380. Late-Breaking Abstracts Session: New mechanisms in airway disease

3407

Late-breaking abstract: Comparison of bone marrow derived-mononuclear cells with mesenchymal stem cells on inflammatory and remodeling processes in experimental chronic allergic asthma

Soraia Abreu[1], Mariana Antunes[1], Debora Xisto[1], Milena Vasconcellos[1], Julia Crossetti[1], Vera Capelozzi[2], Marcelo Morales[3], Patricia Rocco[1]. *[1]Laboratory of Pulmonary Investigation, Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; [2]Department of Pathology, Faculty of Medicine, University of Sao Paulo, Sao Paulo, Brazil; [3]Laboratory of Cellular and Molecular Physiology, Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*

Recently, we demonstrated the beneficial effects of bone marrow-derived mononuclear cells (BMDMCs) on lung morphofunction in experimental chronic allergic asthma. Mesenchymal stem cells (MSCs) have the potential to serve as a universal source for replacement of specific cells in several diseases and thus may offer hope as a potential therapeutic intervention for the treatment of the chronic remodeling changes that occur in asthma. This study aimed to compare the therapeutic effects of BMDMCs with MSCs on inflammatory and remodeling processes in experimental chronic allergic asthma. Thirty-six C57BL/6 mice were assigned to two groups. In the OVA group, mice were sensitized and repeatedly challenged with ovalbumin. Control mice (C) received saline under the same protocol. C and OVA groups were further randomized to receive saline (SAL), BMDMCs or MSCs (1×10^5) intratracheally 24 h after the last challenge. After one week, airway resistance, viscoelastic pressure, static elastance, as well as the degree of lung inflammation and remodeling (light microscopy and immunohistochemistry) were analyzed. BMDMC and MSC therapies led to a reduction in eosinophil infiltration and fibrosis in airway and lung parenchyma compared to OVA leading to a reduction in airway resistance and viscoelastic pressure. These parameters were more reduced after BMDMCs compared to MSCs. In conclusion, in the present model of chronic allergic asthma, both BMDMC and MSC therapies were effective at modulating the inflammatory and fibrogenic processes, however, BMDMC administration led to greater beneficial effect.

Supported by: PRONEX, CNPq, FAPERJ, CAPES.

3408

Late-breaking abstract: Changes in cortisol levels in the plasma of asthmatic individuals undergoing allergen inhalation challenge differentiate isolated early from dual responders

Scott Tebbutt[1], Sarah Kam[1], Amrit Singh[1], Louis-Philippe Boulet[2], Mark FitzGerald[3], Gail Gauvreau[4], Paul O'Byrne[4]. *[1]The James Hogg Research Centre, University of British Columbia, Vancouver, BC, Canada; [2]Centre de Pneumologie de l'Hopital Laval, Universite Laval, Sainte-Foy, QC, Canada; [3]Vancouver Coastal Health Research Institute, University of British Columbia, Vancouver, BC, Canada; [4]Department of Medicine, McMaster University, Hamilton, ON, Canada*

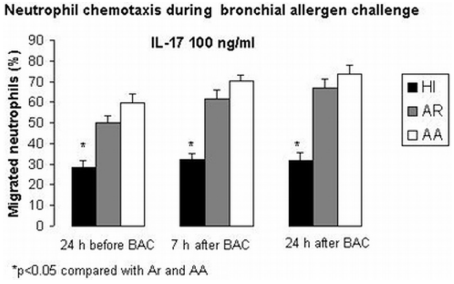
Allergen inhalation challenge in mild asthmatic subjects induces airflow obstruction, airway hyperresponsiveness and inflammation, and provides a model for hypothesis-generating experiments to understand molecular regulation of these responses. Adult asthmatic subjects (18-55 years of age, with stable, mild allergic asthma, n=14) underwent cat allergen inhalation challenges. All subjects had an early asthmatic response of ≥20% fall in FEV1, and six individuals also had a late phase response of ≥15% fall in FEV1 (dual responders). Blood samples were collected just prior to, and two hours after allergen challenge. We have evaluated the differential changes in genome-wide gene expression in peripheral blood cells and changes in the plasma metabolome, post-challenge compared to pre-challenge. Amongst other findings, we have demonstrated significantly reduced cortisol levels in the plasma of mild asthmatic subjects post-challenge, compared to pre-challenge (p=0.013). Importantly, this reduction in plasma cortisol was only significant in subjects who had an isolated early asthmatic reaction, rather than in subjects who also went on to develop a late phase asthmatic reaction. Interestingly, this is consistent with gene expression data demonstrating that in isolated early responders only, there is a significant increase in RNA transcript levels for the hydroxysteroid (11-beta) dehydrogenase 2 gene (HSD11B2), post-challenge compared to pre- (p=0.026). The HSD11B2 enzyme converts cortisol to inactive cortisone. Thus, allergen inhalation challenge may improve understanding of pathways and underlying genes associated with asthma.

