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Recent Advances in Allergen-Specific Immunotherapy in Humans: A Systematic Review

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ABSTRACT

Allergen-specific immunotherapy (AIT) is presumed to modulate the natural course of allergic disease by inducing immune tolerance. However, conventional AITs, such as subcutaneous immunotherapy and sublingual immunotherapy, require long treatment durations and often provoke local or systemic hypersensitivity reactions. Therefore, only <5% of allergy patients receive AIT as second-line therapy. Novel administration routes, such as intralymphatic, intradermal and epicutaneous immunotherapies, and synthetic recombinant allergen preparations have been evaluated to overcome these limitations. We will review the updated views of diverse AIT methods, and discuss the limitations and opportunities of the AITs for the treatment of allergic diseases in humans.

Keywords: Allergy; Immunologic desensitization; Intradermal injection; Transcutaneous administration; Intralymphatic injection; Allergens

INTRODUCTION

Allergen-specific immunotherapy (AIT) was first introduced by Leonard Noon and John Freeman in the early 20th century as a "prophylactic inoculation against hay fever" (1). Allergic diseases were found to be orchestrated by type 2 helper T cells (Th2 cells) that modulate eosinophilic inflammation and the IgE-mediated hypersensitivity reaction. At present, the primary mechanism of AIT is presumed to rely on activation of the adaptive immune response mediated by non-Th2 T cells (e.g., Th1 and Treg cells). These cells inhibit Th2 immunity and generate the neutralizing antibodies IgG and IgG4, which compete with IgE and are induced by allergens at higher doses than natural exposure, thus leading to immune tolerance (2). Various types of AIT were evaluated in the 20th century, including subcutaneous immunotherapy, oral immunotherapy, local bronchial immunotherapy, local nasal immunotherapy, and sublingual immunotherapy (3). Subcutaneous immunotherapy, sublingual immunotherapy, and oral immunotherapy remain in use for patients with allergic diseases (3).

Although AIT is regarded as a robust treatment for allergic diseases via induction of immune tolerance and modification of the natural course of allergic diseases, only <5% of patients receive AIT, making it a second-line therapy after pharmacotherapy and allergen

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Conflict of Interest
The authors declare no potential conflicts of interest.

Abbreviations
AIT, allergen-specific immunotherapy; APC, antigen-presenting cell; CI, confidence interval; CLR, C-type lectin receptor; CSMS, combined symptom-medication score; DC, dendritic cell; Eos, eosinophil; EPIT, epicutaneous immunotherapy; IDIT, intradermal immunotherapy; ILIT, intralymphatic immunotherapy; LC, Langerhans cell; LN, lymph node; MC, mast cell; PC, plasma cell; SAE, serious adverse event; sIgE, allergen-specific IgE; Syk, spleen tyrosine kinase; TEAE, treatment-emergent adverse event.

Author Contributions
Conceptualization: Lee SP, Shin YS, Kim TB, Lee SM; Data curation: Lee SP, Shin YS, Kang SY, Lee SM; Formal analysis: Lee SP, Kang SY, Lee SM; Funding acquisition: Kim TB, Lee SM; Investigation: Lee SP, Kang SY, Kim TB, Lee SM; Methodology: Lee SP, Kang SY, Kim TB, Lee SM; Project administration: Kim TB, Lee SM; Resources: Lee SP, Shin YS, Lee SM; Supervision: Shin YS, Kim TB; Validation: Lee SP, Shin YS, Kim TB; Visualization: Lee SP, Lee SM; Writing - original draft: Lee SP, Shin YS, Lee SM; Writing - review & editing: Lee SP, Shin YS, Kang SY, Kim TB.

avoidance (4). This can be attributed to the frequency of local or systemic hypersensitivity reactions during AIT, as well as the substantial commitments of time, money, and effort from patients. Moreover, subcutaneous immunotherapy and sublingual immunotherapy have reportedly failed to reduce the use of medication in patients with perennial allergic rhinitis, which is regarded as the main therapeutic application for AIT (5). To overcome the limitations of conventional AITs, novel AITs have been developed using alternative routes of administration, such as intradermal immunotherapy (IDIT), epicutaneous immunotherapy (EPIT), and intralymphatic immunotherapy (ILIT); novel AITs have also included alternative synthetic recombinant allergen preparations.

