



ELSEVIER

DRUG DISCOVERY
TODAYDISEASE
MODELS

Drug Discovery Today: Disease Models

Vol. 6, No. 4 2009

Editors-in-Chief

Jan Tornell – AstraZeneca, Sweden

Andrew McCulloch – University of California, San Diego, USA

Asthma and respiratory diseases

Sheep as a model species for the study and treatment of human asthma and other respiratory diseases

Els N. Meeusen¹, Kenneth J. Snibson², Stuart J. Hirst³, Robert J. Bischof^{1,3}

¹Biotechnology Research Laboratories, School of Biomedical Sciences, Monash University, Australia

²Centre for Animal Biotechnology, School of Veterinary Science, The University of Melbourne, Australia

³Airway Pathophysiology Laboratory, Department of Physiology, Monash University, Australia

Classic studies in sheep have contributed greatly to our understanding of lung physiology in health and disease. Similarities in size and structure between sheep and human lungs allow testing of new treatments using the same equipment and procedures used in human medicine, thereby facilitating translation of findings into the clinic. Sheep models of respiratory diseases, in particular sheep models of asthma, continue to be refined and provide unique opportunities for both basic and applied studies of respiratory diseases and their treatments.

Introduction

Mice are the most widely used animal disease model, while rats are generally used as a standard model for testing new drug and delivery therapies before proceeding to clinical trials. As models for respiratory research, however, these rodents have the disadvantage of small body size and fundamental differences in lung structure, physiology and development compared to humans (reviewed in [1,2]). For the treatment of pulmonary disease, direct drug administration into the lung by inhalation is the most appropriate route to reduce systemic effects and dosage required [3]. However, drug deposition in the lung is strongly affected by breathing patterns (respiratory frequency and breathing volume),

Section Editor:

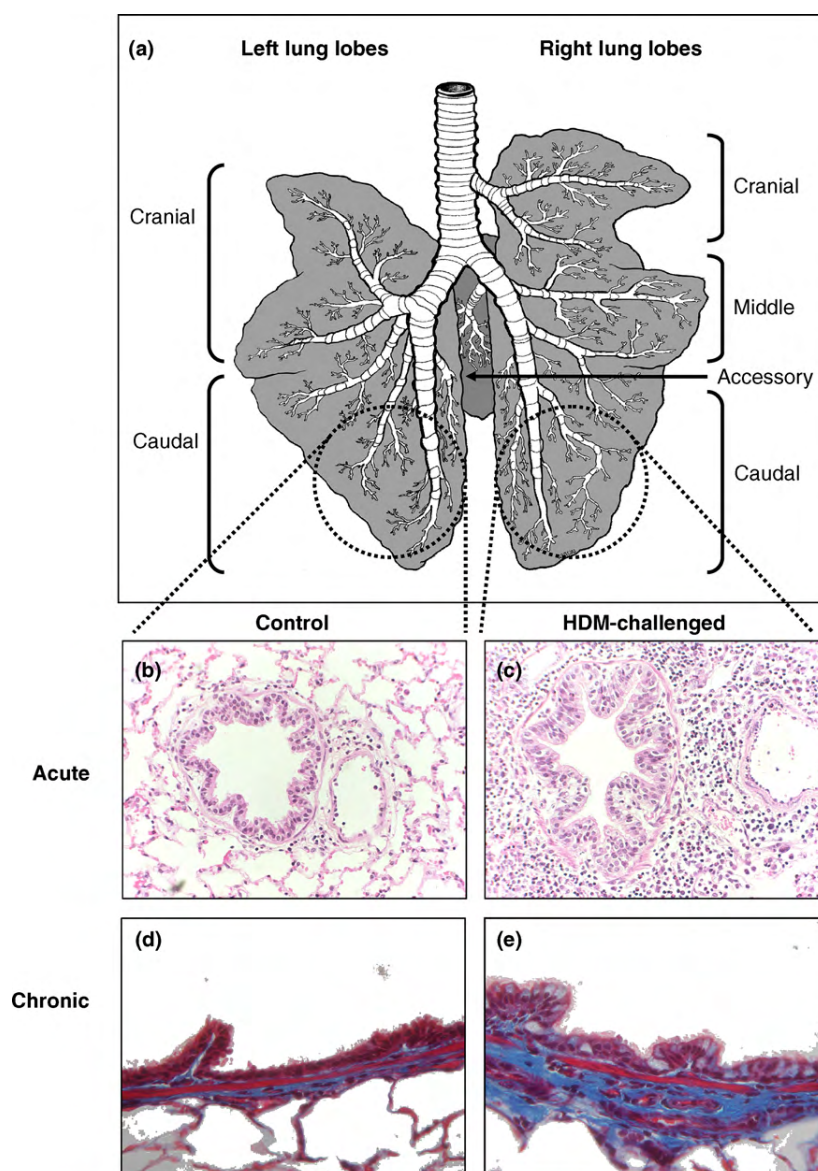
Michelle Epstein – Department of Dermatology, DIAID, Experimental Allergy, Medical University of Vienna, Vienna, Austria

