256. Mechanisms and modulation of allergic inflammation in the lung

P2320

Continuous airway inflammation is related to the development of airway remodeling

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In bronchial asthma, airway remodeling leads to refractoriness to treatment. Recent studies suggest that mechanical stress induces remodeling independently of inflammation. For this hypothesis, we examined the effects of continuous mechanical stress and airway inflammation in the mouse.
Mice were divided into 3 groups: an airway inflammation group (group A), a methacholine-inhalation-induced mechanical stress group (group B), and a control group (group C). On days 14 to 29 after sensitization by intraperitoneal injection of ovalbumin, the mice in each group inhaled physiological saline, ovalbumin, or methacholine. Counts of eosinophils and other inflammatory cells, smooth-muscle/basement-membrane thickening, and goblet-cell hyperplasia were compared among the groups.
Eosinophil counts increased with time in group A, but not in group B or C. Smooth-muscle thickening was slightly greater in group A and group B than in the control group up to day 10 after sensitization. Group A showed a continuous trend toward increased smooth-muscle thickness up to day 15. In contrast, group B showed a slight decrease in smooth-muscle thickness. The difference was more marked in the peripheral than in the central airway. Basement-membrane thickening progressed with time in group A, but was not evident in group B or group C. Goblet-cell hyperplasia calculated on the basis of mucus scores significantly increased in group A, but was unchanged in group B and group C.
Conclusion: Mechanical stress was transiently associated with thickening and proliferation of airway smooth muscle, but this effect decreased with time, suggesting that chronic, continuous airway inflammation plays an important role in remodeling.

P2321

Blocking costimulatory signal for treating steroid-resistant asthma model

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Background: We have constructed steroid sensitive (SS) and resistant (SR) murine asthma models by transferring SS and SR helper T (Th) clones into unprimed mice, respectively. Effect of CTLA4-Ig was analyzed both *in vitro* and *in vivo*.
Method: For *in vitro* experiments, ovalbumin (OVA) reactive Th clones were cultured with antigen presenting cells, OVA, and various concentrations of dexamethasone (DEX). The proliferative response of each Th clone was measured by ^{3}H-thymidine uptake. For *in vivo* experiments, unprimed BALB/c mice were transferred with Th clones, challenged with OVA, and administered with DEX subcutaneously. CTLA4-Ig was administered through nasal inhalation or venous injection. The number of infiltrating cells in bronchoalveolar lavage fluid (BALF) was measured.
Results: SS and SR clones were selected in terms of the *in vitro* effect of DEX on the proliferative responses of antigen-stimulated clones. Airway infiltration of eosinophils and lymphocytes of mice transferred with SS clones were effectively inhibited by the administration of DEX. In contrast, those of mice transferred with SR clones were not significantly inhibited by DEX. Administration of CTLA4-Ig significantly suppressed *in vitro* proliferation of DEX-treated SR clones, and *in vivo* eosinophil infiltration of SR asthma model transferred with SR clones.
Conclusion: Steroid sensitivity of Th clones measured *in vitro* were consistent with that of adoptively transferred asthma model measured *in vivo*. Steroid resistant asthma models can be treated by blocking costimulatory signal mediated through CD28-CD80 and 86.

P2322

The effects of inspiratory muscle training on interleukin-6 concentration during cycling exercise and volitional hyperpnoea

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The plasma concentration of interleukin-6 (IL-6) increases during cycling exercise (EX) (Starkie et al. J. Physiol 2001; 533:585-591) and inspiratory resistive breathing (IRB) (Vassilakopoulos et al. Am. J. Physiol 1999; 277:R1013–R1019). Whether inspiratory muscle training (IMT) can attenuate the magnitude of the IL-6 response to EX and volitional hyperpnoea (VH) rather than IRB is unknown. Therefore, we tested the hypothesis that IMT would reduce the IL-6 response to EX and/or VH.
Twelve male participants performed either 6 weeks of pressure-threshold IMT (n=6) or placebo (PLA) training (n=6). Before and after training, participants undertook three 1 hour experimental trials on separate days: (i) passive rest; (ii) EX; and (iii) VH. EX was performed at maximum lactate steady state power. In VH, participants voluntarily mimicked at rest the breathing and respiratory muscle recruitment pattern attained during EX.
IL-6 peaked immediately after EX for both the IMT and PLA groups (6.75±1.6 and 5.64±1.76 pg·mL-1). Following training, this response was reduced (-33%; P=0.027) for the IMT but not the PLA group. Blood lactate concentration ($[Lac^-]_B$) during EX was also reduced (-35%; P=0.009) for the IMT group only. IL-6 and $[Lac^-]_B$ increased ($P<0.05$) during VH in both groups, but there was no effect of training on these responses. There were no increases in IL-6 or $[Lac^-]_B$ over time for either group during passive rest.
In conclusion, 6 weeks of IMT reduces IL-6 during EX but not VH. The reduction in IL-6 concentration following IMT may be related to a decreased carbohydrate utilisation as indicated by the post-IMT reduction in $[Lac^-]_B$ during EX but not VH.