MECHANISMS OF AITS WITH ALTERNATIVE ROUTES OF ADMINISTRATION

Because they share downstream pathways, IDIT, EPIT, and ILIT are presumed to induce immune tolerance by enhancing Th1 and Treg immune responses, which suppress Th2 immunity via cytokines and generate IgG and IgG4 neutralizing antibodies, both of which compete with IgE (**Fig. 1**) (6).

In IDIT, intradermally injected allergens can drain into lymph nodes (LNs) in a more rapid and efficient manner, compared with subcutaneous injection. The rate of lymph perfusion is much higher in the dermis than in the poorly perfused subcutis, and dendritic cells (DCs) as antigen-presenting cells (APCs) are present at high densities in the dermis (6-8). Therefore, low allergen doses delivered to the dermis can induce allergen tolerance (9).

In EPIT, allergens delivered to the epidermis diffuse into the dermal compartment, where they can be captured by DCs or taken up by Langerhans cells (LCs) (10). These two types of APC move to skin-draining LNs and activate non-Th2 adaptive immune cells (e.g., Th1 and Treg cells), thus modifying T-cell polarization and inducing immune tolerance. In addition, adhesive tape stripping performed before allergen placement on the skin during EPIT enhances allergen penetration by removing the stratum corneum. It also acts as a physical adjuvant by activating keratinocytes and causing them to secrete various cytokines (e.g., IL-1, IL-6, IL-8, TNF-α, and IFN-γ), thus inducing the maturation and emigration of DCs to draining LNs (6,11-14).

In ILIT, allergens are directly delivered to LNs, where the high density of APCs, T cells, and B cells enhances antigen presentation and the subsequent activation of adaptive immune responses (6). Indeed, ILIT delivers 100-fold more allergen to LNs, compared with any other route. It enhances the secretion of IL-2, IL-4, IL-8, IL-10 and IFN-γ, thereby stimulating adaptive immunity; it also induces the proliferation of plasmablasts that secrete non-IgE immunoglobulins, increasing the serum levels of IgG and IgG4, as well as the affinity of IgG4 to allergens (4,15-29).

THERAPEUTIC EFFICACY OF AITS WITH ALTERNATIVE ROUTES OF ADMINISTRATION

IDIT

Only two double-blinded placebo-controlled trials with conflicting results have been conducted regarding IDIT (30,31). The first of these studies reported that daily combined

