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Immuno-localization of type-IV collagen in the blood-gas barrier and the epithelial–epithelial cell connections of the avian lung

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The terminal respiratory units of the gas exchange tissue of the avian lung, the air capillaries (ACs) and the blood capillaries (BCs), are small and rigid: the basis of this mechanical feature has been highly contentious. Because the strength of the blood-gas barrier (BGB) of the mammalian lung has been attributed to the presence of type-IV collagen (T-IVc), localization of T-IVc in the basement membranes (BM) of the BGB and the epithelial–epithelial cell connections (E-ECCs) of the exchange tissue of the lung of the avian (chicken) lung was performed in order to determine whether it may likewise contribute to the strength of the BGB. T-IVc was localized in both the BM and the E-ECCs. As part of an integrated fibroskeletal scaffold on the lung, T-IVc may directly contribute to the strengths of the ACs and the BCs.

1. Introduction

‘Historically, the avian respiratory system is highly ranked among the controversial organ-systems’. Farner [1, p. 1656]

Regarding certain aspects of the functional and structural designs of the avian lung, the statement above is as valid now as when it was made approximately five decades ago. Since the discoveries of the astonishing rigidities of the air capillaries (ACs) [2] and the blood capillaries (BCs) [3] approximately three decades ago, diverse views have been expressed to explain the structural basis of their strengths. It has been suggested that: (i) pairs of epithelial cell processes (retinacula) support the ACs [4], (ii) the presence of a lipoproteinaceous trilaminar substance that forms a three-dimensional web-like system and anchors and reinforces the ACs and the BCs [5] (iii) close-packaging of the ACs and the BCs, a so-called ‘honeycomb-like arrangement’ confers stability [6], (iv) the epithelial–epithelial cell connections (E-ECCs) couple the BCs (figures 1a–f and 2a–k) and strengthens them [7–10], and (v) the arrangement of the collagen and elastic-tissue fibres, connective tissue elements with different mechanical properties, may form a tensegrity system that dissipates tension [11–14]. Despite intensive efforts, the particular structures and mechanisms that impart rigidity to the ACs and the BCs remain elusive.

The structure of the blood-gas barrier (BGB) of the avian lung is well known [7,15,16], but that of the E-ECCs are largely indeterminate. While an early study reported a lack of a basement membrane (BM) in the E-ECCs [15], recently, it was shown that these sites contain extracellular matrix (ECM) or its highly specialized form, the BM, at least in sites close to the BCs [6,10]. The non-fibrillar type-IV collagen (T-IVc) is only found in the BMs [17] where, together with other forms of collagen, it serves as a scaffolding protein [18]. Cogent but implicit evidence [19–21] has shown that the strength of the BGB of the mammalian lung stems from BM, explicitly from the T-IVc [16]. Categorization of
collagen fibrils requires techniques such as immuno-labelling with antibodies raised against a specific collagen type [22]. No previous attempt has been made to find whether T-IVc collagen exists in the BGB and the E-ECCs parts of the gas exchange tissue (ET) of the avian lung. Here, T-IVc was localized in the two sites, and the solidity of the BCs and the ACs implicitly associated with its presence.

2. Material and methods

After approval by the University of the Witwatersrand’s Animal Ethics Committee (Clearance no. 2007/53/01), three adult chickens, *Gallus domesticus* were used in this study: two for immuno-electron microscopic study and one for transmission electron microscopy (TEM). They were killed by injection with 5 ml of Euthanase (thiopentone sodium, 25 mg cm\(^{-3}\)) into the right brachial vein. For immuno-localization of T-IVc, the tissues were dehydrated in methanol, infiltrated with lowicryl [18], treated with polyclonal primary antibody raised against T-IVc, and exposed to gold particles conjugated with secondary antibodies which bound to the primary antibodies. For TEM, the lungs were fixed by vascular perfusion with 2.3 per cent buffered glutaraldehyde and tissues processed by standard laboratory techniques, while others were macerated in sodium hydroxide [23,24]. A total of 96 micrographs were prepared for immuno-electron microscopy and an equivalent number taken for TEM. Preparations treated with phosphate buffered saline only and secondary antibody, but without the primary antibody, served as controls.

