The efficacy of peptide immunotherapy for cat-induced respiratory allergy

ABSTRACT:

Allergen immunotherapy (AIT) can be defined as the repeated administration of specific allergens to patients with IgE-mediated inflammatory diseases, with the ultimate goal of providing protection against allergic symptoms and inflammatory reactions associated with natural exposure to these allergens. Specifically, the therapy primarily strives to establish long-term tolerance against allergens by inducing allergen-specific regulatory B and T cell responses, in addition to modulating the mast cell and basophil activation thresholds to mitigate allergic pathogenesis. AIT is conventionally administered to patients both subcutaneously and sublingually; however, additional routes of administration (ie. intralymphatic immunotherapy) are under investigation. AIT is suitable for both adults and children for a variety of allergens including pollen, pet dander, house dust mite, venom, and a number of food allergens including peanut, egg, and milk. Nevertheless, more research is needed to elucidate many of the direct mechanisms in which AIT suppresses inflammatory immune responses.

INTRODUCTION

Respiratory Allergy

The prevalence of allergic diseases, including but not limited to allergic respiratory diseases, is increasing globally, particularly in developing nations. Allergic respiratory diseases, including rhinitis and asthma, are complex inflammatory diseases associated with significant quality of life disruption, a decrease in work productivity, missed school, and increased health care costs. Allergic rhinitis (AR) is characterized by inflammation of the nasal membranes, while allergic asthma (AA) is characterized by inflammation of the large airways of the lungs. Worldwide, over 400 million people are affected by AR and over 300 million people by AA. According to the World Health Organization, the number of people with AA is expected to rise to 400 million by 2025. Furthermore, there are clear links between the upper and lower airways as AR and AA are frequently comorbid conditions. More than 80% of patients with AA also have AR, whereas around 40% of patients with AR have asthma comorbidly.

Symptoms of AR are mostly nasal and include sneezing, itching, rhinorrhea, and/or nasal congestion. In addition, AR is frequently accompanied by symptoms involving the eyes, ears, and throat, including postnasal drainage. AR is often diagnosed clinically through the results of a careful history and physical examination. AR is strongly suspected when two or more symptoms out of watery rhinorrhea, sneezing, nasal obstruction, and nasal pruritus persist for ≥1 hour on most days. The skin prick test or the serum-specific immunoglobulin E (IgE) level can be used to confirm the diagnosis. The frequency of AR increases with age, and other risk factors include positive allergy skin tests, higher socioeconomic class, family history of allergy, and being born during the pollen season.

AA is characterized by airway inflammation, remodeling, and hyperresponsiveness, and symptoms include shortness of breath, cough, chest tightness, and/or wheezing. AA is often clinically diagnosed through the results of the medical history and physical examination. The diagnosis is made when the patient reacts positively to a skin prick test and presents with episodic symptoms of airflow obstruction or airway hyperresponsiveness which are partially reversible. Confirmation of diagnosis is often done using spirometry to demonstrate obstruction and assess reversibility, in which reversibility is determined by an increase in forced expiratory volume in 1 second (FEV1) of greater than or equal to 12% from...
baseline after inhalation of a short-acting bronchodilator. Atopy, the genetic tendency to develop allergic diseases, is frequently identified as a risk factor for the development of AA. Early dust mite sensitization and maternal asthma are also risk factors of AA, while microbial exposure has shown to be inversely correlated with the development of asthma and atopy. As such, it is likely that AA develops in individuals through a combination of genetic susceptibility and environmental exposures.

The pathophysiology of AR and AA begins with sensitization to allergens (Figure 1). The presentation of processed antigen from allergen to Cluster of Differentiation (CD)4+ T cells leads to the differentiation of naïve T cells into allergen-specific type 2 T helper (Th2) cells. Interleukin (IL)-3, IL-4, IL-5 and other type 2 cytokines produced by activated Th2 cells induce isotype switching of B cells to produce specific IgE that binds onto high-affinity IgE receptors (FcεRI) on mast cells or basophils. At this stage, IgE-sensitized individuals do not display any clinical symptoms, but the mast cells and basophils will readily release mediators upon subsequent encounters with allergen.

