

Review article

## The avian lung-associated immune system: a review

Sven REESE<sup>a</sup>, Grammatia DALAMANI<sup>a</sup>, Bernd KASPERS<sup>b\*</sup>

<sup>a</sup> Institute for Animal Anatomy, Faculty of Veterinary Medicine, University of Munich, Germany

<sup>b</sup> Institute for Animal Physiology, Faculty of Veterinary Medicine, University of Munich,  
Veterinärstr. 13, 80539 München, Germany

(Received 13 June 2005; accepted 21 November 2005)

**Abstract** – The lung is a major target organ for numerous viral and bacterial diseases of poultry. To control this constant threat birds have developed a highly organized lung-associated immune system. In this review the basic features of this system are described and their functional properties discussed. Most prominent in the avian lung is the bronchus-associated lymphoid tissue (BALT) which is located at the junctions between the primary bronchus and the caudal secondary bronchi. BALT nodules are absent in newly hatched birds, but gradually developed into the mature structures found from 6–8 weeks onwards. They are organized into distinct B and T cell areas, frequently comprise germinal centres and are covered by a characteristic follicle-associated epithelium. The interstitial tissue of the parabronchial walls harbours large numbers of tissue macrophages and lymphocytes which are scattered throughout tissue. A striking feature of the avian lung is the low number of macrophages on the respiratory surface under non-inflammatory conditions. Stimulation of the lung by live bacteria but not by a variety of bacterial products elicits a significant efflux of activated macrophages and, depending on the pathogen, of heterophils. In addition to the cellular components humoral defence mechanisms are found on the lung surface including secretory IgA. The compartmentalisation of the immune system in the avian lung into BALT and non BALT-regions should be taken into account in studies on the host-pathogen interaction since these structures may have distinct functional properties during an immune response.

**chicken / lung / BALT / avian respiratory phagocytes / IgA**

### Table of contents

1. Introduction .....	311
2. Anatomy of the avian respiratory system .....	312
3. Bronchus-associated lymphoid tissue .....	315
4. The interstitial immune system .....	317
5. The phagocyte system of the avian lung and air sacs .....	317
6. Particle uptake in the avian lung and air sacs .....	319
7. Secretory immunoglobulins – the IgA system .....	320
8. Concluding remarks .....	320

### 1. INTRODUCTION

Respiratory diseases play an important role in poultry and result in substantial eco-

nomical losses to the poultry industry [27, 85]. In addition, several important poultry pathogens enter the host through the lung surface and subsequently disseminate to

\* Corresponding author: Kaspers@tiph.vetmed.uni-muenchen.de

their target organs in the body. Therefore, animal health significantly depends on the successful control of pathogen invasion and pathogen replication at the bronchus-associated mucosal surface and in the lung tissue. This implicates that vaccination strategies should induce effector mechanisms which help to prevent pathogen invasion in the first place. One way of achieving this goal is the vaccination through the mucosal surface itself, a technique frequently used by the poultry industry. It allows for an economical, efficient and reliable way of vaccination of large poultry flocks independent of the animal's age. Today most of these vaccines rely on live attenuated micro organisms but the development of non infectious vaccines is highly desired. The rational development of novel vaccines requires a comprehensive knowledge of the structure and function of the lung-associated immune system in birds in order to target vaccines appropriately and to provide efficient mucosal adjuvants. There is very limited knowledge about the lung-associated immune system in the chicken and in poultry in general which might be a consequence of the unique and complex anatomy and function of the avian lung.

In birds, the body cavity is not divided by a diaphragm and ventilation is achieved through a unique way of gas transport which requires the action of the air sacs [23]. As a consequence the avian lung is rigid, fixed at the thoracic walls and comprises a highly complex structure [62]. For a better understanding of the lung-associated immune system in birds we will first introduce the basic features of the avian lung and subsequently discuss the structure and function of the bronchus-associated lymphoid tissue (BALT), the interstitial immune system and additional immunological effector systems of the lung.

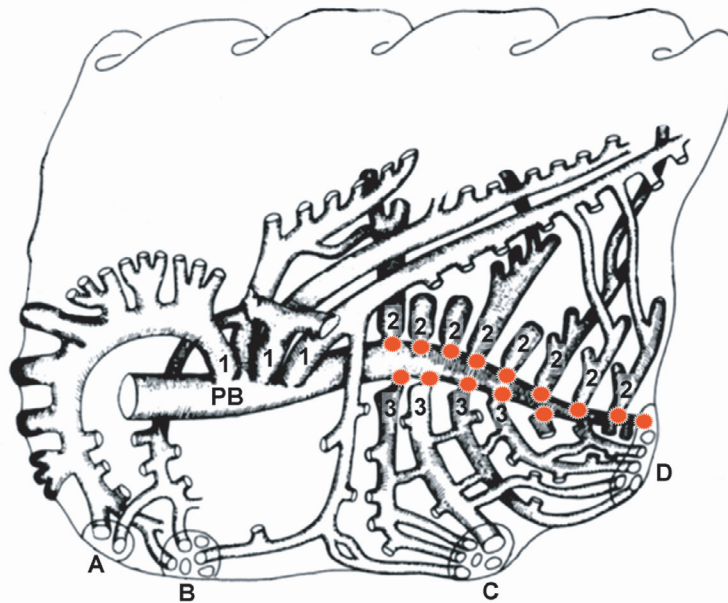
## **2. ANATOMY OF THE AVIAN RESPIRATORY SYSTEM**

The avian parabronchial lung differs morphologically from the mammalian

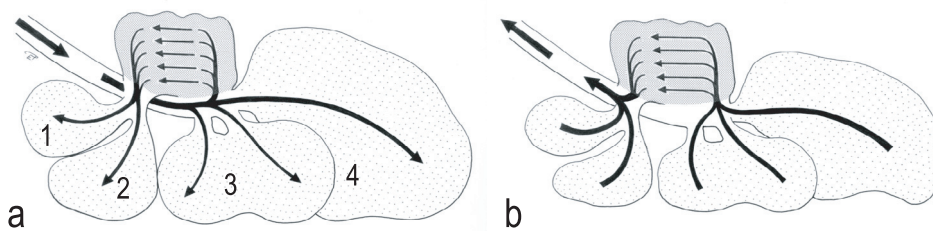
bronchioalveolar lung in certain key aspects [18, 41, 44, 49]. While the arrangement of the bronchial airway system of the mammalian lung displays iterating, commonly dichotomous bifurcations which terminate in blind-ended air conduits [31, 48] in the avian lung, a highly intricate anastomotic system exists [6, 44, 90]. In complete contrast to the tidally ventilated mammalian respiratory system, where fresh inhaled air is mixed with residual stale air in the respiratory airways, the avian lung is a flow-through system [42]. Through the system of air conduits, the primary bronchus, secondary bronchi and tertiary bronchi (parabronchi), the avian specific air sacs ventilate the lung continuously and unidirectionally like a pair of bellows [23, 43, 71]. Gas exchange takes place in the parabronchial tissue mantle, which is the fundamental functional part of the avian lung [40, 68].

