### 70. Mechanisms of asthma

#### 358

## The role of histone deacetylase 9 in the imbalance of Th17/Treg in bronchial asthma

<u>Xiaoxia Hou</u>, Guochao Shi, Xiangyan Ai, Yingmeng Ni. Department of Pulmonary Medicine, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

**Rationale:** Studies have shown that histone deacetylase 9 can affect Tregs function and histone deacetylase inhibitor can inhibit conversion of Tregs into IL-17producing cells.

**Methods:** GATA3, IL-4 and HDAC9 mRNA expression level were measured by SYRB Green RT PCR. IL-17 and TGF- $\beta$  were measured by ELISA. BALB/c mice were randomly divided into three groups, the control group, asthma model group and TSA group. The mice in TSA group were given TSA (1mg/kg) i.p. every other day during sensitization and challenge. The plasma IgE were measured by ELISA, observed the inflammation by HE staining, HDAC9, ROR $\gamma$ t and Foxp3 mRNA expression in lung tissue were measured by PCR.

**Results:** HDAC9 mRNA expression was associated with severity of disease (p<0.01). HDAC9 mRNA expression was correlated with GATA3 mRNA expression positively (p<0.01, r=0.482), the same with IL-4 mRNA expression (p<0.01, r=0.432) and IL-17 (p<0.01, r=0.538), but negatively correlated with TGF- $\beta$  (p<0.01, r=-0.417). In patients with moderate-severe asthma, HDAC9 mRNA expression was negatively correlated with FEV1% (p<0.01, r=-0.657).



Figure 1. HDAC9 mRNA expression was associated with severity of disease.

**Conclusions:** The relationship between expression of histone deacetylase 9 and imbalance of Th17/Treg has been confirmed in asthma. The histone deacetylase inhibitor TSA can control asthma by regulating activity of histone deacetylase.

#### 359

## Sputum fluid endotoxin is associated with the $\mathrm{FEV}_1$ response to oral steroids in non-smokers with asthma

Charles McSharry<sup>1</sup>, Mark Spears<sup>2</sup>, Rekha Chaudhuri<sup>2</sup>, Euan Cameron<sup>2</sup>,

Neil Thomson<sup>3, 1</sup>Immunology, Glasgow University, Glasgow, United Kingdom; <sup>2</sup>Respiratory Medicine, Gartnavel General Hospital, Glasgow, United Kingdom; <sup>3</sup>Respiratory Medicine, Glasgow University, Glasgow, United Kingdom

**Background:** Asthma patients who smoke have a reduced sensitivity to corticosteroids. The mechanisms for this are not well understood. Increased bacterial endotoxin has been found in BAL fluid of non-smokers with steroid-resistant asthma and cigarette smoke is a rich source of endotoxin. We compared sputum endotoxin concentration in smokers and non-smokers with asthma, with the FEV<sub>1</sub> response to oral corticosteroids.

**Methods:** Sputum was induced from 31 non-smokers and 22 smokers with asthma. Endotoxin was quantified by ELISA (LAL-QCL<sup>®</sup>, Lonza Biologics plc), and cytokines by luminex (InVirogen). Spirometry and exhaled nitric oxide measurements were recorded. The response to oral dexamethasone was the change in FEV<sub>1</sub> compared with baseline.

**Results:** Non-smokers had improved FEV<sub>1</sub> after steroids; p=0.015, but smokers were refractory; p=0.591. The steroid response decreased with increasing sputum endotoxin in non-smokers; r= -0.422, p=0.032, but not in smokers; r= -0.126, p=0.585.

The steroid response increased with pre-steroid FeNO in non-smokers; r=0.479, p=0.015, but not in smokers; r=0.310, p=0.226. The endotoxin and IL-1RA concentrations correlated in non-smokers; r=0.633, p<0.001, but not in smokers; r=0.359, p=0.120.

**Conclusions:** Higher endotoxin in sputum fluid was associated with an impaired  $FEV_1$  improvement after steroids only in non-smokers; and was associated with decreased FeNO and increased sputum IL-1RA. We suggest that smoking establishes a chronic refractory state to both steroids and to endotoxin. Identifying intracellular signalling pathways common to both may help understand and potentially reverse these processes.

