Use of Alternative Animals as Asthma Models

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Abstract: This review focuses on the availability, advantages and non-advantages of asthma models in non-laboratory animals (cats, dogs, sheep, swine, cattle, horses, and monkey). Physiology and pathophysiology of the respiratory system as well as methodological aspects differ significantly between species and must be taken into account before evaluating the usefulness of a single species to serve as model for either asthma or chronic airway obstruction. Allergic asthma models have been described in cats, dogs, pigs, sheep, and monkeys. Among these species, the feline one is of particular interest because cats spontaneously develop idiopathic asthma. Currently available allergic feline models are well characterized with respect to lung function, bronchial responsiveness, airway inflammation and lung morphology (remodeling). Other species lacking for collateral airways (i.e. porcine and bovine lungs) are most sensitive to functional consequences of airway obstruction and are therefore suitable to study any obstructive lung disease. Animals of body weights comparable to humans (pigs, sheep, calves) offer the possibility to evaluate pulmonary functions using the same principles and techniques that are applicable to either children or adults during spontaneous breathing (generating lung function data in a directly comparable range). Despite the known disadvantages of being expensive and time consuming and despite limited availability of immunological or molecular tools, large animal models offer the great potential to perform long-term functional studies allowing a simultaneous within-subject approach of functional, inflammatory and morphological changes. This may add valuable information to the present knowledge about the complexity of asthma or other chronic airway diseases.

Key Words: Animal models of asthma, cat, dog, sheep, pig, cattle, horse, monkey.

1. INTRODUCTION

Asthma has been recognized to be a chronic inflammatory airway disease affecting not only the whole lung but having also a considerable impact on general health in humans, quality of life and economic societal burden. Despite the pathobiology of asthma is still relatively poorly understood, an enormous number of studies in humans as well as in various animal models implies that the complexity of this disease embraces heterogeneous processes at cellular, molecular, and genetic levels [1]. There is no doubt that animal models are indispensable to study detailed aspects of pathogenesis and to develop therapeutic strategies.

In literature of the last decade, there are a number of review papers addressing 'pros', 'cons', and limitations of different animal models of human asthma [1-7]. Summarizing the present knowledge, however, leads to the following conclusions: (i) there is no animal with a natural disease perfectly mimicking asthma and (ii) no animal model is available that completely reproduces the multiple features of human asthma. Consequently, many novel candidate drugs for asthma therapy have been shown to work perfectly in animal models, but not in clinical studies [6]. Undoubtedly, this conflicting situation makes clear that the present view on animal models urgently necessitates amplification.

Smaller laboratory animals represent the majority of models available at present, and especially mice are thought to be most useful for immunological research and genetics. In the long term, however, the use of inexpensive and convenient animal models with vast 'tool-kits' cannot compensate for biological irrelevance [8]. Unexpected results and the failure to translate reductionist hypotheses into the biological reality of humans have shown that (i) oversimplification of biological complexity is becoming a problem in medical research, and (ii) there is an urgent need to re-open the area of functional research in complex biological systems (Walker 2007)[1].

Furthermore, present models using laboratory animals are clearly deficient to study the mechanisms of chronic asthma or persistent airway obstruction [1]. Current models are mainly based on allergic exposure or sensitization. However, even those models named 'chronic' do not reflect many of the well known features of human asthma (for example spontaneous deterioration of airflow obstruction, persistent airflow obstruction or chronic airway inflammation). Also, special forms of asthma (non-allergic, aspirin-induced, exercise-induced) are usually not modeled in rodents or small laboratory animals.

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The challenge of the future is therefore to identify the most relevant animal model for each question to be answered. This challenge requires the dialog between medicine and veterinary medicine with respect to comparative and species-specific aspects of anatomy, physiology, immunology, pathology and clinics related to the specific model required; and the availability and applicability of technical solutions. This review focuses on the availability as well as advantages or non-advantages of asthma models in non-laboratory animals (cats, dogs, sheep, swine, cattle, horses, monkey) taking the physiological background and species-specific peculiarities of the respiratory system as well as methodological aspects into account. It is an attempt to open the dialog and to provide information about the usefulness of large animal to serve as models for either asthma or chronic airway obstruction.

2. PATHOPHYSIOLOGICAL FEATURES OF ASTHMA THAT NEED TO BE ADDRESSED IN AN ANIMAL MODEL

Human asthma is defined as an inflammatory disease of the airways that is associated with bronchial hyper-responsiveness leading to episodes of airway narrowing. From an inflammatory point of view, chronic lower airway inflammation, characterized by the presence of eosinophils and neutrophils, and a Th2-cell driven inflammatory response, are cornerstones of asthma. From a functional point of view, (allergic) asthma is diagnosed by bronchoconstriction (in response to allergen exposure) as well as either specific or non-specific hyper-responsiveness to inhaled stimuli. More recently, structural changes of the peripheral airways, i.e. airway remodeling has been considered as an important feature of asthma [9].

The "classical" animal models of asthma, i.e. mice, guinea pigs and rats, have become an indispensable tool for investigating the molecular background of the inflammatory process developing in asthma. With regard to lung function, these models are limited by the size of the animal and the available tests. The more specific the lung function test is, the more invasive it becomes, which renders repeated or long-term measurements often difficult. Poorly invasive test, such as whole body barometric plethysmography (BWBP), are appropriate for repeated measurements but provide less specific information, which has led to debates about its validity [10-12]. Investigation of airway remodeling in these models always implies sacrifice of the animal, which increases the number of animals needed and which renders the follow-up of the same individual impossible. Consequently, these classical asthma models do not allow a simultaneous approach of functional, inflammatory and morphological changes occurring during asthma.

If large animal species present the inconvenient of poorly developed molecular tools limiting the characterization of the inflammatory response, they offer nevertheless the opportunity of an approach integrating functional, inflammatory and structural changes occurring during an asthma-like disease of the respiratory tract.

Ideally, an allergic animal model of asthma should develop *(1)* an acute bronchoconstriction immediately in response to allergen exposure (early phase or immediate response), *(2)* a late phase bronchoconstriction several hours after allergen exposure, *(3)* non-specific bronchial hyperresponsiveness to various stimuli, *(4)* an eosinophilic and neutrophilic bronchial inflammation, *(5)* changes in mucus secretion and quality, *(6)* changes of airway and lung histology, and *(7)* spontaneous increase in airways' resistance after prolonged or repeated allergen exposure [3]. Non-allergic forms of asthma have to be modeled appropriately taking specifications of human diseases into account.

Furthermore, these pathophysiological changes need to be characterized by investigation techniques which should ideally be similar to those used in human respiratory medicine. Some models show clinical symptoms of respiratory disease, such as exercise intolerance, breathlessness, cough, wheezing etc. which can also be of interest from a comparative point of view.

Due to their size, alternative animal models of asthma bear advantages with regard to simultaneous and serial investigations, which are indispensable for a good pathophysiological characterization of the model and which bear an interest for therapeutic trials where specific features such as bronchodilation, inflammation, mucus secretion, mucociliar clearance, etc. can be addressed.