P2323

Assessment of the time course of chronic inflammation in the murine house dust mite model

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House dust mite (HDM) allergens are associated with allergic disorders and the use of this clinically relevant allergen is increasing in animal models. We assessed the chronic inflammatory time course and the anti-inflammatory efficacy of a phosphodiesterase 4 inhibitor and a corticosteroid. BALB/c mice were challenged intranasally with HDM for 5 days/week for 5 weeks. Animals were sacrificed weekly 24 hours after final challenge and recruitment of inflammatory cells assessed in bronchoalveolar lavage fluid (BALF). Lung tissue was stained for the evaluation of a histopathological response. Roflumilast (10 mg/kg) and prednisolone (10 mg/kg) were administered orally twice daily from week 3. Chronic HDM extract exposure resulted in significant recruitment of eosinophils, neutrophils, lymphocytes and macrophages as early as week 1, peaking (1.13±0.32, 0.31±0.05, 0.66±0.10 and 0.33±0.04 x10^6cells/animal respectively) between Weeks 3 and 5. Within the lymphocyte population the proportion of B cells increased from 4 to 46% over the 5 week exposure period. Mice developed perivascular, peribronchiolar and alveolar inflammation which increased in severity during the five week exposure period. The inflammation was accompanied by epithelial and mucus cell hypertrophy/hyperplasia in the bronchi and bronchioles which reached maximum severity during weeks 3 to 5. Perivascular/peribronchiolar fibrosis peaked in week 5. Therapeutic treatment with prednisolone and roflumilast significantly ($P<0.001$) inhibited BALF cell recruitment and reduced the severity of the airway remodelling suggesting this model, in our laboratory, has the potential to test novel compounds for the treatment of allergic disorders.

P2324

Effects of exercise on lung inflammation in ovalbumin-sensitized and single challenged mice

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Background: Studies suggest that physical exercise reduces lung function decline and risk of exacerbations in asthmatic patients. However, the inflammatory lung response involved in exercise during sensitization remains unclear.
Aims: To evaluate the effects of aerobic exercise in an experimental model of sensitization and single ovalbumin (OVA) challenge.

Methods: Male Swiss mice were divided into 4 groups: mice non-sensitized, non-exposed to OVA or exercise (Control, n=7); animals submitted to moderate treadmill exercise (Exercise, n=6); animals sensitized (OVA 10 μg) and single exposed to aerosolized OVA 1% (30 min) (OVA, n= 6) and animals sensitized, submitted to exercise and single exposed to OVA (OVA+Ex, n=6). 24 hours after a single OVA/saline exposure, anesthetized mice were euthanized and we performed measures of inflammatory cells from bronchoalveolar fluid (BALF), IL-4, IL-5, IL-10, IL-1ra from lung tissue by enzyme-linked immunosorbent assay and qualitative measures of IgG1 and IgE OVA-specific by Passive Cutaneous Anaphylaxis.
Results: Exercise decreased total number of cells, as well as eosinophils, neutrophils, lymphocytes and macrophages from BALF in OVA+Ex group ($p<0.05$). The median of titles of IgE and IgG1 OVA-specific in OVA+Ex group was significant lower than OVA group, and IL-4 and IL-5 were also decreased in OVA+Ex when compared with OVA group ($p<0.05$). Levels of IL-10 and IL-1ra did not reach significant difference.
Conclusion: Our results showed that aerobic physical exercise attenuated the acute lung inflammatory response induced by a single OVA-challenge in sensitized mice, suggesting immunomodulatory properties of exercise on sensitization process in asthma.

