

319. Novel mechanisms in asthmatic inflammation

P3458

A membrane bound IL-15 present on P.B. CD34+ progenitors triggers the generation of NK2 cells

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Th2 cells play a pivotal role in the pathogenesis of asthma. Natural killer (NK) cells are also thought to divide into NK1 and NK2 subsets. However, whether NK2 cells represent a truly distinct subset or an immature stage of NK cell differentiation is not certain. Since NK2 seem to be involved in the inception of asthma, we investigated the existence of a specific reservoir of pre-NK2 cells. We show that only normal peripheral blood CD34+ cells are the natural reservoir of NK2 progenitors. Indeed, PB progenitors expanded in STEM α A medium supplemented with r-SCF and r-Flt3-L express a membrane bound IL-15 that has the potential, in the total absence of exogenous lymphokines, to generate: 1) NK cells expressing massively a Th2 phenotype (85-90%). 2) Adherent cells with a bi-lineage commitment potential co-expressing both NK and DC1 markers (10-15%) that produce IL-12 and IFN γ and are expanded in the presence of the soluble IL-15R α chain.

The balance between these subpopulations (NK2 cells and NK/DC1) could play a role in the control of the allergic response and constitute new targets for the anti allergic therapy.

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Plasminogen activator inhibitor (PAI)-1 is expressed in human eosinophils stimulated by TGF- β

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Plasminogen activator inhibitor (PAI)-1 is the main inhibitor of the fibrinolytic system and is known to play an essential role in tissue remodeling. Recent evidence indicates that chronic asthma may lead to tissue remodeling such as subepithelial fibrosis and extracellular matrix (ECM) deposition in the airways. However, the role of PAI-1 in asthma is unknown. Recently the mast cell (MC), which plays a major role in asthma, was found as a novel source of PAI-1, and a large number of MCs expressing PAI-1 are infiltrated in the airways of patients with severe asthma. Furthermore, PAI-1-deficient mice show reduced ECM deposition in the airways of a murine model of chronic asthma by inhibiting MMP-9 activity and fibrinolysis. Therefore, we investigate whether express in eosinophils which play a major role of airway remodeling stimulating with TGF- β . We found that PAI-1 was expressed in purified human eosinophils stimulated with TGF- β . These data suggest that in view of the findings that TGF- β stimulation is associated with elevated PAI-1 level in the lung may contribute to the development of asthma. In summary, PAI-1 may play an important role in the pathogenesis of asthma and further studies evaluating the mechanisms of PAI-1 action may lead to the development of a novel therapeutic target for the treatment and prevention of asthma.

P3460

Interaction of macrophages and airway epithelial cells

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The airway epithelium plays a direct role in the inflammation- and immune response by secretion of cytokines, chemokines and antimicrobial peptides. The aim of this study was to characterize the interaction of airway epithelial cells and macrophages in the event of an infection.

Airway epithelial cells (AEC) were stimulated with typical macrophage cytokines (1 ng/ml IL-1 β , 10 ng/ml TNF- α or combination of these cytokines) for 18 hours and challenged with 1 μ g / ml LPS from *Pseudomonas aeruginosa* or the TLR2 ligands MALP2 (1 μ g/ml) for 6 hours. Thereafter the airway epithelial cell line A549 was co-cultured with different numbers of U937 cells and stimulated with heat inactivated *Pseudomonas aeruginosa* for 18 hours. This experiment was also made with primary AEC's and freshly isolated monocytes. The concentration of IL-8 and IL-6 in the supernatant was measured by ELISA. The expression of hBD-2, hTLR-2 and Psoriasin was determined by Real-Time RT-PCR. Preincubation with TNF- α and following stimulation with the ligand MALP2 leads to an increased expression of hTLR2, hBD-2 and Psoriasin. This increase was significant higher than after stimulation with TNF- α or MALP2 alone. The secretion of the pro-inflammatory cytokine IL-6 was also significantly upregulated. Stimulation of co-cultured AEC and macrophages with heat inactivated *Pseudomonas aeruginosa* leads to a synergistically elevated secretion of IL-6 correlated to the number of monocytes in the co-culture.

We could show that stimulation of AEC with typical macrophage cytokines sensitizes the cells for a better recognition of bacterial components. Our results indicate that an interaction of these cells is necessary for the efficient recognition of microorganisms.

