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Specific Immunotherapy in Atopic Dermatitis

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Allergen specific immunotherapy (SIT) using house dust mite (HDM) extracts has been performed mainly with patients of asthma and allergic rhinitis. In the meanwhile, there has been a long debate on the efficacy of SIT in atopic dermatitis (AD) with only a few double-blind placebo-controlled trials. However, several randomized controlled trials of SIT in AD revealed significant improvement of clinical symptoms and also, positive result was shown by a following meta-analysis study of these trials. In order to predict and evaluate the treatment outcome, finding a biomarker that can predict treatment responses and treatment end-points is critical but it is very challenging at the same time due to the complexity of causes and mechanisms of AD. Other considerations including standardization of the easiest and safest treatment protocol and optimizing the treatment preparations should be studied as well. This review summarizes the basics of SIT in AD including the brief mechanisms, treatment methods and schedules, and also highlights the clinical efficacy of SIT in AD along with mild, controllable adverse reactions. Immunologic effects and studies of various biomarkers are also introduced and finally, future considerations with upcoming studies on SIT were discussed.

Key Words: Specific immunotherapy; subcutaneous immunotherapy; atopic dermatitis; clinical efficacy; biomarker

INTRODUCTION

Atopic dermatitis and allergen

Atopic dermatitis (AD) is a chronic, inflammatory skin disease with intractable pruritus. As one of the leading skin diseases in Westernized countries, its prevalence is increasing steadily world-wide,[1-3] AD affects approximately 20% of pediatrics and 1%-3% of adults,[4] and 40%-60% of pediatric AD patients continue on as adult-forms later in their lives.[1,5,6] Its pathogenesis is multifactorial with roots in a combination of genetic, environmental, skin barrier, and other immunological factors. Although there is no single gene responsible for onset of the disease, family history contributes in predicting prognosis of AD along with interplays between environmental and individual factors. Furthermore, abnormalities of the skin barrier have been extensively studied in the pathogenesis of AD in several studies,[7-12] and these barrier dysfunctions lead to dry and rough surfaced skin of AD patients. Consequently disrupted barrier leads to increased rate of secondary infection and penetration of foreign antigens through damaged stratum corneum.

AD can be classified into either intrinsic or extrinsic AD depending on co-existence with allergic features; barrier dysfunction and increased penetration of foreign allergens of food and environment are closely associated with aggravation of extrinsic AD. In an acute stage, allergen penetrates through damaged skin barrier and binds with an epidermal dendritic cell (DC) expressing FcεRI which plays a role in recruiting cutaneous lymphocyte antigen-bearing T cell to initiate cutaneous inflammation[13] and activate Th2 polarization.[14,15] Also, interleukin (IL)-16 and monocyte chemotactic protein 1 (MCP-1) produced by these epidermal DCs induce differentiation of monocytes into inflammatory dendritic epidermal cells (IDECs), which produce IL-1, IL-6, and tumor necrosis factor α (TNF- α). Other cytokines in AD pathogenesis such as IL-12 and IL-18 aid in transformation of inflammatory responses from Th2 to Th1/0 and enter chronic phase.[16] Through above mechanisms, allergens in environment are important in both acute phase from repetitive exposure and also in chronic status of disease; hence, it is imperative for extrinsic patients who have elevated serum and specific IgE for allergens to avoid possible exacerbating factors. And one of the most frequently noted allergens for AD exacerbation is a house dust mite (HDM).

