

Airway Remodeling in the Asthma Model: Is there Standardization in this Evaluation?

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ABSTRACT

Asthma is a chronic inflammatory disease characterized by obstruction of airflow causing respiratory difficulties at various intensities. Airways inflammation has an important role in the pathophysiology of asthma. The inflammatory process in the airways promotes significant changes in the respiratory structures called airway remodeling, which represents a late and permanent response to tissue injury. This review aimed emphasize the importance of histological evaluation of airway remodeling in the asthma experimental model. There are several methods of histopathological analysis available today, but the implementation of a standardized methodology would help in the damages caused by the allergens and future assessments of possible therapies used to treat the disease.

Keywords: Asthma; Airway; Airway Remodeling; Histological Evaluation

Abbreviations: TH2: T helper 2; IgE: Immunoglobulin E; OVA: Ovalbumin; H&E: Hematoxylin & Eosin; PAS: Periodic-Schiff Acid; MT: Masson's Trichrome

Introduction

Asthma is a chronic inflammatory disease characterized by obstruction of airflow causing respiratory difficulties at various intensities [1]. It represents a major cause of physical disability, affecting 300 million people worldwide [2]. Airways inflammation has an important role in the pathophysiology of asthma, which is characterized mainly by the TH2 type immune response. The inflammatory process in the airways of individuals with asthma promotes significant changes in the respiratory structures, induced by repeated lesions and repairs [3]. This review aimed emphasize the importance of histological evaluation of airway remodeling in the asthma experimental model.

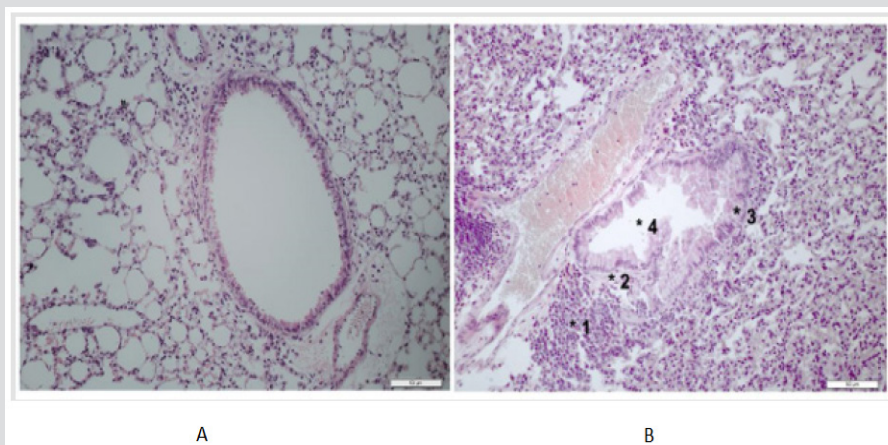
Airway Remodeling

Airway remodeling represents a late and permanent response to tissue injury, mainly caused by primary irritants. It is characterized by tissue and cellular changes in the bronchioles, such as increased

collagen deposition, hyperplasia and smooth muscle hypertrophy, increased vascularization, basement membrane thickening and epithelial layer and epithelial desquamation, goblet cell metaplasia, mucus production and hypersecretion [4]. Inflammatory cells participate of this process in different intensities (Figure 1) [5]. In the normal airway, the distribution of collagen and other components of the extracellular matrix is in equilibrium due to regulation of synthesis and degradation. In asthma, interruption of homeostasis results in increased deposition of extracellular matrix, especially in the reticular lamina, lamina propria and submucosa [6]. Smooth muscle thickening is primarily responsible for bronchoconstriction of the airways and persistent airflow obstruction. Muscle layer thickening may be caused by hyperplasia, hypertrophy or migration and differentiation of progenitors such as myofibroblasts [7]. Further to the center of the bronchiole is located the epithelium, which is considered a physical barrier that protects the body

against environmental influences. Its function is dependent of cell integrity and protein interaction in junctional intercellular complexes, especially the Tight Junctions. Failure in this complex makes the epithelium permeable to allergens and other agents to airway tissue [8]. In the case of asthma, due to the metaplasia and hyperplasia of the goblet cells, the mucus hypersecretion has contributed intensively to the morbidity and mortality due to this

disease, because before an inflammatory process, the increased mucus production causes the narrowing of the airways and asphyxia, which may result in death [9]. The currently available treatments minimize the symptoms in the patients, reducing the inflammation. However, there is still no therapy that prevents or reverses the changes resulting from airway remodeling.



- 1) Presence of inflammatory infiltrate,
- 2) Muscle thickening,
- 3) Epithelial thickening,
- 4) Epithelial cells desquamation into the lumen of the bronchioles.

Figure 1: Lung histological sections of Balb/c mice. A. Airway showing aspect of normality. B. Airway with an intense lung remodeling. Magnification: 200x.

