255. Asthma and COPD: understanding through mechanisms

P2300

T-reg cells levels in smoking and non-smoking patients with asthma

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Background: Tobacco smoking is associated with severity of asthma, response to basis therapy and control achievement. Cigarette smoke reduces the pool of T-regulatory cells in healthy smokers but the role of T-regs in the development of allergic diseases still unclear.

Objective: To assess the level of CD4 + CD25high T-reg in healthy and asthmatic with different status of smoking.

Materials and methods: Were enrolled 55 patients with asthma: mild asthma n=14, (34,5 (32,0:40,0) years), mean FEV1=100,9 (94,3:111,5)%, moderate asthma (n=20), (54,5 (50,5:60,5) years), FEV1=80,24 (72,4:89,5)%, severe asthma (n=21), (45,0 (41,0:45,0) years), FEV1=66,6 (48,9:70,7)%, and 17 healthy (30,0 (28,0:35,0) years), FEV1=104,0 (98, 0:111,0)%. Peripheral blood mononuclear cells were isolated in Ficoll density. To study the phenotypic characteristics of regulatory T cells was assessed variation of CD markers (CD4 +, CD25 +) by flow cytometry (FACSCalibur Becton Dickinson, USA) using appropriate monoclonal antibodies.

Results and discussion: The levels of CD4 + CD25high peripheral blood was lower in smokers (0,84 (0,59:1,20)%) compared with nonsmokers (2,07 (1,20: 2,92)%) ($p < 0,05$). In current smokers patients and patients with history of smoking the levels of CD4 + CD25high peripheral blood were lower (0,62 (0,22:0,94)) compared with nonsmokers (2,20 (0,71:3,47)) ($p < 0,05$). A negative correlation between pack-years and the level of CD4 + CD25high ($r = -0,51$; $p < 0,05$) in healthy volunteers was shown.

Conclusion: Cigarette smoke may have an independent influence on asthma course not only as trigger (irritant) but also as factor that leads to reducing the population of CD4 + CD25high T-reg and inadequate suppression of Th2-response.

P2301

Peripheral Thelper1/Thelper2/Thelper17/regulatory T cell imbalance in asthmatic pregnancy

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Asthma and pregnancy show bilateral clinical interactions with mostly unknown immunological mechanisms. Healthy gestation is characterized by a sensitive balance of Th1/Th2/Th17/regulatory T (Treg) cells which may be altered in asthmatic pregnancy. The aim of this study was to describe the prevalence of these cell subsets in asthmatic compared with healthy pregnancy.
The prevalence of Th1, Th2, Th17 and Treg lymphocytes was identified by cell surface and intracellular marker staining in 24 healthy non-pregnant (HNP), 23 healthy pregnant (HP), 15 asthmatic non-pregnant (ANP) and 15 asthmatic pregnant (AP) women using flow cytometry.
HP and ANP were characterized by increased Th2/Th1 ratio compared to HNP, but no further increase was observed in AP. Healthy pregnancy increased Treg cell prevalence compared with HNP data (4.64% vs. 2.98%; $p<0.05$), and this pregnancy-induced elevation was absent in AP women (2.52% vs. HP; $p<0.05$). Th17 cell prevalence was similar in the HP and HNP groups (2.78% vs. 3.17%; $p>0.05$). Asthma increased Th17 prevalence in non-pregnant patients (3.81% vs. HNP; $p<0.05$), and this asthma specific increase of Th17 cell prevalence was also observed in AP patients (AP vs. HP: 3.44% vs. 2.78%; $p<0.05$). As a result, Th17/Treg ratio was decreased in HP, but not in AP women, compared with HNP data.
Peripheral Thelper1/Thelper2/Thelper17/regulatory T cell imbalance may play a role in the interrelationship and compromised immune tolerance characterizing asthmatic pregnancy.