The allergic response in AR and AA can be divided into immediate- or early-phase response, and late-phase response (Figure 2). In the early-phase response, mediators released by mast cells and basophils cause blood vessels to leak and produce mucosal edema as well as watery rhinorrhea in patients with AR. These responses occur within minutes of allergen exposure, leading to characteristic symptoms such as sneezing, itching, and clear rhinorrhea. In the case of AA, these mediators lead to acute bronchoconstriction and airway hyperresponsiveness. Early asthmatic response develops 10-15 minutes after allergen exposure, reaches a maximum within 30 minutes, and resolves by 1-3 hours.

The late-phase response is hypothesized to be caused by mediators produced by mast cells and basophils in the early-phase response and the infiltration of leukocytes into the tissue. Congestion, irritability, and fatigue distinguish the late-phase response from the early-phase response in patients with AR. Mediators released during the early-phase response are thought to act on postcapillary endothelial cells to promote the expression of vascular cell adhesion molecule and E-selectin, which facilitates the adhesion of circulating endothelial cells. Furthermore, cytokines released from Th2 and other cells may circulate to the hypothalamus, resulting in symptoms of fatigue and irritability. This late-phase response occurs 4-8 hours after allergen exposure, and clinical symptoms may be similar to the immediate response but with congestion more dominant. In terms of AA, the bronchoconstriction that occurs during the late-phase response is thought to be caused by cysteinyl leukotrienes and histamine release. Cytokines released by Th2 cells lead to an increase in airway eosinophils, basophils, and neutrophils. These prolonged responses occur after 3-4 hours and could last several days to weeks. However, late asthmatic response does not always take place and occurs in approximately 60% of adults and 80% of children.

Role of Regulatory T and B cells

Regulatory T (Treg) cells encompass a heterogeneous group of T cell subsets with suppressive capacity to impair excessive immune responses to pathogens, control the development of autoimmune and allergic diseases, and induce immune tolerance. Regulatory B (Breg) cells encompass a heterogeneous group of different immunosuppressive B cell subsets that regulate the immune system by different mechanisms. Abnormal functions or imbalances in Treg and Breg cells have been implicated in the development of allergic diseases. For example, it has been observed that in the umbilical cord blood of newborns at genetic risk of allergy, Treg cells were already defective.

Treg cells can prevent and inhibit ongoing allergic inflammation by four main groups of suppressive mechanisms—suppressive cytokines, metabolic disruption mechanisms, suppression of dendritic cell (DC) activation by membrane-bound molecules, and cytokolysis. Treg cells can directly or indirectly suppress almost all cell types involved in allergic responses through these mechanisms. On the other hand, Breg cells can suppress allergic responses through suppressing effector T cells, promoting the generation of Treg cells and tolerogenic DCs, and producing blocking IgG4 antibodies. By releasing anti-inflammatory cytokines (such as TGF-β and IL-10), Breg cells can suppress effector T cell responses and favor Treg cells induction. Through isotype switching, Breg cells are also able to produce IgG4 blocking antibodies which compete for the same epitopes (reactive sites of antigen molecules that antibodies bind) as IgE, thus inhibiting the activity of IgE.

Treatments for Respiratory Allergy

Therapeutic options for respiratory allergy mostly focus on alleviating symptoms (Figure 3). Options such as avoidance measures, leukotriene receptor antagonists, anti-IgE antibody, and corticosteroids, by topical nasal preparations for rhinitis and inhalation for asthma, are effective methods/agents for both AR and AA. β2-agonists are specific to the treatment of AA, while antihistamines and nasal decongestants are specific to the treatment of AR. In the Allergic Rhinitis and its Impact on Asthma (ARIA) document and Global Initiative for Asthma (GINA) guidelines published in 2017, intranasal and inhaled corticosteroids are still recommended as the most effective drugs for treating AR and AA. Intranasal or inhaled corticosteroids alone usually induce few systemic side effects in patients. However, for patients with concomitant AR and AA using both inhaled and intranasal corticosteroids, and thus receiving higher doses, potential side effects may be more likely to occur. Another therapeutic approach for both AR and AA is allergen immunotherapy (AIT), but the treatment has typically been reserved for patients in which optimal avoidance measures and pharmacotherapy are insufficient to control symptoms. Unlike pharmacotherapy that alleviates mediator- or receptor-related symptoms, AIT is the only disease-modifying treatment for allergy with sustained clinical response after discontinuation of treatment.
IMMUNOTHERAPY

