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LIBRO degli ABSTRACT

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Fine characterization of immunological mechanisms mediated by the major allergens of *Parietaria judaica* and by a hypoallergenic hybrid, rPjEDcys

D. Di Blasi[1,3], F. Gervasi[2], C. Uasuf[1], M.R. Melis[1], M.A. Ragusa[3], P. Colombo[1] and A. Bonura[1]

1. Istituto di Biomedicina e di Immunologia Molecolare del CNR Palermo, Italy 2. Laboratorio Specialistico Oncologia Ematologia e Colture Cellulari per uso clinico, Ospedale Civico, Palermo, Italy 3. Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche (STEBICEF-UniPa), Università degli Studi di Palermo, viale delle Scienze Ed. 16 - 90128 Palermo.

Allergy is a hypersensitivity disease IgE-mediated, affecting more than 25% of the population. Actually the only curative treatment of allergies is Allergen-Specific Immunotherapy (SIT). Recombinant hypoallergenic allergen derivatives with reduced allergenic activity have been engineered to reduce side effects during SIT. *Parietaria judaica* (P*j*) pollen contains two major allergens, Par j 1 and expressing disulphide bond variants of Par j 1 and Par j 2, was generated. The aim of this research project is to compare the immunological mechanisms activated by the major allergens of *Pj* and by rPjEDcys. *In vitro* analysis suggested that rPjEDcys has a reduced allergenity and maintains T cells reactivity. In particular we showed that PBMC of P*j* allergic patients stimulated *in vitro* with the hybrid and the *wild-type* recombinant allergens scored a percentage of proliferating $CD4^+$ and $CD56^+$ cells higher than unstimulated samples. Furthermore, cytokine secretion assays on $CD4^+$ cells demonstrated that rPjEDcys reduces the secretion of two Th2 cytokines that are critical in the development of allergy such as IL-5 and IL-13. Furthermore we observed the selection of a putative pTreg cell subset (defined as $CD4^+ CD25^{++} CD127^-$) in both the w.t. mixture and the rPjEDcys. We characterized these cells at molecular level by REAL-TIME PCR. Moreover, we addressed the kinetic of functional surface marker expression, such as GARP (Glycoprotein A Repetitions Predominant), LAP (Latency-Associated Peptide) and CD39 on $CD4^+$ cells. Our analyses demonstrated that rPjEDcys induces a number of GARP-LAP-CD39 co-expressing cells higher than *wild-type* recombinant allergens. These results suggest that rPjEDcys represents a useful approach for immunotherapy of allergic disease.

Cigarette smoke extract promotes Acetylcholine mediated inflammation and oxidative stress by PEBP1/Raf-mediated MEK and ERK pathway in human bronchial epithelial cells

G. D. Albano[1], A. Bonanno[1], L. Riccobono[1], R. Gagliardo[1], MP. Pieper[2], M. Gjomarkaj[1], M. Profita[1]

1. Institute of Biomedicine and Molecular Immunology "A. Monroy" (IBIM), National Research Council of Italy (CNR), Palermo, Italy; 2. Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany.

Acetylcholine (ACh) promotes oxidative/nitrosative stress in bronchial epithelial cells of COPD patients. The use of anticholinergic and long-acting β2 agonists in the treatment of COPD maximize bronchodilatation and regulate oxidative/nitrosative stress in bronchial epithelial cells. Cigarette smoke extracts (CSE) and Acetylcholine generate the increased expression and activation of m3 muscarinic receptors (mAChR M3), through PEBP1 dissociation. Tiotropium decrease the proinflammatory activity of Acetylcholine and reduces the activity of ACh in guinea pig model of neutrophilic inflammation and remodeling in COPD. β2-Adrenergic receptors are coupled to Gs, where stimulation by a β-agonist activates adenylate cyclase and increases 3,5_adenosine monophosphate (cAMP) level. We aimed to investigate whether the long-term exposure to CSE (0 to 20% for 7 days) promotes inflammation and oxidative/nitrosative stress production in bronchial epithelial cell line (16HBE), via PEBP1 Raf-mediated MEK and ERK pathway activation, by autocrine Acetylcholine. We evaluated the ACh expression and Ros production by flowcytometry, ChAT, M3, NOX4, p-β2AR, PEBP1and ERk1/2 phosphorylation by western blot analysis; the IL-8 release and cAMP levels by ELISA. The 16HBE were preteated with CSE for long-term exposure and Tiotropium and Olodaterol with and without The Hemicholinium (HCh), a potent choline uptake blocker. We showed increased levels of p-β2AR, pPEBP1, M3, ChAT, ACh expression, pERK1/2, Ros, IL-8 and NOX4 in CSE treated 16HBE for long-term exposure compared to untreated cells. HCh reducing levels of ACh synthesis