420. Experimental modulation of airway inflammation

P4091

Inhibition of collagen receptors: α1β1 and α2β1 integrins, has no impact on lymphocytes, but decreases eosinophil transmigration through human extracellular matrix and collagen I coated inserts

Stanislawa Bazan-Socha, Joanna Zuk, Cezary Marcinkiewicz, Jacek Musial. *II Department of Internal Medicine, Jagiellonian University Medical College, Cracow, Poland II Department of Internal Medicine, Jagiellonian University Medical College, Cracow, Poland Department of Biology, College of Science and Technology, Philadelphia, United States II Department of Internal Medicine, Jagiellonian University Medical College, Cracow, Poland*

Introduction: T helper lymphocytes are likely to play a pivotal role in directing disease development and progression in asthma. We recently described presence of α1 and α2 integrins on blood eosinophils and α2 on blood CD4 and CD8 T lymphocytes in chronic asthma. We hypothesize that collagen receptors: α1β1 and α2β1 integrins may play an important role in cell transmigration to the sites of asthmatic inflammation.
Methods: We studied effects of functional active, mouse anti-human monoclonal antibodies against I-domain of α1 and α2 integrin subunit (Chemicon, USA) on eosinophil and lymphocyte transmigration through human extracellular matrix and collagen I coated cell culture inserts (Beckton Dickinson Falcon Cell Culture Inserts – 3 μm pore size). In 6 atopic asthmatics lymphocytes were separated from peripheral blood by gradient centrifugation and eosinophils by gradient centrifugation and negative magnetic separation (Vario MACS, Miltenyi Biotec).
Results: None of the antibodies used influenced lymphocyte transmigration through human extracellular matrix and collagen I coated inserts. However, movement of peripheral blood eosinophils through human extracellular matrix was inhibited by both antibodies (% of inhibition: 30±26 for anti-α1, 25±24 for anti-α2 mAb). Anti-α2 mAb was also active on collagen I coated inserts (37 ± SD33); anti-α1 mAb was not effective.
Conclusion: The α2β1 integrin is present on lymphocytes, but seems to play no role in lymphocyte infiltration. However, both α1β1 and α2β1 integrins might be important in eosinophil transmigration and support airway inflammation.

P4092

Suppressive effect of a mint aqueous extract on IL-13 production

Fatemeh Hajighasemi. *Department of Immunology, Faculty of Medicine, Shahed University, Tehran, Islamic Republic of Iran*

Background: Medicinal plants have been broadly used in treatment of various diseases. Mints are a group of plants belonging to labiatae family have anti-bacterial, anti-tumoral and anti-inflammatory effects. Peppermint is a mint specious extensively used in therapy of several disorders such as common cold and bronchitis. The anti-bronchospasmodic and anti-allergic effects of peppermint have also been shown. Elevation of interleukin-13 (IL-13) (a Th2- type cytokine) level is a well known indicator of allergy. In the present study the effect of aqueous extract of peppermint on IL-13 production in human peripheral blood mononuclear cells (hPBMCs) has been assessed in vitro.
Methods: The hPBMCs were isolated from the venous blood of healthy volunteers by ficoll-hypaque-gradiant centrifugation. Then the PBMCs were cultured in complete RPMI medium. The cells at logarithmic growth phase, were incubated with different concentrations of aqueous extract of peppermint leaves (0.01-10 mg/ml) in triplicate for 24 hours. Afterward the cell culture supernates were collected and the IL-13 concentration was measured using a standard ELISA kit.
Results: Peppermint aqueous extract significantly decreased the IL-13 production in hPBMCs dose-dependently.
Conclusions: The results of this study indicate that peppermint aqueous extract down-regulates the production of IL-13 in hPBMCs. Regarding the important role of IL-13 in atopic allergy, the anti-allergic activity of peppermint and also its inhibitory effect on bronchospasm (a symptom of respiratory allergy), may be partly due to its inhibitory effects on IL-13 production.

P4093

Role of thrombin-activatable fibrinolysis inhibitor in lipopolysaccharide-induced acute lung injury

Masahiro Naito[1], Osamu Taguchi[1], Takehiro Takagi[1], Tetsu Kobayashi[1], Corina D'Alessandro-Gabazza[2], Esteban Gabazza[2]. *[1]Department of Respiratory Division, Mie University School of Medicine, Tsu, Japan; [2]Department of Immunology, Mie University School of Medicine, Tsu, Japan*