AIT is traditionally conducted with whole allergen extract and given in gradually increasing doses13,18. The goal of AIT is to reduce symptoms of allergy by developing tolerance to the allergen in the individual13,18. After commencing AIT, studies have shown that the susceptibility of basophils and mast cells to degranulation is reduced within hours, which is likely linked to the rapid upregulation of histamine receptor H2 (H2R) on these cells13. As opposed to histamine receptor H1, H2R is considered to induce tolerance and suppress functions of allergen-specific T cells and basophils19. Following these changes, Treg and Breg cells are generated while allergen-specific Th2 cells are suppressed18. This leads to immune deviation from a Th2 to a Th1 response with increased synthesis of IL-12 and interferon gamma (IFNγ) and decreased synthesis of IL-418. Release of suppressive factors, such as IL-10 and TGF-β, by Treg cells also induce immunological tolerance and skew B-cell production of allergen-specific IgE to IgG418. Overall, these changes lead to sustained immunological and clinical tolerance to the allergen18.

Routes of Administration for Allergen Immunotherapy (AIT)

Currently, approved routes of administration for AIT in clinical practice are either subcutaneous (SCIT) or sublingual (SLIT) routes13,18. SCIT involves repeated injections of allergen extract subcutaneously to induce immunological tolerance, while SLIT involves keeping the allergen extract drops or tablets under the tongue for 1–2 minutes and then swallowing them13,18. SCIT has been in use for many years in several countries for the treatment of AR and stable asthma, and is considered the most effective form of AIT13,18. However, its widespread use is limited by the risks of systemic allergic reactions and the inconvenience of repeated supervised injections often over long periods in a medical facility13,18. In comparison, SLIT is safer and has fewer serious adverse events than SCIT, allowing home administration, which is more convenient13,18. Despite widespread use in Europe, the effectiveness of SLIT is still limited by troublesome local side effects and poor adherence due to long treatment regime and the need for daily use13,18.

In addition to SCIT and SLIT, other routes of administration are under investigation in order to improve the efficacy of AIT13,18. Research into oral immunotherapy has been increasing over recent years, particularly in food allergy20. The epidermis is another attractive site of research due to the presence of antigen-presenting Langerhans cells and the indirect modulation of Treg cells when the epidermis is injured21. An allergen-containing patch is the most popular form of epitaxic immunotherapy, and has been investigated in seasonal AR13. The direct administration of allergen into lymph nodes is based on the concept that small amounts of allergen in secondary lymphoid organs may elicit significant beneficial immune changes, and has been investigated in grass pollen and cat allergy22. In addition to novel routes of administration, modification of allergens is another research focus to improve the efficacy of AIT13,18.

T Cell Epitope Peptides

The use of whole allergen extract faces certain problems related to its efficacy, including possible severe treatment-related reactions, low patient adherence, and long duration of treatment13,18. In order to solve these problems, major efforts have been directed toward modifying allergen extracts12,14. AIT with short allergen-derived peptides is an important advancement based on the induction of T cell tolerance with reduced allergenicity13,18. Short allergen-derived peptides are carefully selected and designed to represent the dominant T cell epitopes with the removal of IgE epitopes from the whole allergens13,18. Peptides can induce T cell tolerance more efficiently than whole allergens by directly binding to major histocompatibility complex (MHC) on antigen-presenting cells (APCs) in the absence of pro-inflammatory signals13,18. This allows peptide immunotherapy to induce clinical tolerance to allergens in a much shorter time frame than whole allergens and without allergic side effects13,18. Similar to conventional AIT, the mechanisms of action for peptide immunotherapy involve immune deviation and induction of Treg cells13,18. In the absence of pro-inflammatory signals, peptide immunotherapy also exploits a tolerance pathway, leading to specific T cell anergy and allergen-specific Th2 cell deletion13,18.