The primary bronchus enters the lung at the border of the anterior and medial third of the ventral lung surface and extends to the caudal margin, where it opens into the abdominal air sac [18, 51]. Four groups of secondary bronchi (e.g. medioventral (Fig. 1), lateroventral (Fig. 1), mediiodorsal (Figs. 1 and 3a) and laterodorsal secondary bronchi) originate from the primary bronchus [36]. The inspired air completely bypasses the cranial lying openings of the medioventral secondary bronchi, a process which is termed inspiratory aerodynamic valving (IAV) [4, 5, 12, 47, 69, 86, 87]. In contrast, during the inspiratory phase as well as the expiratory phase air flows in the mediiodorsal and lateroventral secondary bronchi. This flow pattern results in a continuous ventilation of the parabronchial lung in a caudocranial direction (Fig. 2) [70]. A more detailed discussion and illustrations of the functional morphology can be found in Duncker [19], McLelland [51], Maina [42], Scheid and Piiper [70].

The wall of the primary bronchus and the initial parts of the secondary bronchi are covered by a mucosa, which is longitudinally folded and ciliated [52]. The epithelium of



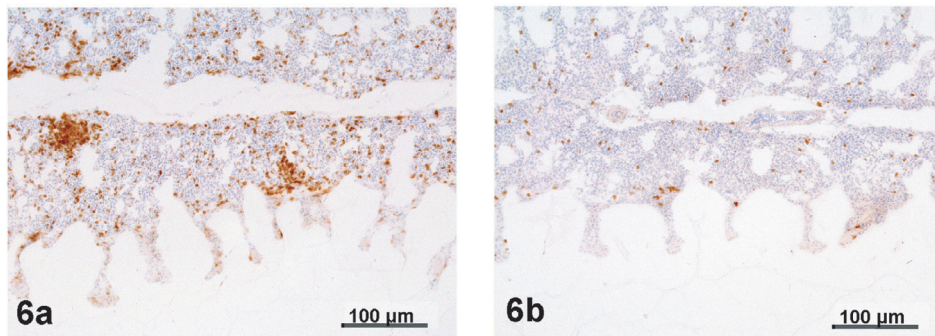
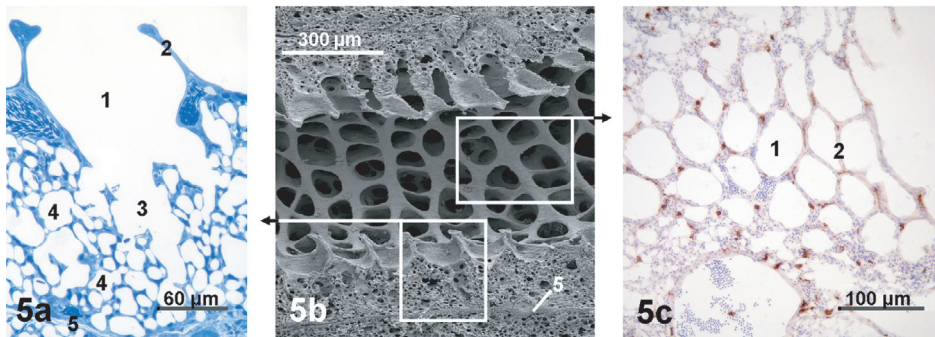
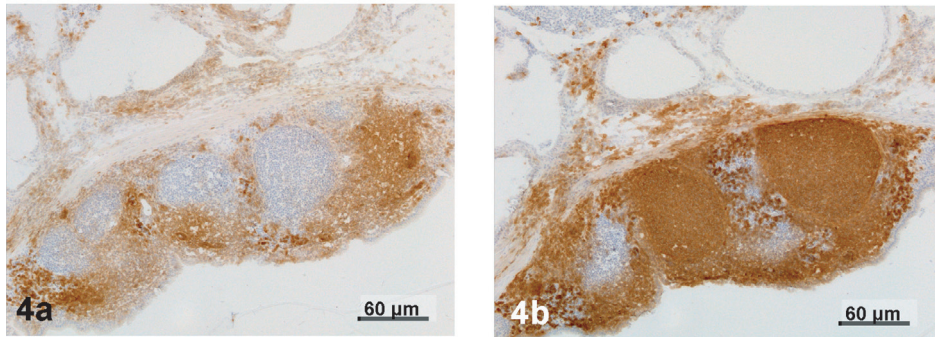
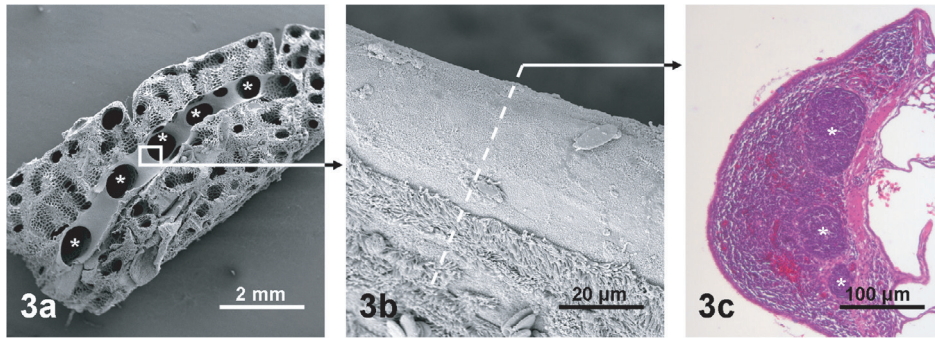
**Figure 1.** Medial view of the right lung of a chicken showing the main bronchi (modified after King [35]). Dots mark the localization of the BALT nodules in the primary bronchus. Legend: PB primary bronchus; 1: medioventral secondary bronchi; 2: mediodorsal secondary bronchi; 3: lateroventral secondary bronchi; A: ostium to the clavicular air sac; B: ostium to the cranial thoracic air sac; C: ostium to the caudal thoracic air sac; D: ostium to the abdominal air sac. (Reprinted from Form and Function in Birds, Vol. 4, A.S. King and J. McLelland, p. 226, 1989, with permission from Elsevier.) (A color version of this figure is available at [www.edpsciences.org](http://www.edpsciences.org).)



**Figure 2.** Air flow pattern in the avian respiratory tract during inspiration (a) and expiration (b). 1: Clavicular air sac; 2: cranial thoracic air sac; 3: caudal thoracic air sac; 4: abdominal air sac (mod. after König H.E., Liebich H.G., *Anatomie und Propädeutik des Geflügels*, Stuttgart, New York, 2001, p. 253, reproduced by permission of Schattauer, Stuttgart, New York).

the thin walled, poorly vascularized air sacs consists of squamous and cuboidal cells, with only a few ciliated columnar and non-ciliated columnar cells [14, 51]. The entrances to the secondary bronchi with the

exception of the medioventral bronchial openings show a modified mucosa with a non-ciliated flat zone as demonstrated by scanning electron microscopy [52] (Fig. 3b). This zone was found to be the primary location



of the BALT in the avian lung [21, 84] (Fig. 1).