P2325
Effects of swimming on lung inflammation in ovalbumin-sensitized and challenged mice
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Background: Epidemiologic studies have suggested that aerobic exercise decreases asthma prevalence and severity, improving aerobic capacity. However, the effects of high intensity exercise during sensitization on lung inflammation in asthma are still unclear.
Objective: To evaluate the effects of high intensity exercise during sensitization process on lung inflammation in an experimental model of allergic pulmonary inflammation.
Methods: Male Swiss mice were divided into 4 groups: mice non-sensitized, non-exposed to ovalbumin (OVA) or submitted to exercise (Control, n=12); animals submitted to swimming (30 min/day for 21 days) (Exercise, n=7); animals OVA-sensitized (OVA 10 μg) and exposed to aerosolized OVA 1%, (30 min, each 48 hours during four days) (OVA, n= 9) and animals sensitized, submitted to swimming and exposed to OVA (OVA+Ex, n=11). 48 hours after last exposure to OVA/saline, anesthetized mice were euthanized and we performed measures of total inflammatory cells from bronchoalveolar fluid (BALF), IL-4, IL-5, IL-10, IL-1ra and immunoglobulin IgE by enzyme-linked immunosorbent assay (ELISA).
Results: Swimming sessions decreased total number of cells from BALF, as well as IgE, IL-4 and IL-5 levels in OVA sensitized and challenged mice ($p<0.05$). On the other hand, levels of IL-10 and IL-1ra showed a decrease in OVA+Ex group when compared with OVA group ($p<0.05$).
Conclusion: In this experimental model exercise decreased pro-inflammatory cytokines, but also decreased regulatory/anti-inflammatory cytokines, maybe suggesting that during high intensity exercise, anti-inflammatory effects are not mediated by regulatory cytokines in sensitization process in allergic pulmonary inflammation.

P2326
Poly:IC causes exacerbation in a murine allergic inflammation model driven by house dust mite in Freund's complete adjuvant
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Rationale: RNA viruses are major causes of respiratory infections and known to exacerbate asthma and other respiratory diseases. The objective of the study was to use poly I:C, a synthetic analogue dsRNA, to elicit exacerbation in a model of allergic inflammation driven by house dust mite (HDM) in Freund's Complete Adjuvant (FCA). This model, developed in partnership with UCB as part of *UBIOPRED WP6*, is characterized by airway hyperresponsiveness (AHR) and a mixed T-helper phenotype [1].
Methods: BALB/c mice were sensitised subcutaneously on day 0 with HDM (100μg) in FCA as previously described [1]. On day 14, mice were exposed to saline or HDM (25μg) via intranasal instillation (i.n.). Poly I:C (30 μg) was administered i.n. 24hrs before (-24hr), at the same time (0hrs) or after (+6hours, +24hours) HDM challenge. 24 hours post-challenge, non-invasive whole-body plethysmography was used to assess AHR stimulated by aerosolised methacholine (MCh, 0-16mg/ml). 48 hours after HDM challenge, the bronchoalveolar lavage fluid (BALF) was collected to measure inflammatory cells.
Results: Poly I:C exacerbated BALF neutrophils (-24, 0, +6), macrophages (-24, 0, +6) and lymphocytes (-24, 0) in the HDM challenged animals. At -24hrs or +6hrs, the AHR associated to MCh was also significantly exacerbated.
Conclusions: Poly I:C exacerbates the inflammation and AHR in a murine model that mimics certain aspects of persistent asthma. This model could be used to investigate new mechanisms of action underlying viral exacerbation in persistent asthma and for the assessment and evaluation of novel therapies for such condition.
Reference:
[1] Nasra J. et al. Am J Respir Crit Care Med 181;2010:A2842.

