

Institutionen för Medicin, Solna  
Enheten för klinisk immunologi och allergi  
Karolinska Institutet

# NOVEL TREATMENT STRATEGIES AND REGULATION OF IgE-MEDIATED ALLERGIC DISEASE

Sarah Thunberg

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*Huvudhandledare:*  
Docent Guro Gafvelin,  
Karolinska Institutet,  
Inst. för Medicin, Solna

*Fakultetsopponent:*  
Medicine Doctor Marek Jutel,  
Wroclaw Medical University, Poland,  
Dept. of Internal Medicine and Allergology

*Bihandledare:*  
Professor Marianne van Hage,  
Karolinska Institutet,  
Inst. för Medicin, Solna

*Betygsnämnd:*  
Docent Lena Palmberg, Karolinska Institutet,  
Inst. för Miljömedicin  
Docent Anna Rudin, Göteborgs universitet,  
Avd för reumatologi och inflammationsforskning  
Docent Ola Winqvist, Karolinska Institutet,  
Inst. för Medicin, Solna



**Karolinska  
Institutet**

## ABSTRACT

Allergic symptoms such as rhinoconjunctivitis, asthma or gastrointestinal symptoms, triggered by inhaled or ingested allergens cross-linking allergen-specific IgE on mast cells or basophils, are defined as *IgE-mediated allergy*. The major allergens from birch pollen (Bet v 1) and cat dander (Fel d 1) are two common allergens eliciting allergic disease. Allergen-specific immunotherapy (SIT) is the only curative treatment for IgE-mediated allergy. It is long-lasting and involves repeated injections of crude allergen extracts. Successful SIT modifies a number of allergen-associated immunological responses. SIT has been shown to induce IL-10 producing regulatory T-cells (Treg), allergen-specific T- and B-cell anergy as well as blocking antibodies. Although effective, SIT is associated with a risk for treatment side effects. This has led to the development of novel treatment strategies, such as modified recombinant allergens with reduced allergenicity (hypoallergens) and new means of antigen delivery. The general aim of this thesis is to investigate regulation of allergic immune responses and how novel strategies for SIT affect those responses.

The first article describes an eight injection short-course SIT study with Bet v 1 hypoallergens; where 27 birch pollen allergic patients participated. The major findings were that SIT with genetically modified Bet v 1 hypoallergens induced allergen-specific neutralizing antibodies and reduced immediate skin reactivity as well as the number of IL-5 and IL-13 producing cells. Even though rBet v 1 hypoallergen treatment exhibited typical immunological features of successful allergen-specific immunotherapy, there was no increase in the number of IL-10 producing cells after treatment. In the second study we therefore decided to evaluate the role of the suppressive cytokines IL-10 and TGF $\beta$  as well as natural FOXP3<sup>+</sup> Treg cells in immune-regulation of allergic immune responses. We found that unlike Treg cells from non-allergic controls, Treg cells from birch pollen-allergic patients displayed an impaired ability to suppress birch-pollen stimulated effector cells. Neutralization of IL-10 in CD4<sup>+</sup>CD25<sup>+</sup> Treg cell and CD4<sup>+</sup>CD25<sup>-</sup> effector cell co-cultures induced a significant increase of TNF $\alpha$  secretion, suggesting that IL-10 and TNF $\alpha$  may have counter-acting properties in the periphery, where IL-10 promotes tolerance and suppression by Treg cells and TNF $\alpha$  promotes inflammatory responses.

In the third and fourth article, recombinant (r) Fel d 1 was coupled to the novel adjuvant carbohydrate based particles (CBPs) and investigated in a mouse model sensitized to Fel d 1. Pre-treatment with CBP-rFel d 1 was able to induce antigen-specific T-cell tolerance and shift immunoglobulin production from an IgE to an IgG2a type of response. Antigen-coupled CBPs also demonstrated improved antigen depot-effects with prolonged antigen-exposure, when compared to the most commonly used adjuvant in vaccine preparations for humans; aluminum hydroxide. Furthermore, CBP-rFel d 1 was tested in a treatment protocol for SIT, where it was able to modulate the allergic immune response in rFel d 1 sensitized mice without adverse effects. Thus, CBPs ability to promote induction of potent immune responses and to deliver allergens without risk of systemic allergen spreading are beneficial properties of an adjuvant aimed to be used in allergen-specific immunotherapy. Possibly, CBPs coupled to infectious or auto-immune antigens could be applied as an adjuvant to prevent other types of diseases.

In conclusion, the work presented in this thesis has shed new light on *in vivo* function of two conceptually different approaches to improve allergen-specific immunotherapy. The thesis has also contributed to increased understanding regarding regulation of allergic immune-responses, thus providing a basis for further research.