sponsiveness *in vitro* correlates with the severity of airway remodelling among individual animals. As shown in Figure 9, the severity of the functional abnormality (ie, airway constrictor hyperresponsiveness), manifest as lowering of the ED200ACh (the estimated dose of acetylcholine needed to double respiratory system resistance), does correlate with several indices of the magnitude of airway remodelling, including the smooth muscle layer thickness. Furthermore, when hyperoxia-exposed immature rats are removed from 8 days' oxygen exposure, both their airway constrictor hyperresponsiveness and their airway wall layer thickening resolve toward normal in parallel (Fig 10 and 11). This constant association between the magnitudes of hyperoxia-induced structural and functional airway abnormalities during both peak and resolution phases supports, though does not prove, the notion that hyperoxia-induced airway remodelling causes airway constrictor hyperresponsiveness in immature rats. A more definitive test of this potential causal relationship may come when, after identifying the precise nature of the mitogenic activity in hyperoxic animals' BAL fluid, specific interventions are designed to neutralize this activity during oxygen exposure. Still, it seems unlikely that airway remodelling can entirely explain hyperoxia-induced airway constrictor hyperresponsiveness, as we have also demonstrated epithelium-dependent increases in tracheal smooth muscle stress generation *in vitro*, in tissues obtained from hyperoxia-exposed rats (Fig 12). Thus, hyperoxia-induced airway constrictor hyperresponsiveness appears to be the complex result of multiple simultaneous mechanisms, which may include airway remodelling as a principal contributor.

### References

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### Chronic Allergic Inflammation Induces Replication of Airway Smooth Muscle Cells *In Vivo* in Guinea Pigs*

*Tony R. Bai, MD; Zang-Ling Wang, MD; Blair Walker, MD; and Peter D. Paré, MD*

The hypothesis that chronic antigen challenge induces airway smooth muscle proliferation in guinea pig airways was tested. We quantified proliferating airway smooth muscle cells incorporating the thymidine analogue 5'-bromo-2'-deoxyuridine (BrDu). The BrDu, 25 mg/kg, was administered intraperitoneally 2 times a week for 5 weeks and daily in the sixth week to 10 control (CON) adolescent guinea pigs and to 16 guinea pigs sensitized to ovalbumin (OA). The OA animals were exposed twice weekly to nebulized 0.5% OA and control animals were given nebulized saline solution. Lungs were processed for histologic findings after physiologic measurements had been obtained.

Maximal pulmonary resistance increased twofold and the acetylcholine (ACh) concentration causing a tenfold increase in pulmonary resistance decreased fourfold in the OA group as compared to the CON group. *In vitro* maximal smooth muscle stress values in response to 1 mM ACh increased twofold in the OA group as compared to the CON group, but the smooth muscle mass was not increased. Serial sections of membranous airways were stained with hematoxylin-eosin or for BrDu by immunohistochemistry. The airway smooth muscle area and nuclei were point-counted, and the smooth muscle cell proliferation index was calculated as the percent of BrDu-labelled nuclei/total nuclei. The smooth muscle proliferation index was increased (OA = 24.4 ± 6.2% (SEM) vs CON = 3.9 ± 1.5%, p <0.02). An increase in BrDu-positive granulocytes (predominantly eosinophils) was noted in the mucosa and adventitia but not in the smooth muscle.

These results are consistent with the notion that airway smooth muscle proliferates as part of the chronic allergic inflammatory response. Increased smooth muscle DNA replication without a measurable increase in muscle area suggests either an early hyperplastic response or increased cell turnover.

*From the University of British Columbia, Pulmonary Research Laboratory, St. Paul's Hospital, Vancouver, BC, Canada.