P2302

Activin-A induces human regulatory T cells that control allergic asthma

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Activin-A is a cytokine involved in essential biological processes.Our previous studies have uncovered activin-A as a controller of experimental asthma through the induction of mouse regulatory T cells (Tregs).Here, we studied the role of activin-A in the induction of human Tregs suppressive against allergic responses in asthmatics.Peripheral blood CD4+ T cells were stimulated with a clinically-relevant allergen in the presence of activin-A (or control).The phenotype of activin-A-treated T cells and their suppressive function on human T cell responses and in asthma protection in a humanized mouse model were investigated.Our data reveal that activin-A greatly inhibits human T cell proliferation and Th2 cytokine release.Activin-A-treated T cells remain hyporesponsive after allergen restimulation and do not express effector cytokines. Still, they express significantly increased amounts of immunosuppressive IL-10.Notably, activin-A-treated T cells are suppressive against allergic responses of atopics and asthmatics, pointing to the generation of a Treg subset (act-A-iTregs). Act-A-iTregs also restrain Th2 responses in the bronchoalveolar lavage (BAL) of severe asthmatics.Using a humanized model of asthma, we show that, co-transfer of act-A-iTregs with human T effectors confers protection against asthma in vivo, as shown by greatly decreased airway hyperresponsiveness, BAL, lung inflammation and Th2 responses in the lungs and draining lymph nodes.Our data reveal that activin-A generates IL-10-producing Tregs that suppress human allergen-driven responses and protect against asthma.Our findings may facilitate the use of act-A-iTregs in adoptive-transfer therapies aiming to re-establish tolerance in asthma.

P2303

4-1BBL mediated balance of Th17/Treg in patients with allergic asthma

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Objective: To investigate the expression of 4-1BBL in peripheral blood of patients with allergic asthma and its role in the balance of Th17/Treg cells.
Methods: We detected the plasma soluble 4-1BBL (s4-1BBL) levels with ELISA and membrane expression of 4-1BBL (m4-1BBL) on monocytes with flow cytometry in patients with allergic asthma and controls. The peripheral blood mononuclear cells (PBMCs) of patients with asthma were in vitro stimulated with or without 4-1BB agonistic mAb for 72h, the concentrations of IL-17 and TGF-β in supernatant were measured with ELISA and the percentages of Th17 and Treg cells were measured with flow cytometry.
Results: The s4-1BBL concentrations of patients with asthma (202.47±60.68 μg/L) were decreased than controls (298.29±40.32 μg/L, $p<0.01$). The m4-1BBL on monocytes of patients with asthma (2.69±1.85%) was decreased than the controls (9.06±6.79%, $p<0.01$). The supernatant concentrations of TGF-β (655.81±476.26 ng/L) and IL-17 (5202±4143.74ng/L) in PBMCs stimulated with 4-1BB mAb were different from the isotype controls (TGF-β: 410.50±368.03 ng/L, IL-17: 8377±3839.98 ng/L, both $p<0.05$). There was a lower proportion of Th17 cells (1.298±0.53% vs 1.536±1.01%) and a higher proportion of Treg cells (3.45±1.03% vs 2.76±0.97%) in the 4-1BB mAb group than isotype control, but no difference between the two groups ($p>0.05$).
Conclusions: Both s4-1BBL and m4-1BBL decreased in peripheral blood of patients with allergic asthma. In vitro agonistic 4-1BB antibody stimulation enhanced TGF-β but inhibited IL-17 production by PBMCs. Thus 4-1BBL/4-BB may restore Th17/Treg balance in allergic asthma more likely by changing the function of Th17 and Treg cells.

P2304

Efficacy of basis therapy and level of immunoregulatory T-cells (T-regs) in COPD

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Background: COPD is heterogeneous disease with variable clinical and radiographic signs, different response to therapy and rate of decline in lung function and survival of patients. The levels of T-regs could influence on the efficiency of the basis therapy of COPD.
Aim and objectives: Compare the data of COPD clinical course dynamics during basic therapy and levels of inducible (CD4+FOXP3+) T-regs.
Methods: Were included 60 patients with COPD stages II-IV, mean age was 57,8±1,09 years, m/f=50/10, mean duration of disease was 10,6±1,05 years. The observation period was 24 weeks after administration of appropriate basis therapy (GOLD reccomendations). At visits were performed spirometry, index BODE, SGRQ. Peripheral blood mononuclear cells were isolated from heparinized blood on Ficoll density gradient (1,077 g/ml). The circulating percentage of CD4+FoxP3+ T-regs in peripheral blood was estimated by the flow cytometry analysis (FACSCalibur Becton Dickinson, USA) using appropriate monoclonal antibodies.
Results: Persons with the levels of CD4+FoxP3+ T-regs cells less than 7% in peripheral blood, were characterized by significant reductions in SGRQ-scores from 53,29±0,36 points at visit 1 to 48,24±4,13 points at visit 2 ($p<0,05$); stable increase in FEV1 from 37,7±3,08% (visit 1) to 46,63±3,39% (visit 2) ($p<0,05$); decreased BODE index from 4,10±0,37 points (visit 1) to 3,46±0,43 points (visit 2) ($p<0,05$). COPD patients with the the level of CD4+FoxP3+ more then 7% didn't have the positive dynamics of clinical and functional parameters as a result of a 24-week treatment ($p>0,05$).
Conclusions: High level of CD4+FoxP3+ T-regs (>7%) is associated with impaired response to basis therapy COPD.

