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

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Purpose: Allergy is a hypersensitivity disease IgE-mediated, affecting more than 25% of the population. The symptoms of IgE-mediated allergies reactions can be transiently ameliorated pharmacologically, but 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* (Pj) pollen contains two major allergens belonging to the family of Lipid Tranfer Proteins (Par j 1 and Par j 2). By means of DNA recombinant technology, a hybrid hypoallergenic (PjEDcys), expressing disulphide bond variants of Par j 1 and Par j 2, was generated. The aim of this research project is to study the immunological mechanisms activated by the major allergens of *Parietaria judaica*, Par j 1 and Par j 2, and hypoallergenic hybrid rPjEDcys. Moreover, the project I am involved is trying to address the question whether this engineered hypoallergenic derivative can be a potential products for safer Allergen Specific Immunotherapy (SIT).

Methods: Par j 1, Par j 2 and PjEDcys were produced as recombinant proteins. Human Peripheral Blood Mononuclear Cell (PBMC) from *P. judaica* allergic patients were stimulated *in vitro* with *wild-type* recombinant allergens and hybrid. PBMC proliferation assay, cytokine secretion assay, magnetic cell sorting of different subset of regulatory T cells, multiparametric flow cytometric analysis and molecular characterization using Real Time-PCR on sorted cells allow to study the biological properties of *wild-type* recombinant allergens and hybrid hypoallergenic derivate.

Results: *In vitro* analysis suggested that PjEDcys have a reduced allergenity and maintained T cells reactivity. PBMC of P. *judaica* allergic patients stimulated *in vitro* with the hybrid and the *wild-type* recombinant allergens scored a percentage of proliferating $CD4^+$ and $CD56^+$ cell higher than unstimulated sample. Consistent with these data, cytokine secretion assay on $CD4^+$ cells demonstrated that PBMC stimulation with rPjEDcys showed a percentage of IL-5 and IL-13 secreting T $CD4^+$ cells lower than the *wild-type* allergens. Both rPjEDcys and *wild-type* stimulation promote the secretion of IFN- γ and IL-10 by T $CD4^+$ cells. Finally whit the aim to study which subset of regulatory cells respond to *wild-tipe* allergens and hypoallergenic hybrid new experiment are setting.

Discussion: In this experimental setting, the use of the major allergens of Pj and the hybrid polypeptides, rPjEDcys allows me to study the immunological mechanisms activated by the two different antigen stimulation and to investigate differences between the *wild-type* allergen and the hypoallergenic mutant rPjEDcys. Our data showed that $CD4^+$ cells are clearly the predominant cell population proliferating in response to mixture of Par j 1 and Par j 2 allergens. The hypoallergenic derivate rPjEDcys retain the ability to stimulate $CD4^+$ cells proliferation like the mixture of allergens (rPar j 1 and rPar j 2). Moreover these

results highlighted a particular interesting datum; the mixture of allergens and the rPjEDcys hybrid showed the ability to stimulate an innate immune response, inducing $CD56^+$ cells proliferative response. Cytokine secretion assay demonstrate that rPjEDcys reduce the secretion of IL-5 and IL-13, Th2 cytokines with a critical role in the development of allergy, compared to *wild-type* allergens. This may reflect the different biological function exerted by rPjEDcys.

Conclusion: Collectivelly, our findings demonstrate that PjEDcys show a reduced allergenicity but maintained its immunogenicity and maybe it is also capable to regulate and redirect the immune response. These results suggest that PjEDcys represent a useful approach for immunotherapy of allergic disease.