A large number of parabronchi originate from the internal surfaces of the secondary bronchi connecting the mediodorsal and lateroventral secondary bronchi with the medioventral bronchi [17, 18, 51]. The wall of the parabronchi and most parts of the secondary bronchi are formed by the gas exchange tissue of the avian lung [18, 40]. The inhaled air flows through the parabronchial lumen and then centrifugally into the exchange tissue through the atria, the infundibula, and the network of air capillaries (Figs. 5a and 5b). The air capillaries are closely surrounded by a network of blood capillaries, which together constitute the most efficient gas exchanger unit among air-breathing vertebrates [41, 43].

The blood-gas barrier essentially consists of an endothelium, a single basal lamina, and a very thin squamous epithelial cell layer [45]. The epithelial lining cells are extremely thin, their cytoplasm being so attenuated that the thickness is often not greater than that of the unit membrane [1]. The blood-gas barrier in the avian lung is approximately 56–67% thinner than that of a mammal of the same body mass while the respiratory surface area is approximately 15% greater [49]. While large surface area and thin tissue barrier enhance respiratory efficiency, these structural features predis-

pose birds to pulmonary injury from environmental toxicants and invasion by pathogenic micro organisms [42].

### 3. BRONCHUS-ASSOCIATED LYMPHOID TISSUE

Organized lymphoid structures in the bronchial mucosa of the chicken lung were first described by Bienenstock et al. in 1973 [8]. These lymphoid aggregates were found to be highly similar to Peyer's patches and other gut-associated lymphoid tissues (GALT) and were designated BALT. In the original report, finger-like mucosal projections containing lymphoid nodules with germinal centers (Fig. 3c) were described. They were found far more frequently in the chicken than in any other species examined. However, these early observations have not been followed up for nearly 20 years and only recently new interest in respiratory diseases has led to a more detailed analysis of the lung-associated immune system in birds [84].

BALT structures of the chicken and turkey are located at the junctions of primary and secondary bronchi [21, 84] (Fig. 1) as well as at the ostia to the air sacs [54] and are found regularly in birds raised under conventional conditions [20, 21]. In the chicken they are confined to the openings of the most caudal secondary bronchi, the

---

**Figure 3.** (a) Scanning electron micrograph of the primary bronchus of a chicken (longitudinal section) showing the openings to the mediodorsal secondary bronchi (\*). (b) Scanning electron micrograph showing the non-ciliated FAE around the opening to a mediodorsal secondary bronchus. (c) Transverse section (hematoxylin and eosin staining) of this nonciliated zone showing a BALT nodule with germinal centers (\*).

**Figure 4.** BALT nodules in the primary bronchus of a 13 week old chicken. (a) Immunohistochemical demonstration of CD3<sup>+</sup> T-lymphocytes; (b) immunohistochemical demonstration of BU1<sup>+</sup> B-lymphocytes.

**Figure 5.** (a) Longitudinal section (methylene blue staining) of the parabronchus wall of a chicken. 1 – Atrium; 2 – Interatrial septum; 3 – Infundibulum; 4 – air capillaries; 5 – interparabronchial septum. (b) Scanning electron micrograph of a parabronchus of a chicken cut in longitudinal section. (c) Horizontal section of the atria demonstrating macrophages in the interatrial septa by immunohistochemistry (stained with mab Kul01).

**Figure 6.** Lymphocytes in the parabronchial wall of a 13-week-old chicken. (a) Immunohistochemical demonstration of CD3<sup>+</sup> T-lymphocytes; (b) immunohistochemical demonstration of BU1<sup>+</sup> B-lymphocytes.

mediodorsal, lateroventral and laterodorsal secondary bronchi [21], while a wider distribution pattern including the regions of longitudinal foldings of the mucosa has been observed in turkey [20]. Mature BALT structures consist of lymphocyte aggregates which are covered by a distinct layer of epithelial cells harbouring considerable numbers of lymphocytes [22], the lymphoepithelium or follicle-associated epithelium (FAE) [7, 20, 21]. The FAE is made up of ciliated and non-ciliated cells, the relative numbers of which differ significantly at different stages of BALT development [20, 21]. Four epithelial cell types have been described in the FAE [20, 21] including cells which have irregular microvilli on their luminal surface and close contact to lymphocytes and therefore display some features of microfold (M) cells. However, particle uptake by these cells has not been observed and characteristic organelles such as apical vesicles are absent [21]. It has been suggested that these cells may not be involved in antigen uptake and processing, but participate in tissue repair [20]. More detailed studies are required to understand the role of the non ciliated epithelium in the development and function of BALT nodules in birds.

Light and transmission electron microscopy revealed a similar cellular composition of the lymphoid nodules in chickens and turkeys. In 6 to 8-week-old birds germinal centers are found in most nodules and occasionally plasma cells are seen under the FAE. Macrophages, cells with dendritic cell morphology and heterophils are distributed in considerable numbers throughout the BALT nodules [20, 21]. Due to the availability of an extended set of leukocyte specific monoclonal antibodies in the chicken system, immuno-histological studies on the composition of BALT structures were carried out (Figs. 4a and 4b). This work confirmed the presence of large numbers of B cells, with IgM<sup>+</sup> cells in extend of IgG<sup>+</sup> cells and these again in extend of IgA<sup>+</sup> lymphocytes [22]. In cases where germinal centers are not present in the BALT struc-

ture, B-cells are confined to the edge of the lymphoid tissue, whereas lymphoid nodules are composed of aggregates of T-cells in the centre surrounded by B cells [22, 32].

The development of the BALT depends on the age and on environmental stimulation [32]. In day old chickens and turkey no or very few infiltrating lymphocytes are seen in the primary bronchus [20–22]. Within the first week after hatch CD45<sup>+</sup> leucocytes start to migrate into the primary bronchus [33] and small lymphocytic infiltrates are found consistently at the openings of the secondary bronchi [20]. Within the next 3–4 weeks lymphoid nodules develop at these locations and IgM-, IgG- and IgA-producing cells can be detected [20, 22]. CD4<sup>+</sup> and CD8<sup>+</sup> T cells are found at the same time localized in distinctive patterns. In the absence of germinal centers CD4<sup>+</sup> cells form single clusters while CD8<sup>+</sup> lymphocytes are distributed throughout the nodules. Germinal centers can first be detected in 2 to 4-week-old birds [20, 22, 33]. They are composed of blast-like cells which stain positive for either of IgG, IgA or IgM and with the pan B cell markers HIS-C1 or CB3 [22, 33]. In addition, IgM and IgG bearing cells with long cytoplasmic processes resembling follicular dendritic cells are present in the germinal centers [22, 33]. In these BALT structures CD4<sup>+</sup> T cells form large parafollicular caps around the germinal centres while CD8<sup>+</sup> cells are scattered throughout the tissue [22]. The number of IgG-, IgA- or IgM-producing cells continues to increase during the following weeks and BALT nodules can be found surrounding the entire openings to the secondary bronchi in 18 weeks old birds [20]. In contrast to the situation in healthy humans and similar to the rabbit and rat [83], BALT is a constitutive structure in the avian lung of specific pathogen free (SPF) and conventionally reared birds [20–22, 33].