P2305

Peripheral blood neutrophil activity during D. pteronyssinus induced late-phase airway inflammation in asthma and rhinitis patients

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Background: Recent investigations suggest that neutrophils may play an important role in the late-phase allergen-induced inflammation in allergic airway diseases.
Aim: To evaluate neutrophil chemotaxis, phagocytosis, and reactive oxygen species (ROS) production in patients with allergic asthma and rhinitis challenged with inhaled D. pteronyssinus.
Methods: Twenty eight patients with allergic asthma and 27 with rhinitis, all sensitized to D. pteronyssinus, as well as 10 healthy subjects underwent bronchial challenge with D. pteronyssinus. Neutrophils from peripheral blood were isolated 24 h before as well as 7 h and 24 h after challenge. For chemotaxis analysis neutrophils were stimulated with interleukin-8, and for ROS analysis as well as for phagocytosis cells were stimulated with S. aureus bacteria. Neutrophil functions were analyzed flow cytometrically.

Results: Neutrophils chemotaxis and ROS production were increased, while phagocytosis was decreased 24 h before challenge in patient groups compared with healthy subjects ($P<0.05$). After challenge, neutrophil chemotaxis and phagocytosis increased after 7 h and 24 h, when ROS production – only after 24 h. Bronchial allergen challenge had no influence for neutrophil functions in healthy subjects ($P<0.05$).
Conclusions: Results show that peripheral blood neutrophil activity is impaired in allergic asthma and rhinitis patients. D. pteronyssinus induced late-phase airway inflammation enhance their chemotaxis, phagocytosis and ROS production.