P3461

Neuropeptides modulates Smad protein expression in cultured epithelial cells

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Transforming growth factor-beta (TGF-beta) plays an important role in the pathogenesis of allergic asthma and other airway diseases. Signals from the activated TGF-beta receptor complex are transduced to the nucleus of airway cells by Smad proteins, which represent a family of transcription factors that have recently been implicated to play a major role as intracellular mediators of inflammation. Chronic inflammatory airway diseases such as bronchial asthma or chronic obstructive pulmonary disease (COPD) are major contributors to the global burden of disease. Although inflammatory cells play the central role in the pathogenesis of the diseases, recent observations indicate that also resident respiratory cells represent important targets for pulmonary drug development. Especially targeting airway neuroeffectors offers a possible mechanism by which respiratory diseases may be treated in the future. Among numerous peptide mediators such as tachykinins, calcitonin gene-related peptide, substance P or neurokinin A is one of the most abundant molecules found in the respiratory tract. Neurogenic inflammation might contribute to selective downregulation of Smad7 through unknown mechanisms. We therefore examine whether neuropeptides modulate Smad protein expression. In this study, we found that Smad7 was significantly decreased in the cultured epithelial cells (BEAS 2 B) stimulating in neuropeptides substance P, Neurokinin A and CGRP. These data suggest that neuropeptides are key role of airway inflammation and plays an important role in airway remodeling in asthma. Smads may prove to be an important target for future development of new therapeutic strategies for asthma and chronic obstructive pulmonary disease.

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Airway allergy in pregnant mice: reduced Th2 responses but enhanced airway hyperresponsiveness

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Since pregnancy may worsen asthma we examined the influence of pregnancy on allergic airway responsiveness in mice. Female C57BL/6 mice were sensitized twice by i.p. injection of ovalbumin (OVA), followed by 3 inhalational exposures to OVA on days 28-30. Mice were mated on days 37-38. On day 55, both pregnant (P, n=7) and non-pregnant (NP, n=9) females were exposed to a single, secondary inhalational OVA challenge. Analyses were carried out on day 56 (gestation day 19). Control groups included non-sensitized but challenged P (n=5) and NP (n=9) females. In the absence of sensitization, OVA exposure did not induce airway inflammation or difference in airway responsiveness to inhaled methacholine (MCh). Following sensitization both P and NP mice developed airway inflammation as well as hyperresponsiveness (AHR) after the secondary OVA challenge. However, AHR was two times higher in OVA-sensitized P compared to NP mice (p<0.05). Analysis of BAL fluid revealed more neutrophils (2-fold increase, p<0.05), and more macrophages but similar numbers of eosinophils in P versus NP OVA-sensitized and challenged mice. These differences were associated with reduced levels of IL-5 (53 \pm 12 pg/ml vs 120 \pm 21, p<0.05) and IL-13 (9 \pm 3 vs 21 \pm 6, p<0.05) in the BAL fluid of P vs NP challenged mice, respectively. In conclusion, airway allergen challenge resulted in reduced Th2 responses but increased AHR and airway neutrophilia during pregnancy in sensitized mice. Supported by OTKA 42609, HL36577 and HL61005.

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Design-based stereological methods to analyse animal models of asthma
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Small animal models are widely used to study various pathologies. Chronic asthma is characterized by airway remodelling, which comprises goblet cell metaplasia, increase in smooth muscle cell layer and peribronchial fibrosis. These features can only be analysed by histopathology. Quantitative analyses are important to unequivocally demonstrate differences between experimental groups. Design-based stereology has moved an additional important step forward in quantitative respiratory research.

The following steps are of major importance for reliable acquisition of quantitative morphological data in studies of chronic asthma models: 1. fixation according to standard protocol, 2. systematic uniform random sampling, 3. an orientator approach to generate isotropic sections, 4. discrimination of distal versus proximal airways, 5. analyses of goblet cells, smooth muscle cell and peribronchial fibrosis. We used such methods to analyse a chronic ovalbumin (OVA) mouse model of asthma. A prominent increase in subepithelial collagen along the bronchial tree including airways was revealed by Sirius red staining. This was supported at the ultrastructural level by a considerable increase in collagen micro fibrils and elastin in the subepithelial lamina propria of lungs chronically exposed to OVA aerosol compared with control lungs. These observations were evidenced by quantitative stereological analysis which revealed a significant increase in arithmetic mean thicknesses of airway collagen and alpha-Smooth Muscle Actin positive cell layer.