Before selecting an animal species as asthma model, two important points need to be addressed, i.e. the anatomical and physiological characteristics of the lung as well as the species-specific response to airway obstruction (see section 3).

3. COMPARATIVE ASPECTS OF THE RESPIRATORY SYSTEM IN NON-LABORATORY ANIMALS

3.1. Anatomical and Physiological Characteristics

3.1.1. Anatomy of the Tracheo-Bronchial Tree and Dead Space Ventilation

Mammalian tracheo-bronchial airways are complicated and can not be defined by one idealized branching system. The branching pattern of the conducting airways is significantly asymmetrical in the human, and asymmetry is believed to have an important effect on air flow. As given in Table **1**, three idealized branching systems are commonly recognized in different species [13]: (i) monopodial (at the branching point a small segment may branch from the main, or parent, stem), (ii) dichotomous (the parent segment may divide into two equal daughter segments), and (iii) polychotomous (the parent segment may divide into many daughter segments). In addition, a comparison of lungs from 5 species (sheep, goat, cat, rabbit, and bonnet monkey) revealed different branching patterns between lobes (cranial *versus* caudal) of the same species and between the same lobe in different species. Furthermore, marked differences in epithelial population distribution within the airway tree were found between the same lobe of different species (e.g. cranial lobes of rabbit and sheep) and between different lobes in the same species (e.g. cranial and caudal lobes of the sheep) [14]. This knowledge demands a high level of standardization in pulmonary airway morphologic studies taking species-specific aspects into account.

Table 1. Airway Branching in Different Animal Species [13, 185, 186]

Species	Branching System of Tracheo-Bronchial Airways
Mouse	monopodial
Rat	monopodial
Hamster	strictly monopodial
Rabbit	primarily irregular dichotomous
Guinea pig	irregular to the pulmonary region but regularly dichotomous thereafter
Human	irregularly dichotomous
Dog	monopodial (within a lobe) follows an irregular dichotomized pattern with fractal features
Rhesus monkey, Baboon	irregularly dichotomous

Due to the anatomy of extrathoracic airways and the lenghth of the tracheo-bronchial tree, the ratio between dead space volume and tidal volume (Vd/Vt) differs between different species as shown in Table **2**. Evaluating models of airway obstruction, this ratio is important to know because the risk of alveolar hypoventilation is higher in species with physiologically higher dead space volume in relation to tidal volume.

Table 2. Ratio between Dead Space Volume (Vd) and Tidal Volume (Vt) in Different Species [187-190]

Species	Vd/Vt Ratio
Guinea pig	38 %
Human	29 - 40 %
Dog	33 %
Sheep	58 %
Pig	53 - 59 %
Cattle	40 - 55 (up to 75 % in adults)
Horse	49 - 75 %

3.1.2. Lobation of the Lung

A lung lobe is defined as "a large area of pulmonary tissue which is ventilated by a large bronchus arising either from a main bronchus or from the trachea; it is separated from neighboring lobes by interlobar fissures which may be continued by connective tissue planes" [15]. Using this definition, the left lung of dogs, cats, cattle, pigs and sheep is composed of two lobes (*lobus cranialis* which is divided into two segments[2] and *lobus caudalis*) while the right lung of these species is composed of four lobes (*lobus cranialis*, *lobus medius*, *lobus caudalis*, *lobus acessorius*). Ruminants (sheep, cattle, goat) and pigs differ from other species because the right cranial lobe bronchus arises directly from the right lateral side of the trachea rather than from the mainstream bronchus. Although the horse lung is not obviously divided by fissures into lobes, 2 parts of the left lung and 3 parts of the right lung are named "lobes" [16]. Based on the fundamental structure of bronchial ramification, the lung of chimpanzee consists of 5 lobes [17].

3.1.3. Lobulation / Segmentation

In human pulmonary anatomy, portions of a lobe ventilated *via* a lobar bronchus are known as broncho-pulmonary segments. On the basis of subgross anatomy defined by McLaughlin *et al*. [18, 19], other mammalian lungs can be divided into three types (Table **3**) taking especially secondary lobules, but also pleura, peripheral airways, bronchovascular relationships and bronchial arterial distribution into account. A secondary lobule is defined as the smallest discrete portion of the lung which is surrounded by connective tissue septa. Especially in the lungs of pigs and cattle, only limited interdependence exists between secondary lobules as a result of the high degree of lung lobulation. Furthermore, tissue resistance is greater (i.e. lung tissue is less compliant) due to a greater degree of lobulation by connective tissue septa in these species.

The presence or absence of respiratory bronchioli is physiologically linked with different mechanisms of particle deposition and clearance. This aspect may play a significant role in animal models that include administration of aerosols (for example inhalation of therapeutic substances). Unfortunately, there is no much information in literature whether existing animal models of asthma have taken this aspect into consideration.

3.1.4. Collateral Airways

Within an anatomical region of lung parenchyma, ventilation normally occurs through a given bronchus *via* the standard airway branching pattern from larger to smaller airways. In species with collateral airways, collateral ventilation refers to any ventilation to this given region that arrives from a neighboring airway [20]. In species lacking for col-

[2] Segments do not anatomically constitute lobes because their bronchi do not arise directly from a mainstream bronchus. However, they may function like lobes because they are surrounded almost completely by visceral pleura.

Table 3. Types of Lungs According to Secondary Lobulation (Modified According to [16, 18])

	Type I	**Type II**	**Type III**
	Cattle, Sheep, Pig	**Dog, Cat, Monkey**	**Horse**
Lobulation of the lung	extremely well developed (secondary lobules are separated by fascial planes)	absent (no subdivision into secondary lobules)	imperfect development (incomplete connective tissue septa between secondary lobules)
Pleura	thick (and supplied by the bronchial artery)	thin (and supplied by the bronchial artery)	thick (and supplied by the bronchial artery)
Distal airways			
Terminal bronchioles	present (predominant distal airways)	absent	present
Respiratory bronchioles	few (infrequently observed and poor developed)	present (very well developed)	present (but poorly developed)
Collateral airways	absent in cattle and pigs partly present in sheep	well developed	partly present
Circulation			
Termination of the bronchial artery	distal airways	distal airways	distal airways and alveoli
Pulmonary veins	follow the bronchi and pulmonary arteries to the periphery	course through the lung parenchyma at some distance from the bronchi and pulmonary arteries	follows the bronchus and artery in the periphery but departs from these structures as it approaches the hilum
Shunts between bronchial artery and pulmonary artery	present (not, however, demonstrated in the pig)	not demonstrated	present

lateral ventilation, atelectases frequently occur in any case of airway obstruction. Three possible pathways for collateral ventilation have been considered in the human lung: (i) epithelialized tubular communications through the bronchiolar wall to adjacent alveoli (i.e. bronchiole-alveolar communications or 'channels of Lambert'), (ii) alveolar pores ('pores of Kohn'), and (iii) interbronchial connections that occur at the level of the respiratory bronchioli and/or connections near the alveolar duct ('channels of Martin') [20-22].