#### 360

## Cigarette smoke exposure facilitates allergic sensitization to house dust mite and aggravates the asthmatic phenotype in mice

Ellen Lanckacker<sup>1</sup>, Kurt Tournoy<sup>1</sup>, Gabriele Holtappels<sup>2</sup>, Hamida Hammad<sup>3</sup>, Bart Lambrecht<sup>3</sup>, Guy Joos<sup>1</sup>, Tania Maes<sup>1</sup>. <sup>1</sup>Laboratory for Translational Research in Obstructive Pulmonary Diseases, Ghent University, Ghent, Belgium; <sup>2</sup>Department of Oto-Rhino-Laryngology, Ghent University, Ghent, Belgium; <sup>3</sup>Laboratory of Immunoregulation and Mucosal Immunology, Ghent University, Ghent, Belgium

Background: Cigarette smoke (CS) exposure has been associated with increased sensitization and asthma development in children and adults.

**Aims and objectives:** We wanted to design a novel mouse model to unravel the impact of CS on the different stages of asthma pathogenesis, specifically during the initial sensitization and acute asthma development.

**Methods:** Mice were exposed to 25µg house dust mite (HDM) extract (intranasaly, 1/week) for 3 consecutive weeks, combined with air or CS exposure (3 times/day, 5 days/week) during 3 weeks or only during the first week.

**Results:** Mice concomitantly exposed to HDM and CS for 3 weeks, showed a significant increase in eosinophils, goblet cells, airway hyperresponsiveness and HDM-specific serum IgG1, compared to sole HDM or CS exposure. Interestingly, exposure to CS only during the first week was sufficient to induce an aggravated asthmatic phenotype after rechallenge with HDM allergens. To further investigate the effect of CS during mucosal sensitization, mice were exposed to HDM (just once) and 3 days of CS, followed by the assessment of DC trafficking and early Th2 responses in the lymph nodes. This short CS exposure amplified DC-mediated transport of HDM allergens to the lymph nodes and was sufficient to generate a Th2 response, characterized by IL-4, IL-5 and IL-13 production in the draining lymph nodes.

**Conclusions:** CS facilitates the development of HDM-induced allergic asthma. Only a few days of smoke exposure are sufficient to facilitate allergic sensitization to common aeroallergens.

Funding: IUAP - Belgian Science Policy P6/35 and BOF/GOA 01251504.

#### 361

# Increased numbers of alveolar mast cells with an altered phenotype are linked to peripheral airway remodelling in patients with allergic asthma <u>Cecilia Andersson<sup>1</sup></u>, Maria Weitoft Lundström<sup>2</sup>, Anders Bergqvist<sup>1</sup>,

Michiko Mori<sup>3</sup>, Leif Bjermer<sup>1</sup>, Gunilla Westergren-Thorsson<sup>2</sup>, Jonas Erjefält<sup>3</sup>. <sup>1</sup>Dept. of Respiratory Medicine & Allergology, Lund University, Lund, Sweden; <sup>2</sup>Lund University, Lung Biology Unit, Lund, Sweden; <sup>3</sup>Lund University, Unit of Airway Inflammation, Lund, Sweden

**Background:** A significant proportion of asthmatics have symptoms despite treatment with inhaled corticosteroids. Recent studies have revealed an expansion of highly FccRI-expressing alveolar mast cells (MCs) in asthma. The aims were to further phenotype alveolar MCs and explore their connection to peripheral tissue remodelling in different asthma cohorts.

Methods: Bronchial and transbronchial biopsies from controls, patients with

rhinitis, mild and uncontrolled asthma were processed for immunohistochemical identification of MC subtypes and expression of pro-fibrotic markers. MC alteration in relation to tissue remodelling (density of collagen, versican, decorin and biglycan) in peripheral lung was studied.

**Results:** The alveolar parenchyma (AP) in uncontrolled asthmatics had increased densities of  $MC_{TC}$  (p=0.05) and  $MC_T$  (p=0.003). The expression of FccRI on alveolar MCs was increased in mild (p=0.01) and uncontrolled (p<0.001) asthma compared to controls. The density of collagen (p=0.01) and decorin (p=0.03) was significantly increased in AP of uncontrolled asthmatics compared to controls. The number of alveolar  $MC_{TC}$  in the uncontrolled asthmatics was positively correlated to the density of collagen in the AP (rs=0.71, p=0.03). MCs in the AP of asthmatics expressed increased levels of pro-fibrotic markers.

**Conclusions:** Our data show that the alveolar region in patients with asthma is infiltrated by activated MCs that correlate to increased alveolar matrix deposition. This may reflect an important involvement of MCs in the peripheral inflammation in asthma and underscores the need to target peripheral lung inflammation in this patient group.