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Tolerance repair for asthma treatment does not require airway remodelling
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Background: Failure of tolerance is now proposed as the immune-biological paradigm underlying asthma. Previously, we showed that prolonging allergen exposure in sensitized mice lead to tolerance repair, as subsequent immunisations and exposures were unable to re-induce allergy [ERS 2005, 233s (A1593)]. Here, we investigated the role of remodelling in tolerance repair.

Methods: Sensitized mice were exposed to ovalbumin (OVA) for different periods, followed by re-immunisation and acute challenge with hen egg lysozyme (HEL), an unrelated allergen. Allergic airway inflammation and remodelling were assessed.

Results: Mice challenged for 2 weeks (w) with OVA developed Th-2 airway disease without evidence for airway remodelling. Continued OVA exposures (8w) resulted in the disappearance of inflammation along with the emergence of remodelling and fibronectin (FB) deposition (PBS: 3.1±0.2 vs OVA 5.6±0.4 µm² FB/µm, p<0.05). Subsequent re-immunisation and acute challenge (1w) with HEL did not induce eosinophilic airway disease or HEL IgE, while FB deposition remained present. To elucidate the role of remodelling, we developed a rush protocol of tolerance repair by shortening the initial OVA exposures to only 2w before HEL immunisation and challenge. We show that in these mice, at a time point where no remodelling is detectable, tolerance was already largely repaired. The airway eosinophilia (PBS/HEL challenged: 48±10 vs OVA/HEL challenged 14±8%, p<0.05) and Th-2 cytokines (IL-13) were significantly suppressed. There were no arguments for a Th-1 driven counterbalancing mechanism.

Conclusion: The immune system is capable of restoring its default tolerogenic bias in the absence of detectable airway remodelling.

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Expression of complement proteins in the lung following allergen sensitization and challenge in mice

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Following allergen challenge activation of the complement system occurs in the lungs of sensitized hosts. Different animal models have demonstrated that activation of the complement cascade and the production of complement split-products are pivotal for the development of allergic airway disease. However, little is known about the expression of complement proteins in the lung. To further investigate the expression of complement factors in the lung, BALB/c mice were sensitized on day 0 and 14 with ovalbumin (OVA, 20 µg in alum hydroxide). Mice were then challenged on day 28, 29 and 30 for 20 minutes with nebulized OVA solution (1% in saline). Bronchoalveolar lavage (BAL) and lung tissue were collected at 4 hours following the first and second challenge and 4 hrs and 24 hrs following the final challenge. From whole lung tissue RNA was isolated using a standardized Trizol protocol and cDNA was synthesized. Using specially designed primers and realtime-PCR analysis, relative expression of complement factor genes to a housekeeping gene and a calibrator was determined. Following allergen challenge, expression of complement factors ©3, C3a receptor, and factor B was increased in the lungs from sensitized and challenged mice compared

to non-sensitized but challenged mice. No difference was detected in the levels of C5 and C5a receptor expression in sensitized and challenged mice compared to challenged only animals. This data shows an increased expression of certain complement factors in the lung following allergen challenge of sensitized mice. To identify the cellular source of this production further studies are needed. This study was supported by DFG (SFB 548, A11) and MAIFOR.

P3466

Oral tolerance attenuates airway remodelling in a model of chronic allergic inflammation

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Objectives: We investigated the effects of induced oral tolerance in controlling airway remodelling in guinea pigs (GP) with chronic airway inflammation. **Material and Methods:** GP were exposed to repeated ovalbumin aerosols twice a week for 4 weeks. At the same period oral tolerance was induced either by offering GP ad libitum 2% ovalbumin in sterile drinking water during 4 weeks (OT1 group) or starting oral ovalbumin after the 4th exposure to ovalbumin aerosols (OT2 group). **Controls:** 1. GP inhaled with ovalbumin and drinking sterile water (OVA group), 2. GP inhaled with normal saline and drinking sterile water (NS group). Collagen, elastic, elaunin and oxytalan fibres content was quantified using a Image Analyser (Image J) and expressed as a percentage of these fibres in the total area of airway wall.

