INBRED MOUSE STRAINS RESPOND DIFFERENTLY TO ALLERGEN (OVALBUMIN) SENSITIZATION AND CHALLENGE. G. S. Whitehead, J. K. L. Walker, K. G. Berman, W. M. Foster, D. A. Schwartz. Duke University Medical Center, Durham, NC.

To better understand the role of genetics in allergen-induced asthma, we investigated airway hyperreactivity and inflammation in the lungs of 9 inbred strains of mice (129/SvIm, A/J, BALB/cJ, BTBR+(T)/tf/tf, C3H/HeJ, C57BL/6J, DBA/2J, FVB/NJ and CAST/EI) following sensitization and aerosolized challenge with ovalbumin (ova). Male mice (6-8 weeks) from each strain were sensitized on days 0 and 14 with i.p. injections of either ova/alum or alum alone. On day 20, airway hyperreactivity to methacholine was measured and whole-lung lavage was performed for each strain. On day 21, mice of each strain were challenged for 1 hour with ova aerosol. At 24, 48, and 72 hours post-exposure, airway hyperreactivity and lung inflammation were assessed for each strain. Prior to the ova challenge, the ova- and alum-sensitized mice of the A/J, 129/SvIm, and BTBR+(T)/tf/tf strains demonstrated enhanced airway responses to inhaled methacholine compared to the other strains. Following ova exposure, the FVB/NJ, BTBR+(T)/tf/tf, and BALB/cJ strains were the only strains in which the airway hyperreactivity significantly increased. Comparatively, the A/J strain reactivity decreased. Further, the 129/SvIm, BALB/cJ, and DBA/2J strains were the only strains in which the ova-sensitized mice were significantly more responsive than the alum-sensitized mice. The inflammatory responses (enhanced eosinophilia and neutrophilia) of the various strains following ova challenge ranged from relatively unresponsive (A/J, BALB/cJ, C3H/HeJ, and DBA/2J) to very responsive (C57BL/6J, 129/SvIm and BTBR+(T)/tf/tf). Our results indicate that physiologic and biological lung responses after ova sensitization and challenge are different among inbred strains and support the hypothesis that genetic factors contribute to the development of allergen-induced airway disease.

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AIRWAY HYPERRESPONSIVENESS IN MICE WITH TARGETED DELETION OF THE TACHYKININ 1 GENE. Stephanie A. Tuck, Saloni Shah, and James G. Martin. Meakins-Christie Laboratories, McGill University, Montreal, Quebec, Canada.

Neurokinins (NK's) are involved in the development of bronchial inflammation and airway hyperresponsiveness. The TAC1 knockout mouse (TAC1-/-), in which the tachykinin1 gene encoding for the NK's substance P (SP) and neurokinin A (NKA) is eliminated through targeted gene disruption, provides a novel model in which to study the effects of NK's on airway function. We investigated airway responsiveness to methacholine (Mch, 10-320μg/kg i.v.) in 10 TAC1-/- and 10 wildtype mice (TAC1+/+). Baseline resistance (R)and elastance did not differ between groups, but the log [Mch] required to increase R to twice the baseline value was significantly lower in TAC1-/- (1.93±0.18 vs 2.07±0.12, $p<0.05$). To determine if differences in responsiveness between TAC1+/+ and -/- mice were due to NK receptor stimulation by released NK's during Mch challenge, NK_1 and NK_2 selective antagonists (CP 99994 and SR 48968 respectively, 0.5 mg/kg i.v.) (n=7) or vehicle (n=7) were administered to TAC1+/+ mice prior to Mch challenge. NK receptor blockade did not affect responsiveness to Mch. To determine if differences in *in vivo* responsiveness were related to differences in contractility of airway smooth muscle, we measured Ca^{2+} mobilization in response to 10 μM 5-hydroxytryptamine(5-HT) and SP in cultured tracheal smooth muscles cells from TAC1-/- and +/+ mice. 5HT and SP increased intracellular Ca^{2+} in both groups, with no significant differences in peak [Ca^{2+}] between groups (peak [Ca^{2+}] to 5HT=319±78 vs 532 ±161 nM for TAC1-/- and +/+ and 61±8 vs 60±3 in response to SP). We conclude that the absence of the tachykinin1gene results in airway hyperresponsiveness but not increased airway smooth muscle contractility. Hyperresponsiveness was not mimicked by NK receptor blockade in wildtype animals.