P2306
Upregulation of myeloid derived suppressor cells (MDSCs) in chronic obstructive pulmonary disease and its relationship with disease severity
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MDSCs have received growing interest as suppressors of immune responses in cancer, induced in the attempt to escape immune surveillance. MDSCs have been recently implicated in immune modulation in chronic inflammatory diseases, particularly autoimmune. Since we proposed an autoimmune component in COPD, we examined the induction of MDSCs in peripheral blood of smokers with COPD with or without lung cancer. In particular, we evaluated the α chain of the IL-4 receptor (IL4Rα, which has been proposed as a marker for MDSCs) in patients with COPD (n=32, 8 with concomitant cancer) compared to subjects with a similar smoking history who did not develop COPD (n=8) and non-smokers (n=10). The expression of IL-4Rα was increased in monocytes from smokers with COPD (17±2%) compared to smokers without COPD (10±1%) and non-smokers (9±1%; $p<0.05$ for both). This increase was particularly evident in COPD patients with concomitant cancer (23±3%) but was also present in those without cancer (16±1%). A similar IL-4Rα pattern was observed in the granulocytic fraction of blood leukocytes (8±1 vs 3±2 vs 4±2%). Of note, IL4Rα expression was not linked to smoking status or cumulative history, but was correlated with the degree of airflow limitation ($p=0.0003$,$r=0.55$). In conclusion, our study shows that IL4Rα expression is upregulated in smokers with COPD, either with or without lung cancer, but not in smokers who despite a similar smoking history did not develop the disease. These results indicate that the upregulation of MDSCs observed in patients with COPD is not due to smoking itself, but is rather related to the severity of the disease.
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P2307
Expression of matrix metalloproteinases and TIMP inhibitors in circulation/lung in patients with chronic obstructive pulmonary disease
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In chronic obstructive pulmonary disease (COPD), increased expression of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) has been repeatedly reported. Some of MMPs/TIMPs, e.g. MMP9, have been investigated both in serum and lung; others were measured only in one of these localisations.
To extend the profile of MMPs/TIMPs systemic/local expression in COPD, we created two pilot cohorts of COPD patients and control healthy subjects, both comprising 20 subjects. Protein expression of MMP2,8,9 and TIMP1,2,3,4 was determined in parallel in serum and in BronchoAlveolar Lavage Fluid (BALF) by a microsphere multiplex assay. To complement protein measurements, mRNA expression was evaluated in BAL cells which were available from 13 COPD patients and 18 controls.
Compared with controls, in patients MMP9 levels were increased systemically (serum protein: $p=0.03$) as well as locally (BALF protein: $p<0.001$, mRNA in BAL cells: $p=0.002$). MMP2 and 8 mRNA were upregulated in BAL cells ($p=0.001$ and $p=0.03$, respectively); MMP8 protein was elevated in BALF ($p<<0.001$). Serum concentrations of TIMP4 paralleled increase of MMP2 and 9 mRNA in BAL cells ($p=0.005$ and $p=0.007$, respectively). The number of TIMP1 transcripts correlated with the number of months free of exacerbation(s) during 2-years follow-up after the BAL sampling ($p=0.03$)
In conclusion, distinct expression profiles of MMPs/TIMPs were observed at systemic and local level in our COPD patients. These pilot data will be subject to further extension and verification, including subanalyses according to the GOLD stage.
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P2308
Circulating mature and progenitor eosinophils in patients with stable asthma express all major traffic related receptors
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Background: Eosinophils differentiate in the bone marrow from CD34+ cells released to the blood with possible traffic to the lung tissue. The known data about eosinophil traffic mostly refer to mature eosinophils and have originated mainly from animal models or from asthma patients after allergen exposure. Thus, we investigated whether mature and progenitor blood eosinophils in patients with stable asthma express similar trafficking related receptors.
Methods: Participants, 13 patients with stable asthma; 7 with high (≥0.3x109/L) and 6 with low (≤0.2x109/L) blood eosinophils, and 5 healthy controls were selected from the West Sweden Asthma Study. Airway eosinophils were studied in induced sputum. Mature (CD45+IL-5Rα+SSChigh) and progenitors (CD45+CD34+IL-5Rα+SSClow) eosinophils and their expression of selectin (PSGL-1), integrins (VLA-4:CD49d+CD29+, Mac-1:CD11b+CD18+), eotaxin(s) receptor (CCR3+), and activation (CD69+, CD25+) were quantified in fresh blood by flow cytometry.
Results: Asthma patients with high blood eosinophils had increased sputum eosinophils and blood eosinophil progenitors compared to the healthy controls ($p<0.05$). Mature and progenitor eosinophils expressed similar levels of PSGL-1 and VLA-4. Mac-1 was expressed in all mature eosinophils but was reduced in progenitors, in all groups (<0.01). Mature eosinophils expressed high levels of CCR3 compared to progenitors ($p<0.05$). However, the CCR3+ eosinophil progenitors were more activated in all groups (<0.01).
Conclusion: Both mature and progenitor blood eosinophils in patients with stable asthma express all major trafficking related receptors important for transfer into the lung tissue.

P2309
A comparison between the diagnostic value of bronchodilator response in spirometry and questionnaire in determining asthma
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Background and aim: Because of unknown physiopathology of asthma the diagnosis of this common respiratory disorder is a challenging issue. In this study we compared the usefulness of a short questionnaire and response to bronchodilator in spirometry for differentiating asthma from other causes of chronic dyspnea.
Method: 208 patients suffering from chronic dyspnea (>6 months) and had definite clinical diagnosis of asthma, chronic obstructive pulmonary disease, pulmonary fibrosis or bronchiectasis were enrolled. A questionnaire was designed by using the questions showed the best sensitivity and specificity in previous researches for diagnosing asthma. 9 of 43 questions were selected for final questionnaire by regression analysis. All of the patients were interviewed to complete questionnaire and spirometric response to bronchodilator was assessed. SPSS 18 and EPI 6 software were used for statistical analysis.
Results: 53.8% of cases had asthma. In determining asthma, bronchodilator test had 48.2% sensitivity, 78.1% specificity, 72% and 56.4% positive and negative predictive values, 2.2 and 0.66 positive and negative likelihood ratios, 21.9% and 51.8% false positive and negative error rates and 62.01% accuracy. 9-question questionnaire showed 97.3% sensitivity, 77.1% specificity, 83.2% and 96.1% positive and negative predictive values, 4.24 and 0.03 positive and negative likelihood ratios, 22.9% and 2.7% false positive and negative error rates and 87.98% accuracy.
Conclusion: According to our findings, this 9-question questionnaire has better diagnostic values than response to bronchodilator in spirometry for defining asthma between patients with chronic dyspnea.