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Pyroglyphidae *Dermatophagoides farinae* (Der f), *Dermatophagoides pteronyssinus* (Der p) and Euroglyphus maynei are the most common types of HDM. The antigenically active particles contain high enzymatic activity and act through destroying tight junction of epidermis, enhancing penetration of allergens deep into the skin.[17,18] One of enzymes that HDM possesses is serine cysteine proteinase, and these enzymes are able to activate proteinase-activated receptors (PARs). Among many PARs, PAR-1, and PAR-2 are known to be most populated in respiratory, gastrointestinal systems and skin.[19] When PAR is activated, various inflammatory mediators such as IL-6 and IL-8 are secreted, leading to increase vascular permeability, infiltration of leukocytes, increased airway hypersensitivity, and other effects by HDM that preceded clinical symptoms of allergic diseases.[20]

Allergen specific immunotherapy (SIT)

Mechanisms of allergen SIT

HDM avoidance has been practiced as a part of lifestyle modification with extrinsic AD patients for quite a period. Yet as a more active treatment modality, SIT is receiving more attention. SIT was initially practiced in allergic rhinitis or asthma patients. Up until now, SIT is the only disease-specific treatment modality that suppresses allergic responses for a long period of time. SIT aims to induce allergen-specific tolerance otherwise known as allergen vaccination[21] through acquiring immune tolerance with induction of allergen-specific regulatory T cells (Tregs).

The acute phase of AD is closely associated with production of Th2 cytokines and commonly observed Th2-biased profiles are suggested to be results of increased clonal expansion or differentiation of Th2 cells or increased tendency to activation and apoptosis of high IFN-γ producing Th1 cells.[22] These Th1 cells are known to be involved in apoptosis of epithelium in AD. Thus, induction of Treg cells during the SIT consequently increases suppression of allergen-induced T-cell proliferation, and Th1 and Th2 cytokines.[23] Thereby, we may observe clinical improvement of AD as a result of skin inflammation reduction and a diminution in epithelium apoptosis.

Tregs involved in mechanisms of SIT express IL-10, transforming growth factor β (TGF-β) to elicit early phase desensitization of mast cell, basophil, and eosinophil. These allergen-specific Tregs also suppress Th2 cells, thereby inhibiting IgE production, while at the same time stimulating expression of IgG4, a non-inflammatory immunoglobulin isotype. Also, cytokines such as IL-3, IL-4, IL-5, IL-9, and IL-13 that are expressed from Th2 play an important role in survival, activation, and differentiation of mast cells, basophil, and eosinophils, but SIT suppresses cytokine axes as well.

Treatment methods and schedules

SIT can be divided into 2 major groups depending on the route of administration: sublingual (SLIT) and subcutaneous (SCIT) methods. While the routes may differ, both equally affect peripheral allergen-specific Tregs through similar mechanisms for inducing T-cell tolerance, inhibitory functions of IL-10, TGF-β, and reduction of mast cell and eosinophil. However, in early stages of treatment, expression of Treg, reduction in IgE or increase in IgG4 might not be evident in SLIT compared to SCIT.[24]

The most important factor to consider while choosing candidates for immunotherapy is finding those who are actually sensitized to HDM. Therefore, majority of previously reported studies also enroll patients who have positive allergen sensitization to HDM. Standards for choosing candidates for SIT in our institution is first selecting extrinsic AD patients with serum total IgE above 150, and then additionally selecting only those who have positive reactions (over 3+) to HDM on CAP-test or skin prick test. We initially start the therapy in weekly regimen for 16-18 weeks as initial build-up phase and slowly escalates dosage of HDM extract, and when the maintenance dosage is reached, the patient visits the clinic biweekly for four times. Afterwards, the monthly regimen can be installed. Depending on clinical response, the patient can continue on with the treatment for 3 to 6 years.

There is no exact consensus for treatment period, interval time between treatments, and follow-up period after termination of SIT, but most literature generally agree upon 3 years as an ideal treatment period.[25] Our institution also maintains one year of treatment for all those started on SIT, and continues for 3 years unless complete remission is reached.