As given in Table **3**, bronchiole-alveolar communications are present in lungs of cats and sheep, but also in rabbits. Alveolar pores may be found in all the common laboratory mammals and man [23], however, because they provide a very high resistance to airflow, it is unlikely that they are a major pathway for collateral ventilation [16, 20]. Channels of Martin have been found in canine lungs first [22]. Due to their size of 120 to 200 μm, they are thought to be the primary pathways for collateral ventilation connecting normal airways at the level of the alveolar duct [20].

Taking anatomical findings together, the presence of collateral ventilation has been proven in dogs, cats, rabbit, ferret, sheep and horses [20]. Consequently, collateral ventilation may serve partially to bypass airway obstruction in these animal species. However, differences exist among species and those differences have clear physiological significance. In dogs, cats, rabbits and ferrets, collateral ventilation is greater compared to sheep or horses. For example, in a dog with a respiratory rate of 20 breathing cycles per minute, 96 % of normal tidal volume is supplied by adjacent airways to a region of the lung where the primary airway is completely obstructed. In contrast, only 16 % of the normal tidal volume is supplied to the obstructed lung segment *via* collateral ventilation in the horse [16]. Cat lungs are probably similar to dog lungs. In sheep, a significant decrease in collateral resistance (i.e. improvement of collateral ventilation) was observed with maturation [24] indicating that collateral resistance may change with age. Also in dogs, the number of interalveolar pores increases with age [16].

Furthermore, significant lobar variations have been observed in the ability of collateral ventilation to maintain tidal volume in dog lungs. In caudal lobes, collateral ventilation should provide over 80 % of the normal tidal volume; while in the middle, cranial, and accessory lobes, collateral ventilation will provide less than 50 % [16]. This may be one reason why the incidence of alveolar hypoventilation or atelectasis (leading to alveolar hypoxia and consequently to a higher risk of pneumonia) is greater in cranial, middle and accessory lobes than in caudal lobes [16].

Despite the presence of alveolar pores the pig has no collateral ventilation [20]. The same is true for the bovine lung. Since pigs and cattle lack normal pathways for collateral airflow, atelectases frequently occur in bovine and porcine

lungs in any obstructive condition. In consequence (since atelectatic lobes cannot be inflated by air through collateral airways), pigs and cattle easily develop ventilatory asynchronisms or regional inhomogeneities in alveolar ventilation. On the other hand, both the lack of collateral ventilation and the presence of connective tissue septa between lobules often limit inflammatory processes to a lobule in these species. Consequently, consolidated lobules may be adjacent to healthy lobules within the same lobe.

3.2. Species Variation in Responses to Obstructive Airway Diseases

Airway obstruction causes inequalities of time constants between different regions of lung (segments or lobules). Consequently, the level of FRC (functional residual capacity) and the presence of atelectatic lung regions do clearly correlate with collateral ventilation in obstructive airway diseases. Species variation in lobulation of the lung and in collateral ventilation results in different responses to airway obstructions. In species with limited collateral ventilation, regions with long time constants cannot empty adequately during the expiratory time and become hyperinflated [16].

In humans, a species with high collateral resistance (i.e. low collateral ventilation), increasing FRC due to airway obstruction is a well known phenomenon. Animals are very variable in this reaction. While species with little or no collateral ventilation have an increased FRC in response to airway obstruction, other ones with good collateral ventilation do not.

In pigs and cattle, airway obstruction easily causes inhomogeneities in ventilation with the consequences of alveolar hypoventilation and atelectases in some parts of the lung while other parts are hyperinflated. Due to mismatches in the ventilation-perfusion-ratio, gas exchange impairments lead to hypoxemia ($PaO_2\downarrow$), hypercapnia ($PaCO_2\uparrow$), an increase in alveolar-arterial oxygen difference ($AaDO_2\uparrow$) and an increase in right-to-left vascular shunt.

In the horse, there is some collateral ventilation that prevents atelectasis. However, collateral time constants are so long that FRC increases in airway obstruction, and collateral ventilation cannot completely prevent gas exchange abnormalities and hypoxemia. In the presence of considerable peripheral airway obstruction, atelectases might be prevented by collateral ventilation, and perfusion can be matched to ventilation. In case of severe airway obstruction (as observed in ovalbumin-sensitized ponies) severe hypoxemia may occur [16].

In contrast, in dogs or monkeys collateral ventilation may provide a way for regions with obstructive airways to empty through regions with less severely obstructed airways thus preventing an increase in FRC. The excellent collateral ventilation of the dog maintains ventilation distal to obstructed airways. Thus, collateral ventilation protects to a certain extent against atelectasis and shunts [25]. In so far, small airway obstruction does not affect arterial gas tension in such a strong way as it does in other species [26]. This may account for the relative lack of hypoxemia documented in dogs and cats with mild to moderate chronic bronchial disease when compared to humans with a similar degree of airway narrowing or obstruction. Nevertheless, severe bronchoconstriction can impair gas exchanges in dogs, such as documented in an allergic model of asthma where dogs developed airway obstruction, hypoxemia, acidosis but no increase of FRC or pulmonary hypertension in response to inhaled allergens [27]. Despite collateral ventilation does maintain ventilation distal to obstructed airways, it does not maintain necessarily normal ventilation-perfusion ratios. The dog seems to have a limited ability to redistribute blood flow from poorer to better ventilated regions of the lung. Wanner *et al*. [28] demonstrated a loss of pulmonary vascular response to hypoxia in antigen-challenged dogs and Rodriguez-Roisin *et al*. [29] described a methacholine-induced V/Q mismatch due to bronchoconstriction and decreased pulmonary vascular resistance. Gas exchanges remained nevertheless within physiological limits due to an increased cardiac output.

Antigen-challenged sheep and histamine-challenged sheep developed similar functional abnormalities as antigen- or histamine-challenged dogs [16]. In sheep exposed to aerosol antigen, airway resistance and FRC increased and transient hypoxemia developed [30].

4. AVAILABILITY OF ALTERNATIVE ANIMAL MODELS FOR ASTHMA

This section will review animal species that are already used or that might be used as alternative models of asthma. The possibilities of assessing changes of lung function, bronchial responsiveness, airway inflammation and changes of lung morphology, i.e. remodeling, will be addressed for each animal species and are summarized in Tables **4-6** for inter-species comparisons.

