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Pathology of diacetyl and 2,3-pentanedione airway lesions in a rat model of obliterative bronchiolitis

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ABSTRACT

Inhalation of diacetyl vapors by workers has been associated with obliterative bronchiolitis (OB), a poorly understood fibroproliferative disease of the small airways. Significant insights into the pathogenesis of OB have been obtained through the use of a rat model. Inhalation exposure of rats to diacetyl or 2,3-pentanedione, a related flavoring agent, can cause severe injury to the airway epithelium and underlying basement membrane. Repeated exposure to diacetyl or 2,3-pentanedione leads to aberrant repair, fibroproliferation and partial to complete occlusion of the airway lumen. Fibroproliferative lesions in rat airways were found to include both intraluminal polyps and circumferential intramural lesions. Intraluminal polyps have been observed to form secondary attachments spanning the airway lumen causing increasing obstruction. These airway lesions in rats are accompanied by inflammation in the form of peribronchial and perivascular infiltrates of lymphocytes, eosinophils and neutrophils. Diacetyl-induced OB lesions in the rat are similar to OB lesions in humans and provide a good model for studying the pathogenesis of this disease.

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1. Introduction

Obliterative bronchiolitis (OB) or bronchiolitis obliterans is a rare disorder most often recognized to occur in the setting of lung or heart-lung transplant with chronic allograft rejection (Myer 2016; Weigt et al., 2013) or hematopoietic stem cell transplant with graft versus host disease (Afessa and Peters 2006; Gunn et al., 2008). OB has also been diagnosed following acute exposure to soluble irritant gases and vapors such as ammonia, chlorine, methyl isocyanate, mustard gas, and nitrogen dioxide (King, 2003). More recently, obstructive lung disease consistent with OB has been diagnosed in microwave popcorn packaging and flavoring industry workers exposed to artificial butter flavoring vapors containing 2,3-butanedione (diacetyl) (Kreiss et al., 2002). Subsequent studies in rats have shown that inhalation exposure to diacetyl, or to the chemically-related flavoring 2,3-pentanedione, causes airway lesions that are histopathologically similar to OB lesions in humans (Morgan et al., 2016, 2012). Although innate and adaptive immune responses, tissue ischemia associated with transplantation, and chronic epithelial injury are known to contribute to the development of OB lesions (Babu et al., 2007; Fernandez et al., 2004; O'Koren et al., 2013; Ropponen et al., 2011; Snell and Westall 2010), the etiology and pathogenesis of this disease are unclear.

2. Epithelial injury

Injury of the bronchiolar epithelium occurs in almost all human cases of transplant-related OB, and in animal models of chemical-induced OB resulting from inhalation of toxic gases or vapors, and is considered to be a critical factor in the pathogenesis of OB (Babu and Nichols 2006; O'Koren et al., 2013). Reaction of the respiratory tract epithelium to the inhaled vapors of diacetyl or 2,3-pentanedione is presumably related to the highly reactive vicinal diketone groups in these chemicals, with the severity of the reaction being impacted by the concentration of the chemical, the local solubility, and the sensitivity of each epithelial type. Since basal cells are critical to epithelial cell renewal following injury, O'Koren et al. (2013) suggested that regions of persistent epithelial denudation following basal cell loss may predispose to the development of airway fibrosis. In vitro studies in our laboratory

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have suggested that basal cells might be more sensitive than differentiated cells, or that damage to desmosomes and hemidesmosomes might be initial events in epithelial injury (unpublished data). Although these details are unclear, the initial event in butter flavoring chemical exposure seems to be epithelial cell injury, which in its most severe form results in epithelial cell necrosis and ulceration (Fig. 1A). This results in epithelial cell regeneration in a local attempt to repair the damage and cover the denuded surfaces. Stem cells in the residual epithelium, whether basal cells or club cells, are presumably the cells attempting this repair, which initially takes the form of a single layer of outstretched cells having a spindled appearance similar to that of superficial squamous cells (Fig. 1B). With time, these regenerating cells proliferate and become cuboidal and often multilayered, but remain recognizable as immature cells because of their high nucleus/cytoplasm ratio, their large hyperchromatic nuclei, and their large nucleoli. At this stage, the regenerating epithelium is histologically indeterminate, and must undergo further differentiation before transforming either to respiratory epithelial hyperplasia with or without cytologic atypia (Fig. 1C), or squamous metaplasia (Fig. 1D), or before returning to morphologically normal respiratory epithelium.

