Co-operation between mast cells and neurons is essential for antigen-mediated bronchoconstriction

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"Online Data Supplement"

Supplemental Methods

Experimental animals. PAR2$^{-/-}$ mice and wild-type C57BL/6 and BALB/c mice were purchased from Jackson Laboratory. Mice deficient in 5-lipoxygenase (5LO$^{-/-}$) were generated as previously described.(1) All experiments were carried out using 8-12 week old mice.

Cell counts. Cells present in the BAL were determined using a hemocytometer. A differential cell count was conducted on a cytospin prepared from 150 µl of BAL fluid and stained with Diff-Quik solution (Sigma Chemical).

Histology. For histopathologic examination, lungs were fixed by inflation (20 cm pressure) and immersion in 10% formalin. To evaluate airway eosinophilia, fixed lung slices were subjected to hematoxylin and eosin (H & E) staining. H&E stained lung slices were digitally imaged midway along the length of the main axial airway, as well as, at both ends of the same airway. Each of the three images was then divided into 4 quadrants and the levels of inflammation in each quadrant were semi quantitatively assessed based on a score of 0 (no inflammation) to 3 (severe inflammation). Scores from all quadrants for each of the 3 pictures representing 1 airway were then averaged together to represent the average H&E score of that airway.(2) To assess goblet cell hyperplasia, serial sections of the left lobes of the lungs that yield maximum longitudinal visualization of the intrapulmonary main axial airway were analyzed following Alcian-blue/periodic acid-schiff reaction (PAS) staining. To avoid bias for a certain region, and to consistently view the identical region in all slides, a 2-mm length of airway, located midway along the length of the main axial airway, was digitally imaged. Using ImageJ software (NIH, National Technical Information Service, Springfield, VA), the area and

length of the PAS/AB-stained region in the sections were measured and the data expressed as the mean volume density (V_s = nl/mm^2 basal lamina + SEM of PAS/Abstained material within the epithelium) as previously described.(3)

MCh treatment in vagotomized mice. Anesthetized, paralyzed, vagotomized, mechanically ventilated mice were treated with i.v. MCh (20 µg/ml, Sigma Chemical) administered through a jugular catheter in increasing doses (50 µl, 100 µl, and 200 µl) or an aerosolized dose response of MCh (12.5-50 mg/ml) delivered via a nebulizer through a side port in the ventilator circuit for 30 s at a rate of 200 breaths/min with a tidal volume of 0.15 ml. Immediately following the aerosol challenge, the nebulizer was isolated from the inspiratory circuit and the original mechanical ventilation was resumed.

Inhibition of PGD_2. To asses the role of prostaglandins in IgE-dependent anaphylactic airway constriction, mice were pretreated with 10 mg/kg indomethacin (1 mg/ml, i.p., Sigma Chemical) 1 hour prior to airway measurements. To determine the efficacy of prostaglandin depletion, levels of PGD_2 were measured by EIA (Assay designs) from harvested gut samples.

Antagonism of histamine receptors. To determine the contribution of HR1 and HR2 to airway responses, passively sensitized mice received a pre-treatment i.p. injection of pyrilamine (40mg/kg, Sigma Chemical) or cimetidine (25mg/kg, Sigma Chemical), respectively.

Supplemental References

1. Goulet, J. L., J. N. Snouwaert, A. M. Latour, T. M. Coffman, and B. H. Koller. 1994. Altered inflammatory responses in leukotriene-deficient mice*Proc Natl Acad Sci U S A* 91:12852-12856.

2. Kelly-Welch, A. E., M. E. Melo, E. Smith, A. Q. Ford, C. Haudenschild, N. Noben-Trauth, and A. D. Keegan. 2004. Complex role of the IL-4 receptor alpha in a murine model of airway inflammation: expression of the IL-4 receptor alpha on nonlymphoid cells of bone marrow origin contributes to severity of inflammation.*J Immunol* 172:4545-4555.

3. Cressman, V. L., E. M. Hicks, W. K. Funkhouser, D. C. Backlund, and B. H. Koller. 1998. The relationship of chronic mucin secretion to airway disease in normal and CFTR-deficient mice.*Am J Respir Cell Mol Biol* 19:853-866.

