Repeated Allergen Inhalations Induce DNA Synthesis in Airway Smooth Muscle and Epithelial Cells In Vivo*

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Airway smooth muscle (ASM) mass appears to be increased in the bronchi of patients with chronic severe asthma. Although the precise mechanisms that induce these changes are unknown, studies suggest that the increased smooth muscle mass is, in large part, due to increases in the number of ASM cells. To further dissect mechanisms that regulate ASM mass, we examined whether repeated allergen inhalations in Brown-Norway rats, which have been reported to increase ASM mass, also induced ASM cell proliferation. We studied six Brown-Norway rats that were actively sensitized to ovalbumin (OA). Animals then received either three aerosolized OA challenges or saline solution (n=3) at 5-day intervals (on days 14, 19, and 23 after sensitization). Both groups of animals were injected with 25 mg/kg bromodeoxyuridine (BrDU), a thymidine analog, every 12 h on days 23 and 24. Formaldehyde-fixed lungs from a sample of OA-challenged and saline-solution-challenged animals were then paraffin embedded and 5-μm sections were prepared for immunocytochemistry or were stained with hematoxylin-eosin. DNA synthesis was determined by positive nuclear staining by indirect immunocytochemical techniques using an anti-BrDU antibody and 4,6-diamidino-2-phenylindole (DAPI) staining techniques. A total of 156 airways were examined from consecutive sections from the six animals (36 airways per animal). Airway size was characterized according to basement membrane length and defined as large (>2,000 μm), medium (1,000 to 2,000 μm), or small (<1,000 μm).

Repeated OA inhalations appeared to induce DNA synthesis in both airway epithelial and smooth muscle cells. After repeated OA inhalations, the number of airway ep-

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**Figure 1.** Repeated allergen challenges induced DNA synthesis in airway epithelial and smooth muscle cells. Left, Quantitation of BrDu incorporation in airway epithelial cells. After repeated OA inhalations, the number of airway epithelial cells incorporating BrDu increased approximately 15-fold in large airways, 50-fold in medium, and 40-fold in small airways as compared with that obtained from control animals (Cont). These data represent means±SEM; data from OA-challenged vs cont ± ani-
mals were statistically different (p<0.001) (analysis of variance [ANOVA]; Bonferroni-Dunn). Further, comparison of BrDu incorporation among large, medium, and small airways in OA-treated animals was also statistically different (p<0.001) (ANOVA; Bonferroni-Dunn). Right, Quantitation of BrDu incorporation in airway smooth muscle cells. After repeated OA inhalations, the number of airway myocytes incorporating BrDu increased approximately 9-fold in large airways, 25-fold in medium, and 14-fold in small airways as compared with that obtained from control animals (Cont). These data represent means±SEM; data from OA-challenged vs control animals were statistically different (p<0.001) (ANOVA; Bonferroni-Dunn). Further, comparison of BrDu incorporation among large, medium, and small airways in OA-treated animals was also statistically different (p<0.001) (ANOVA; Bonferroni-Dunn).
ithelial cells incorporating BrDu increased approximately 15-fold in large airways, 50-fold in medium airways, and 40-fold in small airways as compared with that obtained from control animals (Fig 1, left). Increases in DNA synthesis in ASM cells were also observed with approximately 9-fold in large airways, 25-fold in medium airways, and 14-fold in small airways as compared with that obtained in control animals (Fig 1, right).

To determine the relationship between the number of cells incorporating BrDu and airway size, the number of BrDu-positive cells were normalized to basement membrane length (in microns) (Table 1). Interestingly, there appears to be no difference among airway sizes and the number of BrDu-positive epithelial or smooth muscle cells.

Taken together, these data suggest DNA synthesis is induced in both airway epithelial and smooth muscle cells after inhalational antigen challenge.

**REFERENCES**