P2327
Decreased innate immunity proteins in asthmatic respiratory tract lining fluids
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Human respiratory tract lining fluids (RTLF) contain several innate immunity proteins with microbicidal and bacteriostatic properties. High concentrations of lactoferrin and transferrin are also present, limiting the availability of Fe, which would otherwise support bacterial growth. In the present study we hypothesized that these innate defenses would be impaired in asthma, associated with an increased bacterial burden in the airways.
Antimicrobial protein concentrations were determined in bronchial wash (BW) samples obtained from mild asthmatic and healthy volunteers. To examine the relationship between these proteins and bacterial load, measurements of lipopolysaccharide (LPS) and lipoteichoic acid (LTA) were made.
BW samples were obtained from mild asthmatics (n=16, 26.76.2) and healthy controls (n=16, 25.02.6). Lactoperoxidase, lactoferrin, transferrin, and lysozyme concentrations were determined using ELISAs. RTLF concentrations were established using the urea method. LPS measurements were made using the Limulus Amaebocyte Lysate assay and LTA concentrations determined using ELISA.
Significantly lower BW transferrin ($p=0.01$ Mann Whitney U-Test) and lysozyme ($p=0.03$) concentrations were observed in asthmatics. A similar trend was apparent for lactoperoxidase ($p=0.08$), though for this and the remaining proteins considerable intra-individual variation was apparent. Correspondingly BW LPS was significantly greater ($p=0.04$) in asthmatics, concentrations were not simplistically related to transferrin or lysozyme levels.
These results reveal impaired microbicidal defense at the air-lung interface of mild asthmatics. Such a deficiency could render the asthmatic airway more susceptible to infection.

P2328
Helminth extract from opisthorchis felineus suppresses allergic inflammation through modulation of dendritic cells phenotype
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Background: Recent epidemiological and experimental reports have suggested that helminth infection could induce suppression of allergic Th2 responses [Jeong Y. et al., 2011]. Opisthorchiasis caused by Opisthorchis felineus (O.f.) is the major public health problems of Western Siberia in Russian Federation. Dendritic cells (DCs) are critical for controlling the immune response to various types of antigens so we established them to study the effects of O.f. on the development of airway inflammation.
Aim and objectives: To investigate DC immune phenotype during the treatment with O. f. extract in patients with bronchial asthma (BA).
Methods: Mature DCs were cultured from peripheral blood monocytes of healthy control (n=17) and asthmatic patients (mild BA, n=19; severe BA, n=24) using IL-4 and GM-CSF during 6 days. DCs were stimulated with O.f. extract and LPS on 4 day of incubation. After culturing DCs were harvested and labeled for CD86, CD83, CD209 and analyzed using flow cytometry.
Results: O. f. inhibited the expression of co-stimulatory molecules as CD86 in mature DCs. Number of CD86+CD209+ DC was significantly decreased in different severity of BA compared to healthy donors. Patients with mild and severe BA had increased levels of CD209+ DCs compared to control.
Conclusions: Our findings suggest that levels of co-stimulatory molecules expression on DC are important in immune response balancing and polarization. Increased levels of co-stimulatory molecules as CD86 on DC after helminth stimulation with O.f. might suppress airway inflammation in BA.

P2329

Blockade of thymic stromal lymphopoietin receptor (TSLPR) reduces atopic inflammation in a cynomolgus monkey model of asthma

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TSLP pathway blockade is a potential strategy for asthma treatment, as TSLP modulates cytokine production by mast cells and regulates the activation of dendritic cells (DCs), which prime the differentiation of naïve T cells into inflammatory Th2 cells. We thus tested the effects of TSLPR blockade on the development of allergic inflammation and bronchoconstriction in cynomolgus monkeys after Ascaris suum allergen challenge. Antibodies against human TSLPR were generated and confirmed to be cross-reactive to cynomolgus. Animals were dosed weekly with either vehicle (n=8) or TSLPR HuMAb (n=8) for 6 weeks and their responses to A.Suum challenge at baseline, week 2 and week 6 were assessed. TSLPR HuMab treated subjects showed reduced bronchoalveolar lavage (BAL) eosinophil counts (p=0.04), reduced lung resistance (RL) area under the curve (p=0.04), and reduced IL-13 cytokine levels in BAL fluid (p=0.03) in response to challenge at 6 weeks compared to control subjects. To understand the molecular changes underlying these differences, pre- and 8h post-challenge BAL samples from Mab-treated and control subjects were profiled using expression microarrays. Genes up-regulated by allergen challenge overlapped strongly with 11 genes up-regulated in DCs when stimulated by TSLP (TSLP-DC signature). At 6 weeks, treatment with TSLPR HuMAb reduced the overall number of differentially expressed genes and significantly reduced the induction of the TSLP-DC signature relative to control subjects (p = 0.05). These results demonstrate promising efficacy for TSLPR blockade in an allergen challenge model where TSLP activation of DCs may play a key role.