Background: In acute lung injury, excessive coagulation induced by inflammatory responses may feedback to further increase the inflammatory response and promote fibrosis. Thrombin-activatable fibrinolysis inhibitor (TAFI) is mainly synthesized in the liver and circulates in the bloodstream as an inactive proenzyme. It can be activated by the thrombin-thrombomodulin complex to a carboxypeptidase (TAFIa), which acts as an anti-fibrinolytic factor by inhibiting the activation of plasmin through the removal of carboxy-terminal lysines from fibrin. Recent studies have shown that TAFIa can also regulate inflammatory responses by its ability to inhibit complement C3a and C5a, and osteopontin.
Objective: Our aim was to evaluate the effect of TAFI on acute lung injury in the mouse.
Methods: Acute lung injury was induced in wild type and TAFI deficient (KO) C57/BL6 mice by intratracheal instillation of lipopolysaccharide (LPS:5mg/kg). Mice treated with saline served as controls. We compared the inflammatory response to LPS in both groups. Animals were sacrificed 24 hours after LPS injection and broncho-alveolar lavage fluid (BALF) was sampled.
Results: TAFI KO mice had worse acute lung injury as their total protein, total cell count, and levels of IL-1β, IL-6 and TNF-α were increased in BALF in LPS-treated TAFI KO mice compared to wild type. The increase in cells in BALF was due to an increase in neutrophils. Complement C5a in both BALF and plasma was increased in LPS-treated TAFI KO mice compared to wild type.
Conclusions: These results suggest that TAFI protects against acute lung injury at least partially through its ability to inactivate complement C5a.

P4094

Critical immunoregulatory role for activin-A in human allergic asthma

Sofia Tousa[1], Maria Semitekolou[1], Ioanna Christopoulou[1], Konstantinos Samitas[2], Giannis Paraskevopoulos[3], Catherine Hawrylowicz[4], Mina Gaga[2], Georgina Xanthou[1]. *[1]Cellular Immunology Laboratory, Biomedical Research Foundation of the Academy of Athens, Athens, Greece; [2]7th Respiratory Medicine Department and Asthma Center, "Sotiria" Athens Chest Hospital, Athens, Greece; [3]Allergiology and Clinical Immunology Dept, General Military Hospital of Athens, Athens, Greece; [4]Department of Asthma, Allergy and Respiratory Science, Kings College, London, United Kingdom*

Our studies have revealed that activin-A, a cytokine increased in the sera and lungs of asthmatics, is a critical controller of experimental asthma as it suppresses allergic responses and protects against airway hyperresponsiveness and linked disease in mice. Still, the role of activin-A in human asthma remains elusive. Here, we investigated the effects of activin-A in the suppression of allergic responses of atopics and asthmatics. Peripheral blood (PB) was obtained from atopics and mild/moderate or severe asthmatics (n=12/group). Bronchoalveolar lavage (BAL) was obtained from severe asthmatics (n=5). The effects of recombinant activin-A or control on allergen-specific CD4+ T cell proliferation and cytokine release were investigated during stimulation with allergen ex vivo. Activin-A's role in the generation of suppressive human regulatory T cells (Tregs) was studied. Our findings reveal that activin-A significantly suppresses allergen-driven CD4+ T cell proliferation and IL-5, IL-13, IL-17, IFN-γ, IL-10 release in atopics and asthmatics. Activin-A suppresses BAL mononuclear cell responses of severe asthmatics. Importantly, activin-A enhances dexamethasone-induced suppression of allergic Th2 responses of severe, treatment refractory, asthmatics. Activin-A mediated suppression is associated with the generation of human CD4+Foxp3+ Tregs that completely inhibit allergic Th2 responses. Our results uncover activin-A as a novel protective cytokine that controls human asthma. Our findings have important clinical implications for the use of activin-A, or activin-A-induced human Tregs, as novel immunotherapies for asthma.

P4095

Fluticasone propionate decreases MUC5AC expression on airway epithelial cells induced by rhinovirus infection in the airway

Takenori Okada[1], Naoto Fueki[2], Makoto Fueki[1], Sohei Makino[1], Hironori Sagara[2]. *[1]Dept of Internal Medicine, Jobu Hospital for Respiratory Disease, Maebashi, Gunma, Japan; [2]Dept of Respiratory Medicine, Dokkyo Medical University Koshigaya Hospital, Koshigaya, Saitama, Japan*

Viral infections such as a common cold in the airway can potentially exacerbate signs and symptom of asthma, including mucus overproduction, airway hyperresponsiveness, and airway obstruction. These signs and symptoms are associated with progression of airway remodeling. MUC5AC is related intimately related to mucus overproduction in the airway and is localized in airway epithelial goblet cells. Fluticasone propionate and salmeterol are used to treat asthma. These drugs prevent exacerbation of asthmatic symptoms due to common colds. However, the effects of these drugs on viral-infected epithelial cells in the airway are unclear.
We therefore investigated the effects of fluticasone propionate or salmeterol on rhinovirus 14 (RV14)-induced MUC5AC gene expression in a human airway epithelial cell line (NCI-H292). Fluticasone propionate significantly decreased MUC5AC gene expression in rhinovirus-infected NCI-H292 cells. Our results suggest that fluticasone propionate prevents exacerbation of asthma due to rhinovirus infection by decreasing MUC5AC expression in the airway, potentially slowing the progression of airway remodeling.