3. Subepithelial injury

The epithelium is probably capable of repairing itself if only the epithelium is damaged, and if there remain any residual stem cells. That is, epithelial injury alone may not be sufficient for the development of OB. However, continued or repeated exposure of rats to diacetyl or 2,3-pentanedione can result in loss of all epithelial cells within a region, as well as damage to the underlying basement membrane (Fig. 1E). The connective tissue of the lamina

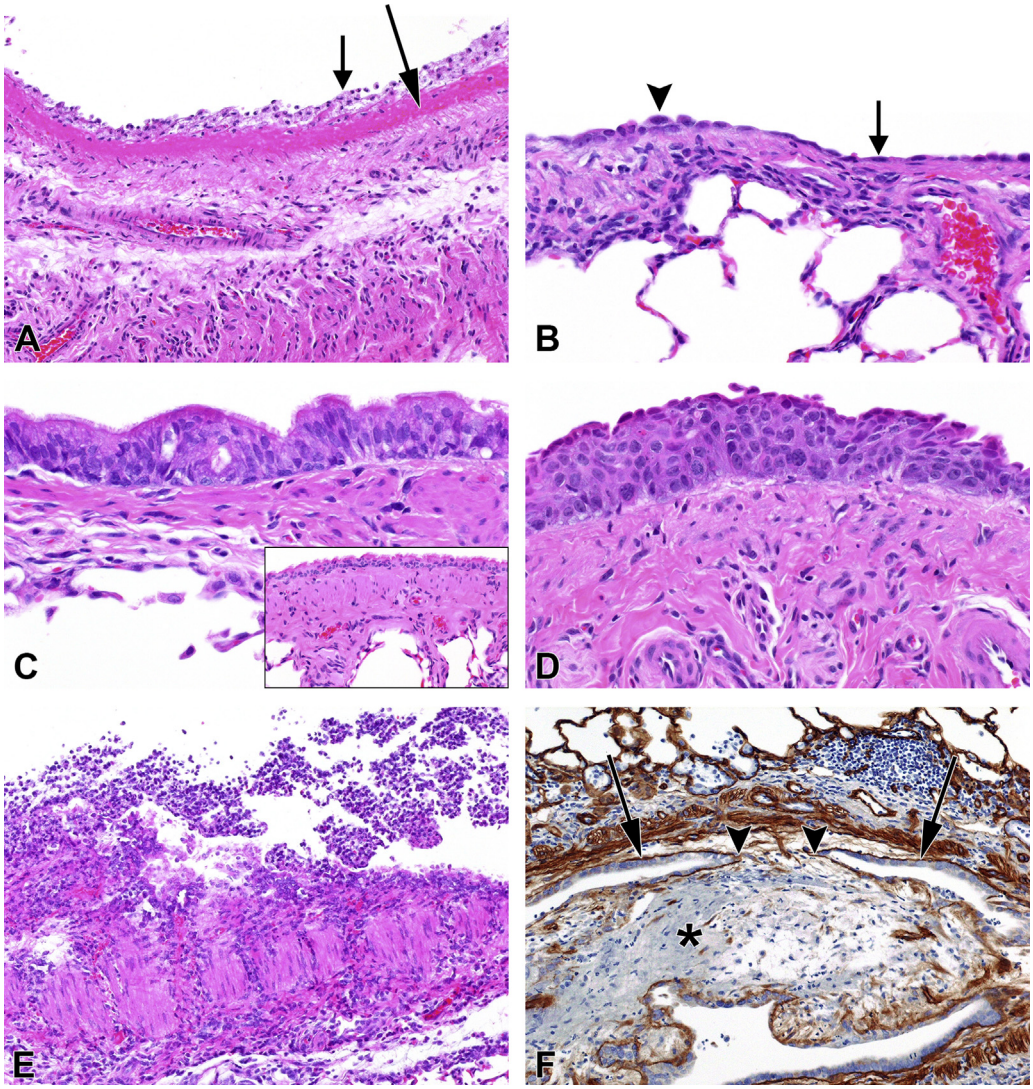

Fig. 1. Epithelial Injury and Response. Male Wistar Han rats were exposed to 200 ppm 2,3-pentanedione 6 h/day, 5 days/week for 2 weeks. A) Ulceration of the bronchial mucosa, with a linear zone of fibrinoid necrosis (long arrow), and a few macrophages and neutrophils on the surface (short arrow). H&E. B) Regenerating bronchial epithelium in the form of elongate, flattened cells on the right (arrow) and cuboidal cells on the left (arrowhead). H&E. C) Respiratory epithelial hyperplasia, characterized by increased height and cellularity (compare to epithelium of control in inset). 200 ppm 2,3-pentanedione followed by 2-week recovery. H&E. D) Squamous metaplasia, with atypia. The multilayered epithelium contains cells with nuclear enlargement and hyperchromasia, and flattening at the surface. H&E. E) More extensive epithelial ulceration, tissue disruption, neutrophilic exudate on surface, and macrophages in tissue. In this type of lesion, it is likely that the basement membrane of the mucosa has been disrupted, which might lead to subsequent fibrosis. H&E. F) Immunohistochemical stain for laminin to highlight the basement membrane of the mucosa (arrows). Note the discontinuity of the basement membrane between the arrowheads, associated with proliferation of fibrous tissue that has protruded through the defect to form a fibrous polyp (asterisk) in the bronchial lumen. Original objective magnification: A, 10×; B, 20×; C, 20×; D, 20×; E, 10×; F, 10×. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)