which is dependent on body size. In addition, although it has been shown that a greater proportion of drug reaches the airways if it is inhaled through the mouth, both rats and mice are obligate nose breathers [3]. Because of these limitations of rodent models, large mammals of similar size and lung structure to humans have an important role to play in the modelling of human lung disease and the testing of new treatment strategies [1,4–7].

General features of the sheep lung model

Much of our understanding of lung physiology under normal and pathological conditions derives from classic studies in sheep [8,9]. Sheep offer the advantage over other commonly used models such as dogs, horses and non-human primates, in that they are less costly to purchase and maintain and pose fewer ethical concerns. Owing to their longevity compared to rodent models, chronic diseases can be modelled in the sheep lung and the effects of long-term therapies assessed. Importantly, owing to their placid nature, sheep are the only animal model where lung delivery and sampling, and detailed measurements of lung mechanics can be performed in relatively non-restrained, un-anaesthetised animals, or under mild sedation. A disadvantage of sheep models is that there are

E-mail address: (els.meeusen@med.monash.edu.au)



Drug Discovery Today: Disease Models

Figure 1. The left and right sides of the sheep lung (a) comprise six distinct lobes separated by tissue septa, which can each be treated as separate tissue segments for treatment/drug delivery (original drawing by Hamish McWilliams). Histological panels show tissue changes following acute (b,c) and chronic (d,e) house dust mite (HDM) challenges in control (left caudal lobe) and challenged (right caudal lobe) lobes of the same sheep lung. Airway inflammation can be seen in the HDM-challenged compared to the control lung lobe (c versus b; H&E, original magnification 200 \times). Structural airway tissue remodelling, including collagen deposition (blue staining) and airway smooth muscle accumulation (red), is seen after repeated HDM challenges (e versus d; Masson's Trichrome, original magnification 400 \times). Adapted from [13,15].

fewer ovine reagents available compared to mice and non-human primates, and as is the case with other large animal models, not all features of the human lung are replicated in sheep [6].

Humans have two lungs, with the left being divided into two lobes (superior and inferior) and the right into three lobes (superior, middle and inferior). Sheep, like cattle and pigs, have highly segmented lungs consisting of two lobes in the left lung and four lobes in the right lung with the bronchus of

the right cranial lobe arising directly from the trachea before bifurcation (Fig. 1). Sheep lobes are well separated by tissue septa with limited connectivity between compartments [6]. This allows for different treatments or infections to be localized, while the remaining lobes of the same animal can be used as controls (Fig. 1). In contrast to the monopodial branching of mice and rats, the sheep tracheobronchial tree has an irregular dichotomous and branching pattern as is seen in humans and other large mammals [6,7] which