BALT development was shown to follow the same time course in SPF and conventional chickens [22] but seems to be influenced by environmental stimuli. Using

a *Bordetella avium* infection model in turkey Van Alstine and Arp showed that the number of BALT nodules significantly increased in infected birds [84]. From these limited experimental data it can be concluded that SPF containment still provides sufficient stimulation by inhaled antigens for the development of the BALT. Whether an antigenic stimulus is essential for the induction of BALT formation in chicken is unknown. Studies by Bang and Bang [3] demonstrating lymphocytic infiltrates in the trachea and the Harderian gland of germ free chickens indicate that BALT structures might also be induced without microbial stimulation. However, the presence of inflammatory substances such as endotoxins in the inhaled air can not be excluded and may play a role in BALT formation. It is therefore conceivable that antigenic stimulation is required for the induction of BALT formation and for the development into fully mature structures in the first few weeks after hatch.

The demonstration of a highly developed and constitutively present BALT structure in the chicken and turkey stimulated some investigators to suggest that these mucosa-associated lymphoid structures may functionally compensate for the lack of lymph nodes in birds [22]. However, it remains to be shown if these structures specifically respond to antigenic stimulation by the generation of memory B and T lymphocytes.

#### 4. THE INTERSTITIAL IMMUNE SYSTEM

In addition to the highly organised BALT structures a diffuse infiltration of leucocytes into the interstitial lung tissue has been described. Using a pan-leukocyte (HIS-C7) marker Jeurissen et al. identified leucocytes located in the tissue surrounding the parabronchi as early as day five after hatch [33]. The majority of these cells stained positive with an antibody specific for monocytes, macrophages and interdig-

itating cells suggesting that these cells are antigen presenting cells. Furthermore, IgM<sup>+</sup> but not IgG<sup>+</sup> or IgA<sup>+</sup> lymphocytes were found at that time point. In older birds, Bu-1 positive B lymphocytes and CD3<sup>+</sup> T cells are found throughout the parenchyma (Fig. 6). The majority of these T lymphocytes were described as CD8<sup>+</sup>  $\alpha/\beta$  TCR expressing cells but few CD4<sup>+</sup> and  $\gamma/\delta$  T cells were also found. Macrophages seem to be abundantly present throughout the interstitial tissue of the parabronchial wall (Fig. 5c). These cells were initially identified by their expression of MHC class II molecules and their reactivity with monoclonal antibodies CVI-ChNL-68.1- and 74.2 [34]. This observation is in good agreement with data obtained from standard histological and TEM studies as discussed below.

#### 5. THE PHAGOCYTE SYSTEM OF THE AVIAN LUNG AND AIR SACS

In the mammalian lung alveolar macrophages constitute an important defence line at the gas-blood barrier [9, 10] that protects the extensive surface area which is only separated by a thin, easily assailable tissue barrier [55]. Relatively little is known about the corresponding cell type in the avian lung-air sac system. In the literature these cells have been designated as "avian respiratory macrophages or phagocytes – ARP" [26, 76] or "free avian respiratory macrophages – FARM" [42, 77]. Alveolar macrophages can easily be collected by pulmonary lavage in mammals, however this procedure yields only few FARM in birds [25, 46, 55, 77, 81]. From these studies it was estimated that alveolar macrophages in mice or rats were 20 times more frequent compared to FARM in the lung of the much larger chicken [46, 79]. Some earlier studies even reported on a complete failure to obtain free avian respiratory macrophages through lung lavage [37, 39, 65]. In this context it should be noted that FARM obtained through lung lavage are not only derived from the surface

of the lung but also from the air sacs as elegantly shown in domestic fowls and ducks [55]. Differences in the sampling technique as well as in the chicken strains and immune status of the birds may account for the variable results obtained within [76] and between studies.

The distribution pattern and the origin of FARM in the avian lung were addressed by several studies using light and transmission electron microscopy. Interestingly, FARM were never found on the surface of the air capillaries which represent the functional equivalent to the mammalian alveoli but were regularly present on the surfaces of the atria and the infundibulae [42, 46]. Thus, macrophages seem to be located at strategic check points where fresh air is distributed into the gas exchange areas and where particles can be trapped and removed. In support of this hypothesis, clusters of macrophages were identified in interatrial septa (Fig. 5c) and the loose connective tissue at the floor of the atria at the entrance to the infundibulae and the gas-exchange tissue proper [37, 46].

Phagocytic cells are also present in the air sacs. Studies using fiberoptic endoscopy identified heterophils as the most abundant cell population followed by macrophages and small numbers of lymphocytes [14]. Additionally, scattered solitary phagocytes were identified in the connective tissue of air sac walls [37].

Some controversy exists as to whether these tissue macrophages can be mobilised under non inflammatory conditions to migrate onto the lung surface. Whereas Klika et al. [37] did not find evidence for the migration of FARM from the interstitial tissue to the surface of the conducting airways other investigators reported the opposite [25, 77]. Nganpiep and Maina [55] compared the number of macrophages obtained by first and subsequent lung lavages and found a clear increase in cell numbers. The authors concluded that these cells emanated through efflux from the subepithelial compartment or even from the vascular system itself.

Inflammatory stimuli applied to the respiratory system efficiently elicit macrophages and heterophils to the respiratory surface. This was clearly shown by the high numbers of macrophages recovered from the lung and air sacs of turkeys after inoculation of incomplete Freund's adjuvant (IFA) into the abdominal air sacs. These macrophages showed rapid adhesion to glass surfaces, phagocytosis of zymosan particles and killing of *Escherichia coli* by in vitro assays [25]. Likewise, intratracheal instillation of heat-killed *Propionibacterium acnes* [79] as well as avirulent *Salmonella typhimurium* [82] induces a rapid and high level influx of avian respiratory macrophages, whereas the intravenous inoculation of *Propionibacterium acnes* resulted in a weak induction of respiratory macrophages [79]. No mobilisation of phagocytes was found after intravenous application of *E. coli* LPS, *Saccharomyces cerevisiae* glucan, IFA or subcutaneous application of LPS-G-IFA [79], compounds which are known to efficiently induce the migration of phagocytes to the mammalian respiratory system [76]. Comparable results were obtained in a *Pasteurella multocida* model, where intratracheal, but not oral administration of an apathogenic vaccine strain efficiently activated the nonspecific cellular defense system of the respiratory tract [28]. Migration of cells to the lungs and air sacs was evident within 2 h and showed peak activity at 8 h after stimulation [56]. Importantly, this response was only seen after administration of a relatively high number of stimulating live bacteria ( $> 10^8$ ) and vanished within 3–4 days [78]. ARP elicited in this way showed increased phagocytic capacities in comparison to those obtained from control chicken [80, 81]. Similar results were found after inoculation of *Pasteurella multocida* into the caudal thoracic air sac in turkeys. The air sac reacted rapidly and intensely with exudation of heterophils (94%) and a smaller amount of macrophages (6%) [24].