Clinical efficacy of allergen SIT in AD

Efficacy of SIT with HDM in AD

In the past, there has been a lack of evidence of SIT in AD compared to that in asthma or allergic rhinitis. However, with increasing reports of comparable efficacy and safety of SIT in AD, researches are actively seeking into the field of SIT in AD as well. Recently published meta-analysis on 8 different randomized controlled trials of SIT on AD showed excellent results of the therapy, strengthening rationale for the treatment.[26]

Results from previously performed randomized controlled SIT are summarized in Table 1. First, in Kaufman and Roth's study in 1974 (United States), quasi-randomized controlled study was performed among total of 52 adult and pediatric AD patients.[27] A total of 26 patients completed the SCIT trial for a period of 2 years, and significant clinical improvement was seen in 81% of the treatment group and 40% of the placebo group. Warner *et al.*[28] conducted randomized, double-blind, placebo-controlled study for children with asthma (United Kingdom) and among 20 children who possessed additional atopic features, there was subjective improvement of clinical eczema features as judged by the patients and parents in active treatment group (77.8%) compared to minimal improvement in the placebo group (27.3%) after 1 year of treatment. Later, Glover and Atherton performed randomized, double-blind, placebo-controlled trials for HDM SCIT for 24 pediatric AD patients.[29] The first study did not reveal

Table 1. Summary of characteristics and results from randomized controlled trials included in this review

Study	Year published	Country	Study design	Total number of patients (treatment, placebo)	Type of SIT	Type of allergens	Total duration (months)	Improvement	Reference
Kaufman and Roth	1974	US	qRCT DB PC	52 (26, 26)	SCIT	dander, HDM molds, pollen	24	(+) by physician	27
Warner *et al.*	1978	England	RCT DB PC	20 (9, 11)	SCIT	HDM	12	(+) by patients	28
Glover and Atherton	1992	England	RCT DB PC	24 (13, 11)	SCIT	HDM	8	(+) by patients	29
Silny and Czarnecka-Operacz	2006	Poland	RCT DB PC	20 (10, 10)	SCIT	dander, HDM pollen	12	(+) by physician	30
Pajno *et al.*	2007	Italy	RCT DB PC	56 (28, 28)	SLIT	HDM	18	(+) by physician	31
Novak *et al.*	2012	Germany	RCT DB PC	168 (112, 56)	SCIT	HDM	18	(+) by physician	32
Qin *et al.*	2013	China	RCT DB PC	107 (58, 49)	SLIT	HDM	12	(+) by physician	33

SIT, specific immunotherapy; qRCT, quasi-randomized controlled trial; DB PC, double-blind placebo-controlled; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; HDM, house-dust mite; (+), Presence.

any statistical difference between the treatment and placebo groups. The second study was conducted with patients who underwent active treatment in the first study and found greater clinical improvement, suggesting that long-term treatment for at least 1 year is necessary. Persistent efforts of SIT in AD continued, and a double-blind, placebo-controlled trial was conducted, for 20 adult and pediatric AD patients.[30] They used W-Atopowe zapalenie skóry (W-AZS), a Polish acronym for atopic dermatitis severity score to assess the extent and severity of skin inflammation index in AD patients concerning pruritus, sleep disturbances and extent and severity of skin inflammation, to evaluate the clinical efficacy. There was a significant decrease in clinical score of W-AZS index after a period of 12 months, supporting growing number of evidence for efficacy. A randomized, double-blind, placebo-controlled trial was performed among a larger population of pediatric patients[31] as a sublingual method for 18 months. Scoring atopic dermatitis (SCORAD) showed a dramatic decrease 9 months after the treatment and disease-control medication for treatment of AD was significantly reduced in treatment group compared to placebo group. In addition, compared to baseline, visual analogue scale (VAS) showed tendency to decrease only in the treatment group, although did not show statistical significance. Another randomized double-blind placebo-controlled trial by Novak *et al.*[32] was conducted with 168 adult AD patients for 18 months. Even though, the study did not reveal the efficacy in overall AD patients, SIT showed statistical significance of SCORAD reduction in subgroup of severe AD patients with SCORAD >50. Median reduction of total SCORAD of 18% was observed. The best outcome was shown during September to February, due to the use of indoor heating and subsequent high HDM exposure. The efficacy was more pronounced with longer duration. Lastly, most recent randomized control trial carried out by Qin *et al.*[33] analyzed 107 patients undergoing SLIT for 12 months. A total of 84 patients finished the trial, compared to the placebo group (53.85%), treatment group (77.78%) showed improvement in symptoms. SIT for AD patients are practiced in Korea as well. But only little clinical studies have been conducted. There was one pilot study of SIT published by Nahm *et al.*[34] Even though 20 AD patients showed significant decreased in SCORAD score with noticeable clinical improvement after 12 months, since it was modified treatment methods combining SIT and histamine-immunoglobulin complex treatment, it was difficult to see the sole and exclusive efficacy of SIT. Our institution performed retrospective review on patients who underwent at least 3 years of HDM SIT for 217 extrinsic AD patients selected through total IgE and CAP test or skin prick test with hypersensitivity to HDM.[35] Clinical improvement was judged based on investigator global assessment (IGA) and patients' subjective assessment of symptoms. In overall, 88.4% of patients showed clinical improvement and among these patients, 63.9% patients showed complete or near-complete remission. Pruritus and loss of sleep was also significantly reduced with 87.2% of patients reporting improvement in pruritus, and 92.7% of patients with only mild or no disturbance of sleep. Hence, although the efficacy of SIT for extrinsic AD patients with positive reactions to HDM was believed to have controversial results for patients in the past, there is a growing trend of thought through many double-blind placebo-controlled trials and meta-analysis, that SIT is indeed an efficient and safe treatment modality for AD patients. While 3 to 6 years of treatment period is generally recommended in literature, there is no set evidence stating long-term efficacy for AD patients receiving SIT for more than 3 years. Yet retrospective review from our institution support the long term efficacy of SIT indicating clinical improvements are most significant when the treatment is continued for a minimum of 3 years.