P2310
T regulatory cells in exacerbation control of chronic obstructive pulmonary disease of mild and moderate stage
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Aim: To investigate the levels of peripheral CD4(+)CD25(++) regulatory T cells and the inflammatory and anti inflammatory cytokines in exacerbation dynamics of mild and moderate stage of chronic obstructive pulmonary disease (AECOPD) patients.
Methods: Peripheral blood samples were collected from 20 patients with mild AECOPD and 21 patients with moderate AECOPD, aged (55,6±3,2; 51±3 respec-

tively) and 20 man from control group. Lymphocytes were isolated by two-color labeled monoclonal antibodies flow cytometry to examine the quantities and percentage of CD4+CD25+ T cell, CD4+CD25++ (CD4(+)Treg), CD3+CD19- T cell, CD3+CD4+ T cell, CD3+CD8+ T cell, respectively. Enzyme immunodetection was used to detect the expression of interleukin-10 (IL-10), tumor necrosis factor alpha (TNF-alpha), procalcitonin (PCT) and C-reactive protein (CRP). Mann-Whitney test was used for comparison between data before and after treatment and control group.
Results: In patients with mild and moderate AECOPD take place significant decreasing of Tregulatory cells (CD4+CD25++) from 3,3±0,8% in control to 1,13±0,17% and 1,3±0,12%%, respectively ($p<0,01$). Interestingly that percentage of Treg didn't change in dynamics of conventional therapy. Reliable increase of TNF-α and decrease of PCT take place in both groups ($p<0,01$). Nevertheless CRP and Il-10 levels of patients with COPD don't differ from the same values of control group.
Conclusion: The decrease of Treg response and the lost balance between inflammatory/antiinflammatory cytokines, suggest a lack of regulation of the systemic inflammatory response that may contribute to pathogenesis in AECOPD of mild and moderate stage.

P2311
The declined CD4+ CD25+Treg cells in patients with moderate to severe asthma associated with over-expressed Th2 response
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Background: Recent studies have showed that Th2 cells can induce the apoptosis of CD4+ CD25+ Treg cells or resist the immunosuppressive effect of Treg cells. We hypothesize that an imbalance of Th2/Treg is presented in patients with allergic asthma.
Methods: Twenty-two patients with mild asthma, 17 patients with moderate to severe asthma and 20 healthy donors were enrolled. All patients were allergic to house dust mites. The proportions of peripheral blood CD4+CD25+ Treg cells and Th2 cells were determined by flow cytometry. The concentration of IL-10, TGF-β and IL-4 in plasma was determined by ELISA. The expression of Foxp3 and GATA-3 mRNA in PBMCs from asthmatic patients and healthy donors was detected by RT-PCR.
Results: Compared with healthy donors and patients with mild asthma, the frequency of CD4+CD25+ Treg cells and plasma IL-10 levels were decreased in patients with moderate to severe asthma. There was no difference of Foxp3 mRNA expression among three groups. However, the frequency of Th2 cells, IL-4 levels and expression of GATA-3 mRNA was higher in patients with mild and moderate to severe asthma than in the control group. The ratio of Th2/Treg and their cytokines was increased in allergic asthma, especially for moderate to severe asthma. The ratio of GATA-3/Foxp3 mRNA was increased in allergic asthma. In patients with moderate to severe asthma, the frequency of peripheral blood Treg cells was negatively correlated to the percentage of Th2 cells and IL-4 levels.
Conclusions: The decline of Treg cells in patients with moderate to severe asthma may play an important role in progress of the disease.

P2312
Peripheral blood Th17 cells and serum IL-17 levels in patients with *D. pteronyssinus*-induced late-phase asthmatic response
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Background: Biphasic cellular immune reaction which follows inhalation of allergen is the specific feature of allergic inflammation. Therefore, Th17 cells and IL-17 may have played a role in the development of the late-phase asthmatic response in patients with allergic asthma.
Objectives: To evaluate the percentage of Th17 cells in peripheral blood (PB) and serum IL-17 levels in patients with *D. pteronyssinus*-induced late-phase asthmatic response.

Table 1. Peripheral blood Th17 cells and serum IL-17 levels in patients with allergic asthma before and after bronchial challenge with *D. pteronyssinus*

Characteristic	24 h before	7 h after	24 h after
Th 17 cells (%)			
EAR	1.50±0.31†	1.54±0.25†	2.42±0.41†
LAR	1.79±0.35†	2.40±0.43†	3.51±0.31†*#
HS	0.60±0.17	0.55±0.19	0.51±0.11
IL-17 levels (pg/ml)			
EAR	4.40±0.61†	6.30±0.76†	9.01±1.51†*
LAR	4.17±0.60†	8.33±0.67†#	13.47±1.85†*#
HS	1.32±0.21	1.58±0.36	2.10±039

Data are expressed as mean ± SEM; †P<0.05, versus HS; *P<0.05, in comparison with the baseline values; #P<0.05, LAR versus EAR.

