506. Asthma mechanisms

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Degree of bronchial hyperresponsiveness (BHR) and new onset of respiratory diseases 9 years later

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In the European Community Respiratory Health Survey, 10933 subjects aged 20-44 years (29 centers, 14 countries) had both a baseline (1991/3) and a follow-up (2000/2) evaluation. The degree of baseline BHR was defined in 8136 (74%) subjects as absent (PD20>1 mg) (n=6852); and low (PD20, mg: (0.5-1]), medium (0.15-0.5] or high (0-0.15], based on PD20 distribution tertiles). Two-level logistic regression models (2nd level: centers) were used to analyze the association between BHR degree and new onset of nasal allergies (allergic rhinitis, AR) and COPD (pre-bronchodilator FEV/FVC<0.70) in non-asthmatic subjects; current asthma and symptoms (wheeze, chest tightness, dyspnea at rest/after exercise/at night); and hospital admissions for breathing problems, in subjects who were free from the respective outcomes at baseline. The analyses were adjusted for gender, age, height, education, pack-years, FEV1 and atopy (IgE >0.35 kU/L to cat, mite, grass or Cladosporium) at baseline.

An increased BHR degree was associated with an increased risk of developing COPD, asthma and asthma-like symptoms 9 years later (see table).

	BHR degree (vs no BHR) (OR; 95% CI)		
New onset outcomes	mild	moderate	severe
AR[#]	1.23 (0.74–2.06)	0.73 (0.39–1.36)	2.47 (1.30–4.71)
COPD[#]	2.12 (0.86–5.23)	4.32 (1.93–9.67)	5.14 (1.60–16.51)
Current asthma	3.17 (2.05–4.90)	4.25 (2.77–6.52)	6.75 (4.08–11.16)
Asthma-like symptoms*	1.12 (0.73–1.74)	1.67 (1.06–2.62)	2.60 (1.33–5.07)
Admissions for breathing problems*	1.84 (1.05–3.24)	1.10 (0.59–2.05)	1.85 (1.03–3.32)

[#]Only in non-asthmatics; *asthma at baseline included in the model.

These preliminary analyses support the interest for BHR degree as a predictor of several adult-onset respiratory diseases.

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Chronic rhinosinusitis and airway inflammation in new onset asthma in adults

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Rationale: In severe asthma, chronic rhinosinusitis (CRS) is associated with increased sputum eosinophils [*Brinke, JACI 2002*]. Many adults with severe asthma have the onset of their disease in adulthood. We hypothesized that the relationship between the severity of CRS and inflammation in the lower airways already exists in the early stages of adult-onset asthma.

Aim: To investigate the relationship between CRS and airway inflammation in adults with new onset asthma.

Methods: Ninety-eight adults (>18yr) with a physician diagnosis of recent (<1yr) onset asthma (age 48 (±16.1)yr, pb FEV_1 99 (±18.8)%pred, 61% female, 43% atopic, 20% steroid naive) were recruited. Diagnosis was based on reversibility in FEV_1 ≥12%pred and/or PC_{20} methacholine < 8 mg/ml. Patients with COPD were excluded.

CT-scanning was performed and blindly scored for sinonasal mucosal thickness using LMK score [*Lund Rhinology 1993*]. Sputum was induced and processed, exhaled nitric oxide (FeNO), pb FEV1 and methacholine challenge were performed and venous blood was taken. LMK scores were related to sputum cell counts, FeNO, lung function and blood using Spearman correlation coefficient.

Results: There was a significantly positive correlation between CT scores and eosinophils in induced sputum (R_s= 0.73, p=<0.001) and peripheral blood (R_s= 0.56, p=<0.001) and level of FeNO (R_s= 0.35, p=0.001). No correlation was found between CT-scan scores and pb FEV_1% pred (p= 0.75) or PC_{20} methacholine (p=0.15).

Conclusion: In adults with recent onset asthma, the degree of CRS and lower airways inflammation are closely related. This implies that patients with recent onset asthma should be checked and treated for upper airways disease, if necessary.

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Association between airway hyperresponsiveness, obesity, and lipoproteins in a young Danish cohort

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Rationale: Epidemiological data have linked obesity with an increased risk of asthma in the community. However, the mechanisms responsible for this relationship remain unknown. The present study investigated the association between airway hyperresponsiveness to methacholine (AHR) and body mass index (BMI) and plasma lipoproteins (LDL, HDL and total cholesterol).

Methods: Associations between AHR, BMI and plasma lipoproteins were assessed in a population-based cohort at ages 14 and 20 years.