#### 362

#### LSC 2012 Abstract – Monocyte-derived dendritic cell recruitment and allergic TH2 responses after exposure to diesel particles are CCR2-dependent Tania Maes, Sharen Provoost, Guy Joos, Kurt Tournoy. Dept of Respiratory Medicine, Ghent University Hospital, Ghent, Belgium

**Background:** Diesel exhaust particles (DEP) inhalation is associated with increased sensitization towards inhaled allergens. Inhalation of DEP increases pulmonary dendritic cell (DC) accumulation and enhances TH2 responses in mediastinal lymph nodes<sup>1</sup>. In inflammatory conditions, DC recruitment is mediated by different CC chemokine receptors (CCR). We hypothesized that CCR2 mediates DC recruitment and TH2 responses upon DEP exposure.

Methods and results: WT and CCR2 KO mice were exposed to saline or DEP, followed by immunological examination. DEP-exposure increased pulmonary expression of CCR2 and MCP-1 in WT mice. DEP-exposure induced a pulmonary recruitment of monocytes and inflammatory (CD11b+) DCs in WT mice, which was abolished in CCR2 KO mice. Adoptive transfer of fluorescently labeled bone marrow derived cells from WT and CCR2 KO mice into DEP-exposed WT mice demonstrated the direct involvement of CCR2 in recruitment of blood monocytes towards the lung upon DEP-exposure. Furthermore, analysis of TH2 cytokine production in mediastinal lymph nodes upon DEP-exposure showed an abolished TH2 response in CCR2 KO mice.

**Conclusion:** These data suggest that monocyte-derived DC, recruited in a CCR2dependent manner, are critical in inducing TH2 responses upon DEP inhalation. **Funding:** Belgian IUAP (P6/35), FWO-Flanders (G.0052.06; G.0329.11N). <sup>1</sup>Provoost et al, J. Immunol 2010, 184:426-32.

#### 363

## Co-ordinate regulation of vectorial cytokine release and paracellular permeability from polarized bronchial epithelium challenged with double stranded RNA (dsRNA)

<u>Jingxi Zhang</u><sup>1</sup>, Emily Swindle<sup>2</sup>, Michelle Hardyman<sup>2</sup>, Stephen T. Holgate<sup>2</sup>, Jane E. Collins<sup>2</sup>, Donna E. Davies<sup>2</sup>. <sup>1</sup>*Respiratory Department, Changhai Hospital, the Second Military Medical University, Shanghai, China;* <sup>2</sup>*Division of Infection, Inflammation and Immunity, University of Southampton School of Medicine, Southampton General Hospital, Southampton, United Kingdom* 

**Background:** Respiratory virus infections, a common cause of asthmatic exacerbations, are associated with increased epithelial permeability and accumulation of inflammatory cells in airway lumen. We hypothesized that the innate anti-viral response of bronchial epithelium controls luminal inflammation by coordinating vectorial cytokine secretion with opening of tight junction (TJ) complexes.

**Methods:** Human bronchial epithelial cells were challenged with dsRNA. Apical and basolateral secretion of IL-8 and TNF- $\alpha$  was measured by ELISA. Macro-molecular and ionic paracellular permeabilities were measured by FITC-dextran and transepithelial electrical resistance (TER) respectively, while TJ integrity was assessed by immunofluorescent staining for ZO-1.

**Results:** dsRNA induced apical and basolateral secretion of IL-8 and TNF- $\alpha$ , with a significant vectorial bias towards the apical surface; this was paralleled by an increase in both macromolecular and ionic permeabilities. These responses were completely blocked by dexamethasone. Neutralization of TNF- $\alpha$  partially inhibited IL-8 and TNF- $\alpha$  release and the increase macromolecular paracellular permeability but had no effect on TER. Inhibition of p38MAPK or JNK also inhibited IL-8 and TNF-a secretion and prevented the increase in macromolecular permeability. Disruption of junctional ZO-1 induced by dsRNA was rescued by inhibition of p38 MAPK and JNK pathways.

**Conclusions:** These findings suggest that bronchial epithelial cells control their inflammatory responses so that changes in macromolecular paracellular permeability are coordinated with vectorial secretion of chemokines and cytokines.

