENT | One-day-old | Four-day-old | Eight-day-old | Adult |
|------------|-------------|--------------|---------------|-------|
| NPT        | PT          | NPT          | PT            | NPT   |
| **SBA staining intensity** | | | | |
| Bronchiolar cell surfaces | + | +++ | + | +++ |
| Respiratory portion/alveolar cell surfaces | + | +++ | + | +++ |

NPT, non-pre-treated; PT, pre-treated; +++, strongly positive; ++, positive; +, faint; –, negative labeling.
studying the pre- and postnatal developing rat respiratory system by means of lectin histochemistry, reported a positive reaction of SBA on the goblet cells of adult rat airway epithelium. After neuraminidase treatment, the same authors did not describe the SBA results but reported an increase in the peanut agglutinin (PNA) binding sites, whose carbohydrate sequence binding specificity is Gal-GalNAc. 

Martins et al.\textsuperscript{33} demonstrated that at the ultrastructural level, the SBA labelling pattern is faint and present on both adult rat alveolar and endothelial cells. SBA staining is much stronger after sialidase treatment than before treatment and predominantly located on the surface of the alveolar epithelial cells. Our present results systematically point towards the presence of GalNAc/Gal residues on the bronchiolar/respiratory tract epithelial cells in the age-moments studied. In all the ages studied, these residues are also much more available for lectin binding after neuraminidase treatment, suggesting that sialic acid moieties are capping GalNAc/Gal residues. These labelling patterns were consistent in 1- to 4-day-old and adult rat lungs. In 8-day-old animals the less evident difference between non-pre-treated and pre-treated sections suggests a lower availability of sub-terminal GalNAc/Gal residues for the lectin. This could be due to the dramatic morpho-biological transformations that the lung experiences at this age. A massive number of alveoli are formed in a period that in rats occurs on postnatal (P) day 3 to P14. After this period, alveoli continue to develop although in a slower pace.\textsuperscript{56,57}

These relevant transformations, which are notably present around P8 may underlie molecular changes that could lead to modifications in the complex structure of surface glycans. Therefore, a transitional period where N-acetylgalactosamine/ galactose residues are less available to SBA in sub-terminal locations could be present. In adult rat lung sections, the binding patterns indicate the presence of GalNAc/Gal residues in terminal, but mainly in sub-terminal positions.

In conclusion, data presented here indicates a systematic increase in the positive reaction of SBA after neuraminidase treatment in 1- and 4-day-old and adult rat lung sections, which advocates a prevailing sub-terminal presence of GalNAc/Gal residues mainly with terminal sialic acids. These glycan compositions are present in glycoproteins, indicating the existence of O- and N-glycans on rat bronchiolar/respiratory tract portion cells immediately after birth and during the postnatal period. In 8-day-old rats, the less obvious difference in reaction intensity between pre-treated and non-pre-treated sections may be due to dramatic changes that occur during the “bulk alveolarization” process on this age-moment. Nevertheless, further studies are required to more clearly understand the lung glyocalyx composition during postnatal lung development and a great deal of research persists to be done before the composition and role of lung surface glycans can be fully understood.