Table 1. Major respiratory disease models in sheep

Disease	Induction stimulus	Features	Refs ^a
Bronchopulmonary dysplasia (BPD) or chronic lung disease of early infancy	Ventilator-induced lung injury (VILI) of premature lambs. Live ureoplasma or endotoxin-induced chorioamnionitis.	Abnormal collagen and elastin deposition; myofibroblast differentiation; early lung maturation.	[35] [36]
Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS)	Saline lavage; intravenous LPS; oleic acid; cotton smoke inhalation injury with/without instillation of <i>Pseudomonas aeruginosa</i>	Severe hypoxemia; progressive decrease in air volume; hyperkinetic cardiovascular response.	[37,38]
Emphysema	Chronic installation of LPS or elastase into lung; nebulised papain exposure	Microscopic emphysema and reduction in TGFβ; Increased collateral ventilation.	[26,39]
Asthma	<i>Ascaris suum</i> , house dust mite (HDM) extract	IgE; Early- and late-phase bronchoconstriction and AHR; airway inflammation and remodelling.	[13,15, 21,24]
Chronic bronchitis	Tobacco smoke; sulphur dioxide	Glandular hypertrophy or hyperplasia.	[40]
Chronic pulmonary hypertension	Sephadex beads injected in the pulmonary circulation	Development of right ventricular hypertrophy.	[41]
Bronchioloalveolar carcinoma (BAC)	Jaagsiekte sheep retrovirus (JSRV) or JSRV envelope protein	Natural and fatal disease of sheep; progressive respiratory distress; impaired alveolar function.	[42,43]
Respiratory syncytial virus (RSV) infection	Infection of neonatal lambs with human RSV	Mild peribronchiolar interstitial pneumonia; induction of surfactant proteins A and D.	[44]

^a Owing to space limitations, this is not a complete list and where possible reviews are cited.

impacts on regional deposition of drugs in the lung [5]. Although respiratory bronchioles are poorly developed in the sheep lung, collateral ventilation, absent in pig and bovine lung, has been documented in sheep [6].

Sheep airway tissues are similar to humans in the distribution of epithelial cell populations [10], mast cells [11] and airway smooth muscle [12]. Like other ruminants, sheep lungs possess a significant population of pulmonary intravascular macrophages that are important in phagocytosing foreign particles and pathogens that pass through the pulmonary circulation [1]. This causes the pulmonary circulation to be sensitive to relatively small doses of intravenously injected endotoxin and to increased pulmonary hypertension after intravenous injection of particles. Humans seem to have few intravascular macrophages in normal lungs; however, they may be induced after endotoxemia or liver injury.

Because of the many similarities in the sheep and human respiratory systems and their unique attributes as experimental animals, sheep have been used as models for a wide range of human respiratory diseases. A summary of the major respiratory diseases modelled in sheep and their characteristics is provided in Table 1. Easy access to ovine airway tissues via endobronchial sampling or at post-mortem provides the opportunity to perform detailed *in vitro* or *ex vivo* studies, using cells or tissues of interest. Cells collected from sheep airways include immune cells [13–15], epithelial cells [16] and airway smooth muscle cells [17]. Tracheal explants display many key features of the airways such as mucus coverage, mucociliary clearance and cell structure, and have been

used to study gene delivery [18], mechanisms of epithelial cell mucus secretion [18,19] and mucosal responses in allergic and non-allergic tissues [20].

Delivery into the sheep lung and tissue sampling

Detailed respiratory disease studies can be undertaken in sheep using similar methodology and equipment used in clinical medicine. For drug delivery, pharmacokinetic and efficacy studies in particular, the following methods can be routinely performed in sheep models.

Liquid instillation in different lung lobes or broncho-pulmonary segments

Using a flexible fibre-optic endoscope or bronchoscope, different treatments can be directed towards different compartments or lobes of the same lung (Fig. 1). Volumes of 1–10 ml can be easily and repeatedly delivered via the biopsy port of the bronchoscope, while deeper regions of the lung can be targeted by using a narrower, paediatric endoscope (5–6 mm in diameter). In addition, aerosolised treatments can be delivered to individual lung segments via a nebuliser catheter inserted to the distal end of the biopsy sampling port of the endoscope.

Intrapulmonary delivery of aerosols by controlled inhalation via a respirator

Sheep can be connected to a respirator via an endotracheal tube passed through the nostrils, and mechanically ventilated during aerosol delivery. As a consequence, the tidal

volume can be varied for each treatment thus mimicking changes in individual breathing depths. Nebulisers can be placed in-line with the respirator to deliver the aerosolised treatment into the lung using a face-mask or via the endotracheal tube.