Side-effects and complications

Both local and systemic complications can occur due to SIT.

Based on a survey of systemic side-effects occurring SCIT in the past 3 years (2008-2011), there were noticeable systemic side-effects in only 0.1% of the total 18.9 million SCIT treatment performed, and there was no single case of fatal complications.[36] Majority of systemic complications occurred within 30 minutes of injection, and some of the delayed type response were mild symptoms such as a flu-like illness.[37]

Common local side-effects that can occur in SCIT are urticaria or pruritus, but majority of these reactions persist for less than 24 hours and are rarely regarded as noticeable complications. Comparing with results from our institution and other RCTs previously performed, mild urticarial eruptions and pruritus occurred in only <1% of patients.[38] Furthermore, in one double-blind placebo-controlled trial, incidence of pruritus lasting for 1-2 days and discomfort, mild exacerbation of atopic lesions, urticaria, headaches or rhinitis were almost similar between the treatment and placebo group,[26,27,31] leading to a conclusion that SIT is relatively a safe treatment modality. From RCTs of those who underwent SLIT,[30,32] fatigue, headache, localized delayed hyper-responsiveness (>1 hour) occurred in first injection of the treatment, and among these side-effects, localized pruritus was most common. Other noted side effects were facial edema and gastrointestinal discomfort. Yet most of symptoms were mild with spontaneous resolution. There were reports of sudden worsening of allergic reactions or generalized pruritus in both placebo and control group, but the patients were all manageable with a brief symptomatic treatment; no other serious adverse events were reported.[39,40]

According to data collected from our institution, we witnessed urticaria, localized eruption, pruritus, exacerbation of previous atopic lesions, and relapse of previously known asthma in <1% of patients. However, the degree of symptoms was very mild, and the symptoms were all controllable with antihistamines. We believe that it is actually very difficult to accurately determine whether such reactions occur due to SIT or by exposure to other exogenous trigger factors. Nevertheless, from evidences collected thus far, SIT is a very safe treatment modality to incorporate in a clinic setting.

Biologic effect of allergen SIT in AD

Immunologic effect and other serologic effect

There are only few reports on immunological changes observed in serum or skin after SIT since most of studies thus far were concentrated on clinical efficacy and safety. Articles elaborating on changes in serum level of IgE and IgG4 are beginning to appear on surface, but works on variety of cytokines and chemokines are lacking. Considering a role of allergen as a potent aggravating factor in AD and complicated axes of immunology in AD pathomechanism, it is an important task to find efficacy of inducing immune tolerance through SIT and acquiring data that shows intricate interplay of immunologic changes before and after treatment.

