

Prompt epithelial damage and restitution processes in allergen challenged guinea-pig trachea *in vivo*

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Summary

Background Little is known about the induction and the morphology of epithelial damage, and of the ensuing epithelial restitution processes in allergic airways.

Objective To examine epithelial damage and restitution in allergen challenged guinea-pig trachea.

Methods Whole-mount techniques, transmission and scanning electron microscopy, cryo-sectioning, and histochemical staining were used. Cell proliferation was monitored by BrdU-immunohistochemistry.

Results Allergen challenge produced patchy, crater-like, and leucocyte-rich epithelial damage sites. At 1, 5, and 24 h damage was associated with poorly differentiated epithelial restitution cells. Already at 1 h the epithelial craters had a floor of flattened restitution cells and the damaged areas comprised <1% of the mucosal surface area (whole-mount preparations). In contrast, cryo sections displayed large areas ($\approx 20\%$, 1 h) of denudation. Epithelial, and subepithelial (fibroblasts, smooth muscle) proliferation was increased 5 and 24 h after challenge ($P < 0.01$).

Conclusion Within 1 h allergen challenge has induced patchy damage sites where epithelial restitution is already advanced; although easily produced by cryosectioning frank denudation was not evident in whole-mount preparations. The present findings may explain the well maintained, functional tightness of allergic airways displaying epithelial damage, shedding, and even denudation. The present data also suggest the possibility that epithelial damage-restitution may be causative to allergic airway remodelling.

Keywords: allergic airways, epithelial damage, epithelial restitution

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Introduction

Epithelial shedding is considered a major characteristic feature and pathogenetic mechanism in inflammatory airway diseases such as asthma [1–3]. The occurrence of shedding is supported by findings of elevated numbers of epithelial cells in sputum and bronchoalveolar (BAL) fluids [4,5], and by histological observations in bronchial biopsies and autopsies [6–9]. Potential mechanisms of epithelial damage have attracted a great deal of interest and cytotoxic

factors, released from activated leucocytes, are now considered the main culprits causing epithelial shedding in asthma [10,11]. However, little attention appears to have been focused on basic *in vivo* paradigms of epithelial damage and restitution processes in allergic airways.

We have recently developed an *in vivo* technique for carrying out low-traumatic (no surgery, no bleeding, and no damage to the basement membrane) and reproducible mechanical removal of the airway epithelium from a defined area of the guinea-pig tracheal mucosa [12]. Using this model we have demonstrated that the epithelial restitution processes involve an early phase, with immediate (within minutes) dedifferentiation and high speed migration of

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explaining phantom phenomena [44]. Perhaps some textbook dogma of epithelial damage in airway inflammation together with the currently overwhelming focus on reductionist approaches have luringly obviated the need for basic, so called 'descriptive', research in this field. Since epithelial damage-restitution processes may be a major mechanism in asthma [45] this aspect deserves to be a significant area for investigation, not least in the characterization of animal models of the disease.

In conclusion, this study has demonstrated that allergen challenge, applied topically on the guinea-pig trachea *in vivo*, induces patchy epithelial damage-sites with crater-like appearances. The epithelial damage sites are shown to be dynamic foci where epithelial repair (dedifferentiation, flattening, migration, and proliferation) is ongoing apparently from the start, and during, the damage process. The abundant occurrence of large, flattened restitution cells at the crater floors may contribute to make the allergic airway mucosa abnormally tight despite the occurrence of epithelial damage and shedding. Importantly, this study provides evidence that conventional cryo-sectioning of airway tissue specimens, previously subjected to allergen challenge, may cause marked artifacts. Thus, the occurrence of frank denudation may be grossly overestimated. Based on the present observations of subepithelial cell proliferation together with our previous data on sequelae to shedding-like denudation *in vivo* [12–14], we suggest that foci of epithelial cell loss may be causative to part of the pathology that is recorded in allergic airways.

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