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Supplementary data
SUPPLEMENTARY INFORMATION

TNF and IL-1β exposure increases airway narrowing but does not alter the bronchodilatory response to deep inspiration in airway segments

Thomas K. Ansell¹,², Howard W. Mitchell², Peter K. McFawn² and Peter B. Noble²,³

¹School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia; ²School of Anatomy, Physiology and Human Biology, University of Western Australia, Crawley, WA, Australia; ³Centre for Neonatal Research and Education, School of Paediatrics and Child Health, University of Western Australia, Crawley, WA, Australia.

Appendix S1- METHODS

Morphometry

Bronchial segments were fixed in 4% formaldehyde solution and processed into paraffin blocks. Transverse sections (5μm) were stained with haematoxylin and eosin. The internal lumen perimeter (Pᵢ) and area (Aᵢ), the area enclosed by the outer ASM perimeter (Aᵅₒ) and area of the ASM layer²⁷ were measured using ImageJ (version 1.45j, National Institutes of Health, MD, U.S.A.). The area of the ASM layer was expressed as √area of the ASM layer/Pᵢ.

Airway analysis and statistics

The volume of the relaxed airway lumen was measured by the volume that could be withdrawn until closure at 5cmH₂O Pᵅₒ²⁸. Airway narrowing to ACh was expressed as %lumen volume. Active lumen pressure to ACh was measured from the change in Pᵅₒ.
Dynamic measurements were made at the troughs of the pressure cycle. Maximum response ($E_{\text{max}}$) and sensitivity ($PD_2=-\log_{10}$, dose producing 50% $E_{\text{max}}$) to ACh was calculated from variable slope sigmoidal curves fitted to individual airways. Specific compliance was calculated from volume strain ($\Delta \text{volume/lumen volume})/\Delta P_{tm}^{24, 28, 29}$.

The ASM strain during breathing manoeuvres ($\Delta P_{tm}$) was calculated from the trough to peak change in the outer ASM perimeter ($P_{mo}$) during DI as a proportion of the $P_{mo}$ immediately prior to DI. The $P_{mo}$ of the bronchial segment in the organ bath was calculated as previously described$^{21, 25, 26}$ and assumes inner wall area is constant at all $P_{tm}$, that $P_{mo}$ is circular and that the lumen is cylindrical. The bronchodilatory response to DI was defined as %reversal of airway narrowing to ACh measured immediately after DI$^{24, 28, 29}$. Bronchodilation to DI was not calculated at doses ($\leq 3 \times 10^{-6}$M), which produced minimal airway narrowing.

Differences between groups were analysed using paired t-tests and 2-way ANOVA. Data analysis and statistical tests were performed using Statistica (version 8.0; StatSoft, Tulsa, OK, U.S.A.) and GraphPad Prism (version 5.0d; GraphPad Software, La Jolla, CA, U.S.A.). Data are presented as means±SEM, where $n=$number of animals.

References


Figure S1

Example traces of transmural pressure and lumen volume to cumulative doses of acetylcholine (ACh, $10^{-7}$ to $3 \times 10^{-3}$M, *arrows*, text labels shown only for whole log doses). Fixed-$P_{tm}$ oscillations were applied to control airways and airways treated with TNF (100ng/mL)/IL-1β (20ng/mL). Traces from the control and TNF+IL-1β treatment groups have been temporally shifted to better distinguish the curves. At the time scale shown, tidal oscillations are not visible but appear as a thick line, the thickness of which indicates the magnitude of the $P_{tm}$ and volume oscillations. Individual DI are visible prior to the subsequent dose of ACh. In response to ACh, lumen volume decreased in a dose-dependent manner. Stiffening of the airway wall produced by ACh appears as a reduced volume oscillation in response to $P_{tm}$ oscillation.
The effect of culture on maximum response ($E_{\text{max}}$, %lumen volume, A) and sensitivity ($PD_2$, B) to ACh under static conditions. There was a non-significant reduction in $E_{\text{max}}$ following 2 days of culture (66.8±14.5% lumen volume), compared with the fresh airway before culture (100.0±0.0% lumen volume i.e. airway closure in all airways, $p=0.11$). There was no difference in $PD_2$ following 2 days of culture (4.37±0.17), compared with the fresh airway before culture (4.21±0.17). $n=6$. Mean±SEM.