Subcutaneous and sublingual allergen specific immunotherapy in experimental models for allergic asthma

Hesse, Laura

DOI:
10.33612/diss.158737284

Chapter 10

Summary

Allergen-specific immunotherapy (AIT) is the only treatment for allergic disorders that is able to achieve prolonged suppression of symptoms of disease, despite re-exposure to the allergen. However, AIT is not effective for all allergic disorders, and treatment for several years is required to obtain long-term protection. Moreover, some forms of AIT have safety concerns, with risk of mild to severe allergic reactions. To improve safety and efficacy of AIT, we have studied the underlying immunological mechanisms in experimental models of allergic airway inflammation. These studies indicate that different parameters of allergic airway inflammation such as airway hyperresponsiveness (AHR), numbers of eosinophils and other inflammatory cells and serum immunoglobulin (spIgE) responses can all be suppressed by AIT, yet they differ in the exact regulatory mechanisms that they respond to. AIT induces desensitization of innate effector cells, neutralizing antibodies (spIgGs) and increased numbers of regulatory T-cells (Treg) as well as changes in the phenotype of allergen-presenting dendritic cells (DCs), all of which appear to play a role in suppressing allergic manifestations upon allergen re-exposure. Experimental models of AIT can be used to increase the safety and effectiveness of AIT on all or specific parameters of allergic inflammation by comparing different routes of delivery of the allergen, or by comparing use of natural extracts, purified major allergens, recombinant allergens or allergen-derived peptides. Moreover, experimental models can be used to improve the effectiveness of AIT by using adjuvants that enhance the tolerogenic phenotype of the allergen- presenting DCs or that disrupt the co-stimulatory interaction between DC and allergen-specific T-cell during treatment. Further research into increasing therapeutic efficiency of AIT to achieve a wider clinical application should therefore focus on obtaining a safe treatment, that is able to efficiently induce tolerance through Treg cell activity and/or a tolerogenic phenotype of the allergen-presenting DC. The optimal treatment modality for AIT, however, is not known, in part due to lack of systematic comparisons of application routes, the most effective form in which the allergen (or its derivatives) should be introduced, and the most optimal adjuvans for use in AIT.

The **aim of this thesis** research project was to compare the use of natural extracts, recombinant allergens and allergen-derived peptides of grass pollen allergens for immunotherapy treatment, to compare delivery via either sublingual or subcutaneous administration, and to evaluate the use of VitaminD3 as an adjuvans. To this end, we first developed a reproducible preclinical grass pollen (**GP**) *Phleum pratense* (Phl p) mouse model of asthma using **BALBc/ByJ mice** and tested whether low dosages of GP could provide symptom relief (**Chapter 2**). Furthermore, we performed an up-dosing experiment for subcutaneous immunotherapy (**SCIT**) for the more anaphylaxis sensitive strain, **C57Bl6/J mice**. Results clearly demonstrated that a natural allergen extract of GP can be used in our experimental mouse model of allergic airway inflammation, wherein sensitization using GP extracts resulted in specific serological responses, and increased ear swelling upon intradermal challenges. Moreover, GP challenges resulted in increased airway resistance and reduced compliance in response to methacholine challenges, induced a clear Th2 cytokine-profile, and led to increased numbers of eosinophilic granulocytes in both BALF and lung cell suspensions. GP-SCIT treatment using **low dosages of GP** were unable to reduce symptoms of allergic airway disease, but in fact resulted in exaggeration of allergic symptoms, as outlined by strong GP-spIgE induction,

low GP-spIgG-levels, increased ear swelling responses to intradermal allergen challenges as well as increased airway resistance, and enhanced eosinophil numbers in BALF and lung in response to inhalation challenges. However, using an updosing treatment regimen for GP-SCIT in C57BL/6J mice, SCIT resulted in successful suppression of allergic inflammation, increased neutralizing antibody responses accompanied by decreased levels of spIgE, and decreased ear swelling and Th2 cell cytokine responses. In his model, signs of systemic anaphylaxis were observed during the initial allergen injections. In chapter 2, we concluded that these experimental mouse models can be used to test improved formulations, adjuvantia and regimens with reduced allergenicity in SCIT treatment using a validated treatment protocol as a reference, making these models a valuable translational research tool for improvement of AIT efficacy and safety in the future.