Supplemental Figure Legends

Figure S1 Airway inflammation in B6 and Wsh/Wsh mice with allergic lung disease. (A) There was no significant difference in serum IgE levels in wild-type (n=8) and mast cell deficient mice (n=6). **(B)** The resulting cell profile was the same for the wild-type (n=6) and mast cell deficient mice (n=8), with the majority of the cells (>80%) being eosinophils. **(C)** IL-13 levels were used to represent the amount of inflammation present in the airways. There was no significant difference in IL-13 between the wild-type (n=8) and mast cell deficient mice (n=9). **(D)** The average H&E score was semi quantitatively measured for each main axial airway, and did not differ between the C57BL/6 (n=9) and C57BL/6 $Kit^{W\text{-}sh}/Kit^{W\text{-}sh}$ (n=9) mice. **(E)** There was no significant difference in mucus levels in the wild-type (n=8) and mast cell-deficient (n=8) mice.

Figure S2 Pharmacological and genetic inhibition of mast cell mediator pathways during passive anaphylaxis. (A) C57BL/6 mice were pretreated with either pyrilamine (40 mg/kg) or cimetidine (25 mg/kg) to block histamine H1 and H2 receptors, respectively. Antagonism of histamine receptors had no effect on anaphylactic airway constriction in passively sensitized mice. Saline- controls, n=3; IgE+DNP, n=13. Pyrilamine- controls, n=3; IgE+DNP, n=10. Cimetidine- controls, n=3; IgE+DNP, n=13 (*P<0.001, $^{\#}P$<0.01, $^{\dagger}P$<0.001). **(B)** Pretreatment of passively sensitized C57BL/6 mice with indomethacin (10 mg/kg), to deplete endogenous prostaglandin production, had no effect on R_{aw} after antigen challenge. Saline- controls, n=3; IgE+DNP+saline, n=6. Indomethacin- controls, n=3;IgE+DNP+indomethacin, n=7 (*P<0.001, $^{\#}P$<0.001). **(C)**

Passively sensitized leukotriene deficient ($5LO^{-/-}$) mice and their wild-type C57BL/6 controls showed no difference in R_{aw} after antigen challenge. $5LO^{+/+}$ - controls, n=3; IgE+DNP, n=6. $5LO^{-/-}$ - controls, n=3; IgE+DNP, n=6 (*P<0.001, $^{\#}P$<0.001). **(D)** Antigen induced airway constriction was equivalent in wild-type and $PAR2^{-/-}$ passively sensitized mice. $PAR2^{+/+}$ - Controls, n=4; IgE+DNP, n=6. $PAR2^{-/-}$ - Controls, n=5; IgE+DNP, n=4 (*P<0.001; $^{\#}P$<0.001).