Asthma Experimental Model

Preclinical models have been widely used in the search for understanding the pathophysiological events responsible for the development of asthma. Several aspects of this complex disease have been investigated experimentally, and it is hoped that further progress in this area will facilitate the development of new effective therapeutic approaches [10]. However, most of the studies used the murine, in which the Balb/c [11,12] and C57BL/6 [13,14] lineage is highlighted. Balb/c and C57BL/6 mice are isogenic animals that develop a strong TH2 immune response with increased IgE levels after exposure to ovalbumin (OVA), a gold standard allergen for assessing the allergic response [15,16]. The experimental asthma models in mice do not accurately reproduce the human disease, since the clinical signs are difficult to investigate. However, animal models have aided in understanding the pathophysiological mechanisms inherent to the disease and in identifying new targets for therapeutic interventions and potential treatments [17].

Airway Remodeling Evaluation

Usually, at the end of the experimental asthma study, the mice are submitted to euthanasia mainly by anesthetic overdose [18] or cervical dislocation [11]. The lungs are then removed and fixed

in formalin or paraformaldehyde. After this time, the lungs are transferred to cassettes and processed by histological techniques. Then, the remodeling could be evaluated in lung histological sections through the staining: Hematoxylin & Eosin (H&E), Periodic-Schiff Acid (PAS) and Masson's Trichrome (MT) but, there are many variables in the procedures used (Table 1). The H&E staining allows the lung morphology evaluation, the presence and inflammatory infiltrate intensity in the peribronchiolar, perivascular and adjacent area, the smooth muscle layer thickening and changes in the epithelium, with emphasis on cellular desquamation for the lumen of the bronchioles. PAS can identify the mucus produced by goblet cells and the distribution of these cells. The MT identifies collagen deposition in the peribronchiolar and perivascular region in the histological sections [19]. Lung histological involvement is a relevant aspect of asthma, there is no established standard of assessment. We believe that all the criteria are important, but the standardization of tissue damage analysis could help researchers to reproduce protocols available in the literature and ensure the effectiveness of the experimental model developed. As each investigator uses a different method of airway remodeling evaluation, it is difficult to compare results involving new therapies for asthma, since the baseline condition of the disease may not be the same for the different conditions evaluated.

Table 1: Evaluation methods of airway remodeling in asthma induction model. Staining: Hematoxylin & Eosin (H&E), Periodic-Schiff Acid (PAS) and Masson's Trichrome (MT).

Article	Histological procedure	Evaluation	H&E	PAS	MT
Dai et al. [18]	10% formalin	5 airway sections	Scoring system: Scale from 0 to 5 for inflammatory infiltration in the peribronchiolar area.	Scoring system: Scale from 0 to 5 for PAS- positive cells	Subepithelial fibrosis was evaluated by the Digimizer software. The area of collagen deposition and the perimeter of basement membrane of bronchioles were measured, and it was evaluated by scoring system: Scale from 0 to 5.
Leite et al. [19]	10% buffered formalin	30 bronchioles (15 from each lung)	Scoring system: Scale from 0 to 3	PAS-positive cells were evaluated into 8 regions and then by scoring system: Scale from 0 to 3.	Scoring system: Scale from 0 to 3.
Goodwin et al. [20]	4% paraformaldehyde	4 airways per animal	Scoring system: Scale from 0 to 3 for the presence and intensity of peribronchial infiltrate	Not investigated	Not investigated
Kang et al. [21]	4% neutral buffered formalin	8 fields	Scoring system: Scale from 0 to 3 for peribronchial and perivascular inflammation, thicknesses of epithelium and smooth muscle layer.	Five-point scoring system was used for evaluating goblet cell hyperplasia. Scale from 0 to 4	Not investigated
Ou-Yang et al. [22]	Formalin	Not specified	Scoring system: Scale from 0 to 4 for peribronchial and perivascular inflammation	Not investigated	Not investigated
Ge et al. [23]	4% paraformaldehyde	Not specified	Image Pro-Plus software was used to identify cellular inflammation. Scoring system: Scale from 0 to 4	Scoring system: Scale from 0 to 4 for PAS- positive goblet cells	The ratio of the area of trichrome staining to the basement membrane perimeter was measured for quantify collagen deposition
Mohammadian et al. [24]	Formalin	5 airway sections	Scoring system: Scale from 0 to 5 for peribronchiolar inflammation.	Scoring system: Scale from 0 to 5. PAS-positive cells	Subepithelial fibrosis was asses using the Digimizer software. The area of collagen deposition and the perimeter of basement membrane of bronchioles were measured, and it was evaluated by scoring system: Scale from 0 to 5.
Ogular et al. [25]	10% formalin	Airways were classified according to their diameter (distal or proximal)	A minimum of 5 points of each airway was measured for evaluate epithelium thicknesses, smooth muscle layers and basement membrane	A total of 500 goblet cells were counted	Not investigated
Royce et al. [26]	10% neutral buffered formaldehyde	Five airways per section	Not investigated	Goblet cell were analyzed using Image Scope software	Scoring system: Scale from 0 to 4 for peribronchiolar inflammation. Epithelial thickness and subepithelial deposition were measured.
Bonfield et al. [27]	10% formalin	Not specified	Not specified	Not investigated	Not specified

Conclusion

Airway remodeling is a result of significant tissue changes induced by repeated injury and repair processes in individuals with asthma. There are several methods of histopathological

analysis available today, but the implementation of a standardized methodology would help in the damages caused by the allergens and future assessments of possible therapies used to treat the disease.

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