3. Results

The most important structural features are: (i) a BM spans the E-ECCs (figures 1b and 2a–d); (ii) the epithelial cells of the E-ECCs sporadically bind together (figure 2b); (iii) the BM of the E-ECCs connects that of the BGB at sites termed triangular areas (figure 2a,c,d); (iv) the triangular areas contain plentiful collagen fibres (CFs; figure 2d); (v) CFs occur in the BGB and in the E-ECCs (figure 2k–h); and, (vi) T-IVc exists in the BM of the BGB (figure 2i) and that of the E-ECCs (figure 2j). To show the reliability of the immuno-localization technique, for the BGB (figure 2i) and the E-ECCs (figure 2k), the control preparations did not bind gold particles. Based on the relative numerical densities of the gold particles, the BMs of the E-ECCs contained fewer or less densely packed T-IVc fibres compared with the BGB.

4. Discussion

The ACs and the BCs respectively measure 5–20 \(\mu\)m [25–27] and 3–12 \(\mu\)m [27] in diameter. It is paradoxical that birds possess BGBs as thin as 0.096 \(\mu\)m [14,28] while they have very high arterial [29] and systolic blood pressures [30]. High pulmonary capillary blood pressure (PCBP) may be directly generated by the large hearts [31], which generate high cardiac outputs and stroke volumes [32]. Under experimental tests of their rigidity, the ACs tolerated a pressure
of 2 kPa [2], and occluding the pulmonary artery to one lung doubled vascular resistance [3]. Increasing the PCBP by as much as 3.3 kPa led to mere 13 per cent change in diameter and the ACs remained open under a 4.7 kPa pressure [7,8]. The mechanical properties of these microscopic structures are unclear.

Existence of a BM between BCs and between the ACs and the BCs has been well documented [15,16] but the state of the E-ECCs has been uncertain and controversial. From studies of different tissues [19–21], it has been persuasively argued, although from circumstantial evidence, that the strength of epithelial tissue derives from the BM and not from other cellular elements. It has been contended that the strength of the mammalian lung’s BGB derives from the BM and specifically from the T-IVc [16]. T-IVc is a distinctively high tensile strength, non-fibrillar, particularly molecularly well-organized form of collagen [22]. The T-IVc localized here in the BMs of the BGB and the E-ECCs, together with other possible forms of collagen, connects to the peripheral and the central parabronchial ‘pillars’ of CFs [13], with the gas ET being effectively ‘suspended’ between the ‘columns’. A closely related family of proteins with similar amino acid sequences and similar chemical and physical properties [33], collagens, form 20 per cent of the total protein in mammals [34] and supporting cells [35]. Collagen- and elastic-tissue fibres, proteoglycans and other glycoproteins are the main structural macromolecules of the connective tissue assemblage of the mammalian lung [24,33]. CFs form 6–20% of the matrix proteins of the dry lung weight and are responsible for the tissue tensile strength and the relative inextensibility of the lung.

Figure 2. (a) E-ECCs connecting BCs. (b) Enlargement of boxed area (a) showing where epithelial cells connect sporadically (circles) and where collagen fibres (CFs) occur (arrows). (c,d) Triangular areas (shown in a) indicating the BM of the BC (dashed line) connecting to that of the (c) E-ECC (dots) and (d) CFs (boxed areas) (d). (e–g) CFs in macerated tissue preparations in the BGB (thin arrows) and in the E-ECCs (bold arrow). (h) Immuno-gold stained T-IVc fibres (arrows) in the BGB. (i) Control preparation showing non-localization of T-IVc fibres in the BGB. (j) Immuno-gold stained T-IVc fibres in the E-ECCs (arrows). (k) Control preparation showing non-localization of T-IVc labelled fibres in the E-ECC. General symbols: BM, basement membrane; BC, blood capillaries; AC, air capillaries; stars, epithelial cells; asterisks, endothelial cells; RBC, red blood cell; TA, triangular area; dot, BM; CFs, collagen fibres; BGB, blood-gas barrier; T-IVc, type-IV collagen. Scale bars (a–k): 2, 0.1, 0.5, 0.1, 1.0, 0.2, 2, 0.1, 0.1, 0.2 μm. The collagen fibres shown in figure h, k–m may include other types of collagen fibres: maceration and TEM techniques cannot distinguish the collagens.
A CF of a diameter of 1 mm can support a 0.5 kg weight before breaking [36]. West et al. [10] reported that the BM of the BGB continues into the E-ECCs, but that the contacting cells lack intercellular junctions. Here, we noted that T-Ivc exists within the relatively much thinner and less conspicuous BM of the E-ECCs and the epithelial cells connect periodically. The E-ECCs appear to form a part of an integrated support system of the avian lung [13], directly strengthening the ACs and indirectly strengthening the BCs.

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References