Methods: We studied 21 patients with EB, 16 asthmatic patients and 12 healthy subjects. We analyzed EP2 and EP4 receptor expression by Real-Time PCR and protein expression by confocal microscopy and flow cytometry in induced sputum and peripheral blood eosinophils. Moreover, we tested the PGE2 effect on eosinophilic apoptosis and bronchial smooth muscle cells (BSMC) proliferation.

Results: Patients with EB showed higher mRNA expression levels of EP2 (1.8-fold and 2.03-fold higher) and EP4 (1.2-fold and 5.43-fold increase) in sputum cells and peripheral blood eosinophils than asthmatic patients, the differences were statistically significant in EP2 sputum samples and EP4 peripheral blood eosinophils ($P < 0.05$). We assessed surface expression of EP2 and EP4 on peripheral blood eosinophils by confocal microscopy. An elevated expression of these receptors in eosinophils of EB patients with respect to asthmatic patients were obtained by flow cytometry. Otherwise, an increase of eosinophilic apoptosis with sputum supernatants of EB patients was observed in comparison with eosinophil cultures with asthmatic patients sputum supernatants ($P < 0.05$). When we evaluated the effects of PGE2 in BSMC proliferation, we observed that EB sputum supernatants significantly increased the percentage of inhibition of BSMC proliferation as compared with asthmatic sputum supernatants (60.33% *versus* 48.92%; $P < 0.05$).

Conclusions: PGE2 acts through EP2 and EP4 receptors in sputum and peripheral blood eosinophils in eosinophilic bronchitis. PGE2 is able to enhance eosinophilic apoptosis which may decrease eosinophilic infiltration and inflammation in eosinophilic bronchitis. Furthermore, PGE2 may inhibit bronchial smooth muscle cell proliferation reducing smooth muscle hyperplasia and preventing airflow obstruction. These results suggest that PGE2 could act like a novel therapeutic strategy in eosinophilic bronchitis, asthma and others inflammatory respiratory diseases.

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Distribution of T-bet+, GATA-3+ and Foxp3+ cell in lung tissue after allergen exposure in a mouse model of allergic inflammation

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Background: Allergic asthma is characterized among others by IgE- and eosinophilia-associated lung inflammation. T cells plays a critical role in this process, leading to the concept that allergic asthma is a disease of immune dysregulation. Naïve T cells differentiate to subsets of T helper (Th) cells regulated by specific transcription factors such as T-bet/Th1, GATA-3/Th2 and Foxp3/T regulatory cells (Tregs). Recent reports suggest an inter-regulation among them; emphasizing the need to study the entire population. In this context, our aim was to investigate the allergen induced accumulation of all three subsets in different parts of the inflamed lung during allergic inflammation.

Methods: C57BL/6 mice were sensitized to ovalbumin (OVA) followed by exposure to OVA or PBS. Lung tissue was collected 24 h after the final exposure. May-Grünwald-Giemsa stained cytospin preparations of lung cells was used to evaluate number of eosinophils. Number of T-bet +, GATA-3 + and Foxp3 + cells per square millimeter (mm^2) was evaluated in the peribronchial, perivascular and alveolar tissue sections using immunohistochemistry.

Results: As expected, the mice acquired a significant lung eosinophilia after OVA exposure (OVA/PBS; $55.78/3.10 \times 106$/ml; $P < 0.001$) which was accompanied by a significant accumulation of T-bet +, GATA-3 + and Foxp3 + peribronchially (OVA/PBS; 0.21/0.02, 0.22/0.06, 0.27/0.04 mean cell number/mm^2 respectively; $P < 0.01$) as well as in the alveolar space (OVA/PBS; 0.52/0.06, 0.21/0.01 and 0.21/0.04 mean cell number/mm^2 respectively; $P < 0.01$). However, only GATA-3 + and Foxp3 + cells were significantly increased perivascularly after OVA exposure (OVA/PBS; 0.53/0.03 and 0.71/0.09 mean cell number/mm^2; $P > 0.01$).

Conclusion: Our data show that allergen exposure result in an equal increased accumulation of Th1, Th2 and Treg cells in peribronchial tissue and also an increase in the perivascular lung tissue, but here with a dominance of Th1. Finally, perivascularly only Th2 and Treg cells increase. Our data argue that during allergic lung inflammation we have an extended network of effectors T cells that can have the capacity to counterregulate each other and other inflammatory cells.

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$CD4^+$ $CD86^+$ expression on experimental allergic murine model

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Background: CD86 is a well-known costimulatory molecule in its interaction with CD28 and/or CTLA present on T cells, and is essential for full activation of naïve T-cell and differentiation. Usually the B7 molecules are expressed mainly on APCs and B cells and in specific conditions on others activated cells.

Objective: To evaluate the expression of CD86 on $CD4^+$ cells during an allergic IgE reaction induced on an experimental murine model, based on a relevant human allergen.

Methods: Induction of systemic allergic response was achieved by three subcutaneous administrations of *Parietaria judaica* (Pj) extract (Bial-Aristegui, Spain) in 27 inbreed male BALB/c mice. Allergic reaction was triggered by ultrasonic nebulization of a 50% Pj solution for 5 min in a glass camera. Different study groups of mice were established according to the elapsed time after challenge: 60 min (I), 120 min (II), and 360 min (III) respectively. Control groups included 5 non-sensitized (N) and 6 sensitized mice (A). After sacrifice, blood was collected by intracardiac puncture. The study of the expression of CD86 and CD25 (BD Biosciences, USA) on $CD4^+$ cells present on peripheral mononuclear cells was performed by flow cytometry on a FACScan (Becton Dickinson, San Jose, CA), and the results expressed in percentage of total $CD4^+$ cells. Statistic analysis was performed using One-Way ANOVA and Tuckey tests.

Results: Our results showed no differences for the total number of $CD4^+$ cells on peripheral blood for the distinct groups: N = 81.66 ± 6.5%; A = 81.66 ± 1.63%; I = 82.33 ± 2.29%; II = 82.33 ± 3.42%; III = 80.55 ± 3.39%. The inhalation of specific allergen induced an increase in $CD4^+$ $CD86^+$ expression compared to control groups: N = 12.66 ± 0.57%; A = 12.66 ± 3.88%; I = 17.33 ± 3.96%; II = 18.77 ± 5.86%; III = 21.55 ± 11.01% ($P < 0.05$). We also confirmed that all the $CD4^+$ cells that expressed CD86 were activated cells ($CD25^+$).

Conclusions: Our results confirmed that a subset of $CD4^+$ cells on peripheral blood also expressed CD86. During specific allergic reaction there was a rapid increase of CD86 T cells in the first 60 min after challenge, with a slight increase at the 4th hour after allergen exposure. All these cells were activated ($CD25^+$). These data suggest that these cells could be relevant in IgE mediated allergic reaction possibly by an autocrine costimulation via CD28/CTLA activation pathway.

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