Repetitive sampling of broncho-alveolar fluid and lung tissue biopsies

Using the biopsy sampling port of a fibre-optic endoscope, 5–10 ml of saline can be delivered into an individual lung lobe and gently aspirated (50–60% liquid sample recovery) to collect the washing from the underlying bronchial and alveolar area. This procedure can be rapidly and repetitively performed by a skilled operator with minimal restraint of the animal. By the same route, bronchial brush biopsies using a flexible wire brush, or endobronchial biopsies using flexible biopsy forceps can be easily and repeatedly taken from different lung compartments.

Tracheal and bronchial mucus velocity

Measuring mucus velocity as a surrogate marker of whole lung mucociliary clearance can be performed by direct observation of marked particle movement with the aid of a bronchoscope or a fluoroscope [21]. Sheep are an ideal animal model to study mucus clearance in response to physical and chemical agents because they have similar airway mucus gland profiles in the tracheobronchial tree and mucus viscosity and velocity rates as human subjects. Sheep also tolerate bronchoscopy well without the use of topical or general anaesthetic agents known to influence mucus velocity.

Peripheral blood sampling

Single blood samples can be taken from the jugular vein of un-anaesthetised sheep by venipuncture, but repeated sampling is usually performed through a catheter implanted in the jugular vein. For longer term studies, the carotid artery can be enclosed in loops of skin in the neck.

Imaging drug deposition and kinetics in the lung

A variety of non-invasive procedures can be used to measure drug deposition in sheep lungs, including gamma scintigraphy, single photon emission computed tomography (SPECT) and positron emission tomography (PET).

Recent advances in microimaging using confocal endomicroscopy [22] offer the opportunity of real-time observations of microscopic structures and cell types, including the bronchial vasculature and collagen extracellular matrix, to be made during disease progression and therapy.

Measuring respiratory mechanics in the sheep

To gain useful information in disease settings, animal models for respiratory disease require accurate and informative methods to assess lung function. While airway obstruction and lung function decline in humans is routinely and conven-

tionally measured as FEV₁ (forced expiratory volume in 1 s) and FVC (forced vital capacity) by spirometry, there is no satisfactory method to measure FEV₁ or FVC in conscious animals. However, an equivalent lung function measurement of airway resistance can be gathered in large animals using minimally invasive procedures. The procedure most often used relies on the insertion of tracheal and oesophageal catheters to obtain respective pressure measurements during the respiratory cycle which, together with airflow measurements, can be used to calculate airway resistance. Compared to most other large animals, sheep tolerate this technique when fully awake (un-anaesthetised) [23,24], so that respiratory mechanics can be evaluated without having to take into consideration the modulatory effects of anaesthetic agents on key respiratory parameters.

The Forced Oscillation Technique (FOT) is another method that has been successfully used in sheep to characterise the mechanical properties of airway resistance and inertance, including tissue resistance and elastance [25]. The FOT is a non-invasive method which employs small-amplitude pressure oscillations superimposed on the normal breathing, and has the advantage over conventional lung function techniques for infants and small children that it requires minimal patient cooperation. The value of using FOT in sheep is that it not only gives valuable information on airway function, but also allows extrapolation of important information on lung tissue mechanics. Given the comparative size and structure of the respiratory system in sheep and humans, this information can be reasonably correlated to the human lung.

In contrast to smaller animals, the sheep respiratory system is large enough to allow the measurement of local airway function in individual lung segments. One procedure designed to examine this relies on wedging the distal end of a bronchoscope in a segmental airway and supplying a continuous airflow through the bronchoscope biopsy channel to assess peripheral airway resistance in the subtended segment. This technique was originally developed for humans to examine small airway function and has been successfully adapted for use in sheep [26]. In experimental sheep research, the wedged bronchoscope procedure lends itself well to segmental allergen challenges and treatment strategies delivered to specific lung lobes via bronchoscopy [27]. Moreover, the effects of allergen and/or treatment on local segmental lung function can be directly assessed using this technique, and can be directly compared with that of untreated lung segments in the same animal. This allows for studies to be conducted with strong internal controls that require the overall use of lower animal numbers to achieve statistical significance.