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INITIAL CHARACTERIZATION OF LUNG FUNCTION IN MICE DEFICIENT IN NEUTROPHIL PROTEINASES

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Rationale: Mice deficient in cathepsin G (CG-/-) and neutrophil elastase (NE-/-) have been shown to differ in the severity of fibrosis induced by bleomycin administration. The forced oscillation technique can be used to determine the effects of lung injury on airway and tissue mechanics. To establish the appropriate conditions for using this method with mice deficient in neutrophil proteinases, baseline assessments of lung collagen, morphometry and mechanics were evaluated. **Methods:** Lung function was measured in untreated adult CG-/-, NE-/-, dual CG-/-/NE-/-, and 129Sv wild-type mice. Mice were tracheostomized and ventilated (450 bpm, *flexiVent*®). Respiratory impedance (Z_{rs}) was measured at a range of trans-respiratory pressures from 0 to 20cmH_2O. The oscillatory signal (1-25Hz) was generated by a loud speaker-in-box and delivered to the mice via a wave tube (l=100, id=0.116 cm). Mechanical parameters were obtained by fitting the constant phase model to the multi-component spectra. Bronchoalveolar lavage was performed and lungs were frozen in liquid nitrogen for measurement of lung collagen or fixed for histology. **Results:** No differences were seen in airway or tissue mechanics between any of the four genotypes. Total cell counts and total lung collagen were not significantly different between genotypes. **Conclusion:** Baseline lung mechanics are similar in wild-type, cathepsin G, neutrophil elastase and dual knockout mice. Future studies will evaluate lung mechanics in bleomycin-treated neutrophil proteinase-deficient mice.

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THE INCREASED AIRWAY HYPERREACTIVITY OBSERVED IN OVALBUMIN-SENSITIZE AND -CHALLENGED MICE IS ENHANCED IN MICE LACKING GRK5. J. K. L. Walker, B. Lawson, R. T. Premont, D. A. Schwartz, R. J. Lefkowitz. Duke University Medical Cente Durham, NC.

G protein-coupled receptor kinases (GRKs) comprise a family of proteins that are crucial the desensitization of G protein-coupled receptors (GPCRs). GPCRs transduce extracellul signals into intracellular responses that must eventually be terminated, or desensitized, ensure physiologic homeostasis. Our previous studies showed that GRKs contribute to th regulation of airway responsiveness in mice (*Am. J. Physiol.* 276: R1214-R1221, 1999). Oth studies demonstrated that GRKs regulate chemokine receptors. Thus, GRKs may play regulatory role in the pathophysiology of allergen-induced asthma. Airway hyperreactivity ar lung inflammation were investigated in homozygous GRK5 knockout (GRK5-KO) mice ar littermate wild-type (WT) mice following sensitization and aerosolized challenge with ovalbum (ova). Mice were sensitized on days 0, 7 and 14 with i.p. injections of either ova/alum (ova alum alone (alum), or no injection (untreated). On days 21, 22 and 23 mice that receive injections were challenged for 1 hour with ova-aerosol. On day 24, the increase in trache pressure over time (airway pressure time index) was measured in response to i.v. methacholir (MCh) followed by whole-lung lavage. The increase in airway pressure time index (APT resulting from MCh injection was similar in untreated WT and GRK5-KO mice. Ova sensitizatic caused eosinophilia, neutrophilia and increased APTI in both the WT and GRK5-KO mic however, the increase in APTI was enhanced in GRK5-KO mice. Interestingly, the alum treate GRK5-KO mice showed a significant increase in APTI relative to untreated GRK5-KO mice, y this was not the case for similarly treated WT mice. These data indicate that GRK5 contribute to the *in vivo* regulation of airway hyperreactivity in a mouse model of allergen-induced asthm and suggests that GRK5 plays a role in dampening the response to allergens.