Results: OVA group showed greater amount of collagen, elastic, elaunin and oxytalan fibres around airways compared to the NS group (P<0.05). Both models of oral tolerance (OT1 and OT2 groups) had a significant reduction in airway collagen, elastic, elaunin and oxytalan fibres in airway wall compared to OVA group (P<0.05).

Conclusions: Airway remodelling may be effectively controlled in animals with chronic allergic inflammation that have been rendered tolerant. Supported by: FAPESP, CNPq, Brazil

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High intense aerobic conditioning has beneficial effects on lung inflammation and airway remodeling in chronically sensitized mice

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Background: Aerobic conditioning have been suggested as a therapeutic strategy to asthmatic patients, however its effects on lung allergic inflammation and airway remodeling remains unknown.

Objectives: We evaluated the effect of high intensity aerobic conditioning (HIAC) on allergic lung inflammation and airway remodeling in mice.

Methods: Twenty-four animals were divided in three groups: control (n=8), OVA (sensitized with ovalbumin; n=8) and OVA+HIAC (sensitized with ovalbumin and trained; n=8). OVA and OVA+HIAC groups were sensitized with i.p. injections of OVA and chronically submitted to OVA inhalation (50 days). OVA+HIAC group was submitted to exercise conditioning. Total and differential cell counts in BAL fluid and morphometric analysis to quantify smooth muscle tickness and collagen and elastic fibers were performed.

Results: OVA+HIAC group presented a decrease in eosinophil cell counts in BAL fluid (Table 1), collagen and elastic contents and smooth muscle tickness (Table 2).

Table 1. Number of cells in BAL fluid

	Control	OVA	OVA+HIAC	p value
Eosinophils	0.10±0.00	4.17±2.04*	2.09±2.48	< 0.01

*compared with control and OVA+HIAC groups

Table 2. Components of airway remodeling

	Control	OVA	OVA+HIAC	p value
Collagen (%)	11.23±6.71	43.55±9.62*	10.35±4.75	< 0.001
Elastic (%)	29.65±3.63	48.58±7.27*	29.27±6.68	< 0.001
Smooth Muscle (points/BM)	0.27±0.19	1.32±0.71*	0.34±0.26	< 0.001

*compared with control and OVA+HIAC group; BM-basal membrane

Conclusions: Our results suggest that HIAC decreases lung eosinophil migration and airway remodeling in chronically sensitized mice.

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P3468**Plasma uteroglobin-related protein 1 (Ugrp1) levels is associated with Ugrp1 -112G/A polymorphism and the severity of asthma**

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Background: Ugrp1 is an airway specific protein having similarity to clara-cell secretory protein (CCSP) and is speculated to have anti-inflammatory properties. We previously reported the association between -112G/A polymorphism and asthma in Japanese adult population.

Objectives: The study was designed to establish an ELISA system for Ugrp1 to examine the relations between plasma Ugrp1 and -112G/A polymorphism, asthma phenotype, and its severity.

Methods: 141 asthmatics and 103 controls were involved. Monoclonal antibodies were obtained from hybridoma cell-lines. A sandwich ELISA system was established and plasma Ugrp1 was measured.

Results: An ELISA system that allows measurement of Ugrp1 within the range of 9.6-1250 pg/ml was established. The mean plasma Ugrp1 for the subjects having -112A allele was significantly lower than those without it (45.1 and 62.6 pg/ml, respectively, p=0.014). The plasma Ugrp1 for asthmatics and controls were 50.1 and 61.4pg/ml, respectively (p=0.12). The mean plasma Ugrp1 for mild, moderate, and severe asthmatics were 66.4, 50.9 and 28.6pg/ml, respectively, and severe asthmatics had significantly lower plasma Ugrp1 levels compared to mild asthmatics and controls (p=0.014 and 0.009, respectively).

Conclusion: The ELISA system to measure ugrp1 protein was established. Plasma Ugrp1 was associated with the Ugrp1 G-112A polymorphism and the severity of asthma.

P3469**Effect of nasal allergen challenge on non-invasive markers of inflammation in allergic rhinitis**

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Rationale: Nasal allergen challenge (Nach) is a validated tool inducing features of allergic rhinitis (AR). We investigated the effect of Nach on non-invasive markers of nasal inflammation.

