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

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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 (1×10^7) intra-tracheally 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, FAPEI, CAPEES.

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

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Allergic 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 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 with mesenchymal stem cells 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 (1×10^7) intra-tracheally 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.

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

Leva Bajoriuniene1, Kestutis Malakauskas2, Simona Lavinskienė2, Edita Gasiuniene1, Jolanta Jeroch1, Astra Vinkuskiene1, Raimundas Sukalaukas3,4, 1Laboratory of Pulmonology and Immunology, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania; 2Institute for Biomedical Research, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania; 3Laboratory 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 inflamed 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 neutrophils chemotaxis were increased in allergic patients challenged with DP. IL-17 is significant regulating neutrophil chemotaxis in patients with AR and AA.

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

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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 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-FTIC was used to detect early apoptotic cells. Apoptosis was measured using FACSCalibur cytometer 24 h before BAC, 7 h and 24 h after BAC.

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

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

Leva Bajoriuniene1, Kestutis Malakauskas2, Simona Lavinskienė2, Edita Gasiuniene1, Jolanta Jeroch1, Astra Vinkuskiene1, Raimundas Sukalaukas3,4, 1Laboratory of Pulmonology and Immunology, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania; 2Institute for Biomedical Research, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania; 3Laboratory Medicine, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

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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.

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

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**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 cysteinyi 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 cysteinyi 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

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**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 re-colonized mice by sensitization and challenge with ovalbumin (OVA).

**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 cysteinyi 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 cysteinyi leukotrienes, and this axis is key for the initiation of allergic airway inflammation. Dectin-2 is associated with asthma.

### 3413 Late-breaking abstract: Relationship with hemoglobin A1c and exacerbations of COPD. A preliminary study

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**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 association 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 not different from the patiens levels of AECOPD patients were significantly higher on entry point compared with other patients (5.78±0.2638; 4.98±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 (1y); 23days (1.3y); 30days (2.3y), 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.