An explanation is needed for serum IgE level changes in response to SIT in regards to highly activated B cells and deregulation of IgE synthesis,[41] but there is no clean-cut evidence. Studies up until now show trend of allergen-specific IgE level gradually decreasing after SIT.[31,42-46] For total IgE, there was a general trend for decrease, but statistically, the results were conflicting with those showing significance[31,46] and those that did not.[27,29] Serum IgE begins to change relatively at a slow rate with no noticeable drop in its levels; moreover, since there is no evident correlation between clinical improvements after SIT treatment, it is hard to explain loss or decrease of response to specific allergen only through changes in IgE.[47] To many scholars, the role of IgE as a measurement of clinical sensitivity remains questionable in reality.[43] Other works have stressed a significant decrease in Der p-specific IgG4,[31,32,45,48,49] and in one pilot study, treatment with SIT led to decrease in markers of AD activity such as IL-16 and thymus and activation regulated chemokine (CCL17) in accordance with clinical improvement.[44]

Examples of biomarker studies

Although finding a biomarker that can accurately predict treatment response is a necessary task, it is a challenging process considering multifaceted and intricately woven immunologic mechanisms and axes involved in allergic patients. Since the late 1990s, there have been many attempts to find biomarker candidates. In the early years, most studies concentrated on endothelial cell adhesion molecules,[50-54] and works on chemokines were published in 2000.[55-58] Brief summary on history of biomarker studies are summarized in Table 2. Reviewing studies on biomarker up until now, there have been reports of allergen-specific non-IgE antibody increasing through SIT,[59] and several studies have shown that serum antibodies can reduce *in vitro* responses mimicking allergic reactions, such as IgE binding to allergen, IgE-facilitated antigen presentation and basophil activation.[60-62] In a double-blind placebo controlled study of grass pollen SIT, there was increase in IgG4, IgE blocking factor along with suppression of facilitated allergen binding.[63] The authors stressed that not only IgG4, but combined assessment of IgG4 and IgE blocking factor can be done in order to more comprehensively observe functional and clinical efficacy.

Recently, grass pollen SLIT experiment was performed in experimental exposure chamber, and the results showed complement component 1 and the receptor stabilin-1, 2 protein induction from tolerogenic DC also known as regulatory DC has correlation with clinical tolerance induced by SIT.[64] In addition, the study opened a possibility into readily selecting clinically responsive and unresponsive group through proteins that are easily detected through quantitative polymerase chain reaction in peripheral blood mononuclear cells, and explained relationship of short-term efficacy with regulatory immune response.

What exact immunologic mechanisms underlie changes induced in SIT is a field of excitement that raises many questions.