Methods: We studied 28 patients with allergic asthma who developed early-phase asthmatic response (EAR) (n=16) and late-phase asthmatic response (LAR) (n=12) after bronchial challenge with *D. pteronyssinus*. The control group included 10 healthy subjects (HS). PB collection was performed 24 h before as well as 7 h and 24 h after challenge. The percentage of Th17 cells was analyzed by FACS. Serum IL-17 levels were determined by ELISA.
Results: See Table 1.
Conclusions: *D. pteronyssinus*-induced late-phase asthmatic response in patients with allergic asthma is associated with increased percentage of Th17 cells in PB and elevated serum IL-17 levels.

P2313
Activin-A expression is increased in severe asthma and is involved in tissue angiogenesis
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Background: Our recent studies show that Activin-A (Act-A), a cytokine belonging to the TGF-β superfamily, suppresses mouse allergic responses; however its effects on human asthma remain unknown.
Objectives: To determine Act-A expression in healthy controls (CTRL) and asthmatics, identify its cellular sources and signaling mediators, and examine correlations with disease severity and airway remodelling.
Methods: Serum samples were obtained from 46 mild-to-moderate asthmatics (MMA), 27 severe asthmatics (SA) and 41 CTRL. 55 subjects (18 CTRL, 18 MMA, 19 SA) underwent bronchoscopy with endobronchial biopsy and BALF collection. Act-A levels in the serum/BALF were examined. Expression of Act-A and its principal signaling mediator ALK4 in the bronchial tissue were assessed by confocal microscopy. Basement membrane thickness, goblet cell hyperplasia and angiogenesis (vessels/mm^2) were also determined.
Results: Act-A levels were significantly increased in MMA in the serum and in MMA and SA in BALF. Serum Act-A was further increased during asthma exacerbation. Bronchial tissue Act-A expression was significantly increased in asthmatics, especially in the subepithelium in SA, while ALK-4 expression decreased with disease severity. Act-A was mainly expressed by mast cells, neutrophils, macrophages and smooth muscle cells. Act-A and ALK-4 were also localized in endothelial cells, particularly in SA. Subepithelial Act-A expression correlated with angiogenesis and disease severity.
Conclusions: Our data suggest that Act-A plays a crucial role in asthma inflammation and participates in the regulation of angiogenesis in SA. Ongoing in vitro studies will further elucidate its specific role.

P2314
Relevance of measurement of serum periostin for diagnosing bronchial asthma and estimating its lung function abnormalities
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Bronchial asthma is diagnosed by combination of presence of characteristic symptoms and measurement of reversibility of lung function abnormalities. Non-invasive markers such as levels of exhaled nitric oxide (FeNO) and sputum eosinophilia have potential usefulness for diagnosis of bronchial asthma and determination of optimal treatment; however, these biomarkers contain several problems in specificity in diseases. Periostin, an extracellular matrix protein downstream of IL-4/IL-13 signals, has emerged as a novel biomarker for bronchial asthma. Particularly, it has been recently shown that efficacy of anti-IL-13 antibody can be predicted using serum periostin levels in steroid-resistant asthma patients. However, it still remains undetermined how measurement of serum periostin level is relevant for diagnosing bronchial asthma. The study group comprised 37 patients with asthma and 30 healthy subjects. In both groups, serum concentrations of SCCA1, SCCA2, periostin (SS18A SS17B) were measured. In asthmatic patients, serum IgE concentrations, eosinophil counts, and exhaled NO levels were also measured. Serum concentrations of periostin (SS18A SS17B) was significantly higher in the asthmatic patients than in the control subjects. Serum concentrations of both types of periostin strongly correlated with eosinophil counts and exhaled NO levels. In contrast, serum SCCA concentrations did not differ between the control subjects and asthmatic patients or significantly correlate with any variable studied.Our results suggested that serum periostin concentrations may be a significant diagnostic marker of bronchial asthma that correlates with eosinophil counts.