P4096

Standard therapy increases the sensitivity of peripheral blood lymphocytes to glucocorticoids in cystic fibrosis but not in severe asthma

Alexander Pukhalsky[1], Galina Shmarina[1], Tamara Vylegzhanina[2], Tamara Chervinskaya[2], Olga Kozyreva[2], Nikolai Kapranov[1], Vladimir Alioshkin[3]. *[1]Department of Cystic Fibrosis, Research Centre for Medical Genetics, Moscow, Russian Federation; [2]Department of Asthma, NRC Institute of Immunology, Moscow, Russian Federation; [3]Laboratory of Cytokines, G.N. Gabrichevsky Insitute of Epidemiology and Microbiology, Moscow, Russian Federation*

Usually the asthma symptoms are well controlled with inhaled glucocorticoids (GCs) or β_2-agonists, but a minority of patients continue to have uncontrolled asthma despite taking high doses of inhaled GCs, sometimes together with oral GCs. It is known that inflammatory response in such patients is relatively resistant to suppression. The aim of this study was to evaluate peripheral blood lymphocyte (PBL) sensitivity to antiproliferative effect of GCs in cystic fibrosis (CF) and asthmatic patients in the course of exacerbation treatment. Individual susceptibility of PBL to GCs was evaluated by Δ_h value calculation: an integrative parameter, including the level of mitogen-induced lymphocyte proliferation and inhibition degree of such stimulation by dexamethasone. In healthy subjects the mean Δ_h level was -0.24±0.30. Negative values of Δ_h correspond to high cell sensitivity to GC. CF patients with lung exacerbations demonstrated low cell sensitivity to GCs (Δ_h = 0.36±0.23), but after antibiotic treatment the sensitivity significantly increased (Δ_h = -0.54±0.24; p=0.014). On the contrary, the cell sensitivity of asthmatic patients remained extremely low. The therapy did not affect high Δ_h level, which averaged before and after exacerbation treatment 2.67±0.27 and 2.65±0.31, respectively. In conclusion, patients with severe asthma have stably diminished GC sensitivity of PBL when compared with CF patients and healthy subjects. We believe that such resistance is a consequence of GC treatment during a long period of time and may be associated with both immune cell selection and epigenetic changes of GC receptors.

P4097

Cultured normal human bronchial epithelial cells may produce collagen type I stimulation with TGF-b

Kenya Kohyama[1], Naoto Fueki[1], Risako Seki[1], Akemi Kohyama[1], Takenori Okada[2], Hironori Sagara[1]. *[1]Dept of Respiratory Medicine, Dokkyo Medical University Koshigaya Hospital, Koshigaya, Saitama, Japan; [2]Internal Medicine, Jobu Hospital for Respiratory Disease, Maebashi, Gunma, Japan*

The clinical characteristics of asthma can be logically explained by asthma-specific airway inflammation and airway remodeling, the details of which have been recently elucidated. Airway remodeling is an important feature of chronic airway disease, but the mechanisms involved remain unclear.
Since TGF-b has been implicated in the development of airway remodeling in asthma based on its strong capacity to induce extracellular matrix (ECM) production, it is possible that Smad7 may also play some roles in the regulation of the process.
Method: We analyzed using by real time RT-PCR and Western blotting method.We thought to determine the relationships between Collagen type I production in normal human bronchial cell line (NHBE).
Normal human bronchial epithelial cells (NHBE) cells stimulated with Th2 type cytokine TGF-beta or regulately cytokine IL-10. Production levels of Type I collagen was expressed in cultured epithelial cells NHBE stimulation with TGF-b. Furthermore, collagen type I production in NHBE cells stimulation with TGF-b up-regulate in a dose and time dependent manner. And also we found that protein levels of collagen type I were increased from activated NHBE cells stimulating with TGF-b.
In contrast, IL-10 decreased collagen type I expression in NHBE cells.
In conclusion, these findings suggested bronchial epithelial cells may produce collagen type I production and IL-10 is a key cytokine possible make an important role of airway remodeling.

P4098

Investigation of the effect of histone deacetylase 2 function on Wegener's granulomatosis

Dai Takagi, Yuji Nakamaru, Satoshi Fukuda. *Otolaryngology Head and Neck Surgery, Hokkaido University Graduate School of Medicine, Sapporo, Japan*

Backgrounds: It has been reported that anti-neutrophil cytoplasmic antibodies (ANCA) activate neutrophils result in induction of glomerulonephritis in Wegener's Granulomatosis (WG) by released hydrogen peroxide.
Histone deacetylase (HDAC) deacetylates histone and deacetylation of histone associates with gene repression.
In this study, we investigated whether HDAC2 function decreased in WG patients, and effect of oxidative stress on function of HDAC2.
Patients and methods: A549 cells (lung epithelial cells) were stimulated by H_2O_2 to induce oxidative stress and expression of HDAC 2 and HDAC2 activity were measured.
Six patients of WG diagnosed according to American College of Rheumatology criteria were examined.
Fresh PBMCs were isolated from heparinized blood and then whole cell protein was prepared. Target proteins were detected by Western blot analysis. We also measured HDAC 2 activity on patients.
Results: Treatment of A549 cells with H_2O_2 did not suppress expression of HDAC2. However, treatment of H_2O_2 significantly decreased total HDAC2 activity ($p < 0.05$).
We found that HDAC 2 activity was significantly decreased in WG patients compared with healthy subjects (HS), 75.5±7.4 Arbitral Units at HS, 35.2±12.3 AU at WG ($p < 0.05$). Furthermore, we found negative correlation between HDAC 2 activity and titer of c-reactive protein and titer of PR3-ANCA.
Discussion: These results suggest that function of HDAC 2 is reduced in WG and that this reduction affects inflammation and vasculitis of WG. Oxidative stress may worsen the disease via reduction of HDAC2 function. Thus, HDAC2 may serve therapeutic targets by modulating the function, which eventually regulates the development of WG.