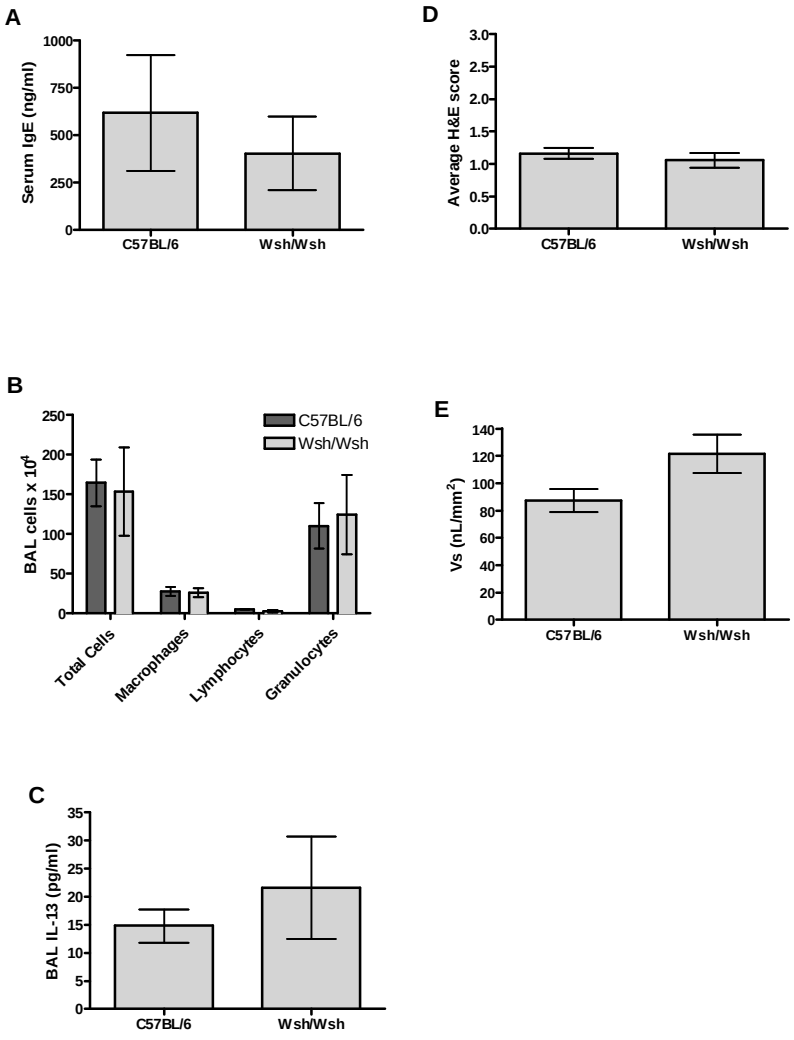
Figure S3 Serotonin mediates antigen-induced central airway constriction in BALB/c mice. Vehicle treated mice sensitized with OVA and challenged with either PBS (Controls, n=2) or OVA (OVA, n=5). OVA challenged mice had a significant increase in R_{aw}. Pretreatment with 150 mg/kg PCPA (OVA+PCPA, n=4) abolished anaphylactic airway constriction in actively sensitized mice challenged with OVA (**P<0.001 compared to all other groups).

Figure S4 Antigen-induced bronchoconstriction following vagotomy and atropine in BALB/c mice. Passively sensitized and challenged wild-type BALB/c mice after dissection of the vagus nerve or pretreatment with atropine. Antigen challenged mice (n=6) showed a significant increase in R_{aw} compared to controls (n=3). Airway constriction was abolished in vagotomized animals (n=5). Blockade of mAChRs with atropine (10 µM/kg, n=6) resulted in complete loss of central airway constriction after antigen challenge in passively sensitized mice. No significant change in G was observed. (**P<0.001 compared to all other groups).

Figure S5 Airway mechanics in response to MCh challenge after vagotomy. (A) Vagotomy also did not significantly affect the response to iv MCh. MCh,*n*=3; MCh+vagotomy, *n*=3. **(B)** Constriction of airway smooth muscle following aerosolized MCh was not altered following dissection of the vagus nerve. MCh,*n*=7; MCh+vagotomy, *n*=7.