4.1. Cats

The feline species is a particularly interesting animal model because cats spontaneously develop idiopathic asthma, which is characterized by episodes of coughing, airway obstruction due to bronchoconstriction and mucus hypersecretion (wheezing), bronchial hyperresponsiveness and eosinophilic bronchial inflammation [31]. Although some allergens are suspected, e.g. Bermuda grass, the precise allergens of the naturally occurring disease are not identified. One percent of the feline population suffers from asthma [32], which is probably an underestimated percentage because of the lack of easily applicable diagnostic tools in veterinary medicine and the difficulty to treat animals showing only mild to moderate symptoms such as sporadic cough. In veterinary practice, feline asthma is diagnosed based on respiratory symptoms, characteristic radiographic features (essentially an increase of bronchial and interstitial pattern, lung hyperinflation), bronchoscopic findings (mucus hypersecretion, airway wall edema and congestion, increased airway wall reactivity during bronchoscopy) and cytologic findings (eosinophils) in bronchoalveolar lavage fluid (BALF) [33]. Asthmatic cats are treated with steroids and bronchodilators, which generally allows a successful symptom control [31, 34]. Based on these asthma-like characteristics, the feline species has been used as asthma model by applying sensitization protocols using either *Ascaris suum* antigens [35, 36] or Bermuda grass antigens [37].

Table 4. Characteristics and Approaches of the Functional Response to Allergen Exposure in Alternative Animal Models of Asthma

	Early Phase Bronchoconstriction		Late Phase Bronchoconstriction		Bronchial Hyperresponsiveness	
Cat	yes	[35-37]	yes	[36]	yes	[35-37]
Dog	yes	[51]	yes	[51]	yes	[50, 52, 53]
Sheep	yes	[64]	yes (~50%)	[64]	yes	[64]
Pig	yes	[3, 88]	yes	[89]	yes	[92]
Cattle	no	-	no	-	yes	[132]
Horse	no	-	yes	[141, 143]	yes	[143]
Monkey	yes	[168-171]	yes	[168-171]	yes	[169, 170]

Table 5. Characteristics and Approaches of the Inflammatory Response to Allergen Exposure in Alternative Models of Asthma

	Bronchoalveolar Lavage or Bronchial Brushing/ Biospy Cytology in Response to Allergen Exposure		Markers in Exhaled Breath Condensate Detected in Response to Allergen Exposure	
Cat	eosinophils and neutrophils	[35, 36]	H_2O_2	[41]
Dog	eosinophils and neutrophils	[59]	not documented	-
Sheep	eosinophils and neutrophils	[65, 70]	not documented	-
Pig	eosinophils	[90, 94]	not documented for asthma model, but technique validated	[93]
Cattle	eosinophils and neutrophils (circulating blood and bone marrow)	[101]	not documented for asthma model, but technique validated	[93]
Horse	neutrophils	[142]	H_2O_2	[149]
Monkey	eosinophils and neutrophils	[169-171]	not documented	-

Table 6. Characteristics and Approaches of the Structural Response to (Repeated) Allergen Exposure in Alternative Models of Asthma

	Radiography		Computed Tomography		Biopsy		Post-Mortem Histology	
Cat	yes	[36, 45]	yes	not documented for asthma model	yes	not documented for asthma model	yes	[35, 46]
Dog	yes	not documented for asthma model	yes	not documented for asthma model	yes	[63]	not documented for asthma model	-
Sheep	yes	not documented for asthma model	yes (~50%)	not documented for asthma model	yes	not documented for asthma model	yes	not documented for asthma model
Pig	yes	not documented for asthma model	yes	not documented for asthma model	yes	not documented for asthma model	yes	not documented for asthma model
Cattle	yes	not documented	no	-	yes	[134]	yes	[112, 115, 135]
Horse	yes	[139]	no	-	yes	[163]	yes	[160, 161]
Monkey	yes	not documented for asthma model	yes	not documented for asthma model	yes	not documented for asthma model	yes	[168, 172]

Respiratory function can be assessed in cats by different methods. Respiratory mechanics allowing measurement of pulmonary resistance and dynamic compliance can only be performed under general anesthesia but provide most precise information about lung function and airway responsiveness if bronchoconstrictive agents such as methacholine, histamine or carbachol are administrated by inhalation [33]. The major inconvenient of this technique is the need for anesthesia, which does not allow a prolonged or repeated follow-up of pulmonary function. Flow-volume loops can be performed in conscious cats wearing a facemask and allow to a certain extent detection of airway obstruction [38]. A well tolerated but less precise method of assessing pulmonary function is barometric whole body plethysmography (BWBP), which allows measurement of enhanced pause (Penh) as well as determination of bronchial reactivity by use of bronchoconstrictive agents which can be administrated by inhalation [39, 40]. The major advantage of BWBP is the possibility of repeated and long-term investigations which do not interfere with other tests such as chest radiography or bronchoscopy. By using this technique, the acute and late allergic response to inhaled allergens has been evidenced in *Ascaris suum*-sensitized cats. Indeed, five to ten minutes after allergen exposure, an increase of Penh, suggestive of bronchoconstriction, occurs and second increase of Penh as well as of respiratory rate is recorded six to eight hours after allergen exposure [36].

Allergen-sensitized cats develop bronchial hyperresponsiveness in response to allergen challenge that can be assessed using respiratory mechanics [35, 37] or BWBP [36, 40]. The persistence of bronchial hyperresponsiveness depends on allergen challenge protocols; regularly challenged cats develop within several weeks persisting and even increasing hyperresponsiveness [35], whereas bronchial hyperresponsiveness induced by a single allergen challenge resolves within less than a week.

The best method of assessing airway inflammation is based on sampling of BALF, which occurs under anesthesia. Volumes ranging from 5 to 15 ml of BALF can be obtained, allowing cytological analysis and measurement of inflammatory markers, such as total proteins, F_2-isoprostanes and matrix metalloproteinases (MMP-9) [35-37]. Hydrogen peroxide (H_2O_2) determined in exhaled breath condensate has also been described as a non-invasive method of assessment of airway inflammation [41].

From an immunological point of view, increased levels of specific IgE, IgG and IgA levels have been detected in serum and BALF of Bermuda grass sensitized cats, suggesting a Th2 lymphocyte driven immune response [42, 43]. Rush immunotherapy has been described in this model, allowing to dampen the eosinophilic airway inflammation and to change BALF cytokine profiles [44].

The feline asthma model provides different ways of approaching airway remodeling. Given the size of this animal species, bronchial biopsies might be performed and chest radiographs can also provide information about airway thickening [45]. Although not yet described, CT-scans could also be of interest for remodeling assessment. Histologic alterations sharing features with human asthmatic airways have been evidenced *post-mortem* in chronically-challenged cats and cyclosporine A was shown to prevent bronchial hyperresponsiveness and airway remodeling [46].

Asthmatic cats might also be evaluated from a clinical point of view, allowing a scoring system based on appetite, behavior, respiratory symptoms (cough, wheezing), breathing strategy and lung auscultation.

4.2. Dogs

The "naturally" occurring lower airway diseases in dogs include neutrophilic chronic bronchitis in older animals [47] and eosinophilic bronchopneumopathy in young dogs [48]. From a functional point of view, these diseases are poorly characterized whereas the inflammatory and immune components have been partly described and are still under investigation [49].