Apart from disease modelling and drug delivery studies, sheep models have also been extensively used for the testing of surgical interventions before clinical application, including tracheal occlusion [28] and bronchoscopic lung volume

reduction (BLVR), and for optimising lung-targeted gene therapy in both adult and foetal sheep [29,30].

The following sections describe in more detail the development and use of sheep as a major animal model for human asthma pathogenesis and treatment.

Sheep models of asthma

Allergic asthma and airways inflammation have been studied extensively in sheep naturally or actively sensitised to the nematode, *Ascaris suum* and challenged in the lung with antigens derived from the worm (reviewed in [21]). More recently, a sheep asthma model has been developed using sensitisation and challenge with the major human allergen, house dust mite (HDM) [13,15,24]. As animal models are more likely to mimic true pathogenetic pathways when they are based on the known insult that causes the human disease, the use of HDM offers an improvement to the sheep asthma model compared to the *Ascaris* model and allows for a more standardised sensitisation protocol [31,32]. The immunological consequences of allergen exposure on the airways observed in human asthma are replicated in the sheep asthma model and are associated with allergen-specific IgE responses, immune-related activation and recruitment of eosinophils and lymphocytes, local mast cell activation and mucus hypersecretion of the airways (Fig. 1) [13,15,33]. Airway allergen challenge induces both early- and late-phase bronchoconstriction and airway hyper-responsiveness (AHR) in a proportion of sensitised sheep [23,24]. Irritant stimuli such as cholinergic agonists, histamine and pollutants, each known to affect AHR in human airways, have also been induced responses in the sheep model of asthma (reviewed in [21]).

Structural and functional changes that develop in the airways of chronic asthmatics after long-term exposure to allergens have also been observed in sheep repeatedly exposed to allergen. Chronic exposure to HDM in a proportion of sheep leads not only to a deterioration of lung function but also to an increase in key indices of structural airway remodelling, including collagen deposition, airway smooth muscle mass, and mast cell number in the smaller peripheral airways [15,24,34]. Mucociliary dysfunction is another feature of chronic asthmatic disease in humans and has also been reported in sheep following chronic allergen challenges [21].

Evaluation of anti-asthma therapies in the sheep asthma model

Drugs currently used for the treatment of asthma such as corticosteroids and leukotriene antagonists have proven to be equally effective in blocking the inflammation and physiological symptoms of asthma in sheep. In addition, the sheep asthma model has enabled testing of many new pharmacological agents that can block or interfere with key pathways associated with the development or progression of the asthma phenotype (reviewed in [21]). Most of these drugs

dampen the allergic inflammation and prevent the decline in lung function that accompanies asthma. Some agents shown to be effective in the sheep model have, however, been disappointing in human trials [6] and may reflect some differences in the sheep and human respiratory system. Nevertheless, the many similarities in sheep and human lungs, and the ability to use similar equipment and sampling procedures as in humans does facilitate direct translation of new drugs and interventions to the clinic.

Conclusion

Although studies undertaken in humans continue to further our understanding of airways disease, there are practical as well as ethical limitations with respect to the frequency upon which airway sampling and measurements can be performed in patients. Studies using large animal models such as sheep offer a wider scope for repeated sampling of airway cells/tissue and measures of airway function. In addition, unlike human and most animal studies, the sheep respiratory models allow for greater control and study of the onset, progression and resolution of experimental disease and the delivery and evaluation of new therapies.

Sheep are more closely related to humans than rodents, resulting in stronger homologies in protein structures, growth factors and mediators. With the recent sequencing of the bovine genome (>95% similarity) and the availability of bovine/ovine microarrays, sheep models of human respiratory diseases may also become valuable platforms for detailed molecular analysis and drug discovery.

Acknowledgements

The authors would like to thank Hamish McWilliams for the lung drawing in Fig. 1. EM, KS and RB are materially involved in a company (Allergenix Pty Ltd) commercialising a sheep asthma model. Dr. Stuart Hirst passed away on Dec 26, 2009. His co-authors would like to sincerely acknowledge his great enthusiasm and support, as well as his friendship, during the time he worked at Monash University.