Supplemental Figures

Figure S1



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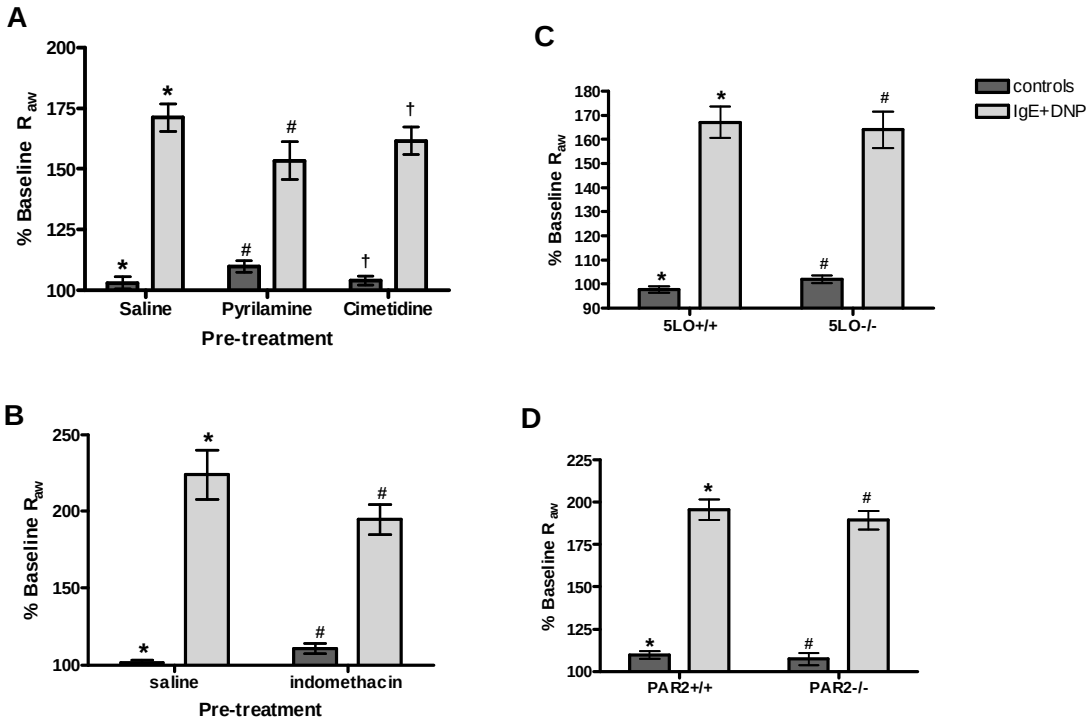
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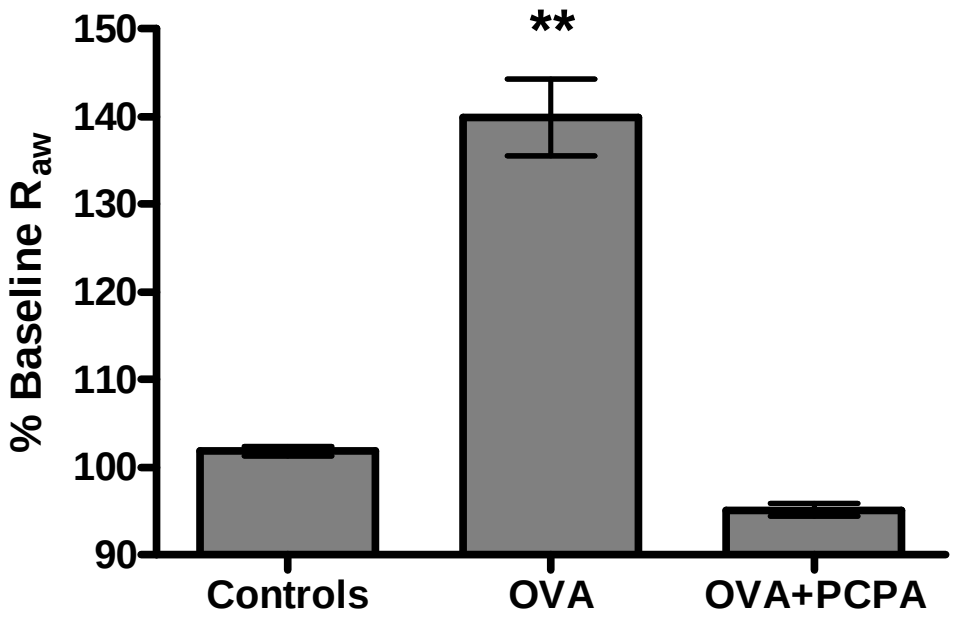
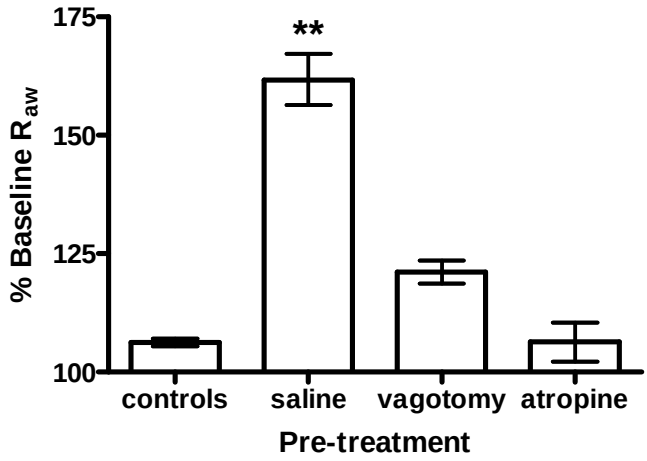