3409

Late-breaking abstract: Interleukin 17 expression and peripheral blood neutrophils chemotaxis in patients with allergic rhinitis and asthma challenged with *D. pteronyssinus*

Leva Bajoriuniene[1], Kestutis Malakauskas[2], Simona Lavinskiene[2], Edita Gasiuniene[1], Jolanta Jeroch[2], Astra Vitkauskiene[3], Raimundas Sakalauskas[1]. *[1]Pulmonology and Immunology, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania; [2]Institute for Biomedical Research, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania; [3]Laboratory Medicine, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania*

Background: Increasing evidence suggests that interleukin (IL)-17 is important in neutrophil migration and function. A mouse-model study showed that allergen inhalation enhanced IL-17 expression and recruitment of neutrophils into inflammed airways. But the role of IL-17 in patients with allergic airway diseases is unknown.
Aim: To investigate influence of bronchial allergen challenge (BAC) with *D. pteronyssinus* (DP) on IL-17 expression and peripheral blood (PB) neutrophils chemotaxis in patients with allergic rhinitis (AR) and asthma (AA).
Methods: 8 AR, 4 AA patients and 6 healthy individuals (HI) were investigated. All patients had positive skin prick tests to DP and underwent BAC with standardized extracts of DP. Serum IL-17 concentration was analyzed by ELISA. Neutrophils were isolated from PB and chemotaxis was investigated using different concentrations of IL-17 measured by FACSCalibur cytometer 24 h before, 7 h and 24 h after BAC.
Results: Serum IL-17 levels and PB neutrophils chemotaxis were significantly higher in AR and AA compared to the HI before and after BAC (figure). Serum IL-17 levels significantly correlated with the PB neutrophils chemotaxis (r=0.67, p=0.043).



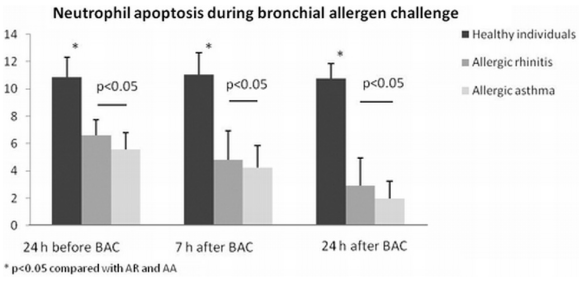
Conclusion: IL-17 levels and PB neutrophil chemotaxis were increased in allergic patients challenged with DP. IL-17 is significant regulating neutrophil chemotaxis in patients with AR and AA.

3410

Late-breaking abstract: Bronchial allergen challenge with *D. Pteronyssinus* inhibit blood neutrophil apoptosis in patients with allergic asthma and rhinitis

Simona Lavinskiene[1], Jolanta Jeroch[1], Kestutis Malakauskas[2], Ieva Bajoriuniene[2], Edita Gasiuniene[2], Jurgita Jackute[2], Raimundas Sakalauskas[2]. *[1]Institute for Biomedical Research, Medical Academy, Lithuanian University of Health Science, Kaunas, Lithuania; [2]Department of Pulmonology and Imunology, Hospital of Lithuanian University of Health Sciences, Kaunas, Lithuania*

Background: Recent investigations suggest that neutrophils can play important role in the pathogenesis of allergic asthma and rhinitis.
Aim: To evaluate blood neutrophil apoptosis in patients with allergic asthma and rhinitis provoked with specific allergen.
Methods: Eight patients with allergic rhinitis (AR), four - with allergic asthma (AA) and six - healthy individuals (HI) were involved in to the study. All AR and AA patients had positive skin prick tests to *D. Pteronyssinus* and underwent bronchial allergen challenge (BAC) with standardized extracts of *D. Pteronyssinus*. Neutrophils were isolated from peripheral blood by density gradient centrifugation and annexin V-FITC was used to detect early apoptotic cells. Apoptosis was measured using FACSCalibur cytometer 24 h before BAC, 7 h and 24 h after BAC.