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4-1BBL/4-1BB costimulation alleviates airway inflammation by restoring Treg/Th17 balance in allergic asthma

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Objective: Recent studies have demonstrated Th17/Treg imbalance in patients with asthma and the effect of 4-1BBL/4-1BB on Th17/Treg balance. This study aimed to investigate whether 4-1BBL/4-1BB costimulation alleviated airway inflammation by restoring Treg/Th17 balance in OVA-induced asthmatic mice.
Methods: BALB/c mice were randomly assigned to three groups (asthma group, 4-1BB group and control group). Mice in the asthma group were sensitized and challenged with OVA. Agonistic 4-1BB mAb was administered in the 4-1BB group. Mice in control group were treated with PBS.
Results: (1) The serum IgE concentration [23.350 (65.945–42.635) ng/L vs. 99.292 (192.185-92.893) ng/L, $P<0.01$], total number of cells [$(17.35\pm3.23)\times10^6$ vs. $(6.44\pm1.90)\times10^6$, $P<0.05$] and eosinophils [$(9.79\pm3.84)\times10^4$ vs. $(0.91\pm0.37)\times10^4$, $P<0.05$] in bronchoalveolar lavage fluid (BALF) and airway inflammation in the 4-1BB group were significantly reduced compared with the asthma group. (2) Agonistic 4-1BB mAb treatment decreased IL-17 [(225.747 ± 15.000) ng/L vs. (265.730 ± 23.121) ng/L, $P<0.05$] but increased TGF-β levels [(24.468 ± 8.229) ng/L vs. (16.047 ± 2.789) ng/L, $P<0.05$] in BALF in asthmatic mice. Agonistic 4-1BB mAb inhibited the expression of Th17 cells [(3.18 ± 0.39)% vs.(6.25 ± 2.62)%, $P<0.01$] and RORγt mRNA [$(10.766\pm3.285)\times10^{-3}$ vs. $(30.853\pm5.686)\times10^{-3}$, $P<0.01$] in lung.
Conclusions: Agonistic 4-1BB mAb treatment partially alleviated airway inflammation by restoring the function of Treg cells and inhibiting the proliferation and function of Th17 cells.

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IL-25 secreted from epithelial cells has the potential to promote airway remodeling in asthma

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Introduction: Interleukin (IL)-25 plays a pivotal role in the pathogenesis of asthma, not only in airway inflammation, but also in airway remodeling.
Objective: To explore the function and significance of IL-25 in the pathogenesis of eosinophilic asthma (EA) and non-eosinophilic asthma (NEA).
Methods: Induced sputum was analyzed from 50 untreated asthmatic patients: 26 with EA, 24 with NEA. Serum and induced sputum from all the participants were collected and the level of IL-25 in the samples was determined by enzyme-linked immunosorbent assay (ELISA). Expression of IL-25 in bronchial epithelium and basement membrane thickening were quantified by immunohistochemistry.
Results: Compared with healthy control subjects, the lung function was impaired in patients with EA and NEA. ELISA results showed that the levels of IL-25 in the serum and induced sputum of asthmatic patients were significantly higher than healthy subjects ($p < 0.05$). But there were no statistic differences between EA and NEA patients ($p > 0.05$). The immunohistochemistry results indicated that higher expression of IL-25 and thickened basement membrane were observed in asthmatic bronchial epithelium. Correlation analysis showed that the level of IL-25 in serum and induced sputum was positively correlated with the average thickness of basement membrane in asthmatic patients.
Conclusion: IL-25 secreted from epithelial cells has the potential to promote airway remodeling in asthma.The increased level of IL-25 in peripheral blood and bronchial epithelium was parallel, eosinophil may not be necessary for airway remodeling in asthma.

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Inhibition of collagen receptors: $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins decreases eosinophil transmigration, but has no impact on peripheral blood mononuclear cell movement through human microvascular endothelial cell lung monolayer