P4099

Polyphenolic compounds and experimentally induced allergic asthma

Sona Franova[1], Marta Joskova[1], Martina Sutovska[1], Olga Pechanova[2], Gabriela Nosalova[1]. *[1]Department of Pharmacology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia (Slovak Republic); [2]Department of Normal and Pathological Physiology, Slovac Academy of Sciences, Bratislava, Slovakia (Slovak Republic)*

Some epidemiological studies related asthma symptoms contain data supporting the idea that health benefits associated with fruits, vegetables and red wine in the

diet are probably linked with polyphenols that most likely reduce the occurrence of asthma complications.
Our experimental work was aimed at influence of polyphenols on airway hyperreactivity and allergic inflammation in experimental conditions of allergic asthma (21 days OVA sensibilization) after their short- or long-term (21 days) administration.
We compared the activities of polyphenolic compounds mixtures from red wine (Provinol) and red fruits (Flavin7) and polyphenolic substances - resveratrol and quercetin.
The changes in reactivity of respiratory system after polyphenols administration we measured: by in vitro method; by in vivo method (used whole-bodyplethysmograph).The degree of inflammation was evaluated by eosinophil calculation and by estimation of inflammatory cytokines IL-4, IL-5 in bronchoalveolar lavage fluid (BALF).
The results of our experiments showed that: Polyphenolic compounds Provinol and Flavin7 posses an efficient antiasthmatic activities. They cause bronchodilation and also suppress asthmatic inflammation in the airways. Quercetin and resveratrol are able to induce only acute bronchodilation without antiinflammatory effects.
Our results demonstrate positive antiasthmatic effect of mixture of polyphenols. This outcome confirm the hypothesis, that may become an additional therapy in prevention of airway hyperresponsiveness in asthma subjects.

P4100
Cigarette smoke extract suppresses the maturation and function of bone marrow derived dendritic cells
Masoumeh Givi, Frank Redegeld, Gert Folkerts, Esmaeil Mortaz. *Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht, Netherlands; Chronic Respiratory Disease Research Center, National Research Institute of Tuberculosis and Lung Disease, Tehran, Islamic Republic of Iran*

Chronic obstructive pulmonary disease (COPD) is characterized by chronic airway inflammation. Cigarette smoke has been considered as a major risk factor in the pathogenesis of COPD. The potential role of DCs in the the respiratory tract of smoker's and COPD patients is poorly understood. In aim of this study is to investigate the effects of cigarette smoke extract (CSE) on the maturation, development and function of mouse bone marrow. Bone marrow derived DCs were developed by culturing isolated cells by femurs of BALB/c mice in presence of GM-CSF (20 ng/ml) for 10 days. CSE was added to cells cultures for 10 days. The surface expression of maturation and co-stimulatory markers were CD11C-MHC11-CD83 and CD86, CD40 and CD80, respectively, as measured by FACS analysis. The functional capacity of DCs was measured by uptake of Dextran-FITC as maesured by FACS analysis. The production of TNF-a, IL-6 and IL-12 was measured by ELISA. After 10 days of incubation with CSE, the DCs maturation was significantly decreased (MHCII/CD11C compared to control, $P \leq 0.05$) and co-stimulatory receptors (CD11C/CD 86, CD11C/CD40 compared to control, $P \leq 0.05$). The cytokine production did not differ between the experimental groups. However, restimulation with CSE resulted is significant less cytokine production of DCs that had been exposed to CSE for 10 days compared to the non exposed cells. In addition, the uptake of FITC-Dextran was significantly decreased in DCs that had been exposed for 10 days to CSE. It cannot be excluded that the exacerbations in COPD patients might be due to a decreased maturation, development and function of DCs induced by cigarette smoke.

P4101
LSC 2011 Abstract: The effect of endothelin-1 on human basophil function in vitro
Katharina Cima, Silvia Blunder, Susanna Desole, Julia Günther, Judith Löffler-Ragg, Christian M. Kähler. *Pneumology, Internal Medicine, Medical University of Innsbruck, Innsbruck, AT Internal Medicine, Laboratory of Rheumatology, Innsbruck, AT*