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OZONE (O_3)-INDUCED AIRWAY HYPERREACTIVITY AND INFLAMMATION LEPTIN-DEFICIENT AND LEPTIN RECEPTOR-DEFICIENT MICE. R.A. Johnston I.N. Schwartzman, G.G. Krishna Murthy, and S.A. Shore. Physiology Program, Departm of Environmental Health, Harvard School of Public Health, Boston, MA 02115-6021.

Leptin is a pro-inflammatory, satiety-controlling cytokine that is increased in the serur obese individuals. The purpose of this study was to examine the hypothesis that the pro-inflammatory effects of leptin account for the increased risk of asthma that is observed in obese. To that end, we examined O_3-induced airway hyperreactivity and O_3-induced pulmonary injury and inflammation in wildtype mice (C57BL/6J) and mice deficient in either leptin (*ob/ob*) or the leptin receptor (*db/db*). *Ob/ob* and *db/db* mice weighed approximately twice as much as wildtype mice. Airway reactivity to aerosolized methacholine, as measured by whole body plethysmography, was unaltered 4 h post-O_3 exposure (0.5 ppm for 3 h) in *db/db* mice, similar to previous results obtained in *ob/ob* mice, while wildtype mice became hyperreactive. In contrast, 4 h post-O_3 (2 ppm for 3 h bronchoalveolar lavage fluid IL-6, MIP-2, and total protein levels were elevated in *ob/ob* and *db/db* mice compared to wildtype mice (Table).

	IL-6 (pg/ml)		MIP-2 (pg/ml)		Protein (μg/ml)	
Mouse	Air	O_3	Air	O_3	Air	O_3
wildtype	9±4	149±41	<1.5	21±3	104±9	202±2
ob/ob	10±5	666±104	<1.5	34±6	90±5	320±3
db/db	3±3	521±152	<1.5	60±5	96±5	290±2

The results indicate that the reduced O_3-induced airway hyperreactivity observed in mice lacking leptin is not the result of the loss of the pro-inflammatory effects of leptin. Instea O_3-induced airway inflammation is increased in obese *ob/ob* and *db/db* mice, perhaps as result of other pro-inflammatory effects of obesity.

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REGULATED OVEREXPRESSION OF NITRIC OXIDE IN THE LUNG INDUCES AIRWAY HYPERRESPONSIVENESS IN MICE

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To explore the role of nitric oxide (NO) in airway disease we have developed a regulatable transgenic mouse capable of overexpressing inducible nitric oxide synthas (iNOS) in an airway specific fashion. The iNOS mouse (FVB) contains two transgenes a rtTA under control of the CC10 promoter; and the mouse iNOS cDNA under contro of a tetracycline response element. Addition of doxycycline (Dox, 0.5 mg/ml) to th drinking water caused an increase in iNOS RNA, protein, and immunohistochemica staining in the epithelium. Dox treatment increased exhaled NO from 8.8 ± 0.4 ppb to plateau of 31.2 ± 2.8 ppb (n=7, $p<0.05$). Increased NO levels were sustained for at leas 2 weeks and returned back to baseline within 24 h after withdrawal of Dox. There wer no differences between Dox treated or untreated iNOS mice and wild type mice in lun histology, BAL protein or BAL cell count. However, measuring airway responsivenes using whole body plethysmography showed that iNOS mice treated with Dox wer hyperresponsive to methacholine (MCh). Following a PBS baseline, the mice wer challenged with increasing concentrations of MCh. Before Dox treatment there was n difference in responsiveness to MCh between iNOS treated or untreated or wild typ mice. After one day of Dox treatment the MCh dose producing 300% PBS Penh was 1 ± 1 mg/ml, compared with 22 ± 3 in the absence of Dox (n=6, $p<0.05$). These dat indicate that increased levels of nitric oxide in the airways can induce airwa hyperresponsiveness in the absence of inflammation.

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ABSTRACTS

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