Although dogs suffering from lower airway disease poorly share features with asthma, this animal species has been used for asthma research, in particular with regard to lung function. *Ascaris suum* sensitized dogs have been used to investigate gas exchange and ventilation-perfusion ratios after bronchoprovocation with allergen, methacholine or histamine [50]. Respiratory mechanics are usually assessed under anesthesia but they even have been measured in conscious dogs by Dain and Gold [51], who documented an acute increase of pulmonary resistance and a decrease of dynamic compliance after allergen challenge. Interestingly, spontaneous and inherited non-specific airway hyperreactivity has been described in some dog breeds; Basenji-greyhounds and Basenjis being significantly more reactive to methacholine and citric acid than mongrel dogs [52, 53]. Other less invasive methods of lung function testing are described: flow-volume loops were applied as in cats [54]; the forced oscillation technique allowed assessment of bronchoconstriction in healthy histamine-challenged dogs [55]; BWBP can be performed in conscious or slightly sedated dogs and allows quantification of airway reactivity [56, 57] and head-out plethysmography allows measurement of airway resistance and FRC [58].

Airway inflammation might be assessed by analyzing inflammatory markers in exhaled breath condensate as well as by bronchoscopy and BALF analysis. The inflammatory airway response of asthma models used in the 70ties and 80ties is poorly documented in dogs. However, in ragweed-sensitized Beagle dogs, BALF eosinophils and total and specific IgE levels significantly increased after allergen challenge [59]. The potential effect of ultrafine carbon particle exposure prior to allergen exposure has been evaluated in ragweed-sensitized dogs. Although particle exposure increased BALF neutrophil count, the immune allergic response remained unchanged [60].

Morphological changes of the respiratory tract of dogs can be assessed by classical techniques such as bronchial biopsies and chest radiographs. Computed tomography appears however as a promising tool for assessment of bronchial wall thickening and even airway narrowing due to bronchoconstriction [61].

Dogs can also be used to study hyperventilation and "ski asthma". It has indeed been documented that repeated hyperventilation with cool, dry air induces a neutrophilic and eosi-

nophilic peripheral airway inflammation and increased bronchial reactivity in dogs [62]. Racing sled dogs even naturally present features of human athletes suffering from "ski-asthma", i.e. intraluminal mucus accumulation within the bronchi and increased macrophage and eosinophil counts in BALF 24 to 48 hours after race [63].

4.3. Sheep

Sheep are naturally prone to viral and bacterial respiratory diseases. However, sheep might encounter in their natural environment *Ascaris suum* antigens and become sensitized. A pulmonary challenge with this allergen induces an acute and sometimes even a delayed bronchoconstriction (~50% of sensitized sheep are "dual" responders). Bronchial inflammatory cell infiltration (eosinophils and neutrophils) and non-specific airway hyperreactivity also occur within hours of allergen exposure [64].

These allergic airway responses, either naturally acquired or experimentally induced by active *Ascaris suum* [64, 65] or house dust mite sensitization [66], can be assessed from a functional point of view in conscious animals by respiratory mechanics, inductance plethysmography [67] and head-out plethysmography [68]. As there is no need for anesthetizing the animals, the allergic sheep is an interesting model for prolonged and repeated investigations of the respiratory function and there is evidence for a progressive decline of lung function in chronically challenged sheep [69].

Bronchoscopy and BALF demonstrate an increased inflammatory cell influx into the airways; characterized by eosinophils, neutrophils and macrophages [65, 70]. Although not yet described, exhaled breath condensate could easily be sampled in this animal model and provide non-invasively evidence of airway inflammation. From an immunological point of view, nearly no data are available in allergic sheep.

Remodeling has been poorly investigated in this animal model, but there is evidence in a house dust mite model that bronchial epithelial hyperplasia, collagen deposition and bronchial smooth muscle increase, and apparition of mast cells in alveolar septa occur after six months of repeated allergen exposure [66].

Due to its size, the sheep offers the possibility for studying mucociliary clearance [71] and therapeutic trials comparing the effect of bronchodilators on lung function and mucociliary clearance have been performed in this species [72, 73]. Several other studies evaluating the effect of anti-inflammatory agents [74, 75], protease inhibitors [76], chemotaxis inhibitors [77] etc. have been performed in allergic sheep models. However, a striking difference between sheep asthma and human asthma has been evidenced when platelet factor antagonists were shown to modulate the late phase allergic response in sheep [78], whilst these drugs are of poor interest for human patients.

4.4. Swine

Despite differences in subgross anatomy of the lung (see section 3), there is evidence that porcine airways and human airways share many structural and physiological similarities with respect to immunological features [79, 80], the composition of airway surface liquid [81], or pulmonary gene transfer [82] enabling the pig to become a useful model of airway diseases.

Naturally, the swine species develops often acute and chronic infectious pulmonary diseases, but spontaneous sensitization to *Ascaris suum* allergens might occur. Beside passive or active *Ascaris suum* sensitization [3], pigs can also undergo active sensitization to ovalbumin [83]. Based on models of allergy, pigs have been used as asthma models, although the establishment of a stable chronic asthma model appears to be difficult because the sensitivity to the antigen declines after repeated allergen exposure [3].

Lung function can be assessed in anesthetized pigs using conventional respiratory mechanics (i.e. pulmonary resistance, dynamic lung compliance) or non-invasively in conscious pigs using either BWBP [84] or impulse oscillometry [85, 86]. The majority of swine asthma models, however, have been investigated invasively measuring conventional respiratory mechanics in mechanically ventilated pigs. An acute bronchoconstrictive response has been documented in several models [83, 87]. In actively *Ascaris suum*-sensitized pigs, this immediate allergic bronchoconstriction was found to be associated to IgE and resolved within 1-2 hours [88], while the late phase pulmonary airways obstruction started to develop 3 hours after antigen challenge and peaked at 9 hours with a magnitude similar to the immediate reaction [89]. Other studies indicate that high endogenous cortisol levels in pigs appear to control the development of allergen-induced late phase reactions in pigs because a delayed bronchoconstriction only occurred if the animals were pre-treated with metapyrone, a cortisol-synthesis inhibitor [87, 90]. Interestingly, cysteinyl leukotriens were not confirmed to be important bronchoconstrictive mediators of allergen-induced acute airway response in the anaesthetized pig [91]. The development of non-specific airway hyperresponsiveness in ovalbumin sensitized/challenged pigs has been shown *in vivo* using acetylcholine, but the presence of non-responders was also reported [92].

Airway inflammation in pig models has been assessed by either BAL or lung biopsy specimens. Recently, the collection of exhaled breath condensate has been validated in pigs [93] and would offer an interesting new approach for quantification of lower airway inflammation non-invasively and repeatable in porcine models. Airway inflammation develops in response to allergen challenge in pigs and is characterized by eosinophils and neutrophils [94]. Inflammatory markers such as eosinophilperoxidase and myeloperoxidase have been specifically developed in order to monitor the inflammatory response [90]. Furthermore, the molecular cloning and expression of porcine interleukin-5 – showing 65% amino acid identity to the human IL-5 sequence – has been realized [95]. A mast cell tryptase inhibitor administrated prior to allergen challenge prevented acute bronchoconstrictive response as well a decrease of histamine release [96], whilst inhaled or systemically administrated budenoside decreased inflammatory cell influx into the airways but did not prevent the delayed bronchial obstruction [94].