Table 2. Prior studies on biomarker candidates of atopic dermatitis

Candidate marker	Action	Clinical results	Reference
sE-selectin	An adhesion molecule on endothelial cells	Reflection of disease severity	50,52-54,66,67
sVCAM-1	An adhesion molecule on endothelial cells	Not correlated with disease severity	51,52,54,67
sICAM-1	An adhesion molecule on endothelial cells	Not correlated with disease severity	52,54
TARC/CCL17	A chemokine that attracts $CCR4^+$ or $CCR8^+$ cells	Reflection of disease severity	55,56,58,68-72
MDC/CCL22	A chemokine that attracts $CCR4^+$ cells	Reflection of disease severity	56,57,68,69,72,73
CTACK	A chemokine that attracts $CCR10^+$ cells	Reflection of disease severity	58
IL-13	An inducer of IgE production	Reflection of disease severity	74
IgE	Primes the IgE-mediated allergic reaction	Reflection of disease severity No significant result	32,46,68,71,75 29,30,72,76
ECP	A basic protein located in the eosinophil primary matrix	Reflection of disease severity No significant result	75,77-80 67
TEC	Eosinophils control mechanisms associated with allergy	Reflection of disease severity	75,79,81
sIL-2R	Expressed by antigen-activated T lymphocytes	Reflection of disease severity	79-81
IL-16	A chemokine that attracts $CD4^+$ cells	Reflection of disease severity	44,73,75
IL-18	An interferon-γ inducing factor	Reflection of disease severity	71,82,83
BDNF	A peripheral neurotrophin	Reflection of disease severity	84,85
NGF	A potent mediator in neuroinflammatory processes	Reflection of disease severity Positive staining on AD skin only No significant result	86 87 88
Substance P	A neurotransmitter and a neuromodulator	Reflection of disease severity Not correlated with disease severity	86 89
CCL18	A chemokine that attracts both innate and adaptive immune cells	Reflection of disease severity Significantly decreased after Tx	90 72
MEC/CCL28	A chemokine that attracts $CCR3^+$, $CCR10^+$ cells	Reflection of disease severity	91,92
PF-4	A platelet chemokine	Reflection of disease severity	93,94
Beta-TG	A platelet chemokine	Reflection of disease severity	93,94
IL-31	Associated with skin-homing CLA-positive T cells	Reflection of disease severity	95
CLSP	A modulator of calcium-dependent proteins	Positive relation in AEAD skin	96
Der p-specific IgG4	A specific IgG molecule for Der p	Reflection of disease severity	32,33,45,48,49

Underlyng bar: studies and results of SIT in AD.
sE-selectin, soluble E-selectin; sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular adhesion molecule-1; TARC, thymus and activation-regulated chemokine; CCL, C-C motif ligand; CCR, chemokine receptor; MDC, macrophage-derived chemokine; CTACK, cutaneous T cell-attracting chemokine; IL-13, interleukin-13; ECP, eosinophil cationic protein; TEC, total eosinophil count; sIL-2R, soluble IL-2 receptor; *BNDF*, brain-derived neurotrophic factor; NGF, nerve growth factor; Tx, treatment; MEC, mucosa-associated epithelial chemokine; PF-4, platelet factor 4; beta-TG, beta-thromboglobulin; CLA, cutaneous lymphocyte antigen; CLSP, calmodulin-like skin protein; AEAD, acute exacerbated.

There still remains a room to search deeper into discovering biomarkers and choose appropriate candidates, mechanism, treatment response, surrogate end points, and clinical trial for new drug development.[65] Hence, it will not be an understatement to say that the new era of AD expects a discovery of biomarker that can assess and standardize treatment response. If biologic marker can show clear-cut correlation with clinical symptoms, it will be an outbreak in the field of science, but considering variegated clinical pictures and associated immunological changes, the quest for a search will not be easily answered upon. But through future endeavors in creating more high-quality standardized experiments that reflect clinical improvement and enable predictions of treatment end-points, we will be building cornerstone for biomarker discovery.

Further considerations and conclusion

The effectiveness of SIT has been proven through many clinical studies recently published and more studies are expected in the future. However, there are still issues that need to be addressed before clinically applying SIT in hospital-settings. Standardized method in selecting candidate patients should be applied for institutions along with objective qualifying criteria. Also, effective treatment modality for those who are not solely sensitized to HDM (polysensitized patients) raises attention. There are different routes and schedules for SIT at the moment, and there is rush or ultra-rush protocol besides the well-known

conventional protocol. In the future, a development for a safe protocol which enables faster immune reaction is promising. If we can perform further studies to see whether early intervention allows for blocking progression into allergic march, we will be opening many doors into prevention and treatment of various allergic diseases. Endeavors in optimizing preparations used and improving treatment response with more refined allergoids, potent adjuvants, or recombinant vaccine are also suggested. Lastly, more work should be done in an attempt to discover biomarkers for SIT that will allow clinicians to predict the outcomes or to judge appropriate treatment duration for the patients. Uncovering a new biomarker shall advance the upcoming development and applications of SIT.

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