Clinical studies **directly comparing SCIT and SLIT** report differences in kinetics and magnitude of immunological changes induced during treatment, with SCIT achieving faster and stronger suppression. Comparative studies into the mechanisms underlying immune suppression in SCIT and SLIT are by and large lacking. In **Chapter 3**, we therefore compared GP-SCIT and GP-SLIT treatments in three dosages in the BALB/cBYJ mouse model, and found both treatments to be effective at these dosages on specific parameters of allergic airway inflammation. We did, however, observe differences in the parameters suppressed by the two treatments: GP-SCIT suppressed Th2 inflammation and induced neutralizing antibodies, while GP-SLIT suppressed the clinically relevant lung function parameters in an asthma mouse model, indicating that the two application routes depend on partially divergent mechanisms of tolerance induction.

To overcome the urgent need for improved AIT efficacy, ideally at lower allergen doses, several strategies have been explored, including the use of adjuvants. Use of 1,25-dihydroxy-vitamin D3 (**VitD3**) as an adjuvant for SCIT in the classical mouse model of OVA-induced allergic airway inflammation resulted in suppression of airway inflammation. VitD3 exerts its immunoregulatory properties through induction of tolerogenic DCs and prevents DC maturation via downregulation of costimulatory molecules and increased production of IL-10, facilitating the generation of adaptive Treg cells. However, the use of VitD3 as an adjuvans has not yet been tested in GP-driven mouse models of AIT. **Chapter 4** described the first study comparing the **adjuvant effects of VitD3** supplementation in **GP-SCIT and GP-SLIT** treatments in an experimental model for allergic airway disease. Strikingly, and in contrast to previous results using unsupplemented AIT (chapters 2 and 3), we found a prominent Treg cytokine profile in lung tissue after VitD3 supplemented GP-SCIT and GP-SLIT, as demonstrated by increased levels of IL10, and in SLIT also of TGF-β1. In contrast, clear suppression of Th2 cytokine responses by VitD3 supplementation was not observed. On other parameters, we find remarkable similarity in the effects of VitD3 supplemented GP-SCIT and GP-SLIT; an enhanced GP-spIgG2a antibody response and suppression of lung tissue eosinophils. In GP-SLIT, we additionally observed an effect of VitD3 supplementation on GP-spIgG1 levels and GP-spIgA levels, as well as on suppression of ear swelling responses and methacholine-induced airway resistance. These data show that VitD3 increases efficacy of both SCIT and SLIT underscoring the relevance of proficient VitD3 levels for successful AIT.

Next, we aimed to find the **optimal VitD3 dose** in our **GP-SCIT** model for optimal suppression of all parameters of allergic airway disease and tested whether use of the **synthetic lipid SAINT** in

the mixture of GP-SCIT extracts and VitD3 enhances suppression of parameters of allergic inflammation in an experimental mouse model of GP-SCIT (**Chapter 5**). Here, we show that increasing dosages of VitD3 could enhance the efficacy of GP-SCIT treatments in suppressing asthmatic manifestations upon GP challenges in our experimental mouse model and found remarkable effects of high dose VitD3 supplementation in these GP-SCIT treatments when compared to the unsupplemented GP-SCIT group: reduced total IgE and GP-spIgE levels in sera after challenges, an overall enhanced GP-specific IgG1 antibody response, an increased neutralizing activity, suppression of ear swelling responses and airway resistance and compliance, suppression of Th2 cytokine levels after *ex vivo* restimulation, reduced numbers of eosinophils in BALF and lung tissue, reduced type-2 cytokines and levels of eotaxin, GM-CSF, IL-33, and KC in lung tissue homogenates. Addition of SAINT, however, did not provide an improved efficiency of VitD3-GP-SCIT on suppression of the experimental parameters of inflammation and airway hyper responsiveness, even though allergen dosage and adjuvant dosage were optimal. These studies underscore the relevance of VitD3 as an adjuvant to improve clinical efficacy of SCIT treatment regimens.