P2330

Intracellular glutathione redox status in human dendritic cells regulates Th1/Th2 balance through IL-12 and IL-27 production

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Glutathione redox status, changes in intracellular reduced (GSH) or oxidized (GSSG) glutathione, plays a significant role in cellular function. We examined whether intracellular glutathione redox status in human dendritic cells (DCs) regulates the production of polarizing signals, such as IL-27, IL-12, and Th1/Th2 responses.

Human PBMCs were obtained from healthy adult volunteers, and monocyte-derived DCs (MD-DCs) were generated from PBMCs. MD-DCs treated with glutathione reduced form ethyl ester (GSH-OEt) or L-buthionine-(S,R)-sulfoximine (BSO) were stimulated by LPS, and the levels of Th1 and Th2 cytokines were measured. Then, DCs matured by LPS or TSLP were cocultured with allogeneic CD4(+) naive T cells and Th1/Th2 balance was evaluated.

Monocyte-derived DCs exposed to GSH-OEt and BSO had increased and decreased intracellular GSH contents, respectively. LPS-induced IL-27 and IL-12 production was enhanced by GSH-OEt and suppressed by BSO. Mature GSH-OEt-treated MD-DCs enhanced interferon (IFN)-γ production from CD4(+) T cells compared with nontreated MD-DCs, and siRNA against IL-27 and anti-IL-12 mAb suppressed the effect of GSH-OEt on IFN-γ production. Although human myeloid DCs activated by TSLP (TSLP-DCs) prime naïve CD4(+) T cells to differentiate into Th2 cells, treatment of TSLP-DCs with GSH-OEt reduced IL-13 production and enhanced IFN-γ production by CD4(+) T cells. Interleukin-27 p28 siRNAs and anti-IL-12 mAb attenuated the inhibitory effect of GSH-OEt on Th2 polarization. These results indicate that Th1 and Th2 balance are controlled by intracellular glutathione redox status in DCs through the production of IL-27 and IL-12.

P2331

Subcutaneuous injection of monoclonal antibody anti-DEC205 conjugated with ovalbumin atenuates allergic lung inflammation of animals previously sensitized to allergen

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DEC205 (Dendritic Endothelial Cell 205) is an endocytic receptor abundant on DCs in lymphoid tissues. Ovalbumin (OVA) protein can be chemically coupled to monoclonal a-DEC-205 antibody. Here, we sought to study the effect of a-DEC205 monoclonal antibody conjugated to OVA after OVA sensitization in a model of allergic lung disease. BALB/c mice were sensitized twice with OVA/Alum on days

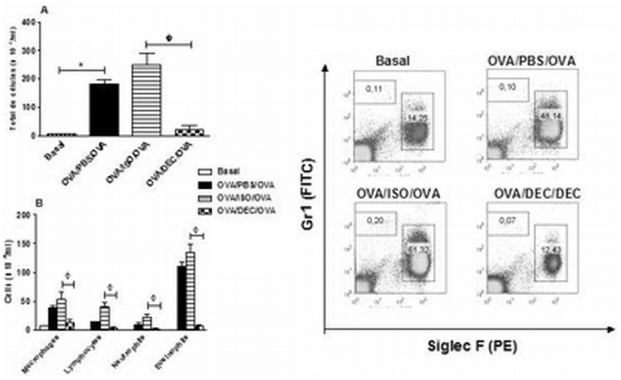

Figure 1

0 and 7 and challenged twice with intranasal OVA on days 14 and 21 (OVA/alum group). The animals received a-DEC-205-OVA treatment after OVA sensitization (OVA/DEC group). The treatment consisted of two injections of a-DEC205 a week apart. We found that treatment with a-DEC205 decreased significantly allergic inflammation as revealed by total cell numbers and eosinophils counts in bronchoalveolar lavage (BAL) fluid.