References

- 1 Matute-Bello, G. *et al.* (2008) Animal models of acute lung injury. *Am. J. Physiol. Lung Cell Mol. Physiol.* 295, L379–399
- 2 Wenzel, S. and Holgate, S.T. (2006) The mouse trap: It still yields few answers in asthma. *Am. J. Respir. Crit. Care Med.* 174, 1173–1176 discussion 1176–1178
- 3 Raeburn, D. *et al.* (1992) Techniques for drug delivery to the airways, and the assessment of lung function in animal models. *J. Pharmacol. Toxicol. Methods* 27, 143–159
- 4 Allen, J.E. *et al.* (2009) Animal models of airway inflammation and airway smooth muscle remodelling in asthma. *Pulm. Pharmacol. Ther.* 22, 455–465
- 5 Cryan, S.A. *et al.* (2007) In vivo animal models for drug delivery across the lung mucosal barrier. *Adv. Drug Deliv. Rev.* 59, 1133–1151
- 6 Kirschvink, N. and Reinhold, P. (2008) Use of alternative animals as asthma models. *Curr. Drug Targets* 9, 470–484
- 7 Scheerlinck, J.P. *et al.* (2008) Biomedical applications of sheep models: from asthma to vaccines. *Trends Biotechnol.* 26, 259–266