Methods: 20 subjects with stable AR (11F/9M, 19-51 y, no anti-AR-therapy) participated in a double-blind, placebo (P)-controlled, parallel study. Subjects randomly received a nasal challenge with a relevant allergen extract (ALK-Abellö, NL) or its diluent (P). The inflammatory response was evaluated by nasal brushes (NAB), nasal lavage (NAL), and nasal nitric oxide (nNO) at prechallenge, and 20min, 7 and 24h postchallenge. Inflammatory cell differentials were performed on May-Grünwald-Giemsa-stained cytopins from NAB. Eosinophil cationic protein (ECP), tryptase and α 2-macroglobulin were measured in NAL by immunoassays. MUC5Ac by immunoblotting. nNO was measured by a chemoluminescence analyzer (Ecomedics CLD88sp).

Results: As compared to P, Nach increased the mean NAB-eosinophils by 144% at 7h postchallenge (p=0.03). Moreover, Nach increased α 2-macroglobulin and tryptase (p=0.002; p=0.004, resp.) at 20min and ECP at 20min (p=0.03) and at 7h (p=0.02) postchallenge. MUC5Ac was detectable in all samples but not affected by Nach. nNO was decreased at 20min (p=0.001), back to baseline at 7h and slightly increased at 24h post-allergen (p=0.04).

Conclusion: Nach induced significant increases in several non-invasive inflammatory and leakage markers in NAB and NAL. Our findings extend previous data and underscore the validity of Nach and monitoring of non-invasive markers in intervention studies in AR. nNO levels may be underestimated by Nach-induced congestion; its applicability needs to be established.

P3470**Low exposure to inhaled formaldehyde: effect on allergen bronchial response in asthmatics sensitized to mite**

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Background: Formaldehyde, a widespread domestic indoor pollutant, has been shown to increase the risk of childhood asthma for concentrations above 60 μ g.m⁻³.

Aim: To evaluate the influence of pre-exposure of low dose formaldehyde

(100 μ g.m⁻³ during 30 min according to the World Health Organization's recommended maximum value for indoor environments) on bronchial response to *Dermatophagoides pteronyssinus*.

Method: Nineteen subjects with an intermittent asthma sensitized to mite were included. Each subject underwent a mite-allergen bronchial challenge test immediately after a standardized exposure in a chamber to formaldehyde or air (random order). Blood samples and induced sputum were collected 24 hours before and after mite challenge to be analysed for ECP.

Results: FEV₁ and clinical symptoms scores before BCT to mite were not different when subject were preexposed to formaldehyde or placebo (p=0.470; 0.959). After formaldehyde inhalation, patients developed an immediate bronchial response with a significant lower dose of mite allergen compared to air exposure (the geometric mean PD₂₀ for Der p 1 was 34.3 ng after formaldehyde and 45.4 ng after placebo, p=0.05). The maximum fall in FEV₁ from baseline in late phase reaction was significantly higher after formaldehyde (15% versus 11%, p=0.046). After formaldehyde, a tendency was observed towards a higher increase in sputum ECP (p=0.094) and in peripheral eosinophil counts relative to baseline (p=0.059).

Conclusion: Our study demonstrated that exposure to low levels of formaldehyde significantly enhanced bronchial responsiveness to mite allergen in mite-sensitized subjects with asthma.

P3471**Reduced bronchial CD4⁺ T cell activation in smokers with occupational asthma**