In the group treated with a-DEC 205, but not with the isotype control, we observed a reduction of total and OVA-specific IgE antibodies as well as IL-4 and IL-5 levels in BAL. Furthermore, treatment with a-DEC 205 decreased the methacholine-induced respiratory pattern associated with allergy.Our results indicate that treatment with anti-DEC205 monoclonal antibody conjugated to OVA is effective in reducing allergic responses in animals previously sensitized to OVA. These results suggest a potential therapeutic use of a-DEC 205 in allergic disorders.

P2332

Thymic stromal lymphopoietin promotes asthmatic airway remodeling in human lung fibroblast cells through stat3 signaling pathway

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Objective: To identify the role and regulation of thymic stromal lymphopoietin (TSLP) in in asthmatic airway remodeling.

Methods: The expression of TSLP, α smooth muscle actin (α-SMA) and collagen I were detected by immunohistochemistry. we silenced or overexpressed TSLP in human lung fibroblast cell (HLF-1) by shRNA approaches or transfection, and TSLPR was detected by ELISA and Western blot. Total signal transducer and activator of transcription 3(STAT3),the phosphorylation of STAT3 and TSLP, α-SMA, collagen I expression were detected by Western blot. The α-SMA,collagen I mRNA expression were determined by quantitative RT-PCR. we inhibited Stat3 activity by targeted small molecules and then detected TSLP-induced expression of α-SMA,collagen I in both mRNA and protein levels by quantitative RT-PCR and Western blot.

Results: Overexpression of TSLP,α-SMA and collagen I were detected in epithelium of asthma patients (A, B1-2). TSLPR was expressed in HLF (C1-2). The phosphorylated STAT3 and upregulation of α-SMA,collagen I were induced by the introduction of TSLP in HLF-1 (D1-3), and the repression of α-SMA, collagen I were detected after TSLP silencing (E1-3). We found that TSLP-induced α-SMA,collagen I upregulation is in a STAT3 dependent manner, (F1-4). See Figure 1, p. 422s).

Conclusion: TSLP functions in asthmatic airway remodeling through STAT3 signaling pathway.

P2333

Low dose diesel exhaust particle exposure exacerbates allergic asthma in young mice

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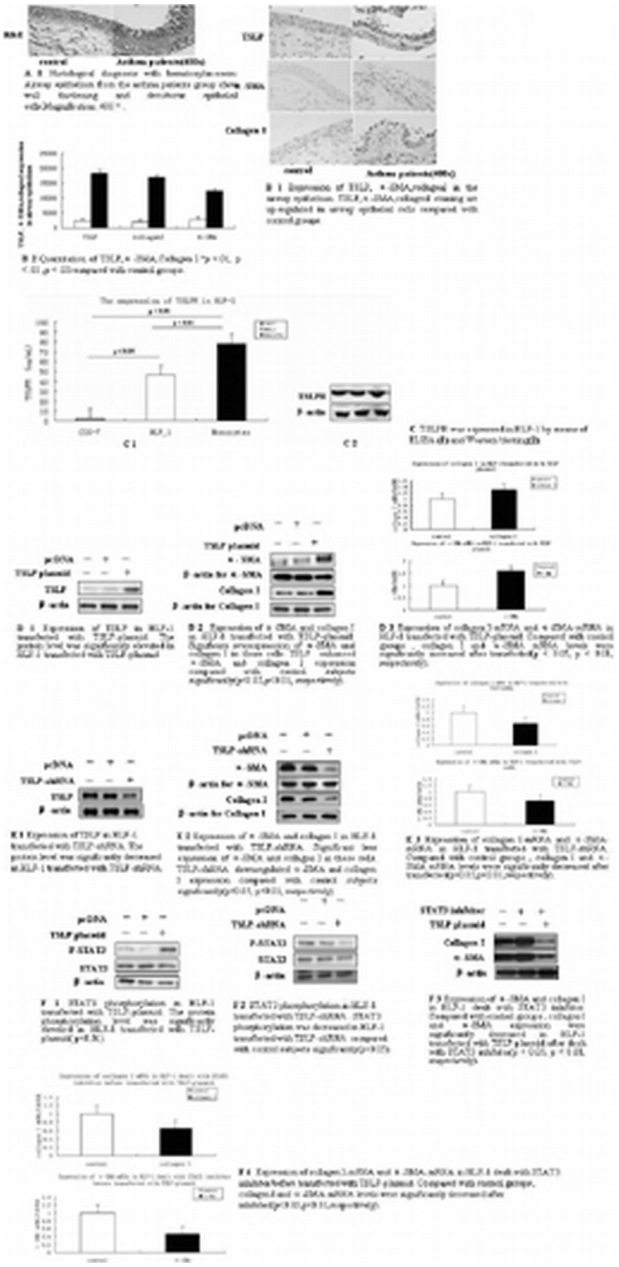
Rationale: Recent epidemiological studies correlate traffic pollution with the development of asthma in a dose dependent manner. Diesel exhaust particles (DEP) comprise the majority of the particulate matter in air pollution, and DEP expo-