Endothelin-1 (ET-1) has proinflammatory properties and contributes to allergic late-phase responses. As basophils play a key role in allergic rhinitis or asthma, we investigated the effect of ET-1 on basophils.
Cells were isolated from venous blood of healthy donors via magnetic cell sorting. To show ETA or ETB receptor expression RT-PCR was performed. The chemotactic effect of ET-1 [10^{-6}-10^{-16}M] was analysed in modified Boyden chambers (positive control MCP-1 [10^{-8}M]). To explore ET-1 signalling, cells were preincubated with BQ-123 [10^{-6}-10^{-16}M] or BQ-788 [10^{-6}-10^{-16}M]. Migration depth was quantified microscopically. Histamine release upon ET-1 [10^{-6}-10^{-12}M] and upon additional stimulation with the secretagogue fMLP [10^{-5}M] was determined by ELISA.
The RT-PCR revealed basophils to express both, ETAR and ETBR. ET-1 [10^{-6}-10^{-8}M] further proved to be a strong chemoattractant for human basophils ($p<0.0001$). Despite basophils express both receptors, only the ETAR antagonist BQ-123 [10^{-6}-10^{-12}M] significantly blocked migration towards ET-1 [10^{-8}M]. Furthermore, the histamine release was increased by 2.46 to 2.6 fold after ET-1 stimulation [10^{-6}-10^{-12}M]. Interestingly, only the evoking of histamine release by additional stimulation with fMLP [10^{-5}M] resulted in a dose dependent effect of ET-1, showing ET-1 [10^{-8}M] to be most effective.
Our observations reveal for the first time that basophils express ETAR and ETBR and ET-1 induces histamine release and basophil migration, which seems ETAR dependent. Considering the fact that ET-1 is involved in the mechanisms of airway inflammation, targeting ET-1 by receptor antagonists may be a new option in the treatment of allergic airway disease.

P4102
Resveratrol impairs the release of steroid-resistant cytokines from bacterial endotoxin-exposed alveolar macrophages (AM) in COPD
Jurgen Knobloch, David Jungck, Katja Urban, Andrea Koch. *Medical Clinic III for Pneumology, Allergology and Sleep Medicine, University Hospital Bochum-Bergmannsheil, Bochum, Germany*

Airway inflammation in COPD might be insensitive to corticosteroids. However, corticosteroids are recommended in COPD (GOLD stages III, IV) with frequent exacerbations. Resveratrol has anti-inflammatory properties and could be an alternative in COPD therapy.
We investigated the effect of dexamethasone (Dex) versus resveratrol on the release of COPD-related inflammatory mediators (IL-6, IL-8, GM-CSF, MCP1) and MMP9 from AM exposed to bacterial endotoxin (lipopolysaccharide, LPS). We compared never-smokers (NS), current smokers without airway obstruction (S) and current smokers with COPD (each n=12). Cytokines and MMP9 were measured in cell culture supernatants with ELISA.
The release of IL-8 and MMP9 from LPS-exposed AM was increased in COPD (each $p<0.001$), the release of GM-CSF and IL-6 was decreased in COPD (each $p<0.01$) and the release of MCP1 was without differences between the cohorts. Dex impaired the release of all cytokines and MMP9 from LPS-exposed AM of all cohorts, but for IL-8 and GM-CSF this effect was reduced in COPD ($p<0.05$). In AM of COPD there was an almost complete reduction of IL-6 release but only a partial reduction of IL-8, GM-CSF, MCP1 and MMP9 release demonstrating a partial corticosteroid-insensitivity. In contrast, resveratrol almost completely reduced the release of all cytokines and MMP9 without significant differences between the cohorts (Emax/COPD: 92±10%-100% reduction).
Our data provide evidence for a corticosteroid-resistance of AM-dependent inflammatory responses induced by gram-negative bacteria in COPD and thus question the utility of corticosteroids in COPD therapy. Instead, resveratrol may prove an alternative.

P4103
Endothelin receptor B (ET_BR) dependent GM-CSF mRNA stabilization explains the higher efficacy of bosentan vs. ambrisentan in the reduction of GM-CSF release from human airway smooth muscle cells (HASMCs)
David Jungck[1], Juergen Knobloch[1], Katja Urban[1], Erich Stoelben[2], Justus Strauch[3], Juergen Behr[1], Andrea Koch[1]. *[1]Medical Clinic III for Pneumology, Allergology, Sleep Medicine, University Hospital Bochum-Bergmannsheil, Bochum, Germany; [2]Department of Thoracic Surgery, Lungenklinik Merheim, Kliniken der Stadt Koeln, Cologne, Germany; [3]Clinic for Heart & Thoracic Surgery, University Hospital Bochum-Bergmannsheil, Bochum, Germany*

Introduction: TNFα and GM-CSF are pivotal in chronic inflammatory airway diseases and lung fibrosis. TNFα-induced Endothelin-1 (ET-1) release is required for full TNFα induced GM-CSF transcription in HASMCs suggesting anti-inflammatory potential of endothelin receptor antagonists (ERA). The MAP-Kinase ERK protects GM-CSF mRNA from degradation. Ambrisentan (ETAR blocker) and bosentan (dual blocker) are available for PAH therapy.
Aim: We compared the anti-inflammatory potential of bosentan vs. ambrisentan.
Methods: HASMC culture, qRT-PCR, ELISA.
Results: TNFα and ET-1 induce transcription and release of ET-1 and GM-CSF (each $p<0.05$). Bosentan reduces GM-CSF release more efficiently than ambrisentan ($p<0.01$; EC50: 4.5 vs. 11×10^{-8}M; EMAX: 63.7 vs. 54.8% reduction; n=9) but both block GM-CSF transcription similarly. Specific ET_BR inhibition (BQ 788) also reduces GM-CSF mRNA. Combined blocking of ET_AR (ambrisentan) and ERK activity (PD 098059) leads to a greater reduction of GM-CSF release than single inhibition (each $p<0.05$). In the presence of actinomycin D which blocks gene transcription bosentan leads to a significantly greater reduction of GM-CSF mRNA than ambrisentan ($p<0.01$).
Conclusion: Following TNFa-induced ET-1 release, ET_AR induces GM-CSF transcription and ET_BR signals via ERK to protect GM-CSF mRNA from degradation. This can explain why bosentan reduces TNFα induced GM-CSF release more effectively than ambrisentan. Thus, bosentan may be superior in the therapy of early stages of chronic airway diseases by preventing the establishment of inflammation.