Results: Neutrophil apoptosis before and after BAC was more intensive in HI group compared to patients with AR and AA (table). Neutrophil apoptosis in AA group was more inhibited after BAC in comparison with AR group.
Conclusions: Our preliminary results show that neutrophil apoptosis was decreased in patients with AA and AR compared to HI before and after BAC. This finding let us suggest, that specific allergen has inhibitory effect on neutrophil apoptosis and neutrophils may play an important role in allergic inflammatory process.

3411
Late-breaking abstract: House dust mite triggering of Dectin-2 is critical for the initiation of allergic airway inflammation
Deborah Clarke[1], Martyn Foster[2], Nicola Davis[1], Stephanie Heasman[3], Arthur Lewis[3], Ian Anderson[1], Matthew Sleemam[1], Richard May[1], Matthew Robinson[1]. *[1]Department of Respiratory, Autoimmunity and Inflammation, MedImmune Ltd, Cambridge, United Kingdom; [2]Department of Pathology, Safety Assessment UK, AstraZeneca R&D Charnwood, Loughborough, Leicestershire, United Kingdom; [3]Research Histology, MedImmune Ltd, Cambridge, United Kingdom*

Introduction: How the immune system senses aeroallergens and triggers an aberrant inflammation is poorly understood. Dectin-2 is a house dust mite (HDM)-sensing pattern recognition receptor, and its expression on dendritic cells is required for the Th2-skewed adaptive response to HDM.
Objective: To define the role of Dectin-2 in HDM-induced allergic airway inflammation and its expression in biopsies from asthmatic patients.
Results: In a 3 week mouse model of repeated intranasal HDM challenge, prophylactic antibody blockade of Dectin-2 potently attenuated the characteristic allergic inflammation and airway hyperresponsiveness. Anti-Dectin-2 did not reduce the inflammation once established, but did prevent neutrophil influx following a single HDM challenge. Together these data suggest a non-redundant role for Dectin-2 in the initiation of the HDM response.
To define the mechanism by which Dectin-2 triggers HDM-induced inflammation, we conducted ex vivo experiments using cultured alveolar macrophages. These indicated that Dectin-2 was necessary for the induction of cysteinyl leukotrienes, as reported for dendritic cells, but not chemokines or cytokines. Furthermore we demonstrated in our single challenge model that zileuton, an inhibitor of leukotriene production, produced a similar effect as Dectin-2 blockade.
Finally we found a marked increase in the number of Dectin-2 positive cells in bronchial biopsies from asthmatic subjects when compared to normal controls.
Conclusions: Alveolar macrophage sensing of HDM by Dectin-2 elicits the production of cysteinyl leukotrienes, and this axis is key for the initiation of allergic airway inflammation. Dectin-2 is associated with asthma.

3412
Late-breaking abstract: Dysregulation of allergic airway inflammation in the absence of microbial colonization
Anke Sichelstiel[1], Tina Herbst[2], Nicola Harris[2], Benjamin Marsland[1]. *[1]Service de pneumologie, CHUV, Lausanne, Switzerland; [2]Global Health Institute, EPFL, Lausanne, Switzerland*

Rationale: The incidence of allergic disorders is increasing in developed countries and has been associated with reduced exposure to microbes and alterations in the commensal bacterial flora.
Objectives: To ascertain the relevance of commensal bacteria upon the development of an allergic response, we utilized a model of allergic airway inflammation in germ-free (GF) mice that lack any exposure to pathogenic or non-pathogenic microorganisms.
Methods: Allergic airway inflammation was induced in GF, specific pathogen free (SPF) or recolonized mice by sensitization and challenge with ovalbumin (OVA). The resulting cellular infiltrate and cytokine production were measured.
Measurements and main results: Our results show that the total number of infiltrating lymphocytes and eosinophils were elevated in the airways of allergic GF mice as compared to control SPF mice, and that this increase could be reversed by re-colonization of GF mice with the complex commensal flora of SPF mice. Exaggerated airway eosinophilia correlated with increased local production of Th2 associated cytokines, elevated IgE production and an altered number and phenotype of conventional dendritic cells (cDC). Regulatory T cell populations and regulatory cytokine levels were unaltered but GF mice exhibited an increased number of basophils and decreased numbers of alveolar macrophages (AM) and plasmacytoid dendritic cells (pDC).

