Asthmatic airway epithelium is intrinsically inflammatory and mitotically dyssynchronous

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Online Data Supplement

Materials and Methods Supplement

Mechanical injury model

An *in vitro* epithelial injury model that allows for the study of epithelial repair processes in the lung was adapted for use in this study (15-17). Briefly, at 0h on the timeline in Figure 1, epithelia were scraped in two perpendicular lines with a p1000 pipette tip and placed in bromodeoxyuridine (BrdU)-containing (10μM) medium. BrdU-containing medium was changed at +24h following the DEX/VEH pulse mentioned previously. Epithelia were incubated at 37°C and 5% CO_2 until +48h. In some experiments, wounds were imaged daily (i.e. 0, +24, +48h) using a 16X phase contrast objective lens and wound area was measured in triplicate by a single operator blinded to the culture conditions using ImageJ Software (18). Contrast was enhanced equally in all images to improve wound visualization.

Although the cultures were kept at an air-liquid interface, depending on interval culture time they generated up to 0.5 mL of apical secretions (including fluid used to wash the apical surface). These apical secretions and all basolateral media (~1mL) were collected at all time points and frozen prior to analysis. No medium was added to the apical surface of the cultures to maintain them at an air-liquid interface.

Analysis of Inflammatory Mediators

Inflammatory (i.e. interleukin [IL]-1β, IL-6, IL-10, and IL-13) and fibrogenic (i.e. transforming growth factor [TGF]-β1) cytokines were measured in apical and basolateral secretions at 0, +24, and +48h by flow cytometry on a FACSCalibur™ System (BD Biosciences, San Jose, CA) using a FlowCytomix Multiplex Kit with FlowCytomix Pro

2.3 software (Bender MedSystems, Burlingame, CA). These cytokines were selected as an initial screening set for these experiments because of their prominent role in asthmatic inflammation and/or remodeling (19).

Cell Cycle Analysis

Epithelia were washed once with PBS and harvested at +48h (Figure 1) for analysis by flow cytometry. A single cell suspension was achieved by exposure for 5 minutes with trypsin – ethylenediaminetetraacetic acid (EDTA) solution (#T3924; Sigma-Aldrich, St. Louis, MO) followed by filtration through a 40 μm strainer. Cells were simultaneously labeled with the following according to the manufacturers' protocols: 1) Carboxyfluorescein FLICA Apoptosis Poly-Caspase Detection Kit (Immunochemistry Technologies, LLC, Bloomington, MN) and 2) APC BrdU Flow Kit containing 7-AAD (amino-actinomycin-D) (BD Biosciences, San Jose, CA). Flow cytometry data were generated on a FACSCalibur™ System (BD Biosciences). Samples were gated to study 7-AAD content in $BrdU^{+}FLICA^{-}$ cells. Data were analyzed by means of the cell cycle analysis feature of FlowJo 7.6 (Tree Star, Inc., Ashland, OR) using a Watson (Pragmatic) model with equal coefficients of variation for the G1/G0 and G2/M peaks.