P4104
Effect of thrombin inhalation in a murine model of bronchial asthma
Takehiro Takagi[1], Masahiro Onishi[1], Masahiro Naito[1], Atsushi Fujiwara[1], Tetsu Kobayashi[1], Hiroyasu Kobayashi[1], Corina D'Alessandro-Gabazza[1], Esteban Gabazza[1], Osamu Taguchi[1]. *[1]Department of Respiratory Medicine, Mie University Graduate School of Medicine, Tsu, Mie, Japan; [2]Department of Immunology, Mie University Graduate School of Medicine, Tsu, Mie, Japan*

Background: Thrombin is the effector enzyme of the coagulation system with important biological functions not only in thrombosis and hemostasis but also in inflammation. The precise role of thrombin in allergy remains unknown but recently there are some reports showing that thrombin plays an important role in the pathogenesis of asthma. In addition, previously we reported that thrombin inhibitor did not improve allergic response in murine asthma model.
Objectives: To evaluate the effect of inhaled thrombin on airway inflammation and hyperresponsiveness in a murine asthma model.

Methods: Bronchial asthma was induced by sensitization and challenge with ovalbumin (OVA). Littermates treated with saline were used as controls. The effect of inhaled different dose of thrombin was assessed by administering prior to OVA exposure. Airway inflammation was evaluated by measuring the number of inflammatory cells and the level of cytokine in bronchoalveolar lavage fluid (BAL). Airway hyperresponsiveness was measured using a plethysmograph.
Results: The levels of IgE, IL-5, and the number of eosinophils in BAL were decreased in low dose of thrombin treated mice compared to saline treated mice. The degree of airway hyperresponsiveness was significantly decreased in low dose of thrombin treated mice as compared to saline treated mice.
Conclusion: These results suggest thrombin exerts a differential effect in bronchial asthma depending on its concentration.

P4105
Efficacy of inhaled anti-IL-13 mAb in a mouse model of asthma
Jonathan Hacha[1], Maud Guéders[1], Kate Tomlinson[2], Geneviève Paulissen[1], Natacha Rocks[1], Agnès Noël[1], Roger Palframan[2], Didier Cataldo[1]. [1]*Laboratory of Tumor & Development Biology (GIGA-Research), University of Liege, Liege, Belgium;* [2]*Immunology Research, UCB, Slough, United Kingdom*

Interleukin-13 (IL-13) is a prototypic Th2 cytokine and a potential cornerstone of asthma pathology. IL-13 is involved in IgE synthesis, bronchial hyperresponsiveness, mucus hypersecretion, subepithelial fibrosis and eosinophil infiltration.
We assessed the potential efficacy of an inhaled high affinity monoclonal antibody (mAb) Fab' fragment neutralizing IL-13 against allergen-induced inflammation and hyperresponsiveness. BALB/c mice were subjected to ovalbumin (OVA) exposure for 1, 5, and 10 weeks referred to as short term (ST), intermediate term (IT) and long term (LT) protocols respectively. The antibody was administered as an aerosol generated by inExpose® in a tower allowing a nose-only exposure.
In a one-week OVA-exposure model (ST), we assessed the effectiveness of different doses of anti-IL-13 (0.5 to 5 mg/ml). We report a dose-dependent increase of the anti-inflammatory effect reaching a maximum at a 5 mg/ml. Airway responsiveness to methacholine was measured by using the flexiVent® system.
In the different protocols used in this study, administration of the anti-IL-13 Fab' by inhalation significantly decreased bronchial responsiveness to methacholine, BALF eosinophilia, inflammatory cell infiltration in lung tissue, mucus cell upregulation, peribronchial collagen deposition and smooth muscle hyperplasia. After 1 and 5 weeks of allergen exposure (ST and IT), levels of pro-inflammatory mediators IL-13, IL-4, IL-5, CCL-11, MMP-2 and MMP-9 were significantly lower in lung parenchyma when mice were treated by the IL-13 neutralizing antibody.
In conclusion, our data generated in a rodent model suggest that inhaled anti-IL-13 Fab' could represent a novel and effective therapy for the treatment of asthma.