Although reports about histological alterations in response to infectious agents are available in pigs [97, 98], no data about morphological or histological alterations in allergen sensitized pigs have been published. For comparative

aspects, structural evidence for neurogenic inflammation (that may occur in asthma) was sought in bronchial airways of pig and humans by three-dimensional mapping of substance P-immunoreactive nerves using immunofluorescent staining and confocal microscopy [99].

4.5. Cattle

In 1970, young calves (aged 7-20 weeks) were sensitized with infective *Ascaris suum* eggs and this challenge resulted in severe respiratory distress accompanied morphologically by atelectases, pulmonary edema, emphysema, and alterations of alveolar architecture. In addition to an increase in the white blood cell count, the involvement of eosinophils was confirmed locally in the lung and systemically by a dose-dependent eosinophil response in the bone marrow and in the circulating blood [100, 101, 102]. Similar findings had already been described before in cattle infected with *Ascaris lumbricoides* and were classified as 'acute atypical pneumonia' or 'diffuse interstitial pneumonia' [103, 104]. After having contact to moldy hay, the development of a 'bovine allergic pneumonitis', based on pulmonary hypersensitivity to allergens of *Micropolyspora faeni*, was reported in cows [105]. Despite these encouraging former data, no asthma model based on allergic sensitization has been developed in cattle so far.

As in other farm animals, the bovine lung is naturally exposed to a variety of viral and/or bacterial agents causing acute and chronic infectious pulmonary diseases. Thus, infectious pulmonary diseases are the main field of interest in veterinary research, and a large variety of infectious models is available focusing mainly on pathogenesis, immunization or therapeutic options. For a number of microorganisms that are currently under discussion being triggers of asthma or with respect to either asthma exacerbation or chronicity – for example respiratory syncytial virus (RSV), *Chlamydophila* spp. or *Mycoplasma* spp. [106, 107, 108, 109] – calves do represent natural hosts because of natural susceptibility, and could therefore serve as 'natural models' of these infections as follows: First, the bovine respiratory syncytial virus (BRSV) is closely related to human respiratory syncytial virus (HRSV) which is an important cause of respiratory disease in young children, and there are many similarities between RSV infection in juvenile cattle and humans [110]. Therefore, experimentally infected calves are suited to the study of RSV-induced chronic bronchiolitis that is typical in both species [111, 112, 113]. Second, respiratory *Mycoplasma (M.) bovis* infection in bovines may share features of pathogenesis with human *Mycoplasma pneumoniae* infections, and experimental lung infection of cattle with *M. bovis* results in a Th2-skewed immune response [114]. Third, infections with *Chlamydophila* spp. are common in cattle. In naturally infected calves, persistent respiratory chlamydial infections were found to be associated with chronic inflammation of lung and airways, and lung function tests revealed significantly increased peripheral airway resistance indicating peripheral airway obstruction on a sub-clinical level for several months [115, 116].

To resume the present situation, there is a need of animal models evaluating both viral and bacterial infections (especially caused by *Mycoplasma* and/or *Chlamydophila* spp.) in human asthma, but at present no models exist in which especially chronic infection or airway remodeling caused by the latter organisms could be studied *in vivo* [117, 118]. Cattle, however, present natural infections with these microbes. Despite none of these naturally occurring infections has been combined to allergic conditions in the bovine species until now, the present infection models might be of interest because they could be developed to combined allergic-infectious models in calves. Due the longer life time compared to laboratory animals, attention could also be paid on the pathogenesis of persisting respiratory infections or on mechanism of chronicity.

Subgross anatomy of the lung and the resulting functional consequences in any case of obstructive airway diseases are comparable between swine and cattle (see section 3). Consequently, airway obstructions in cattle easily lead to ventilatory asynchronisms, atelectases, increases in FRC, and gas exchange disturbances. For investigations of pulmonary function, a variety of methods has been applied successfully to cattle. Conventional measurement of respiratory mechanics (pulmonary resistance, dynamic lung compliance) is possible in conscious cattle of all ages, i.e. from the newborn calf until the adult cow using an oesophageal ballon catheter and a pneumotachograph of the appropriate size [119, 120, 121]. Since ventilatory parameters of calves aged 2-7 months (weighing approximately between 50 and 180 kg) are comparable to those of adult humans, all lung function techniques originally designed for human medicine that do not require active cooperation are applicable to spontaneously breathing calves with body weights less than 150-200 kg. Consequently, different forced oscillation techniques have been validated in awake calves in order to assess changes in respiratory mechanics [122, 123, 124, 125], and especially respiratory impedance measurements below 15 Hz were found to be suitable separating distal and proximal airway obstructions by means of impulse oscillometry [126, 127]. Detection of lung inflation by radiography or measurements of FRC are possible in order to evaluate emphysema *in vivo* [128, 129]. Inhomogeneities in ventilation are detectable non-invasively by applying capnovolumetry to conscious calves[3] or using imaging techniques [130]. Impaired gas exchanges can be easily evaluated either by well established arterial blood gas analyses or non-invasively by pulse oximetry in calves [131].

Thirty years ago, a first assessment of bronchial constriction in response to a specific inhalative allergen challenge in cattle was just done by counting the increase in respiratory rate [105]. In the meantime, non-specific bronchial challenge tests are applicable even to bovines. Using carbachol and conventional measurement of total pulmonary resistance, the development of bronchial hyperresponsiveness was confirmed after RSV infection in calves [132].

Assessment of airway inflammation can be performed by bronchoscopy and broncho-alveolar lavage. BALF can be used for cytological, biochemical, immunological or molecu-

[3] Reinhold, P.; Reissig, S.; Jaeger, J.; Langenberg, A.; Smith, H.-J.: Capnovolumetry is a useful technique to detect peripheral airway obstruction (2007) European Respiratory Journal, **30** (Supplement 51), 8s, Abstract 233.

lar investigations. As in pigs, there is a methodological limitation using BALF for diagnostic purposes: Because of the high degree of lung lobulation, BALF samples obtained from different parts of the lung reflect local conditions and are not necessarily representative for the whole lung [133]. In BALF, a variety of mediators involved in both airway inflammation and airway remodeling have been evaluated in conjunction with acute or chronic airway obstructions caused by infectious noxes; for example concentrations of leukotrien B4 (LTB4) or 8-isoprostane (8-IP), and activities of matrix metalloprotease [115, 132]. Exhaled breath condensate (EBC) offers a new potential method to examine pulmonary inflammation non-invasively in normal breathing conditions, and the EBC collection process itself has been validated in calves [93]. Caused by experimentally induced respiratory infections, the concentration of LTB4 in EBC of calves increased significantly and this increase correlated with deterioration in lung function [132]. Despite this encouraging finding EBC analysis is still a method undergoing further validation in both human as well as veterinary medicine.