Currently, most AIT formulations are based on crude allergen extracts, which can induce IgE-crosslinking and sometimes induce mild to severe side effects. **Allergen-derived peptides** are considered to be a safe alternative, but efficacy of peptide AIT is suboptimal. In **Chapter 6**, we describe experiments that test whether peptide AIT using conjugation of **sialic-acid glycans** to peptides derived from the major grass pollen allergen *Phleum pratense P5a* (Phl p5a)can enhance efficacy of SCIT in a GP-driven mouse model of allergic asthma. Sialylation has been shown to increase uptake and processing of the peptides by DCs, whilst instructing a tolerogenic phenotype onto the DC. Sialylated Phl p5a peptides used for T cell stimulation by bone-marrow derived DCs resulted in increased proliferation, FoxP3 expression and TGF-β1 release by $CD4^+$ T-cells isolated from GP-sensitized mice. Thereafter, we compared SCIT treatment using sialylated and unmodified Phl p5a-derived peptides to the use of a GP extract or a sham treatment in GP-sensitized mice. Our *in vivo* findings indicate that the use of a mix of two peptides of the major allergen Phl p5a was also able to reduce most parameters of allergic airway inflammation after GP challenges, although the suppression on eosinophilic inflammation was not as prominent as with the full GP extract. A direct comparison between unmodified and sialylated peptides used for SCIT revealed a significantly increased induction of $FoxP3^+$ T-cells, and decreased numbers of $GATA3^+$T-cells associated with an enhanced suppression of eosinophilia in both BALF and lung tissue by the sialylated peptides, as compared to the unmodified peptide-SCIT. In conclusion, sialylation of Phl p5a peptides was shown to enhance efficacy of peptide-SCIT in a GP driven mouse model of allergic asthma.

Similar to GP allergic patients, treatment options for house dust mite (HDM)-allergy include allergen avoidance and AIT. However, **HDM extracts** (*Dermatophagoides pteronissinus* (Der p)) are proteolytically active, resulting in variable allergen content, and limited stability. Given the fact that sensitization to Der p 1 and 2 identifies more than 95% of HDM-allergic individuals, and considering the causal role of these two major allergens in initial sensitization to this ubiquitous allergen, we hypothesized that AIT with **purified Der p1 and p2** might be a more attractive therapeutic approach compared to the use of HDM extracts. In **Chapter 7**, we tested the hypothesis that treatment with natural purified Der p 1 and 2 can be an effective treatment in inducing a protective neutralizing

antibody response and in suppressing allergic inflammation in a mouse model of HDM-driven allergic asthma. In a direct comparison of **DerP1/2-SCIT to HDM extracts** we show that the DerP1/2-vaccine results in marked suppression of type-2 cytokine levels in lung and BALF, and increased Der p 1-spIgG responses. The levels of Der p 1-spIgG1 after SCIT were negatively correlated to levels of IL-5, IL-13 and CCL20 after HDM challenge, indicating a protective role for this neutralizing antibody response in our mouse model. Moreover, DerP1/2-SCIT was uniquely able to prevent the HDM-challenge induced increase in CCL20/MIP3α levels, and a similar trend was observed towards preventing the HDM-induced CCL17/TARC and eotaxin response in lung tissue. While these immunological parameters argue in favour of DerP1/2-SCIT, we do not observe differences in the more translational parameters of AHR, ear swelling tests and eosinophilia, where both treatments have similar efficacy in suppressing the allergic responses. Hence, we postulate that DerP1/2-SCIT is at least as effective in suppressing the HDM-induced adaptive and innate response as crude allergen (whole body) extracts. These data warrant clinical studies to explore the safety and efficacy of the use of these purified natural allergens as a novel vaccine for HDM induced allergic disease, including rhinitis and allergic asthma.

Finally, we use our extensive experience with experimental models for allergic airway disease to provide the research community with well-developed protocols for SCIT and SLIT in both BALBc/ByJ and C57BL6 mouse models of allergic asthma using natural allergen extracts (**Chapter 8**). We provided detailed methods to obtain the most important outcome parameters for translational studies, including invasive lung function measurements for AHR, spIgE and spIgG levels in serum, ear swelling tests for the early phase response, and inflammation of lung tissue and airways. Moreover, we described how to re-stimulate lung cells with allergen extracts, perform flow cytometric measurements to identify populations of relevant immune cells, and perform ELISAs and Luminex assays to measure the cytokine concentrations in bronchoalveolar lavage fluid (BALF) and lung tissue. In C57BL/6 mice, we included an adapted SCIT treatment protocol, including monitoring of immediate responses like severity of shock and loss of body temperature after the first injections, to avoid anaphylaxis in this mouse strain.

In conclusion, we show that experimental models of allergic airway disease can be used to test modifications of allergen-specific immunotherapy treatment with the aim of developing allergen vaccines with improved safety and efficacy, thereby increasing the therapeutic potential with the long-term goal of a curative treatment for allergic disease.