- 8 Newnham, J.P. and Moss, T.J. (2001) Antenatal glucocorticoids and growth: single versus multiple doses in animal and human studies. *Semin. Neonatol.* 6, 285–292
- 9 Harding, R. and Hooper, S.B. (1996) Regulation of lung expansion and lung growth before birth. *J. Appl. Physiol.* 81, 209–224
- 10 Plopper, C.G. *et al.* (1983) Comparison of nonciliated tracheal epithelial cells in six mammalian species: ultrastructure and population densities. *Exp. Lung Res.* 5, 281–294
- 11 Miller, H.R. (1996) Mucosal mast cells and the allergic response against nematode parasites. *Vet. Immunol. Immunopathol.* 54, 331–336
- 12 Collie, D.D. *et al.* (1995) Distribution and quantitation of lung parenchymal contractile tissue in ovine lentivirus-induced lymphoid interstitial pneumonia. Do tissue forces limit lung distensibility? *Lab. Invest.* 73, 441–447
- 13 Bischof, R.J. *et al.* (2003) Induction of allergic inflammation in the lungs of sensitized sheep after local challenge with house dust mite. *Clin. Exp. Allergy* 33, 367–375
- 14 Dunphy, J.L. *et al.* (2002) Isolation and characterization of a novel eosinophil-specific galectin released into the lungs in response to allergen challenge. *J. Biol. Chem.* 277, 14916–14924
- 15 Snibson, K.J. *et al.* (2005) Airway remodelling and inflammation in sheep lungs after chronic airway challenge with house dust mite. *Clin. Exp. Allergy* 35, 146–152
- 16 Wanner, A. *et al.* (1986) Ciliary responsiveness in allergic and nonallergic airways. *J. Appl. Physiol.* 60, 1967–1971
- 17 Driska, S.P. *et al.* (1999) A method for isolating adult and neonatal airway smooth muscle cells and measuring shortening velocity. *J. Appl. Physiol.* 86, 427–435
- 18 Ferrari, S. *et al.* (2001) Mucus altering agents as adjuncts for nonviral gene transfer to airway epithelium. *Gene Ther.* 8, 1380–1386
- 19 French, A.T. *et al.* (2007) The expression of intelectin in sheep goblet cells and upregulation by interleukin-4. *Vet. Immunol. Immunopathol.* 120, 41–46
- 20 Abeynaike, L. *et al.* (2010) An ovine tracheal explant culture model for allergic airway inflammation. *Journal of Inflammation* (In Press)
- 21 Abraham, W.M. (2008) Modeling of asthma. COPD and cystic fibrosis in sheep. *Pulm. Pharmacol. Ther.*
- 22 Thiberville, L. *et al.* (2009) Human in vivo fluorescence microimaging of the alveolar ducts and sacs during bronchoscopy. *Eur. Respir. J.* 33, 974–985
- 23 Abraham, W.M. *et al.* (1983) Characterization of a late phase pulmonary response after antigen challenge in allergic sheep. *Am. Rev. Respir. Dis.* 128, 839–844
- 24 Koumoundouros, E. *et al.* (2006) Chronic airway disease: deteriorating pulmonary function in sheep associated with repeated challenges of house dust mite. *Exp. Lung Res.* 32, 321–330
- 25 Hoffman, A. *et al.* (2003) Physiologic responses of sheep to two different methods of papain exposure. *Inhal. Toxicol.* 15, 761–780
- 26 Tsai, L.W. *et al.* (2007) Bronchoscopic measurement of collateral ventilation in a sheep model of emphysema. *Respiration* 74, 565–571
- 27 Collie, D.D. *et al.* (2001) Local lung responses following local lung challenge with recombinant lungworm antigen in systemically sensitized sheep. *Clin. Exp. Allergy* 31, 1636–1647
- 28 Khan, P.A. *et al.* (2007) Tracheal occlusion: a review of obstructing fetal lungs to make them grow and mature. *Am. J. Med. Genet. C: Semin. Med. Genet.* 145C, 125–138
- 29 McLachlan, G. *et al.* (2007) Optimizing aerosol gene delivery and expression in the ovine lung. *Mol. Ther.* 15, 348–354
- 30 Yu, Z.Y. *et al.* (2007) Lentivirus vector-mediated gene transfer to the developing bronchiolar airway epithelium in the fetal lamb. *J. Gene Med.* 9, 429–439
- 31 Sharma, S. *et al.* (2003) Uneasy breather: the implications of dust mite allergens. *Clin. Exp. Allergy* 33, 163–165
- 32 Collie, D.D. (2003) Comparative, complementary and relevant: the immunological basis of ovine lung allergic responses. *Clin. Exp. Allergy* 282–286
- 33 Bischof, R.J. *et al.* (2009) Measurement and impact of remodeling in the lung: airway neovascularization in asthma. *Proc. Am. Thorac. Soc.* 6, 673–677
- 34 Bischof, R.J. *et al.* (2008) Immune response to allergens in sheep sensitized to house dust mite. *J. Inflamm. (Lond.)* 5, 16
- 35 Allison, B.J. *et al.* (2008) Ventilation of the very immature lung in utero induces injury and BPD-like changes in lung structure in fetal sheep. *Pediatr. Res.* 64, 387–392
- 36 Kramer, B.W. *et al.* (2009) Prenatal inflammation and lung development. *Semin. Fetal Neonatal Med.* 14, 2–7
- 37 Murakami, K. *et al.* (2002) A novel animal model of sepsis after acute lung injury in sheep. *Crit. Care Med.* 30, 2083–2090
- 38 Fernandez-Bustamante, A. *et al.* (2009) Regional aeration and perfusion distribution in a sheep model of endotoxemic acute lung injury characterized by functional computed tomography imaging. *Crit. Care Med.* 37, 2402–2411
- 39 Collie, D.D. *et al.* (2006) Local lung responses following endobronchial elastase and lipopolysaccharide instillation in sheep. *Int. J. Chron. Obstruct. Pulmon. Dis.* 1, 189–199
- 40 Nikula, K.J. and Green, F.H. (2000) Animal models of chronic bronchitis and their relevance to studies of particle-induced disease. *Inhal. Toxicol.* 12 (Suppl. 4), 123–153
- 41 Sato, H. *et al.* (2008) Large animal model of chronic pulmonary hypertension. *ASAIO J.* 54, 396–400
- 42 Leroux, C. *et al.* (2007) Jaagsiekte Sheep Retrovirus (JSRV): from virus to lung cancer in sheep. *Vet. Res.* 38, 211–228
- 43 Palmarini, M. *et al.* (1997) Sheep pulmonary adenomatosis: a unique model of retrovirus-associated lung cancer. *Trends Microbiol.* 5, 478–483
- 44 Olivier, A. *et al.* (2009) Human respiratory syncytial virus A2 strain replicates and induces innate immune responses by respiratory epithelia of neonatal lambs. *Int. J. Exp. Pathol.* 90, 431–438