#### 364

## Mucosal explant induced migration of T-cells from severe asthmatics is inhibited by CCR4 antagonism

<u>Thomas Southworth</u><sup>1</sup>, Anna Kuklinska-Pijanka<sup>1</sup>, Justyna Sutula<sup>1</sup>, Paul Cadden<sup>1</sup>, Simon Hodgson<sup>2</sup>, David Hall<sup>2</sup>, Dave Singh<sup>1</sup>. <sup>1</sup>NIHR Translational Research Facility, The University of Manchester, United Kingdom; <sup>2</sup>Respiratory Therapy Area Unit, GlaxoSmithKline, Stevenage, United Kingdom

**Background:** Th2 cells that express chemokine receptor 4 (CCR4) are key to inflammation in asthma. Work in mild asthma patients suggests that CCR4 plays a role in Th2 migration into the lung, making CCR4 a possible therapeutic target. However, little evidence is known on the role of CCR4 on Th2 migration in severe asthma patients on high dose corticosteroids.

Aim: To investigate if chemokines released by mucosal explants, from asthma patients on high dose inhaled corticosteroids, drive Th2 migration and if such a response is CCR4 dependent.

**Methods:** Bronchial explants, from 11 severe (SA) and 9 steroid naïve (SNA) asthma patients, were cultured in media +/– house dust mite extract. The supernatants were used as chemoattractants in migration assays with patient-matched peripheral T-cells. The effects of three CCR4 antagonists, GSK494652A, GSK2239633A and GSK2192991A, on T-cell migration were examined.

**Results:** Bronchial explant conditioned media from SNA induced higher T-cell migration than that from SA (p=0.03). However, house dust mite extract did not enhance chemotaxis in either SNA (p=0.9) or SA (p=0.6). Inhibition of CCR4 reduced T-cell migration in response to unstimulated explant conditioned media (Table 1).

Table 1. Effects of CCR4 antagonists on T-cell migration

	Chemotaxis Index			
	Control	GSK494652A	GSK2239633A	GSK2192991A
SNA Median (range)	2.7 (0.8-17.2)	1.7* (0.6-10.1)	1.7* (0.6-11.2)	1.9* (0.8-7.8)
SA Mean (sem)	2.0 (0.3)	2.2 (0.4)	1.6* (0.2)	1.4* (0.2)

Control v antagonist: \*p<0.05.

**Conclusions:** Targeting CCR4 may prove to be effective in reducing Th2 recruitment into the lung and the subsequent inflammatory response in asthma patients who are on high dose inhaled corticosteroid treatment.

#### 365

#### Apolipoprotein A1 regulate innate immune response and tight junction formation in the airway epithelium to promote the resolution of allergic airway inflammation

<u>Sung Woo Park</u><sup>1</sup>, Eunhee Lee<sup>1</sup>, Ansoo Jang<sup>1</sup>, SooTaek Uh<sup>2</sup>, YongHoon Kim<sup>3</sup>, David Erle<sup>4</sup>, Choonsik Park<sup>1</sup>. <sup>1</sup>Internal Medicine, Soonchunhyang University Bucheon Hospital, Bucheon, Republic of Korea; <sup>2</sup>Internal Medicine, Soonchunhyang University Seoul Hospital, Seoul, Republic of Korea; <sup>3</sup>Internal Medicine, Soonchunhyang University Chonan Hospital, Chonan, Republic of Korea; <sup>4</sup>Lung Biology Center, University of California San Francisco, United States

Apolipoprotein A-1 (ApoA1), main component of HDL have anti-inflammatory effect as well as reverse cholesterol transport. The objective of this study was to determine airway levels of ApoA1 in the asthmatics as well as its effect on innate immune response and resolution of inflammation on experimental asthma. Two-dimensional electrophoresis was performed for differential display proteomics in the bronchoalveolar lavage (BAL) fluids from asthmatics. Intra-nasal ApoA1 was treated (D24,25) after challenged ovalbumin (OVA, D22,23,24) in an established murine model of asthma. Mild to moderate asthmatics had significantly less ApoA1 in BAL fluid than healthy controls. In a murine model of asthma, ApoA1 suppressed the cardinal features of asthma when given after OVA challenge. ApoA1 significantly decreased lung IL-25, IL-33 levels as well as other Th2 cytokines.ApoA1 inhibits the production of IL-25, IL33, and CCL20 in the allergen treated cultured primary bronchial epithelial cells. ApoA1 also increased production of lipoxin A4 (LXA4)in the OVA challnged lung and promote restore the allergen induced disrupted tight junctions proteins Zo-1 and occludin in the bronchial epithelium.Our data demonstrate that ApoA1 regulate both initiation and resolution of airway inflammation. The mechanism includes down regulation of IL-25, IL-33 and CCL20 expression in the epithelium which promote Th2 response. ApoA1 regulate pro-resolution mediator LXA4 and tight junction proteins production. Together, ApoA1 could be therapeutic strategy for chronic airway inflammation such as asthma.