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A recent study revealed that smoking inhibited the asthma associated increase in bronchial CD4⁺ T cell density (1). To study the activation status of subepithelial T cell subsets, multicolour immunofluorescent staining with monoclonal antibodies to CD3, CD4, CD8, CD25, HLA-DR and Ki-67 was performed on bronchial biopsies from 20 workers with occupational asthma (12 smokers and 8 non-smokers), 15 healthy workers (7 smokers and 8 non-smokers) and 10 non-smoking, non-exposed controls. While non-smoking asthmatics had median 61 (range 35-163) CD25⁺ T cells/mm² (5.8% of all T cells), smoking asthmatics had median 0 (0-27, p<0.001) and the control groups varied from median 0-7 cells/mm² (0-53). Triple staining revealed that CD25 was almost exclusively (94%) expressed on CD4⁺ T cells in all groups. In contrast, although both non-smoking and smoking asthmatics tended to have higher percentage of HLA-DR⁺ T cells (3.6 and 3.9% of all T cells respectively) than the control groups (0-1.6%), the density of HLA-DR⁺ T cells tended to be reduced in smoking compared to non-smoking asthmatics (median 15 vs. 37 cells/mm², p = 0, 07). HLA-DR was almost exclusively (83%) expressed on CD8⁺ T cells. The density and percentage of proliferating (Ki-67⁺) bronchial T cells was significantly higher in non-smoking asthmatics (median 14 cells/mm², 1.2% of all T cells) compared to smoking asthmatics and control groups (all median 0). Both CD4⁺ and CD8⁺ T cells proliferated in the asthmatic bronchial mucosa equally. In conclusion, smokers with occupational asthma appear to represent an asthmatic phenotype without any evidence for bronchial CD4⁺ T cells activation. 1. Sjøheim, T. *et al.* Proc Am Thorac Soc 2005 2; A616.

P3472**Cigarette smoking reduces dendritic cells and alters airway immunity in asthma**

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Introduction Cigarette smoke exposure increases the risk of developing asthma, worsens established asthma and renders it less responsive to steroid therapy. We hypothesized that cigarette smoking may act through reduction of the numbers of airway dendritic cells (DCs), considered to be key to Th1 and Th2 differentiation and B cell growth.

Methods Bronchial biopsies from asthmatic(A) smokers(Sm) and non-smokers(NSm), steroid naive(SN) and steroid-treated(ST) and healthy NSm (controls) were obtained and sections stained and counted for Langerhans cells(CD1a), mature DCs(CD83), B cells(CD20), the Th1 cytokine, IFN and the Th2 cytokine IL-4.

Results The table on page 593 shows the data for number of immuno-positive cells/mm² of area presented as median(range) and the ratio IFN to IL-4. Mature DCs and B cells were significantly fewer in the ASm than in the ANSm or controls. There was a trend for decreased IFN in the ASmSN vs ANSmSN.

Conclusion Cigarette smoking reduces bronchial DC and alters airway immunity, which may predispose to airway infections and to the development of difficult to treat asthma.

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Abstract P3472 – Table 1

	TotANSm N=21	TotASm N=24	p	ANSmSN N=6	ASmSN N=9	p	ANSmST N=15	ASmST N=15	p	Control N=10	p
CD83	76 24-464	37 0-131	0.006	99 68-154	48 8-131	0.025	74 24-464	32 0-106	0.04	85 40-294	‡,§,§§,¶¶
CD1a	26 7-302	33 9-165		85 33-302	57 21-165		17 7-194	31 9-115		36 11-127	
CD20	45 10-447	26 4-234	0.014	99 10-207	23 4-111	0.05	42 10-447	33 4-234		34 10-130	‡,§§
IFN γ	82 2-275	72 6-273		144 44-232	70 6-240		65 2-275	75 8-273		13 2-242	†,‡,§,§§,¶¶,¶¶¶
IL-4	162 39-856	174 49-495		145 69-262	174 64-335		177 39-856	161 49-495		128 20-580	
IFN γ /IL-4	0.49 0.02-1.45	0.39 0.02-2.89		0.93 0.49-1.45	0.49 0.02-1.43		0.35 0.02-1.3	0.31 0.06-2.89		0.15 0.03-0.58	†,‡,§,§§

† p<0.05 ANSm vs Control, ‡ p<0.05 ASm vs Control, § p<0.05 ANSmSN vs Control, §§ p<0.05 ANmSN vs Control, ¶ p<0.05 ANSmST vs Control, ¶¶ p<0.05 ASmST vs Control.

P3473**Effect of allergen particle size on inflammatory and cellular profile after mite bronchial challenge test**

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Background: Immediate bronchial response to cat allergen was localized in the large airways and small particles induced late symptoms and decreased peripheral airway calibre (Lieutier-Colas F *et al.*, 2003). Similar results were obtained with mite allergen: the dose of Der p 1 provoking immediate bronchial symptoms was 8-9 times lower with large particle (9.7 μ m) than with small particle (1.1 μ m).