There is a very limited knowledge about adhesions mechanisms of micro organisms to avian respiratory macrophages. In one



investigation it was demonstrated that Sero-group A strains of *Pasteurella multocida* adhere to turkey air sac macrophages. The adhesion of the bacteria was mediated by capsular hyaluronic acid, which was recognized by a specific cell surface glycoprotein [63]. Additional experiments demonstrated that capsular hyaluronic acid bound to an isoform of the CD44 molecule expressed on cultured turkey peripheral blood monocytes [64]. This corresponds to the observation that mammalian alveolar macrophages express the hyaluronan receptor CD44 [15]. In another study where chickens were experimentally inoculated with avian fimbriated *Escherichia coli* it was shown that variations in the expression of the F1 fimbrial phase influenced the potential of avian respiratory macrophages for phagocytosis of these bacteria. *E. coli* isolates which expressed F1 fimbriae were rapidly killed by the macrophages, whereas in the absence of F1 fimbriae they were resistant [61].

A more extended discussion of the non-specific cellular defence system of the avian respiratory tract can be found in a recent review by Toth [76].

## 6. PARTICLE UPTAKE IN THE AVIAN LUNG AND AIR SACS

Inhaled aerosol particles are eliminated from the respiratory system by several independent mechanisms including aerodynamic filtration, mucociliary clearance and phagocytosis. A detailed study by Hayter and Besch [29] showed that the deposition of particles critically depends on their size. Large particles (3.7–7  $\mu\text{m}$  in diameter) are removed in the nasal cavities and the proximal trachea, while smaller particles are deposited throughout the respiratory system. Midsize particles (1.1  $\mu\text{m}$ ) are trapped primarily in the lung and cranial air sacs while smaller particles (0.091  $\mu\text{m}$ ) pass through the entire lung and are finally trapped in the abdominal air sacs. Removal of small inert particles (non-toxic iron oxide aerosol, particle diameter 0.18  $\mu\text{m}$ ) from the

lung was first investigated by Stearns et al. [74] in a duck model. It was shown that these particles were not only phagocytosed by FARM but also removed by the epithelial cells of the atria and the initial portions of the infundibula. Moreover particles were found in interstitial macrophages. These observations were subsequently confirmed in chickens, pigeons and ducks by Maina and Cowley [46] as well as Nganpiep and Maina [55] who observed phagocytosis of putative foreign particulate material and of red blood cells by the epithelial lining of the atrial muscles, the atrial floor and the infundibulae. From this work the picture emerges that the epithelium and the interstitial macrophages of the atrial and infundibular area play an important role in the removal of particles from the air on their way to the thin, extensive and highly vulnerable tissue of the gas exchange area [55]. With surface phagocytic cells at the atrial and infundibular levels [42, 46], subepithelial phagocytic cells and interstitial macrophages [32, 39, 72] and resident pulmonary intravascular macrophages [46], birds appear to possess a highly diversified pulmonary defence armoury [42]. Unfortunately, the pathway of antigen uptake and processing in the chicken lung has not been studied to date. It is therefore unclear, if phagocytosed material is presented to the local immune system in the BALT and parenchyma or transported to other secondary lymphoid organs such as the spleen.

As described above, smaller particles are transported through the lung into the air sacs. The thin air sac walls are primarily covered by squamous and cuboidal epithelial cells. In contrast to the epithelium of the parabronchial atria particle uptake by the air sac epithelium has not been described. In addition, a mucociliary escalator system was only found in the most proximal part of the air sacs right next to bronchial openings [14]. Thus, particle clearance is largely accomplished by phagocytic cells and is significantly slower than in the lung [25, 53].

## 7. SECRETORY IMMUNOGLOBULINS – THE IgA SYSTEM

Adaptive humoral immune responses on mucosal surfaces are primarily mediated by secretory IgA [60]. In the chicken an IgA like immunoglobulin was originally described by Orlans and Rose [58] and was later shown to be present as a polymeric immunoglobulin in serum and in several external secretions [88]. Cloning of the chicken C $\alpha$  gene provided structural data allowing the definite designation of this gene as the avian homologue for the mammalian  $\alpha$  H chain [50]. Even though the existence of a secretory component in chicken IgA was proposed for many years [59], final proof has been provided only recently through the cloning of the chicken polymeric immunoglobulin receptor [89]. IgA can be recovered from intestinal fluid, bile, tears and tracheal as well as lung washings [75]. However, the highest concentration of IgA is found in the bile fluid [66].

The presence of IgA<sup>+</sup> B cells in the chicken respiratory tract was demonstrated by immunohistology. IgA<sup>+</sup> B cells and plasma cells were found beneath the tracheal epithelium in 6-weeks-old birds, but not in younger individuals [33]. In the lung, IgA<sup>+</sup> cells were already present in 2-week-old birds within the epithelium of both BALT and non-BALT areas and a dramatic increase in cell numbers was observed with age [22]. Using haemolytic plaque assays Lawrence et al. [38] demonstrated that these cells actively secrete IgA. Since IgA can be obtained by lung lavage at readily detectable concentrations [13] it seems likely that IgA<sup>+</sup> cells in the tissue are the prime source of secretory IgA in the chicken lung. However, it is still unknown if the poly-Ig receptor is expressed on the lung epithelium and distribution of IgA secreted in the upper respiratory [2, 57] tract into the lung can not be excluded so far.

## 8. CONCLUDING REMARKS

The lung-associated immune system has largely been neglected in avian immunology research despite its importance in the control of respiratory diseases. The morphological studies discussed above clearly show that an organized mucosa-associated lymphoid tissue, the BALT, is constitutively present in normal chicken and turkey lungs. These structures show features characteristic for mucosal inductive sites [11] including T cell zones intervening between B cell follicles, the presence of antigen-presenting cells and a follicle-associated epithelium in contact with inhaled particles [22]. However, functional studies on antigen sampling properties, the transfer and processing of antigen and the stimulation of naïve T and B lymphocytes has not been published to date. Information on mucosal effector sites in the avian lung is even more limited. The presence of intraepithelial and interstitial lymphocytes has been described [33]. More recently, the isolation and functional characterization of CD8<sup>+</sup> effector T cells expressing IFN- $\gamma$  from lung tissue of influenza virus infected birds was described [73]. Unfortunately, this work did not specify whether these cells were obtained from the mucosal inducer or effector sides of the lung tissue. Similarly, a recent study in an infectious bronchitis virus model in chickens applied microarray analysis to investigate the host response in the lung but did not discriminate between BALT structures and the interstitial immune system as the source of RNA [16]. With the chicken genome sequence available [30], future work on host-pathogen interaction will increasingly apply this approach or more targeted gene expression analysis [67] to the lung tissue. Such work should take the distinct anatomical structures of the lung-associated immune system into account which may very well show strikingly different phenotypic properties and functional activities.