References

1. Alescio T, Cassini A. Induction in vitro of tracheal buds by pulmonary mesenchyme grafted on tracheal epithelium. J Exp Zool 1962;150:83-94.
2. Cardoso WV. Lung morphogenesis revisited: old facts, current ideas. Dev Dyn 2000;219:121-30.
3. Spooner BS, Wessells NK. Mammalian lung development: Interactions in primordial formation and bronchial morphogenesis. J Exp Zool 1970;175:445-54.
4. Merkus, PJFM. Ten Have-Opbroek AAW. Quanjer PH. Human lung growth: A Review. Pediatr Pulmonol 1996;21:383-97.
5. Schittny JC. Development of the lung. Cell Tissue Res 2017;367:427-44.
6. Massaro GD, Massaro D. Formation of pulmonary alveoli and gas-exchange surface area: quantification and regulation. Ann Rev Physiol 1996;58:73-92.
7. Mund SI, Stampanoni M, Schittny JC. Developmental alveolarization of the mouse lung. Dev Dyn 2008;237:2108-16.
8. Thurlbeck WM. Postnatal human lung growth. Thorax 1982;37:564-71.
9. Tschanz SA, Salm LA, Roth-Kleiner M, Barré SF, Burri PH, Schittney JC. Rat lungs show a biphasic formation of new alveoli during postnatal development. Am J Physiol 2014;317:L97-95.
10. Zeltinger TB, Caduff JB, Gehr P, Pfenninger J, Burri PH. The postnatal development and growth of the human lung. I. Morphometry. Respir Physiol 1987;67:247-67.
11. Burri PH. The postnatal growth of the rat lung III. Morphometry. Anat Rec 1974;180:77-98.
12. Butler JP, Loring SH, Patz S, Tsuda A, Yablonskiy DA, Mentzer SJ. Evidence for adult lung growth in humans. New Engl J Med 2012;367:247.
13. Narayanan M, Owers-Bradley J, Beardsmore CS, Mada M, Ball I, Gurirov R, et al. Alveolarization continues during childhood and adolescence. Am J Respir Crit Care Med 2012;185:186-91.
14. Cardoso WV, Whitsett JA. Resident cellular components of the lung. Developmental aspects. Proc Am Thorac Soc 2008;5:767-71.
15. Herriges M, Morrissey EE. Lung development: orchestrating the generation and regeneration of a complex organ. Development 2014;141:502-13.
16. Ten Have-Opbroek AAW. Lung development in the mouse embryo. Exp Lung Res 1991;17:111-30.
17. Saisiekharan R, Myette JR. The sweet science of glycobiology: Complex carbohydrates, molecules that are particularly important for communication among cells, are coming under systematic study. Am Sci 2003;91:432-41.
18. Varki A. Evolutionary forces shaping the Golgi glycosylation machinery: why cell surface glycans are universal to living cells. Cold Spring Harb Perspect Biol 2011;3: pii: a005462.
19. Varki A, Gagneux P. Biological functions of glycans. In: Varki A et al., editors. Essentials of glycobiology. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 2017. p. 77-88.
20. Fukuda M. Cell surface carbohydrates: cell type-specific expression. In: Fukuda M, Hindsaul G, editors. Molecular and cellular glycobiology. Oxford: Oxford University Press; 2000.
21. Haltiwanger RS, Lowe BL. Role of glycosylation in development. Annu Rev Biochem 2004;73:491-537.
22. Stanley P. What have we learned from glycosyltransferase knockouts in mice? J Mol Biol 2016;428:3166-82.
23. Tran DT, Ten Hagen KG. Mucin-type O-glycosylation during development. J Biol Chem 2013;288:6921-9.
24. Varki A, Kornfeld S. Glycobiology: Historical background and overview. In: Varki A et al., editors. Essentials of glycobiology. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 2017. p. 1-18.
25. Low FN. Electron microscopy of the rat lung. Anat Rec 1952;113:437-47.
26. Macklin C. The pulmonary alveolar mucoid film and the pneumocytes. Lancet 1954;1:1099-2005.
27. Barkhordari A, Stoddart RW, McClure SF, McClure J. Lectin histochemistry of normal human lung. J Molec Histol 2004;35:147-56.
28. Castells MT, Ballesta J, Pastor LM, Madrid JF, Marin JA. Histochemical characterization of glycoconjugates of the extrapulmonary airways of several vertebrates. Histochem J 1990;22:24-35.