P2332 – Figure 1

sure augments allergic responses in adult animals. However, it is unclear if DEP exposure exacerbates allergic asthma in young animals and whether this is dose dependent.
Objective: To determine if DEP exacerbates allergic asthma in young mice and whether a low dose is as effective as a high dose.
Methods: Three week old Balb/c mice were exposed intratracheally 3 times a week for 3 weeks with DEP alone at a high and low dose (6 and 1.2mg/kg respectively), house dust mite (HDM; 0.8mg/kg) alone, or DEP high or low dose in combination with HDM. Data was collected 24 hours after the last exposure. DEP doses were based on exposure data from children in the Cincinnati Childhood Allergy and Air Pollution Study.
Results: High dose DEP alone increased neutrophilic influx into the lungs. Low dose DEP alone did not. Both high and low dose DEP combined with HDM exposure increased antigen specific IgE, lung inflammatory cell counts, airway hyperreactivity, goblet cells, Th2 cytokine levels, T cell activation, effector T cells, and activated myeloid dendritic cells. Interestingly, both doses of DEP with HDM increased lung IL-17A levels and T cells that stained positive for both IL-13 and IL-17, which have been associated with more severe asthma.
Conclusions: DEP exacerbated allergic responses in young mice and a low dose was as effective as a high dose when combined with HDM, although low dose DEP alone did not increase inflammatory cell counts compared to high dose DEP alone.

P2334
Gender differences in eosinophilic airway inflammation in allergic and non-allergic asthma
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Introduction: Several studies have shown potential gender specific differences in the pathophysiology and clinical presentation of asthma, whose mechanisms are not fully understood.
Aims and objectives: We examined the influence of gender on eosinophilic airway inflammation in steroid-naïve patients with allergic and non-allergic asthma.
Methods: The subjects comprised 280 Japanese patients [101 males and 179 females, median (range) age 53 (18-88) years] with asthma who were untreated with glucocorticosteroids and during attack-free periods. We used the levels of fractional exhaled nitric oxide (FeNO) as a marker of eosinophilic airway inflammation. The FeNO concentration was measured using the recommended online method. We compared the levels of FeNO between males and females, separately for allergic and non-allergic asthma.
Results: In 171 patients with allergic asthma, 70 males had significantly higher FeNO levels compared with 101 females (59.6±57.3 versus 43.3±46.6 ppb, respectively; *P*=0.02); in 109 patients with non-allergic asthma, there was no significant difference in FeNO levels between 31 males and 78 females (46.4±36.3 versus 39.8±38.0 ppb, respectively; *P*=0.2).
Conclusions: Our results indicate that the importance of eosinophils in airway inflammation differs between males and females in allergic asthma, but not in non-allergic asthma. In allergic asthma, female patients may include higher rates of other inflammatory phenotypes than eosinophilic asthma compared with male patients.