P4106
Effects of corticosteroid and montelukast treatment on distal lung parenchyma and airway walls in inflammation in guinea pigs with chronic allergic inflammation
Nathália B. Gobbato[1], Flávia C.R. Souza[1], Carla Máximo Prado[2], Mílton Arruda Martins[1], Iolanda Calvo Tibério[1], Edna A. Leick[1]. [1]*Medicine, School of Medicine of University of São Paulo, São Paulo, Brazil;* [2]*Biological Sciences, Universidade Federal de São Paulo, São Paulo, Brazil*

Rationale: The effects of montelukast or dexamethasone in asthma pathophysiology are barely understood.
Aims and methods: We evaluated the inflammation in distal lung parenchyma and airway walls in guinea pigs (GP) with chronic allergic inflammation. GP were inhaled with ovalbumin (OVA group-2x/week/4weeks). After 4th inhalation, GP were treated with montelukast (M group-10mg/kg/PO/day) or dexamethasone (D group-5mg/kg/IP/day). After 72 hrs of 7th inhalation, GP were anesthetised, lung strips were submitted to histopathological evaluation.
Results: On distal parenchyma both montelukast and dexamethasone were effective in reducing RANTES and NF-kB positive cells compared to OVA group ($p<0.05$). Montelukast was more effective in reducing the eotaxin positive cells compared to dexamethasone treatment ($p<0.05$). There was a more expressive reduction of IGF-I positive cells in D group compared to M animals ($p<0.05$). On airway walls, both montelukast and dexamethasone were effective in reducing IGF-I and RANTES positive cells compared to OVA group ($p<0.05$). Dexamethasone was more effective reducing the number of eotaxin and NF-kB positive cells than Montelukast ($p<0.05$).
Conclusions: In this animal model, both corticosteroid and montelukast treatments contribute to the control of the inflammatory response in distal lung parenchyma and airway walls. Dexamethasone treatment induced a greater reduction of NF-kB expression in airway walls which suggests one of the mechanisms that explains the higher efficacy of this therapeutic approach.
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P4107
Immune modulatory activity in probiotic supernatants
Inge Kepert[1,2], Juliano Fonseca[3], Kerstin Hochwind[4], Oliver Eickelberg[5], Michael Schmid[4], Philippe Schmitt-Kopplin[3], Anton Hartmann[4], Susanne Krauss-Etschmann[1,2]. [1]*Comprehensive Pneumology Center, Ludwig-Maximilians-University, Asklepios Clinic Gauting and Helmholtz Zentrum München, Developmental Immune Biology of the Lung, Munich, Germany;* [2]*Ludwig-Maximilians-University München, Dr. von Haunersches Kinderspital, Munich, Germany;* [3]*Helmholtz Zentrum München, BioGeoChemistry and Analytics, Munich, Germany;* [4]*Helmholtz Zentrum München, Microbe-Plant Interactions, Munich, Germany;* [5]*Comprehensive Pneumology Center, Ludwig-Maximilians-University, Asklepios Clinic Gauting and Helmholtz Zentrum München, Remodeling of Lung Tissue, Munich, Germany*

Aims: Probiotic bacteria were suggested for primary prevention of allergic diseases, but clinical studies targeting infants perinatally were inconclusive. One reason might be the unpredictable individual crosstalk between bacterium and host. Therefore, the use of soluble, biochemically defined probiotic compounds might be advantageous. We aimed to develop biological screening tools to detect immune regulatory activity in probiotic supernatants and further to perform a chemical characterization.
Methods: The human T-cell line KM-H2, which secretes the allergy-associated chemokine CCL17 abundantly, was incubated with crude supernatants of 50 strains. CCL17 levels were then measured by ELISA. Selected supernatants were chemically fractionated (Ultra Performance Liquid Chromatography, Mass Spectrometry MS) and single fractions were retested on KM-H2. Further confirmation of bioactivity was done with human monocyte derived dendritic cells (DCs) that were matured with lipopolysaccharide in the presence or absence of supernatants or fractions. The expression of the costimulatory molecules CD80, CD83, CD86 and CD40 was then assessed by flow cytometry.
Results: 15 supernatants from 50 strains significantly reduced both CCL17 secretion of KM-H2 and LPS-induced upregulation of DC markers. The immune modulatory activity could be assigned to single fractions with different polarity. Moreover, MS analysis indicates the presence of a single bioactive compound.
Conclusion: Supernatants from selected probiotic strains have immune modulatory activity in the screening assays applied. Furthermore, we have preliminary indications of the chemical nature of one soluble probiotic compound with *in vitro* immune modulatory effects.

P4108
The effect of *M. tuberculosis* chaperonin 60.1 on leukocyte migration
Yanira Riffo-Vasquez[1], Anthony Coates[2], Clive Page[1], Domenico Spina[1]. [1]*Sackler Institute of Pulmonary Pharmacology, King's College London, London, United Kingdom;* [2]*Helperby Therapeuthics Limited, Helperby Therapeuthics Limited, London, United Kingdom*