Main results: In unadjusted analyses, higher LDL cholesterol levels at age 14 were associated with AHR to methacholine at age 20 in both sexes (p<0.05). HDL, LDL/HDL ratio and total cholesterol were not associated with AHR. In a multiple regression analysis adjusted for sex, lung function, smoking and asthma, only higher levels of BMI at age 14 or 20 years were significantly associated with increased AHR at age 20 years, while neither LDL, HDL, LDL/HDL ratio nor total cholesterol were significantly associated with AHR to methacholine.

Conclusions: We confirmed that there is a strong association between BMI and AHR to methacholine in young people. This association seems to be independent of the plasma lipoproteins levels and we did not find an independent association between levels of lipoproteins and AHR.

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Effect of vitamin D treatment on antimicrobial peptides in asthma patients and healthy controls

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Vitamin D deficiency has been linked to asthma because of the proposed role of vitamin D in inflammation control and host defense against infection. Antimicrobial peptides (AMPs) are effector molecules of the innate immune system, and their expression may be decreased by allergic inflammation. Vitamin D increases expression of AMP in vitro, but its effects on AMPs levels in asthma patients are unknown.

Hypothesis: AMP levels in nasal secretions of patients with allergic asthma are lower than those in controls and can be restored by vitamin D substitution.

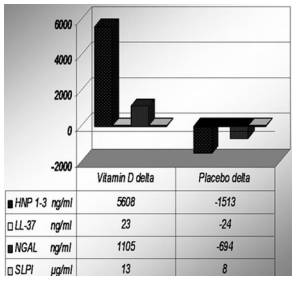
Methods and results: 20 allergic asthma patients and 20 controls (18-45 yrs) were included. The influence of allergic asthma on AMPs was assessed in a case control design, and the effect of 7 days daily oral treatment with 2 mcg 1,25(OH)2D3 active vitamin D (calcitriol) on AMPs was assessed in a placebo-controlled cross-over study.

The levels of the AMPs HNP1-3 and NGAL were significantly lower in asthmatics, whereas there was a trend for LL-37 (table 1).

Table 1. Mean AMP in asthma patients and healthy controls

	Asthma	Controls	p-value
HNP1-3 ng/ml	5215	10845	0.023
LL37 ng/ml	99	186	0.134
NGAL ng/ml	2333	4343	0.007
SLPI μg/ml	893	828	0.840

Treatment with 1,25(OH)2D3 significantly increased HNP1-3 and there was a trend for an increase in LL-37 and NGAL.



Conclusion: Levels of AMPs are lower in nasal secretions in asthmatics, and treatment with active vitamin D increases these levels.

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Chemokine and their receptors mRNA expression in brush-biopsies of nasopharynx in bronchial asthma patients

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Introduction: Mechanism of BA onset and progression at different ages are still poorly understood. System of chemokines in upper airways is a tempting object of investigation BA pathogenesis.
Methods: We examined 70 patients with asthma. The patients age ranged from 16 to 74 years. Were studied 31 men and 39 women. The control group used brush-biopsy 17 healthy volunteers with no history of atopic diseases. Were studied mRNA of eotaxin, eotaxin-2, MIP-1α, MIP-1β, RANTES, CCR1, CCR3, CCR5, CXCR1, and CXCR2.
Results: A study in patients with asthma was revealed a significant increase in eotaxin mRNA expression (p = 0.045), eotaxin-2 (0.036), MIP-1α (0.0003), MIP-1β (0.002) and CXCR1 (0.004) compared with healthy volunteers. At the same time CCR1 gene expression in patients with asthma was reduced compared with control. To study the age dynamics of the asthma patients were divided into 4 groups: first group - 16-25 years (n = 14), the second group - 26-39 years (n = 14), the third group - 40-53 years (n = 21) and the fourth group - 54-74 years (n = 21). Eotaxin-2 had wave-like dynamics depending on patents age. MIP-1α was lowest in the second and third groups, and in the first and fourth - were significantly higher. The values of MIP-1β - the lowest in the first group with increasing age became more and peaked in the fourth group. mRNA of CCR1 and CXCR1 was significantly decreased in the second group compared to the first, meanwhile in the third and fourth ones levels of mRNA of both receptors increases depending on the age of the patients.
Conclusion: The state of chemokines and relative receptors gene expression in upper airways reflects age features of BA pathogenesis.