Figure S4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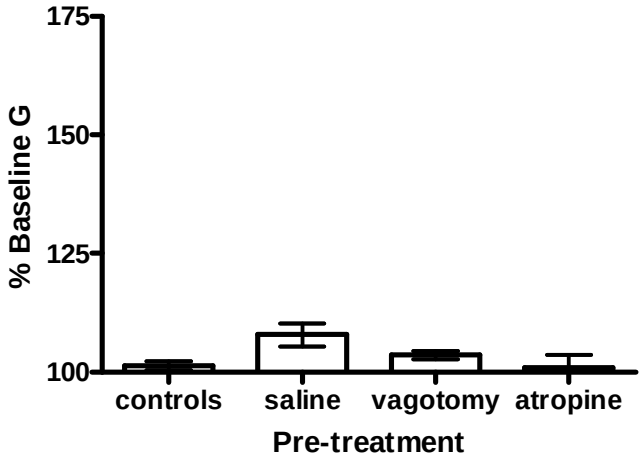
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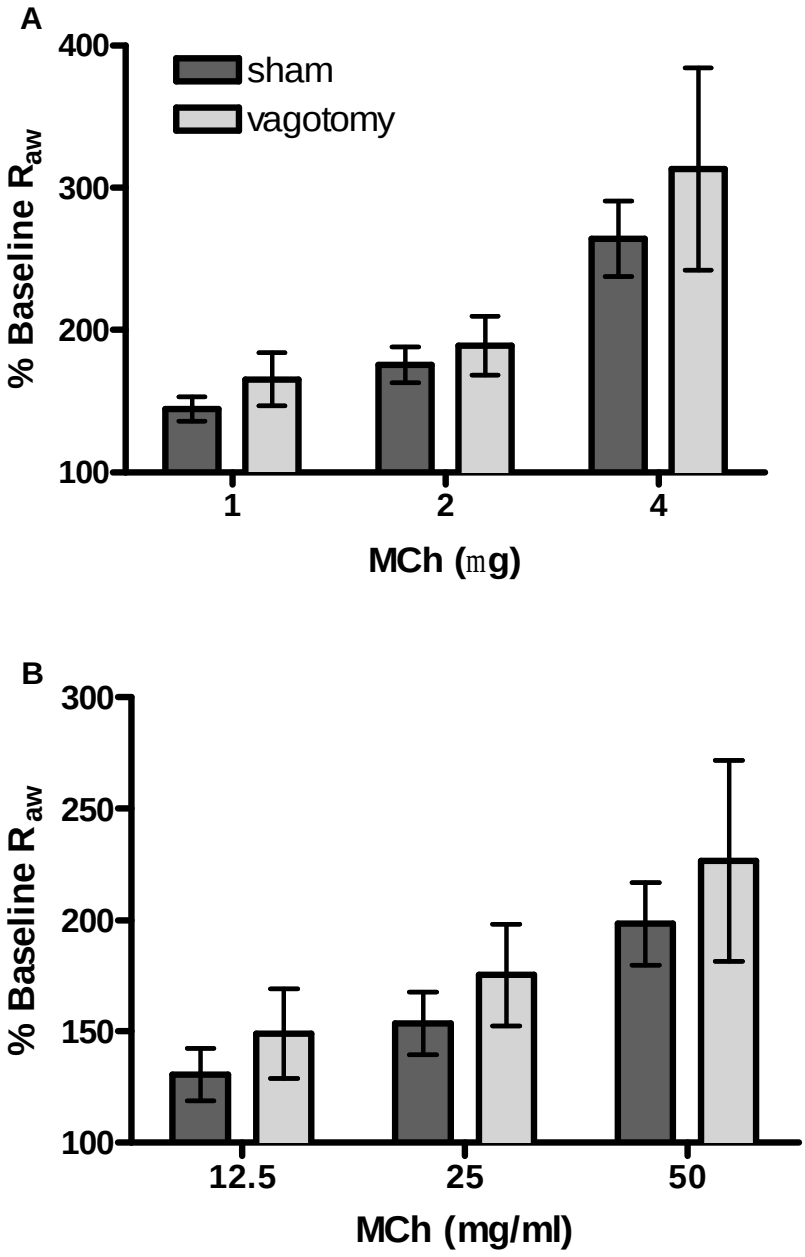
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