For the assessment of structural changes, bovine lung tissue can be obtained either *in vivo* by biopsy [134] or *ex vivo*. Acute airway obstruction was associated histologically with bronchiolitis in RSV infected calves [112, 135]. In contrast, in chronic-persistent airway obstruction the involvement of bronchus-associated lymphoid tissue (BALT) was confirmed in calves persistently infected with *Chlamydiae* [115].

In the bovine species, mechanisms of mucociliary function have been studied *in vivo* [136] and are currently under investigation *in vitro* [137, 138].

4.6. Horses

Recurrent airway obstruction (RAO) or heaves is a common, naturally occurring syndrome of adult horses, which is characterized by neutrophilic chronic lower airway inflammation, reversible airway obstruction and bronchial hyperresponsiveness [139]. Around 12-50 % of all adult horses in Europe and the United States suffer from RAO [140]. RAO-susceptible horses suffer from a respiratory hypersensitivity to inhaled environmental moulds, which develop in organic material such as hay and straw, and to inhaled unspecific irritants. The most commonly implicated antigens are borne by spores of *Aspergillus fumigatus*, *Faenia rectivirgula* and *Thermoactinomyces vulgaris*. Endotoxin has also been shown to play a role in development the disease [141].

RAO is to some extent sharing features with human asthma: airway obstruction, bronchial hyperresponsiveness and airway inflammation completely regress within four weeks after allergen exposure and symptom relief can be achieved by use of corticosteroids and bronchodilators [139]. On the other hand, an acute allergic respiratory response within minutes of allergen exposure does not occur; bronchoconstriction and bronchial influx of inflammatory cells occur within six hours after mold exposure [141]. The predominant cell in BALF is the neutrophil [142], thus a characteristic of chronic obstructive pulmonary disease (COPD).

From a functional point of view, bronchial obstruction and airway hyperresponsiveness can be quantified by measurement of respiratory mechanics [143], by impulse oscillometry [144, 145] or other forced oscillation techniques, by inductance plethysmography [146] and by forced expiration [147]. Impaired gas exchanges can be easily evaluated in horses by determining arterial blood gas tensions of oxygen and pulmonary scintigraphy allows quantification of ventilation/perfusion (V/Q) mismatch that is corrected after appropriated medical therapy [148].

Airway inflammation can be assessed by determination of H_2O_2 in exhaled breath condensate, whose concentration correlates with BALF neutrophil percentage [149]. A sensitive indicator of lung damage in RAO-affected horses seems to be alveolar clearance determined by scintigraphy. Indeed, even symptom free RAO-affected horses have an increased clearance rate in comparison to healthy control horses [150]. The “golden standard” for quantification of airway inflammation remains however bronchoalveolar lavage. Due to their large size, bronchoscopy and bronchoalveolar lavage are commonly performed in sedated horses and important lavage volumes (up to 500 ml) can be obtained, allowing the collection of bronchoalveolar fluid and substantial amounts of bronchial and alveolar cells. Large numbers of cells obtained from bronchial brushings and BALF allowed documenting an increased binding activity of transcription factor NFkappaB and AP-1 when horses showed clinical signs of RAO [151, 152, 153] as well as an enhanced survival of pulmonary granulocytes by delayed apoptosis [154].

Although RAO is characterized by a neutrophilic inflammation, the inflammatory process and cytokine expression of pulmonary cells has been shown to be characteristic of a pulmonary Th2-type immune response [155, 156, 157]. Oxidative stress has also been largely investigated in RAO-affected horses and findings with regard to pulmonary oxidant or anti-oxidant markers, such as glutathione, F2-isoprostanes, uric acid, ascorbic acid, partially mimic changes observed in human asthma patients [158, 159].

Morphological changes of the lower airways of RAO-affected horses are documented and include bronchial smooth muscle remodeling, hyperplasia of epithelial cells and degenerative changes in the larger conducting as well as in the peripheral airways [160, 161, 162]. *In vivo* assessment of remodeling can be partially performed using chest radiographs and bronchial biopsies. Peripheral changes of lung tissue and airways can be safely assessed used thoracoscopy-guided biopsies [163].

The genetic background of RAO benefits from increasing interest and genetic predispositions as well as environmental factors favoring development of RAO have been identified [164]. Molecular studies confirmed a strong up-regulation of the equine CLCA gene (chloride channels, calcium-activated) in the airways of RAO horses, implying a significant role of this gene in the pathogenesis of mucus overproduction [165]. Moreover, micro-arrays become to be used to study gene expression of RAO-affected horses [166] and candidate genes allowing detection of RAO-predisposition in horses start being identified [167].

4.7. Monkeys

Three species, the squirrel (*Saimiri sciureus*), the rhesus (*Macaca mulatta*) and the cynamolgus monkey (*Macaca*

fascicularis), have been described in monkey asthma models [7]. Monkeys are either naturally sensitized to *Ascaris suum* or they can undergo active sensitization with *Ascaris suum*, pollen or house dust mite [7, 168, 169].

Allergen–sensitized monkeys share many features with human asthma; i.e. they develop an early phase of bronchoconstriction in response to allergen inhalation, the so-called dual-responders also develop a late phase of bronchoconstriction, as well as non-specific airway hyperreactivity and eosinophilic and neutrophilic bronchial inflammation [168, 169, 170, 171]. As mentioned in section 3, monkeys' airway and lung morphology is similar to that of humans [172], which renders this animal model particularly attractive. Moreover, the immune system of monkeys is similar to that of humans, as well as the innervations of airways.

Monkeys' lung function can either be investigated under anesthesia for measurement of conventional respiratory mechanics or by use of forced oscillometry to measure respiratory impedance either as input or as transfer impedance [173, 174]. Airway inflammation can be assessed using bronchoscopy and determination of BALF inflammatory markers. Although not yet reported, it should be possible to collect exhaled breath condensate in monkeys as a mean on non-invasive assessment of airway inflammation.

Airway remodeling is poorly documented in monkeys, although mucus cell hyperplasia, hypertrophy of epithelial cells, basement membrane thickening and mucosal eosinophil accumulation have been described [168]. Similar to what is used in human medicine, computed tomography probably bears an interesting potential for non-invasive and longitudinal follow-up of airway remodeling in this asthma model.

The monkey model is also interesting for a neurological approach of asthma, as suggested by Chen and collaborators reporting an increased excitability of neurons of the *Nucleus tractus solitarius*, suggesting that the central nervous system could contribute to asthma exacerbations [175]. A further attractive point is the genomic approach of this asthma model. Indeed, lung tissue of sensitized and allergen-challenged monkeys displays a different micro-array profile than that unchallenged control monkeys [176].