Animal Studies

In transgenic mice with the human MHC class II molecule and lacking endogenous mouse MHC class II, Campbell et al. treated the mice with a specific Fel d 1 peptide to examine the local allergen-specific T cell response within lung tissue22. Compared to control (influenza hemagglutinin peptide), treatment with Fel d 1 peptide reduced total leukocyte and eosinophil recruitment to the lung, thus reducing lung inflammation and improving lung function. A significant increase in IL-10, produced by allergen-specific Treg cells, was reported in lung tissue following peptide AIT22. Blocking the peptide-induced effects with the administration of an anti-IL-10 monoclonal antibody after treatment further demonstrated that IL-10 is a critical cytokine in the resolution of allergic lung inflammation22.
Human Clinical Trials

The Fel d 1 allergen consists of two disulphide-linked heterodimers of chains 1 and 2. Earlier studies in subjects with respiratory allergy to cats were conducted with two 27-amino acid peptides, called IPC-1 and IPC-2, from Fel d 1 chain 1[23-26]. These synthesized peptides contained dominant T cell epitopes and were licensed as Allervax CAT. In the first trial, 95 cat-sensitized patients were blinded and randomized into receiving placebo, 7.5 µg, 75 µg, or 750 µg of Allervax CAT[23]. Six weeks after weekly subcutaneous injections for 4 weeks, improvement in lung and nasal symptoms scores were observed in patients who received higher doses of Allervax CAT[23]. However, treatment with higher doses was associated with many allergic side effects which occurred an hour or more after[27]. In a randomized double-blind placebo-controlled (RDBPC) trial, patients who received four subcutaneous weekly injections of 250 µg Allervax CAT did not tolerate significantly more cat extract in skin tests than controls[28]. In another RDBPC trial involving patients receiving eight subcutaneous injections of 750 µg Allervax CAT, improvement in pulmonary function was only evident at a single time point after treatment[29]. Due to high incidence of adverse events and non-superior clinical outcomes to conventional AIT, development of Allervax CAT was halted[30].

A second generation of synthetic T cell epitope peptides (Cat-SPIRE / Cat-PAD) was developed and comprised seven separate small peptides, derived from Fel d 1 chains 1 and 2, which bound to commonly expressed human leukocyte antigen-D related (HLA-DR) molecules[27-31]. Compared to first generation peptides, Cat-SPIRE are much shorter, such that they do not trigger cross-linking of IgE on basophils and mast cells[25,32]. In a RDBPC escalating, single dose trial, Worm et al. injected 88 patients, either intradermally or subcutaneously, with 0.03 – 12 nmol of Cat-SPIRE and measured late-phase skin reaction as a surrogate marker for clinical efficacy[27]. In the intradermal group, a 40% reduction in late phase skin reaction was observed at 3 nmol, and although none of the changes in reaction were significant, there was a trend toward significance at 3 nmol[27]. Smaller reductions and more adverse allergic symptoms were observed in the subcutaneous group[27]. As such, intradermal was proven to be superior regarding tolerability and selected as the route of administration for later clinical trials[27,32]. In a RDBPC phase 2 study, patients were randomized into two active groups: 4 intradermal doses of 6 nmol given 4 weeks apart or 8 intradermal doses of 3 nmol given 2 weeks apart[30,31]. The treatment effect was apparent at 18 – 22 weeks, and the reduction in symptom severity in the 6 nmol group was significantly different from the placebo and 3 nmol groups at 1 year[30]. In the subsequent follow-up study, a greater reduction in symptom severity was observed in the 6 nmol group compared to placebo 2 years after the start of treatment[31]. Results from the phase 2 study highlight the effects of dosing and frequency of dosing on the efficacy of treatment.

LIMITATIONS

The phase 3 clinical study for Cat-SPIRE was completed in June 2016. In this RDBPC trial, patients were randomized into three groups: 4 intradermal doses of 6 nmol Cat-SPIRE followed by 4 injections of placebo given 4 weeks apart, 8 doses of 6 nmol Cat-SPIRE given every 4 weeks, or 8 doses of placebo given 4 weeks apart[31-34]. Although the results have yet to be published, it was announced that the primary endpoint, a difference in combined score of symptoms and allergy medication use between the treatment and placebo one year after the start of treatment, was not achieved[35].