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Introduction: Recruitment of the inflammatory cells to the airways is mediated by adhesive molecules. Among integrins, the most important in cell trafficking are those containing α_4 and β_2 subunits. We hypothesized that also collagen integrin receptors: $\alpha_1\beta_1$ and $\alpha_2\beta_1$, are involved in asthma pathogenesis. We recently described increased expression of both: α_1 and α_2 subunits on blood eosinophils and α_2 on CD4 T lymphocytes in asthma.
Aim: The aim of the study was to analyse effect of $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrin inhibition on transmigration of eosinophils and peripheral blood mononuclear cells (PBMC) through human microvascular endothelial cell lung monolayer in 12 atopic asthmatics and 12 healthy controls. We analysed also CD4/CD8 ratio in PBMC population before and after transmigration assay.
Methods: PBMC were separated by gradient centrifugation; eosinophils by gradient centrifugation and negative magnetic separation. For inhibition purposes we used snake venom derived anti-adhesive proteins: viperistatin, VP12, VLO5 and VLO4 (potent and selective inhibitors of $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_4\beta_1$ and $\alpha_5\beta_1$ integrins, respectively).
Results: In both groups of subjects all anti-adhesive proteins inhibited eosinophil, but only VLO5 and VLO4 PBMC transmigration; CD8 T cells migrated better than CD4 in control samples, but their transmigration was decreased after incubation with anti-adhesive proteins.
Conclusion: Both collagen receptors: $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins are involved in eosinophil transmigration. The role of $\alpha_2\beta_1$ on lymphocyte is probably different.

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The prognostic value of CD8+ and CD25+ lymphocytes blood content for asthma exacerbation

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Background: The recognition of asthma as an inflammatory disease led to a search for biomarkers for assessing of airway inflammation and for the prediction of asthma exacerbation.
Objective: To evaluate the blood content of CD8+ and CD25+ lymphocytes in patients with asthma and to predict asthma exacerbation.
Methods: 48 patients with asthma exacerbation and 48 with asthma remission were randomly selected and 30 matched control subjects were included. All were submitted to detailed clinical history and examination, pulmonary function testing. Investigation of CD8+ and CD25+ lymphocytes in blood was carried out by flow cytometry.
Results: Patients with asthma exacerbation had significantly lower values of CD8+ lymphocytes than patients with asthma remission (20,41±4,52% vs 23,29±5,08%; p<0,001) and controls (20,41±4,52% vs 23,19±3,37%; p=0,005), they also had significantly higher values of CD25+ cells than patients with asthma remission (10,22 (4,92-13,09)% vs 5,85 (4,14-7,83)%; p<0,001) and controls (10,22 (4,92-13,09)% vs 5,49 (3,69-6,01)%; p<0,001). The regression equation for the prediction of asthma exacerbation is: Z=exp(2,05909+0,280831X-0,20392Y)/(1+exp(2,05909+0,280831X-0,20392Y)), where X – % of CD25+ cells and Y – % of CD8+ lymphocytes. If Z≤0,5, the patient has remission; if Z>0,5, the patient has exacerbation; the above Z, the above asthma exacerbation probability. The value of correct predictions is 81%.
Conclusion: Decrease of CD8+ lymphocytes blood content and increase of CD25+ lymphocytes blood content can be predictor for asthma exacerbation and allows to predict asthma exacerbation with the probability of 81%.

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Leukotriene (LT)C_4 aggravate bleomycin-induced pulmonary fibrosis in mice

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Background: Synthesis of cys-LTs is thought to cause inflammatory disorders such as bronchial asthma and allergic rhinitis. Recent reports have suggested that LTC_4 is an important regulator of pulmonary fibrosis. This study examined the effect of LTC_4 in LTC_4 synthase-overexpressed transgenic (Tg) mice with bleomycin-induced pulmonary fibrosis. We also focused on the function of lung-derived fibroblasts in the Tg mice.
Methods: Prior to administration of bleomycin, pranlukast hydrate, a cys-LT1 receptor antagonist, was intragastrically administered to Tg mice daily from the previous day of the administration. Bleomycin was administrated by intratracheal instillation. Concentrations of interleukin (IL)-4, -13, and transforming growth factor (TGF)-β1 in BAL fluid were measured 14 days after the administration of bleomycin. And lung tissue was examined histopathologically. In addition, lung-derived fibroblasts from Tg and wild-type (WT) mice were cultured for 7 days, and LTC_4 secretion and cell viability were assessed by EIA and MTT assay, respectively. And the expression of TGF-β1 mRNA was measured by real time PCR.
Results: The levels of IL-4, -13, and TGF-β1, and pulmonary fibrosis were greater in Tg than in WT mice. The reduction of LTC_4 function in Tg mice could be decreased both these cytokines and pulmonary fibrosis. Furthermore, continuous LTC_4 secretion from fibroblasts was higher in Tg than in WT mice, while reduction of LTC_4 by pranlukast in fibroblasts from Tg, but not in those from WT mice, decreased cell viability and expression of TGF-β1 mRNA.
Conclusion: These findings first suggest that overexpression of LTC_4 using transgenic mice is responsible for the development of pulmonary fibrosis.