## ACKNOWLEDGEMENTS

This work was supported by the “Bayerisches Staatsministerium für Umwelt, Gesundheit und Verbraucherschutz” and the BMBF program FUGATO.

## REFERENCES

- [1] Abdalla M.A., Maina J.N., King A.S., King D.Z., Henry J., Morphometrics of the avian lung. 1. The domestic fowl (*Gallus gallus* variant *domesticus*), *Respir. Physiol.* 47 (1982) 267–278.
- [2] Avakian A.P., Ley D.H., Protective immune response to *Mycoplasma gallisepticum* demonstrated in respiratory-tract washings from *M. gallisepticum*-infected chickens, *Avian Dis.* 37 (1993) 697.
- [3] Bang B.G., Bang F.B., Localized lymphoid tissues and plasma cells in paraocular and paranasal organ systems in chickens, *Am. J. Pathol.* 53 (1968) 735–751.
- [4] Banzett R.B., Butler J.P., Nations C.S., Barnas G.M., Lehr J.L., Jones J.H., Inspiratory aerodynamic valving in goose lungs depends on gas density and velocity, *Respir. Physiol.* 70 (1987) 287–300.
- [5] Banzett R.B., Nations C.S., Wang N., Fredberg J.J., Butler J.P., Pressure profiles show features essential to aerodynamic valving in geese, *Respir. Physiol.* 84 (1991) 295–309.
- [6] Bellairs R., Osmond M., *The atlas of chick development*, Academic Press, London, 1998.
- [7] Bienenstock J., Befus D., Gut- and bronchus-associated lymphoid tissue, *Am. J. Anat.* 170 (1984) 437–445.
- [8] Bienenstock J., Johnston N., Perey D.Y., Bronchial lymphoid tissue. I. Morphologic characteristics, *Lab. Invest.* 28 (1973) 686–692.
- [9] Bowden D.H., Macrophages, dust, and pulmonary diseases, *Exp. Lung Res.* 12 (1987) 89–107.
- [10] Brain J.D., Macrophages in the respiratory tract, in: Fishman A.P., Fisher A.B. (Eds.), *Handbook of Physiology*, Vol. 1. Circulation and Nonrespiratory Functions, American Physiological Society, Bethesda, Maryland, 1985, pp. 447–471.
- [11] Brandtzaeg P., Pabst R., Let’s go mucosal: communications on slippery ground, *Trends Immunol.* 25 (2004) 570–577.
- [12] Brown R.E., Kovacs C.E., Butler J.P., Wang N., Lehr J., Banzett R.B., The avian lung: is there an aerodynamic expiratory valve? *J. Exp. Biol.* 198 (1995) 2349–2357.
- [13] Cihak J., Hoffmann-Fezer G., Ziegler-Heitbrock H.W.L., Stein H., Kaspers B., Chen C.H., Cooper M.D., Lösch U., T cells expressing the Vbeta1 T-cell receptor are required for IgA production in the chicken, *Proc. Natl. Acad. Sci. USA* 88 (1991) 10951–10955.
- [14] Crespo R., Yamashiro S., Hunter D.B., Development of the thoracic air sacs of turkeys with age and rearing conditions, *Avian Dis.* 42 (1998) 35–44.
- [15] Culty M., O’Mara T.E., Underhill C.B., Yeager H., Swartz R.P., Hyaluronan receptor (CD44) expression and function in human peripheral blood monocytes and alveolar macrophages, *J. Leukoc. Biol.* 56 (1994) 605–611.
- [16] Dar A., Munir S., Vishwanathan S., Manuja A., Griebel P., Tikoo S., Townsend H., Potter A., Kapur V., Babiuk L.A., Transcriptional analysis of avian embryonic tissues following infection with avian infectious bronchitis virus, *Virus Res.* 110 (2005) 41–55.
- [17] Duncker H.R., The lung air sac system of birds. A contribution to the functional anatomy of the respiratory apparatus, *Ergeb. Anat. Entwicklungsgesch.* 45 (1971) 7–171.
- [18] Duncker H.R., Structure of the avian respiratory tract, *Respir. Physiol.* 22 (1974) 1–19.
- [19] Duncker H.R., Vertebrate lungs: structure, topography and mechanics. A comparative perspective of the progressive integration of respiratory system, locomotor apparatus and ontogenetic development, *Respir. Physiol. Neurobiol.* 144 (2004) 111–124.
- [20] Fagerland J.A., Arp L.H., A morphologic study of bronchus-associated lymphoid tissue in turkeys, *Am. J. Anat.* 189 (1990) 24–34.
- [21] Fagerland J.A., Arp L.H., Structure and development of bronchus-associated lymphoid tissue in conventionally reared broiler chickens, *Avian Dis.* 37 (1993) 10–18.
- [22] Fagerland J.A., Arp L.H., Distribution and quantitation of plasma cells, T lymphocyte subsets, and B lymphocytes in bronchus-associated lymphoid tissue of chickens: age-related differences, *Reg. Immunol.* 5 (1993) 28–36.
- [23] Fedde M.R., Relationship of structure and function of the avian respiratory system to disease susceptibility, *Poult. Sci.* 77 (1998) 1130–1138.