3413
Late-breaking abstract: Relationship with hemoglobin A1c and exacerbations of COPD. A preliminary study
Haruka Aoki, Takeshi Hisada, Hiroaki Tsurumaki, Reiko Yoshino, Hidemasa Kuwabara, Tamotsu Ishizuka, Kunio Dobashi, Masatomo Mori. *Department of Medicine and Molecular Science, Gunma University Graduate School of Medicine, Maebashi, Japan*

Background and objective: Hyperglycaemia during hospital admission is associated with poor outcomes in patients admitted with acute myocardial infarction, stroke and pneumonia. Systemic inflammation may represent a possible cause of glycometabolic disorder. We studied that associtation Hemoglobin A1c with an increased risk of acute exacerbation of COPD (AECOPD).
Methods: Patients with COPD were prospectively enrolled and followed between 2010 and 2011. Medical records, HbA1c, fasting glucose and metabolic markers were assessed in 29 patients.
Results: A total of 29 patients (mean age of 78.2 years) were recruited, 11 with AECOPD and 18 without. Clinical data were collected from the patients. HbA1c levels of AECOPD patients were significantly higher on entry point compared with other patients (5.783±0.2638; 4.983±0.1778, respectively p=0.0361). Moreover, there was a trend for increased length of stay and frequency of admissions in patients with higher levels of HbA1c. (4.5%<HbA1c≤5%; 5%<HbA1c≤5.5%; 5.5%<HbA1c≤6%; HbA1c>6%, 15days (0.9/year (y)); 26days (1/y); 23days (1.3/y); 30days (2.3/y), respectively)
Conclusion: Previous study revealed that comorbid diabetes prolongs length of stay and increases risk of death in patients with AECOPD. However, less evidence exists for relationship of HbA1c with AECOPD. For the first time our study demonstrates that HbA1c is a prognostic factor associated with AECOPD. Taken together with a previous study that revealed a similar trend, our study suggests that further studies are now required to elucidate the reasons for these poorer outcomes, in particular whether premorbid glycaemic control or inpatient control is responsible, as these are potentially modifiable factors.

3414
Late-breaking abstract: Further studies on the mechanism of action of doxofylline
Thierry Jolas[1], Clive Page[2], Luigi Allegra[3]. *[1]Scientific Director, CEREP, Celle l'Evescault, France; [2]Sackler Institute of Pulmonary Pharmacology, Institute of Pharmaceutical Science, King's College London, London, United Kingdom; [3]Pneumology, IRCCS Policlinical Hospital, Milan, Italy*

Xanthines such as theophylline have been used in the treatment of lung diseases since the early 1900's, but have a major drawback of a very narrow therapeutic window and many drug/drug interactions. This means that regular plasma levels often have to be obtained and can make the use of theophylline problematic. With the increasing availability of other classes of drugs for the treatment of respiratory diseases, this has limited the use of xanthines, despite their clean clinical benefit in the treatment of patients with asthma and COPD. Doxofylline is a xanthine molecule having both bronchodilator and anti-inflammatory activity, with an improved therapeutic window over conventional xanthines such as theophylline. However, the mechanistic basis of this improved therapeutic window is not understood. The present study has investigated the ability of doxofylline to inhibit human recombinant PDE and HDAC enzymes, and bind to adenosine receptor assays *in vitro* in comparison with theophylline. Theophylline had a significant effect on adenosine receptor binding that was not shared by doxofylline. Our results suggest that doxofylline may have a wider therapeutic window than theophylline due to a lack of adenosine receptor antagonism. Neither drug had any significant inhibitory effect on HDAC enzymes over a wide range.
Additionally, in contrast to doxofylline, theophylline showed a significant effect on PDE3. It has been suggested that the ability of theophylline to inhibit PDE3 may contribute to the unwanted cardiovascular effects observed at higher serum concentrations. The lack of effect of doxofylline on PDE3 may also help explain its improved tolerability profile on the cardiovascular system.