Aim: investigate the influence of mite allergen particle size on systemic and local inflammatory and cellular response after allergen bronchial challenge test.

Method: 19 allergic patients sensitized to mite with mild asthma were included. A mite bronchial challenge test (BCT) was performed blindly on 3 occasions with 3 nebulizers randomly selected (MMAD:1.1; 5.6 and 9.7 μ m). Blood samples were collected and induced sputum were performed 24h before and after mite BCT. A cellular count was performed blindly in blood and induced sputum. The production of cytokines was assessed with flow cytometry.

Results: The level of IL-5 in blood differed after BCT according to particle size and was significantly increased with the 1.1 μ m particles (p=0.015). The level of IL-5 in sputum supernatant was significantly increased 24 hours after BCT only with 1.1 μ m particles (p = 0.046). No difference in sputum eosinophil count was observed according to particle size (p= 0.084).

Conclusion: The increase of IL-5 with 1.1 μ m suggested that eosinophils were more involved when patient allergic to mite inhaled small particles. This suggests that late phase reaction is mostly localized in the small airways and is appeared to be induced by eosinophils.

P3474**Systemic inflammation and lung function in young adults**

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Introduction: Impaired lung function is associated with systemic inflammation and is a risk factor for cardiovascular disease in older adults. It is unknown when these associations emerge and to what extent they are mediated by smoking, chronic airways disease, and/or established atherosclerosis. We studied the association between lung function and systemic inflammation in a population-based birth cohort of approximately 1000 young adults.

Methods: Serum C-reactive protein (CRP) and spirometry were measured at ages 26 and 32. Pregnant women were excluded. Where appropriate, analyses were adjusted for height and sex to account for differences in predicted lung function.

Results: There were inverse associations between FEV1 and CRP at ages 26 (r= -0.13 p<0.001) and 32 years (r= - 0.15, p<0.001). These associations were not significantly different in men and women and were independent of smoking history, diagnosis of asthma, and body mass index. Similar associations were found between FVC and CRP. The association between CRP and the FEV1/FVC ratio was weaker with a significant correlation only in women.

Conclusions: Reduced lung function is associated with systemic inflammation in young adults. The association is independent of smoking, asthma and body mass index. The reasons for the association are unexplained, but suggest that the association between poor lung function and cardiovascular disease may be mediated by an inflammatory mechanism.

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P3475**Decreased T-bet expression and changes in levels of chemokines (IP-10, MIG, RANTES, MCP-1 and IL-8) in peripheral blood of adults with asthma**

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Introduction: T-bet is a novel transcription factor regulating lineage commitment of Th lymphocytes to a predominant Th1 phenotype. Previous studies on T-bet and asthma mainly focused on bronchial biopsy specimens. This study assessed the relationship of T-bet expression and chemokines in peripheral blood of asthmatics.

Methods: Blood was collected from 24 steroid naive asthmatics, 39 asthmatics on inhaled steroid, and 32 age- and sex-matched controls for assay of T-bet expression, specific IgE and chemokines levels. T-bet mRNA expression was assessed by real-time quantitative RT-PCR. Chemokine levels were assessed by flow cytometry (human chemokine cytometric bead array kit, Becton Dickinson Biosciences Pharmingen, CA, USA).

Results: The mean (SD) age and FEV₁% predicted of the asthmatic subjects were 43.6(14.6)yrs and 85.9(20.0)% respectively. The median[IQR] T-bet expression after normalization with β -actin was suppressed in asthma subjects when compared with controls (asthma 1.07[0.59] vs controls 1.07[1.14], p=0.03). The median [IQR] of RANTES was elevated and IP-10 was suppressed in asthmatics when compared to controls (RANTES: 13658.0[13673.3] vs 6299.5[19407.8]pg/ml, p=0.03; IP-10: 1047.6[589.8] vs 1306.4[759.9]pg/ml, p=0.001). T-bet expression correlated negatively with RANTES level in the asthmatics (r=-0.29, p=0.032).

Conclusions: This study showed that T-bet could be measured in peripheral blood but its expression was suppressed in asthmatics. This is in concordance with asthma being a predominantly Th-2 disease. More studies are needed to explore the potential application of peripheral blood monitoring of T-bet.