- [24] Ficken M.D., Barnes H.J., Acute airsacculitis in turkeys inoculated with *Pasteurella multocida*, Vet. Pathol. 26 (1989) 231–237.
- [25] Ficken M.D., Edwards J.F., Lay J.C., Induction, collection, and partial characterization of induced respiratory macrophages of the turkey, Avian Dis. 30 (1986) 766–771.
- [26] Fulton R.M., Reed W.M., DeNicola D.B., Light microscopic and ultrastructural characterization of cells recovered by respiratory-tract lavage of 2- and 6-week-old chickens, Avian Dis. 34 (1990) 87–98.
- [27] Glisson J.R., Bacterial respiratory disease of poultry, Poult. Sci. 77 (1998) 1139–1142.
- [28] Hassanin H.H., Toth T.E., Eldimerdash M.M., Siegel P.B., Stimulation of avian respiratory phagocytes by *Pasteurella multocida*: effects of the route of exposure, bacterial dosage and strain, and the age of chickens, Vet. Microbiol. 46 (1995) 401–413.
- [29] Hayter R.B., Besch E.L., Airborne particle deposition in the respiratory tract of chickens, Poult. Sci. 53 (1974) 1507–1511.
- [30] Hillier L.W., et al., Consortium I.C.G.S., Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution, Nature 432 (2004) 695–716.
- [31] Horsfield K., Pulmonary airways and blood vessels considered as confluent trees, in: Crytal R.D., West J.B., Weibel E.R., Barnes P.J. (Eds.), The lung: Scientific foundations, Lippincott-Raven, Philadelphia, 1997, pp. 1073–1079.
- [32] Jeurissen S.H., Vervelde L., Janse E.M., Structure and function of lymphoid tissues of the chicken, Poult. Sci. Rev. 5 (1994) 183–207.
- [33] Jeurissen S.H., Janse E.M., Koch G., De Boer G.F., Postnatal development of mucosa-associated lymphoid tissues in chickens, Cell Tissue Res. 258 (1989) 119–124.
- [34] Jeurissen S.H., Janse E.M., Kok G.L., De Boer G.F., Distribution and function of non-lymphoid cells positive for monoclonal antibody CVI-ChNL-68.2 in healthy chickens and those infected with Marek's disease virus, Vet. Immunol. Immunopathol. 22 (1989) 123–133.
- [35] King A.S., Structural and functional aspects of the avian lungs and air sacs, in: Felts W.J.L., Harrison R.J. (Eds.), International review of general and experimental zoology, Academic Press, New York, 1966, pp. 171–267.
- [36] King A.S., Apparatus respiratorius, in: Baumel J.J. (Ed.), Handbook of avian anatomy: Nomina anatomica avium, Nuttall Ornithological Club, Cambridge, Massachusetts, 1993, pp. 257–300.
- [37] Klika E., Scheuermann D.W., De Groot-Lasseel M.H., Bazantova I., Switka A., Pulmonary macrophages in birds (barn owl, *Tyto tyto alba*), domestic fowl (*Gallus gallus* f. *domesticus*), quail (*Coturnix coturnix*), and pigeons (*Columbia livia*), Anat. Rec. 246 (1996) 87–97.
- [38] Lawrence E.C., Arnaud-Battandier F., Koski I.R., Dooley N.J., Muchmore A.V., Blaese R.M., Tissue distribution of immunoglobulin-secreting cells in normal and IgA-deficient chickens, J. Immunol. 123 (1979) 1767–1771.
- [39] Lorz C., Lopez J., Incidence of air pollution in the pulmonary surfactant system of the pigeon (*Columba livia*), Anat. Rec. 249 (1997) 206–212.
- [40] Maina J.N., Scanning electron microscope study of the spatial organization of the air and blood conducting components of the avian lung (*Gallus gallus* variant *domesticus*), Anat. Rec. 222 (1988) 145–153.
- [41] Maina J.N., Comparative respiratory morphology: themes and principles in the design and construction of the gas exchangers, Anat. Rec. 261 (2000) 25–44.
- [42] Maina J.N., Some recent advances on the study and understanding of the functional design of the avian lung: morphological and morphometric perspectives, Biol. Rev. Camb. Philos. Soc. 77 (2002) 97–152.
- [43] Maina J.N., Fundamental structural aspects and features in the bioengineering of the gas exchangers: comparative perspectives, Adv. Anat. Embryol. Cell Biol. 163 (2002) III–XII, 1–108.
- [44] Maina J.N., A systematic study of the development of the airway (bronchial) system of the avian lung from days 3 to 26 of embryogenesis: a transmission electron microscopic study on the domestic fowl, *Gallus gallus* variant *domesticus*, Tissue Cell 35 (2003) 375–391.
- [45] Maina J.N., Morphogenesis of the laminated, tripartite cytoarchitectural design of the blood-gas barrier of the avian lung: a systematic electron microscopic study on the domestic fowl, *Gallus gallus* variant *domesticus*, Tissue Cell 36 (2004) 129–139.
- [46] Maina J.N., Cowley H.M., Ultrastructural characterization of the pulmonary cellular defenses in the lung of a bird, the rock dove,

- Columba livia*, Proc. R. Soc. Lond. B Biol. Sci. 265 (1998) 1567–1572.
- [47] Maina J.N., Africa M., Inspiratory aerodynamic valving in the avian lung: functional morphology of the extrapulmonary primary bronchus, J. Exp. Biol. 203 (2000) 2865–2876.
- [48] Maina J.N., van Gils P., Morphometric characterization of the airway and vascular systems of the lung of the domestic pig, *Sus scrofa*: comparison of the airway, arterial and venous systems, Comp. Biochem. Physiol. A Mol. Integr. Physiol. 130 (2001) 781–798.
- [49] Maina J.N., King A.S., Settle G., An allometric study of pulmonary morphometric parameters in birds, with mammalian comparisons, Philos. Trans. R. Soc. Lond. B Biol. Sci. 326 (1989) 1–57.
- [50] Mansikka A., Chicken IgA H chains. Implications concerning the evolution of H chain genes, J. Immunol. 149 (1992) 855–861.
- [51] McLelland J., Anatomy of the lungs and air sacs, in: King A.S., McLelland J. (Eds.), Form and function in birds, Academic Press, London, 1989, pp. 221–280.
- [52] McLelland J., MacFarlane C.J., Solitary granular endocrine cells and neuroepithelial bodies in the lungs of the Ringed Turtle Dove (*Streptopelia risoria*), J. Anat. 147 (1986) 83–93.
- [53] Mensah G.A., Brain J.D., Deposition and clearance of inhaled aerosol in the respiratory tract of chickens, J. Appl. Physiol. 53 (1982) 1423–1428.
- [54] Myers R.K., Arp L.H., Pulmonary clearance and lesions of lung and air sac in passively immunized and unimmunized turkeys following exposure to aerosolized *Escherichia coli*, Avian Dis. 31 (1987) 622–628.
- [55] Nganpiep L.N., Maina J.N., Composite cellular defence stratagem in the avian respiratory system: functional morphology of the free (surface) macrophages and specialized pulmonary epithelia, J. Anat. 200 (2002) 499–516.
- [56] Ochs D.L., Toth T.E., Pyle R.H., Siegel P.B., Cellular defense of the avian respiratory system: effects of *Pasteurella multocida* on respiratory burst activity of avian respiratory tract phagocytes, Am. J. Vet. Res. 49 (1988) 2081–2084.
- [57] Ohshima K., Hiramatsu K., Distribution of T-cell subsets and immunoglobulin-containing cells in nasal-associated lymphoid tissue (NALT) of chicken, Histol. Histochem. 15 (2000) 713–720.
- [58] Orlans E., Rose M.E., An IgA-like immunoglobulin in the fowl, Immunochemistry 9 (1972) 833–838.
- [59] Peppard J.V., Rose M.E., Hesketh P., A functional homologue of mammalian secretory component exists in chickens, Eur. J. Immunol. 13 (1983) 566–570.
- [60] Phalipon A., Cardona A., Kraehenbuhl J.P., Edelmann L., Sansonetti P.J., Corthesy B., Secretory component: A new role in secretory IgA-Mediated exclusion in vivo, Immunity 17 (2002) 107–115.
- [61] Pourbakhsh S.A., Boulianne M., Martineau-Doize B., Fairbrother J.M., Virulence mechanisms of avian fimbriated *Escherichia coli* in experimentally inoculated chickens, Vet. Microbiol. 58 (1997) 195–213.
- [62] Powell F.L., Respiration, in: Whittow G.C. (Ed.), Sturkie's Avian physiology, Academic Press, London, 2000, pp. 233–264.
- [63] Pruimboom I.M., Rimler R.B., Ackermann M.R., Brogden K.A., Capsular hyaluronic acid-mediated adhesion of *Pasteurella multocida* to turkey air sac macrophages, Avian Dis. 40 (1996) 887–893.
- [64] Pruimboom I.M., Rimler R.B., Ackermann M.R., Enhanced adhesion of *Pasteurella multocida* to cultured turkey peripheral blood monocytes, Infect. Immun. 67 (1999) 1292–1296.
- [65] Qureshi M.A., Marsh J.A., Dietert R.R., Sung J., Bolnet C.N., Petite J.N., Profiles of chicken macrophage effector functions, Poult. Sci. 73 (1994) 1027–1034.
- [66] Rose M.E., Orlans E., Payne A.W., Hesketh P., The origin of IgA in chicken bile: its rapid active transport from blood, Eur. J. Immunol. 11 (1981) 561–564.
- [67] Rothwell L., Young J.R., Zoorob R., Whittaker C.A., Hesketh P., Archer A., Smith A.L., Kaiser P., Cloning and characterization of chicken IL-10 and its role in the immune response to *Eimeria maxima*, J. Immunol. 173 (2004) 2675–2682.
- [68] Scheid P., Mechanisms of gas exchange in bird lungs, Rev. Physiol. Biochem. Pharmacol. 86 (1979) 137–186.
- [69] Scheid P., Piiper J., Aerodynamic valving in the avian lung, Acta Anaesthesiol. Scand. Suppl. 90 (1989) 28–31.
- [70] Scheid P., Piiper J., Respiratory mechanics and air flow in birds, in: King A.S., McLelland J. (Eds.), Form and function in birds, Academic Press, London, 1989, pp. 369–392.