5. CHANCES AND ADVANTAGES OF ALTERNATIVE MODELS

Beside the classical features of asthma that should be addressed in a model (see section 2), there is a need to evaluate the significance of co-factors influencing the incidence and severity of asthma. Although the list is not exhaustive, the following co-factors have been discovered so far to be relevant: *(1)* genetic susceptibility, *(2)* age and maturation, *(3)* nutrition, *(4)* infections, *(5)* exposure to air pollution (especially fine and ultrafine particulate material) [2, 117, 177, 178, 179]. In patients, these co-factors may interact with known pathobiological features and may significantly influence both the clinical outcome and the effect of therapeutic measures. Consequently, the most important co-factors that are known at present in humans need to be taken into consideration in animal models as well.

To do so, animal research that focuses excessively on one laboratory species will not succeed to transfer information from basic discovery to clinical application [8]. Models can be improved markedly by placing more emphasis on biological relevance when evaluating the usefulness of different species and by taking greater advantage of the unique experimental opportunities that are offered by large animals [8]. Species-specific peculiarities suggest the use of different animals to answer different questions and to enlarge the tool kit from the elegance of gene manipulation to the very basic measurement of functions at different levels (respiratory tissue, lungs, and the whole organism).

The Natural Host

The presence of natural diseases (see section 4) can be used to develop animal models of airway diseases in natural hosts. While natural asthma occurs in cats, and qualifies this species to serve as an asthma model, recurrent airway obstruction in horses shares many features with human COPD. Bacterial infections may clinically relevant contribute to chronic airway diseases (either causative or related to exacerbation), and atypical bacteria — specifically *Mycoplasma* spp. and *Chlamydophila* spp. — deserve special attention in asthma pathogenesis [117, 177]. Since similar infections naturally occur in calves, this species offer the possibility to analyze host-pathogen interactions under natural host conditions. In a natural host, it is likely that the occurring functional and structural changes which lead eventually to the clinical outcome of a disease mimic the response much closer to the response in humans compared to an artificial response in an unnatural laboratory host.

Immunology and Defense Mechanisms

In many aspects of immuno-physiology, closer similarities exist between large animal species and humans than between rodents and humans. These similarities include especially the development of immunocompetence during fetal ontogeny and in the neonate. In contrast to mice, fetuses from both humans and several large animal species attain a well developed peripheral immune system by the time of birth which undergoes further comparable developments after birth. These similarities support the fact that large animals are valuable models for maternal-fetal interactions and for development immunology [8].

There are also marked differences in the genetically conserved determination and regulation of defense mechanisms. For example, the interleukin-8 (IL-8) has been identified to play an important role in many inflammatory conditions being involved in the recruitment of neutrophils to the inflammatory site. The *Il8* gene, however, is absent in mice but is present in dogs, pigs, sheep, and cattle, and there is substantial cross-species activity with human IL-8 [8, 180]. Such findings underline the necessity taking species-specific peculiarities into strong consideration when assessing the biological relevance of an animal model.

Intra-Subject Follow Up and sample size

Due to the size of non-laboratory animals, large-sized samples of different diagnostic media (blood, fluids, biopsies, etc.) can be sampled *in vivo* and sampling is repeatable. Thus, serial within-subject sample collection becomes easily possible and allows a better monitoring on an individual ba-

sis. Consequently, the effect of inter-individual variability can be minimized leading to a reduction of the unavoidable number of subjects that need to be included in an experiment. Furthermore, the use of large animals might be advantageous to recover suitable amounts from material that is difficult to obtain, for example rare cell populations for proteomic analysis [8].

Age, Lung Maturation, Life Time, and Chronicity

In the pathogenesis of asthma, influences of age and lung maturation are of interest to explain the higher asthma prevalence at a young age compared with adults. Therefore, animals aged a few days have been used to represent the juvenile lung and were compared to animals aged several weeks representing the adult lung in rodent maturational models [181]. In humans, however, the postnatal lung development continues over months or even years [182, 183]. Larger animals have much longer time periods for postnatal development and for the total life times compared to rodents. For example, the functional maturity of the respiratory system is not reached before one year of age or a body weight of 300 kg in the bovine species [119, 184]. These similarities offer the possibility to study influences on lung maturation or aging on certain diseases as well as to compare pathogenetic mechanisms of the juvenile respiratory system with those of the adult respiratory system in models comparable more to the human lung. Furthermore, for studies of long-lasting effects that might be involved in the phenomena of persistence and chronicity of airway diseases in humans for years, long-lived subjects seem to be more appropriate models than short-lived laboratory animals.

Pulmonary Function Tests and Diagnostic Measures

Technical solutions for measuring pulmonary functions or collecting samples from the respiratory tract strongly depend on the size of the subject. Methods and techniques that had been developed originally for humans are applicable to animals fulfilling similar physiological criteria, i.e. similar physiological ranges for variables to be measured (i.e. airflows, pressures, or volumes during respiration). So far, a variety of equipments of human pneumology has been applied successfully to calves, sheep, and pigs in order to perform spirometry, measurements of respiratory mechanics, or capnography for example. Such techniques allow the assessment of airway obstructions using the same parameters as known and evaluated for humans. Furthermore, the use of the same technique enables the investigator to perform bronchial hyperreactivity or bronchodilation tests using comparable protocols as defined for human medicine. In so far, direct comparisons between data obtained in large animal models and data obtained in patients become possible. The same is true for techniques obtaining samples from the respiratory tract or imaging techniques. In a lot of cases, the technical equipment to perform bronchoscopy, BAL, biopsy or EBC collection in animals with body weights comparable to humans are applicable directly or with less modifications (see Tables **4**-**6**).

Nutrition

Interactions between consumption of certain food components and the risk or presence of chronic airway diseases has been postulated by a number of studies. Because nutrition can neither be standardized nor controlled over long lasting studies in human medicine, expected relationships can only be examined retrospectively; mainly based on questionnaires. Large animals offer the possibility to evaluate the influence of nutrition experimentally. Because monogastric pigs are very similar to humans with respect to nutrition and digestion, this species seems to be most appropriate model for studying influences of food compared to either carnivores (dogs, cats) or herbivores (cattle, sheep and other ruminants, or horses).

6. CONCLUSION

Since asthma is a very complex disease with many faces it is unlikely that one animal model will ever be able to reflect all aspects. For different aspects of the disease, the usefulness of different model has to be evaluated separately. Both potentials and analysis aspects of different models have to be clarified taking the strengths and the weaknesses of each animal species and each model design carefully into account - in order to minimize irrelevance and to define the biological usefulness for the question that needs to be answered.

For studies in mice, the most commonly used animal, a broad spectrum of molecular and immunological tools and genetic approaches is available. This is truly not the case for other animal species. Large animal species, however, present unique physiological and natural preconditions as well as experimental advantages that are of great value to develop alternative models of allergic and non-allergic chronic airway diseases. Despite the known disadvantages of being expensive and time consuming, large animal models are worth to be considered for their possible role as 'functional models'. They offer the potential to perform long-term studies allowing a simultaneous within-subject approach of functional, inflammatory and morphological changes, and taking the influence of co-factors into account. Based on close cooperation between research groups with different expertises, alternative models in larger animals may supplement well established asthma models in laboratory species, and may contribute to a better understanding of complex respiratory diseases as asthma or COPD.

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