29. Dorscheid DR, Conforti AE, Hamann SF, McClure J. Lectin histochemistry of the lung epithelium of the extrapulmonary airways. In: Grippi MA, Sherker MA, Hussain A, Ul-haq R. Molecular and physiological determinants of pulmonary developmental biology: a review. Am J Biomed Res 2013;1:13-24.
30. Cardoso WV, Lu J. Molecular regulation of lung development. In: Grippi MA, Editor-in-Chief. Fishman’s pulmonary diseases and disorders. McGraw-Hill Education; 2015.
31. Chen YD, Liu JY, Lu YM, Zhu HT, Tang W, Wang GX, et al. Functional roles of CEBP beta and SUMO-1 modification in lung development. Int J Molec Med 2017;40:1037-46.
32. Gaillard D, Puchelle E. Differentiation and maturation of airway epithelial cells: roles of extracellular matrix and growth factors. In: Gaultier C, Bourbon JR, Post M, editors. Lung development. Oxford: Oxford University Press; 1999. p. 46-76.
33. Wang X, Inoue S, Gu J, Miyoshi E, Noda K, Li W, et al. Dysregulation of TGF-beta 1 receptor activation leads to abnormal lung development and emphysema-like phenotype in core fucose-deficient mice. Proc Natl Acad Sci 2005;102:15791-6.
34. Freeze HH, Baum L, Varki A. Glycans in systemic physiology. In: Consortium of Glyobiology Editors. Essentials of glycobiology. 3rd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2017. p. 521-526.
35. Lau KS, Partridge EA, Grigorian A,
Silvescu CI, Reinhold VN, Demetriou M, et al. Complex N-Glycan Number and Degree of Branching Cooperate to Regulate Cell Proliferation and Differentiation. Cell 2007;129:123-134. doi: 10.1016/j.cell.2007.01.049
46. Cummings RD, Darvill AG, Etzler ME, Hahn MG. Glycan-recognizing probes as tools. In: Varki A et al., editors. Essentials of glycobiology. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 2017. p. 611-25.
47. Mislovicová D, Gemeiner P, Kozarova A, Kozár T. Lectinomics I. Relevance of exogenous plant lectins in biomedical diagnostics. Biologia 2009;64:1-19.
48. Sharon N. Lectins: carbohydrate-specific reagents and biological recognition molecules. J Biol Chem 2007;282:2753-64.
49. Ito T, Nagahara N, Ogawa T, Inayama Y, Kanisawa M. Lectin binding to the luminal surface of distal airway epithelial cells of rodents. J Electron Microsc 1985;34:381-3.
50. Honda T, Hayasaka M, Hachiya T, Ota K, Katsuyama T. Carbohydrate histochecimy of the apical membranes of non-ciliated bronchiolar cells and type II pneumocytes in six mammalian species. Acta Histochem Cytochem 1995;28:107-17.
51. Thom I, Schult-Kronenfeld O, Burkholder I, Goern M, Andritzky B, Blonski K, et al. Lectin histochemistry of metastatic adenocarcinomas of the lung. Lung Cancer 2007;56:391-7.
52. Tsokos M, Anders S, Paulsen F. Lectin binding patterns of alveolar epithelium and subepithelial seromucous glands of the bronchi in sepsis and controls—an approach to characterize the non-specific immunological response of the human lung to sepsis. Virchows Arch 2002;440:181-6.
53. Yi SM, Harson RE, Zabner J, Welsh MJ. Lectin binding and endocytosis at the apical surface of human airway epithelia. Gene Ther 2001;8:1826-32.
54. Shimizu T, Nettesheim P, Mahler JF, Randell SH. Cell type-specific lectin staining of the tracheobronchial epithelium of the rat: quantitative studies with Griffonia simplicifolia I isoelectin B4. J Histochem Cytochem 1991;39:7-14.
55. Castells MT, Ballesta J, Madrid JF, Aviles M, Martinez-Menarguez JA. Characterization of glycoconjugates in developing rat respiratory system by means of conventional and lectin histochemistry. Histochemistry 1991;95:419-26.
56. Meyrick B, Reid L. Pulmonary arterial and alveolar development in normal postnatal rat lung. Am Rev Respir Dis 1982;125:468-73.
57. Lau M. Long-term changes in alveolarization in the postnatal rat following transient inhibition of early “classical” alveologenesis. PhD Thesis. University of Toronto; 2010.