- [71] Scheid P., Slama H., Piiper J., Mechanisms of unidirectional flow in parabronchi of avian lungs: measurements in duck lung preparations, *Respir. Physiol.* 14 (1972) 83–95.
- [72] Scheuermann D.W., Klika E., Lasseel D.G., Bazantova I., Switka A., An electron microscopic study of the parabronchial epithelium in the mature lung of four bird species, *Anat. Rec.* 249 (1997) 213–225.
- [73] Seo S.H., Peiris M., Webster R.G., Protective cross-reactive cellular immunity to lethal A/Goose/Guangdong/1/96-Like H5N1 influenza virus is correlated with the proportion of pulmonary CD8+ T cells expressing gamma interferon, *J. Virol.* 76 (2002) 4886–4890.
- [74] Stearns R.C., Barnas G.M., Walski M., Brain J.D., Deposition and phagocytosis of inhaled particles in the gas exchange region of the duck, *Anas platyrhynchos*, *Respir. Physiol.* 67 (1987) 23–36.
- [75] Tizard I., The avian antibody response, *Sem. Avian Exotic Pet. Med.* 11 (2002) 2–14.
- [76] Toth T.E., Nonspecific cellular defense of the avian respiratory system: a review, *Dev. Comp. Immunol.* 24 (2000) 121–139.
- [77] Toth T.E., Siegel P.B., Cellular defense of the avian respiratory tract: paucity of free-residing macrophages in the normal chicken, *Avian Dis.* 30 (1986) 67–75.
- [78] Toth T.E., Siegel P.B., Cellular defense of the avian respiratory system: dose-response relationship and duration of response in intratracheal stimulation of avian respiratory phagocytes by a *Pasteurella multocida* bacterin, *Avian Dis.* 37 (1993) 756–762.
- [79] Toth T.E., Siegel P., Veit H., Cellular defense of the avian respiratory system. Influx of phagocytes: elicitation versus activation, *Avian Dis.* 31 (1987) 861–867.
- [80] Toth T.E., Veit H., Gross W.B., Siegel P.B., Cellular defense of the avian respiratory system: protection against *Escherichia coli* airsacculitis by *Pasteurella multocida*-activated respiratory phagocytes, *Avian Dis.* 32 (1988) 681–687.
- [81] Toth T.E., Pyle R.H., Caceci T., Siegel P.B., Ochs D., Cellular defense of the avian respiratory system: influx and nonopsonic phagocytosis by respiratory phagocytes activated by *Pasteurella multocida*, *Infect. Immun.* 56 (1988) 1171–1179.
- [82] Toth T.E., Curtiss R. 3rd, Veit H., Pyle R.H., Siegel P.B., Reaction of the avian respiratory system to intratracheally administered avirulent *Salmonella typhimurium*, *Avian Dis.* 36 (1992) 24–29.
- [83] Tschernig T., Pabst R., Bronchus-associated lymphoid tissue (BALT) is not present in the normal adult lung but in different diseases, *Pathobiology* 68 (2000) 1–8.
- [84] Van Alstine W.G., Arp L.H., Histologic evaluation of lung and bronchus-associated lymphoid tissue in young turkeys infected with *Bordetella avium*, *Am. J. Vet. Res.* 49 (1988) 835–839.
- [85] Villegas P., Viral diseases of the respiratory system, *Poult. Sci.* 77 (1998) 1143–1145.
- [86] Wang N., Banzett R.B., Butler J.P., Fredberg J.J., Bird lung models show that convective inertia effects inspiratory aerodynamic valving, *Respir. Physiol.* 73 (1988) 111–124.
- [87] Wang N., Banzett R.B., Nations C.S., Jenkins F.A. Jr., An aerodynamic valve in the avian primary bronchus, *J. Exp. Zool.* 262 (1992) 441–445.
- [88] Watanabe H., Kobayashi K., Isayama Y., Peculiar secretory IgA system identified in chicken. II. Identification and distribution of free secretory component and immunoglobulin of IgA, IgM, and IgG in chicken external secretions, *J. Immunol.* 115 (1975) 998–1001.
- [89] Wieland W.H., Orzaez D., Lammers A., Parmentier H.K., Verstegen M.W.A., Schots A., A functional polymeric immunoglobulin receptor in chicken (*Gallus gallus*) indicates ancient role of secretory IgA in mucosal immunity, *Biochem. J.* 380 (2004) 669–676.
- [90] Woodward J.D., Maina J.N., A 3D digital reconstruction of the components of the gas exchange tissue of the lung of the muscovy duck, *Cairina moschata*, *J. Anat.* 206 (2005) 477–492.