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Which children have the strongest longitudinal associations between early exposure to environmental tobacco smoke and age of asthma development?
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Objective: We sought to understand the effects of risk factors such as atopy on the longitudinal association between early-life exposure to environmental tobacco smoke (ETS) and age of physician-diagnosed asthma (PDA) development in childhood.
Methods: In the Toronto Child Health Evaluation Questionnaire, parents of 5619 grades 1-2 students reported age of PDA development, exposure to ETS during pregnancy and the first year of life, history and family history of atopy and demographic information. Using Cox proportional hazard models, we conducted stratified analyses by potential effect modifiers.
Results: Household ETS exposure prevalence was 8.3% during pregnancy and 10.6% in the first year of life; 15.5% of children developed PDA, 31.2% had a history of atopy and 9.8% had a history of maternal asthma. Children exposed to ETS during pregnancy developed asthma sooner [adjusted hazard ratio (aHR) 1.27, 95% confidence interval (CI): 1.00-1.61]. Stronger associations were seen among children without a history of atopy (aHR 1.92, 95% CI: 1.42-2.61) and without maternal asthma (aHR 1.74, 95% CI: 1.34-2.25); these relationships persisted for ETS exposure in the first year of life (aHR 1.52, 95% CI: 1.12-2.07 and 1.39, 95% CI: 1.08-1.79, respectively).
Conclusions: Longitudinal associations between ETS exposure during pregnancy or the first year of life and age of PDA development are stronger in children without a history of atopy and without maternal asthma. Greater understanding of these associations may guide hypotheses regarding possible mechanisms of association and suggest strategies for exposure reduction in higher-risk children.

P2336
Prognosis of allergic and non-allergic asthma
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Some studies indicate that atopy is less important as a predictor of severe asthma. But, remission is more uncommon in allergic asthma than in non-allergic asthma. The purpose of this study was to investigate the prognosis of asthma and risk factors for asthma onset, especially sensitization of specific allergens. A cohort of three age groups responded to a respiratory questionnaire in 1990 and 2003. At baseline, 2060 subjects who reported respiratory symptoms and 482 controls were investigated with interviews, spirometry and skin prick test. A total of 721 asthmatics and 976 subjects without respiratory disease were clinically verified. At follow-up in 2003, 340 subjects with persistent asthma and 186 subjects with asthma remission were identified while 76 subjects reported new asthma onset. Sensitisation to pets and a high symptom score were significant determinants of persistent asthma (ORs 3.23 [95% CI 1.9-5.67], and 5.76 [2.35-13.34] respectively), and onset of asthma, (ORs 2.65 [1.13-4.86.0], and 1.7 [1.21-2.35],

respectively). A high self-reported responsiveness to airway irritants (OR 1.6 [1.12-2.2]), and more asthma medications (OR 2.0 [1.3-2.9]) were additional risk factors for persistent asthma at the follow-up. Belonging to the older age group decreased the risk of having persistent asthma or asthma onset. In conclusion, the findings of this study show that asthmatics sensitized to pets have a more severe prognosis than asthmatics not sensitized to pets. Sensitization to pets was also a strong predictor for onset of asthma. Our study indicates that special care should be given to asthmatics who report having problems with a high number of airway irritants as such patients are more likely to suffer from persistent problems.

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Standardization, sensitivity and specificity of an ash *(Fraxinus excelsior)* pollen allergen extract

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Background: Ash (*Fraxinus excelsior*), a wind-pollinated tree species causing spring time pollinosis, is the main representative of the Oleaceae family in temperate zones.
Aims and objectives: There is a need to standardize allergen extracts. Here we calibrated the biological activity of an ash pollen in-house reference preparation (IHRP) in allergic subjects and assessed the sensitivity and specificity of a prick-test solution prepared from this IHRP.
Methods: 27 ash pollen allergic subjects, with ash pollen and nOle e 1-specific serum IgEs (sIgE) >2.0 kUA/L and >0.7 kUA/L, respectively and positive ash pollen nasal challenge tests (NCT) participated. Skin prick testing (SPT) with IHRP was performed and the concentration inducing a mean wheal diameter of 7 mm was defined as 100 IR/mL. Subsequently, a 100 IR/mL solution of IHRP was assessed in 30 ash allergic subjects (history of ash allergy and ash-specific sIgE >0.7 kUA/L) and 30 non-allergic subjects (no history of allergy and ash-specific sIgE <0.35 kUA/L).
Results: The 100 IR/mL concentration corresponded to 1/148 weight/volume. All ash allergic subjects had a positive SPT (>3mm) and 29/30 non-allergic subjects had a negative SPT. Therefore, the sensitivity of the 100 IR/mL solution was 100% [88.6-100.0] and its specificity was 96.7% [83.3-99.4].
Conclusions: A 100 IR/mL prick test solution for in vivo diagnosis of ash pollen allergy was shown to be highly sensitive and specific.