We have shown that *M. tuberculosis* Cpn60.1 inhibits allergic lung inflammation and bronchial hyperresponsiveness in mice (Riffo-Vasquez Y et al. Clin Exp All. 2004). Here we have evaluated the effect of lower concentrations of *Cpn*60.1 on lung eosinophilia and leukocyte/endothelial cell interaction *in vivo.* Balbl/c mice were immunized twice with ovalbumin (ova, 10mg/mouse i.p. in alum). From day 14 all mice were exposed to ova (3%) once daily for 3 days and Cpn 60.1 was given i.n.15 min earlier. Lung lavages were performed 24 h after the last exposure. To examine the recruitment of cells in the microvasculature of the cremaster muscle, Cpn60.1 at 1μg/mouse was given s.c. into the scrotal sac of ova-sensitized mice followed by 100ng of eotaxin 10 minutes later. Four hours later the animals were prepared for intravital microscopy. Cpn60.1 at 0.001-1 μg/mouse inhibited the migration of eosinophils into the airways (ova 44.3±8.6 vs ova/60.1: 12.7±1.42; 13.3±3.4; 13.4±4.3 and $9.4\pm2.2\times10^4$/ml, n=10 for 0.001-1 μg of Cpn60.1 respectively, $p\leq 0.05$). Four hours after eotaxin injection, saline treated mice showed a significant accumulation of cells in the extra vascular tissue (saline: 40±5 *vs* Cpn60.1: 2.3±1 cells/50μm^2, n=6) and higher number of cells rolling along the vessel wall in 30 sec (saline: 12±1.12 vs Cpn60.1: 2.8±1.02) compared to Cpn60.1 treated mice. In contrast, the adherence of cells to the endothelial layer was higher in Cpn60.1 compared to saline treated mice (saline adhesion: 15±1.7 vs Cpn60.1: 22.2±4.8 cells/50μm, n=6). Cn60.1 inhibits leukocyte migration to the lung in response to ova and prevented leukocyte rolling along and transmigration across the vessel wall *in vivo*.

P4109
Expression of hyperoxidized peroxiredoxins is enhanced in peripheral blood mononuclear cells of bronchial asthma patients
Yun-Jeong Bae[1], Tae-Bum Kim[2], Jae Hyung Roh[3], Hee-Bom Moon[2], You Sook Cho[2], Keun Ai Moon[4]. [1]*Health Promotion Center, Asan Medical Center, Seoul,* [2]*Department of Allergy and Clinical Immunology, Asan Medical Center, Seoul,* [3]*Internal Medicine, YeongYang-Gun Health Center, Kyung Sang Bukdo,* [4]*Asan Institute for Life Science, Asan Institute for Life Science, Seoul, Korea*

Background and objectives: Increased oxidative stress is related to the pathogenesis of asthma. Peroxireoxin (Prx), ubiquitous antioxidant enzymes, also termed thioredoxin peroxidases. However, its role in the allergic inflammation remains unidentified. The present study investigates the expression and possible role of Prx and hyperoxidized Prx (Prx-SO3) in asthmatic patients.
Methods: At first, the expression of Prx and Prx-SO3 in peripheral blood mononuclear cells (PBMCs) of asthma patients were semi-quantitatively measured by using Western blot methods from asthma patients and control subjects. And then, to evaluate if higher sensitivity to oxidative stress exposure exists in PBMCs from asthmatics, intracellular ROS levels with hydrogen peroxide treatment were also determined by flowcytometry.

Results: The levels of Prx-SO3/Prx in PBMCs of asthma patients were significantly higher compared to those in normal subjects and were also related with asthma severity. Furthermore, the intracellular ROS after hydrogen peroxide treatment were remarkably enhanced and prolonged in PBMCs from asthmatics, while transiently increased intracellular ROS levels were observed in control subjects.
Conclusions: The hyperoxidation of Prx may be related with increases susceptibility to oxidative stress and possibly play a role in the pathogenesis of severe asthma.

P4110
LSC 2011 Abstract: P2Y2 receptor regulates VCAM-1 membrane and soluble forms and eosinophil accumulation during lung inflammation
G. Vanderstocken, B. Bondue, M. Horckmans, L. Di Pietrantonio, B. Robaye, J.M. Boeynaems, D. Communi. *Brussels, Gosselies, Belgium*

ATP has been defined as a key mediator of asthma. In this study, we evaluated lung inflammation in mice deficient for the P2Y2 purinergic receptor. We observed that eosinophil accumulation, a distinctive feature of lung allergic inflammation, was defective in OVA-treated P2Y2-deficient mice compared with OVA-treated wild type animals. Interestingly, the upregulation of VCAM-1 was lower on lung endothelial cells of OVA-treated P2Y2-/- mice compared with OVA-treated wild type animals. Adhesion assays demonstrated that the action of UTP on leukocyte adhesion through the regulation of endothelial VCAM-1 was abolished in P2Y2-deficient lung endothelial cells. Additionally, the level of soluble VCAM-1, reported as an inducer of eosinophil chemotaxis, was strongly reduced in the bronchoalveolar lavage fluid (BALF) of P2Y2-deficient mice. In contrast, we observed comparable infiltration of macrophages and neutrophils in the BALF of LPS-aerosolized P2Y2+/+ and P2Y2-/- mice. This difference could be related to the much lower level of ATP in the BALF of LPS-treated mice compared with OVA-treated mice.
Our data define P2Y2 as a regulator of membrane and soluble forms of VCAM-1 and eosinophil accumulation during lung inflammation.