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microRNA levels in nasal biopsies in allergic and non-allergic rhinitis and asthma

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Introduction: Rhinitis and bronchial asthma are both manifestations of an inflammatory process within a continuous airway system. The upper and lower airways may be considered as a unique entity influenced by a common and often evolving inflammation process.
Aims and objectives: We aimed to study if microRNA (miRNA) and nasal nitric oxide (nNO) levels differentiate in asthma, allergic rhinitis (AR) and non-allergic rhinitis (NAR) patients.
Methods: A 12-years follow-up survey on factors for asthma and atopic diseases was conducted with 6041 subjects aged 30-38 years in year 2007. From them 180 responders were selected to participate in the clinical study, when nNO and nasal biopsies were taken. Based on the survey and clinical data the participants were classified to healthy controls (n=40), AR (n=51), AR combined with asthma (AR+asthma n=37) and NAR (n=42) patients. The levels of 27 inflammatory miRNAs were determined from nasal biopsies.
Results: No significant changes in the levels of 24 miRNAs were observed between groups. However, expression of mir-146a was significantly elevated in AR and AR+asthma groups compared to controls. In addition, the levels of mir-126 were significantly up-regulated in AR+asthma group compared to controls. Similarly, mir-18a levels were increased in AR+asthma group compared to controls and NAR group. Finally, nNo levels were significantly increased in AR and AR+asthma group compared to control or NAR group.
Conclusions: These preliminary findings emphasizes that some differences in the miRNA levels can be found from the upper airways between these patient groups. Further studies are needed to deeper examine the molecular mechanisms and to establish new markers for the diseases.

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The toll-like receptor (TLR) 5 ligand, flagellin, prometes asthma by priming allergic responses to indoor allergens

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Allergic asthma is associated with exposure to allergens and non-allergenic bacterial products residing in common house dust, suggesting that bacterial products can act as adjuvants to promote allergic sensitization to innocuous inhaled antigens. We therefore tested the ability of various microbial products, signaling through different toll like receptors (TLR), to prime allergic sensitization to inhaled antigens. Of the products tested, bacterial flagellin primed the strongest Th2 and Th17 responses to co-instilled ovalbumin (OVA). Following subsequent challenge with aerosolized OVA, these mice displayed strong asthma-like responses, including airway eosinophilia, mucus cell hyperplasia and airway hyperresponsiveness. As with purified flagellin, instillation of HDE into the airways of mice also primed Th2 and Th17 immune responses to OVA, and following OVA challenge, the animals developed strong asthma-like responses. Repeated instillations of HDE without OVA also induced asthma-like responses, indicating that house dust contains both adjuvants and allergens. The adjuvant activity in HDE was not dependent on TLR4, which senses lipopolysaccharide (LPS), but was dependent on TLR5, which senses bacterial flagellin. These findings might be relevant to human asthma because human sera from asthmatics had higher levels of anti-flagellin antibodies than did sera from control individuals. Taken together, these findings suggest that bacterial flagellin, and not LPS, is the major adjuvant in common house dust and that flagellin promotes allergic asthma by priming allergic responses to otherwise innocuous inhaled antigens.

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Rapamycin inhibits IL-33-induced airway inflammation

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Introduction: IL-33, a member of the IL-1 cytokine family, mediates a range of pathologies including asthma and arthritis (Liew F.Y. et al. Nature Reviews Immunology, 2010; 10: 103-110). The mechanism by which IL-33 induces airway inflammation and the associated signalling pathways are incompletely understood. Rapamycin was initially described as a macrolide antibiotic that selectively blocks the mammalian target of rapamycin (mTOR), a serine/threonine kinase involved in numerous signaling pathways.
Aim: To identify the role of mTOR and rapamycin in IL-33-mediated airway inflammation.
Method: BALB/c mice were treated intranasally with 1 µg IL-33 daily with or without 1mg/kg rapamycin for 5 days. Airway inflammation was assessed on day 6 by bronchoalveolar lavage (BAL) cellularity and cytokine analysis.
Results: Intranasal IL-33 induced profound airway inflammation with increased cellular recruitment on BAL consisting mainly of macrophages and eosinophils. Treatment with rapamycin significantly reduced IL-33-mediated cellular recruitment (Figure 1A) and reduced both eosinophil (Figure 1B) and macrophage (Fig. 1C) influx into the airway.

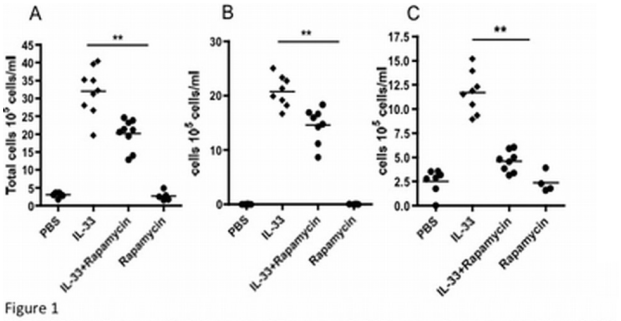

Figure 1
A, Total BAL cell counts. B, BAL eosinophil counts. C, BAL macrophage counts. **, p<0.001

Rapamycin also significantly reduced IL-33-mediated IL-5 and IL-13 production in BAL fluid.
Conclusions: Intranasal IL-33 induces airway inflammation that is partially blocked by treatment with intranasal rapamycin suggesting an important role for mTOR in the signaling pathway of IL-33 in vivo.