FUTURE DIRECTIONS

To reduce T cell-dependent side-effects, some researchers are looking into developing B cell epitope–based allergy vaccines[36,37]. This type of immunotherapy uses genetically engineered non-IgE-reactive peptides (between 20 – 40 amino acids) derived from or close to the IgE-binding sites of the allergen that do not contain T cell epitopes[36,37]. Conceptually, these peptides will not cross-link effector cell-bound IgE and will have no or minimal T cell reactivity[36,37]. Hence, these peptides should not induce immediate allergic inflammation or T cell-dependent side effects[36,37]. Furthermore, the use of a strongly immunogenic carrier (a protein carrier that is covalently linked to the peptides and not related to allergens) should induce allergen-specific IgG responses, which strongly block IgE antibodies from binding to the allergen[36,37]. B cell epitope–based allergy vaccines are a new area of research and hold potential to improve the efficacy of AIT in treating Fel d 1-induced respiratory allergies[36,37].

Additionally, a team in Switzerland is researching the vaccination of cats and dogs against the allergens they produce[38]. Specifically, for cat-related allergies, the team is looking to vaccinate cats against the allergen Fel d 1 and sensitize them to the allergen, thus rendering them hypoallergenic through the immune destruction of Fel d 1 in the cats’ body[38]. Fel d 1 appears not to be of critical importance for cats so the vaccination will not harm the cats[38].

CONCLUSION

Allergen immunotherapy, with research spanning over a century, is a well-established therapy with a primary goal of inducing natural suppressive immune responses upon subsequent allergen exposure and decreasing inflammatory, allergic responses. Although there are many fundamental regulatory mechanisms at play that contribute to the desensitization process during allergen immunotherapy, more research is still needed in this field, particularly in the immunological, cellular and molecular mechanisms inducing tolerance. However, the results published thus far are promising, and the research community needs to push forward in their research ventures so that the millions of people affected by cat-induced allergic exacerbations can be treated and managed accordingly.
References

The efficacy of peptide immunotherapy for cat-induced respiratory allergy


List of Abbreviations:

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<tr>
<td>AR</td>
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<td>AA</td>
<td>allergic asthma</td>
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<td>allergen immunotherapy</td>
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<td>IgE</td>
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<td>FEV₁</td>
<td>forced expiratory volume in 1 second</td>
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<td>dendritic cell</td>
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<td>APC</td>
<td>antigen-presenting cell</td>
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<td>MHC</td>
<td>major histocompatibility complex</td>
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<td>Cluster of Differentiation</td>
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<td>Th2</td>
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<td>Breg</td>
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<td>TGF-β</td>
<td>transforming growth factor beta</td>
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<td>ARIA</td>
<td>Allergic Rhinitis and its Impact on Asthma</td>
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<td>GINA</td>
<td>Global Initiative for Asthma</td>
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<td>H₁R</td>
<td>histamine receptor H2</td>
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<td>IFNγ</td>
<td>interferon gamma</td>
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<td>SCIT</td>
<td>subcutaneous immunotherapy</td>
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<td>sublingual immunotherapy</td>
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<tr>
<td>RDBPC</td>
<td>randomized double-blind placebo-controlled</td>
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<td>HLA-DR</td>
<td>human leukocyte antigen-D related</td>
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**Figures**

**Figure 1.** Immunological mechanisms underlying allergic sensitization.
Dendritic cells in the mucosal surface or airway lining process inhaled allergens and present processed antigen via MHC class II molecules to CD4+ T cells in lymph nodes. This results in the differentiation of naïve CD4+ T cells to allergen-specific Th2 cells. Activated Th2 cells release IL-3, IL-4, IL-5, and other type 2 cytokines, which drive pro-inflammatory processes by inducing isotype switching of B cells to produce antigen-specific IgE as well as infiltration of mast cells, neutrophils, and eosinophils. Secreted IgE binds onto high-affinity IgE receptors on mast cells or basophils, thus leading to the IgE sensitization of individuals against the allergen. DC: dendritic cell; Th: helper T cell; IL: interleukin.

**Figure 2.** Early- and late-phase responses in allergic rhinitis and asthma.
The early-phase response is due to subsequent cross-linking of IgE by allergen, causing mast cell degranulation and the release of mediators such as histamine, prostaglandins and leukotrienes. In the late-phase response, Th2 cells release chemoattractant cytokines, such as IL-5, promoting the infiltration of eosinophils, basophils, and neutrophils into the area. Upon infiltration, these cells release inflammatory mediators and reactivate the reactions of the immediate response. Th: helper T cell; IL: interleukin; AR: allergic rhinitis; AA: allergic asthma.

**Figure 3.** Common therapeutic options that focus on alleviating symptoms for allergic rhinitis and asthma.