

205 Basophil Activation Is a Reliable Biomarker Of Allergic Bronchopulmonary Aspergillosis (ABPA) In CF: One Year Results Of a Longitudinal Cohort Study

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RATIONALE: *A. fumigatus* (*Af*) colonizes the airways of 35% of cystic fibrosis (CF) patients, with some further progressing towards allergic bronchopulmonary aspergillosis (ABPA). We hypothesized that changes on basophil surface activation pattern could discriminate between CF patients with and without ABPA.

METHODS: It is a longitudinal cohort study comparing blood basophil CD203c levels, which were measured at baseline and upon *in vitro* activation with *Af* allergen, in CF-ABPA patients (n=18) and in two control groups: CF patients colonized with *Af* but without ABPA (CF-AC; n=17) and CF patients without *Af* colonization or ABPA (CF; n=15). Patients were tested every six months and when ill with pulmonary exacerbation.

RESULTS: Basophil CD203c surface expression discriminated CF-ABPA from CF-AC and CF over time (repeated measures ANOVA p=0.0003). *Ex vivo* stimulation with *Af* extract (optimal at 10 minutes exposure) or recombinant Asp f1 (optimal at 30 minutes) produced similar results without difference in categorization. CF-ABPA patients experienced more pulmonary exacerbations, were more frequently co-infected with *P. aeruginosa*, and had more diabetes than controls.

CONCLUSIONS: The blood basophil CD203c assay is a suitable diagnostic biomarker of ABPA in CF.

206 Exosomes Secretion By Eosinophils: A Possible Role In Asthma Pathogenesis

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RATIONALE: Eosinophils secrete cytotoxic granules which are involved in initiation and propagation of diverse inflammatory responses as asthma. Our hypothesis is that some of these granules are exosomes which contain miRNAs and participate in the pathogenesis of these diseases. Our aims are to characterize the eosinophils exosomes and to investigate their role in asthma.

METHODS: We study the capacity of eosinophils to generate intracellular precursors of exosomes (multivesicular bodies, MVBs) by electron, confocal and fluorescence microscopy and flow cytometry analysis. Eosinophils were labelled with the specific marker of MVBs (Lysobiphosphatidic Acid, LBPA) and the reporter of endosomal vesicles (CD63). Exosomes derived from eosinophils were characterized by Western Blot (WB), Nanoparticle Tracking Analysis (NTA) to estimate the size distribution and concentration, flow cytometry and by electron microscopy images.

RESULTS: We observed that eosinophils generate intracellular MVBs. It was confirmed by the colocalization of LBPA and CD63 in these vesicles. This result was corroborated by electron microscopy images. Exosomes purified from eosinophils from healthy and asthmatic subjects were CD63+. The amount of exosomes was increased after stimulation with IFN- γ . NTA showed that the size of eosinophils exosomes was into the characteristic exosomal range. Finally, we found that the exosomes production from asthmatic subjects was higher than healthy subjects.

CONCLUSIONS: Our findings provide the first evidence that eosinophils produce MVBs and secrete exosomes. It is possible that they have an important implication in the pathogenesis of asthma. Moreover, they also could be considered as a future biomarker

207 CD49d-Expressing Neutrophils Are Found In The Nasal Lavage During An Acute Upper Respiratory Illness

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RATIONALE: Severe respiratory viral infections are associated with an increased risk for development of asthma and atopic disease. We previously demonstrated that recruitment of CD49d-expressing neutrophils (CD49d⁺ PMN) were critical to the development of post-viral atopic disease in a mouse model. We undertook this study to determine if CD49d⁺ PMN were recruited to human nasal mucosa with acute upper respiratory tract viral infections.

METHODS: Subjects 5-50 years of age within 4 days of onset of acute respiratory infection symptoms were enrolled. Following consent, a brief medical history, rhinitis symptom score (SNOT-20 questionnaire), nasal swab for viral PCR, and nasal lavage were obtained. At least 4 weeks later identical testing was performed (convalescent data). The frequency of CD49d⁺ PMN in the nasal lavages was determined by flow cytometry.

RESULTS: Compared to convalescence, CD49d⁺ PMN were found more frequently in the nasal lavage during an acute respiratory illness (3.0% [1.28-5.46] vs. 1.0% [0-1.92]; illness vs. convalescence; median [IQR], p<0.0001, n=28). SNOT-20 scores were higher during the acute illness as well (1.53 [0.94-1.99] vs. 0.38 [0.05-0.85]; p<0.0001). A positive viral PCR was found in 13 subjects (rhinovirus (n=11), RSV (n=1), or influenza B (n=1)), and all exhibited higher CD49d⁺ PMN frequencies during the viral infection versus convalescence (2.76% [1.16-4.0] vs. 0% [0-0.93]; p=0.0025, n=13).

CONCLUSIONS: These preliminary data demonstrate that the CD49d⁺ PMN are increased in the nasal lavage during an acute viral respiratory infection. Further studies will need to be performed to see if the presence of these cells correlates with development of atopic disease.

208 Estradiol Has a Negative Impact On The Anaphylactic Response In Mice, Independent From Mast Cell Degranulation

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RATIONALE: Epidemiologic studies indicate gender differences in the prevalence of allergic diseases, with adult women suffering more frequently from anaphylaxis. We hypothesized that female sex hormones alter the course of allergen-induced anaphylaxis in mice.

METHODS: Passive systemic anaphylaxis was induced in male as well as in female mice that were either ovariectomized (OVX) or sham-operated. To investigate the role of estradiol (E2), we repeatedly injected naïve female mice with the specific estrogen-receptor antagonist *ICI 182,780*. Additionally, OVX mice were subcutaneously implanted with pellets releasing either E2 or placebo. To explore the effect of E2 on mast cell degranulation *in vivo*, plasma histamine levels were determined after antigen-challenge and anaphylactic reaction in response to histamine-challenge was measured in OVX and sham-operated mice. *In vitro* antigen-induced degranulation and cytokine production were evaluated in bone marrow and peritoneal derived mast cells, in the presence or absence of E2.

RESULTS: Male mice as well as OVX female mice were less susceptible to passive systemic anaphylaxis compared to sham-operated female mice and this effect was reversed by implantation of E2-pellets. Also, the anaphylactic response in naïve female mice was reduced after pre-treatment with *ICI 182,780*. The effect was not accompanied by a decrease in plasma histamine-levels after challenge. Mice that underwent OVX showed a reduced anaphylactic response to histamine. E2 did not enhance antigen-induced mast cell degranulation *in vitro*.

CONCLUSIONS: E2 has a negative impact on anaphylaxis in mice, which is not due to increased mast cell degranulation.