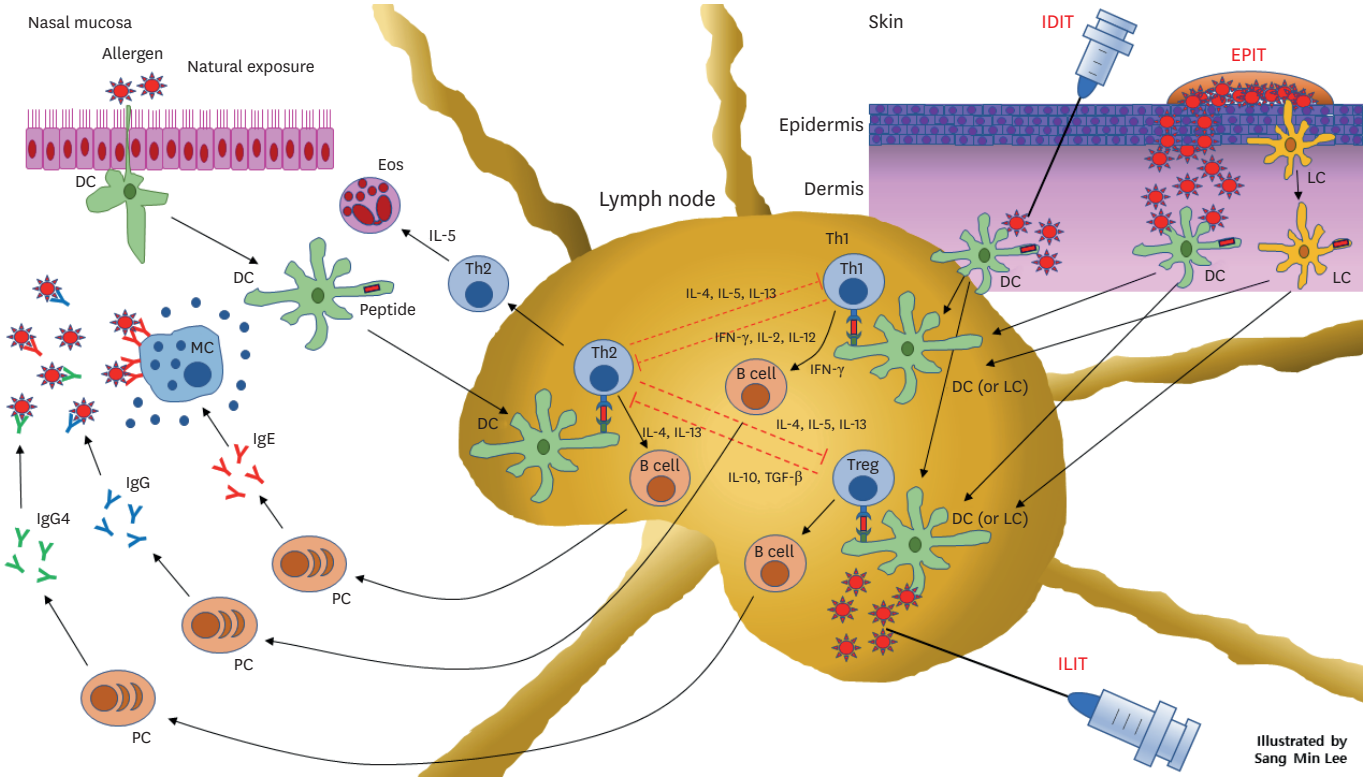

Figure 1. Mechanisms of novel AITs with alternative routes of administration. Similar to conventional AITs, IDIT, EPIT, and ILIT are presumed to induce immune tolerance by enhancing Th1 and Treg immune responses that suppress Th2 immunity and generate the neutralizing antibodies, IgG and IgG4, which compete with IgE.

symptom-medication scores (CSMSs) in the active group, a parameter strongly recommended as a primary end point in evaluating the treatment efficacy of AIT, did not differ from daily CSMSs in the placebo group (30) (**Fig. 2A**). Moreover, nasal symptoms and asthma symptoms were worse in the treatment group, while symptom-free days were fewer. IDIT increased serum allergen-specific IgE and the expression of CRTH2, a Th2 cell surface marker, in T cells that had been cultured from biopsy specimens; these findings indicated that IDIT enhanced Th2 immunity. The small quantity of allergen (7 ng of the major allergen Phl p 5) in a small volume (20 μL) may further induce Th2 rather than Th1 or Treg immunity, resulting in low therapeutic efficacy, although intradermal injection of allergens may promote drainage of allergens into LNs. Conversely, the second study found that IDIT reduced CSMSs and serum allergen-specific IgE levels, while increasing allergen tolerance in the conjunctival provocation test (31). In both studies, grass pollen allergens (*Phleum pratense*) were injected weekly in six total administrations. Compared to a prior report, a higher concentration of allergen (0.3 or 0.6 μg protein/ml) in a larger volume (0.1 ml) may explain the significant reduction of CSMSs in the latter study. However, a volume of 0.1 ml is too large for intradermal injection and can induce severe pain at the injection site. Additionally, no study has compared IDT with conventional AIT such as SCIT or SLIT.

EPIT

Senti et al. (11,32,33) evaluated the outcomes and safety of EPIT in adults with grass pollen (*Phleum pratense*)-induced allergic rhinitis, titrating the concentrations of allergens as well as the number and duration of administrations using abrasion and tape-stripping pretreatments (34). EPIT decreased hay fever symptoms during the pollen season by more than 30%, and

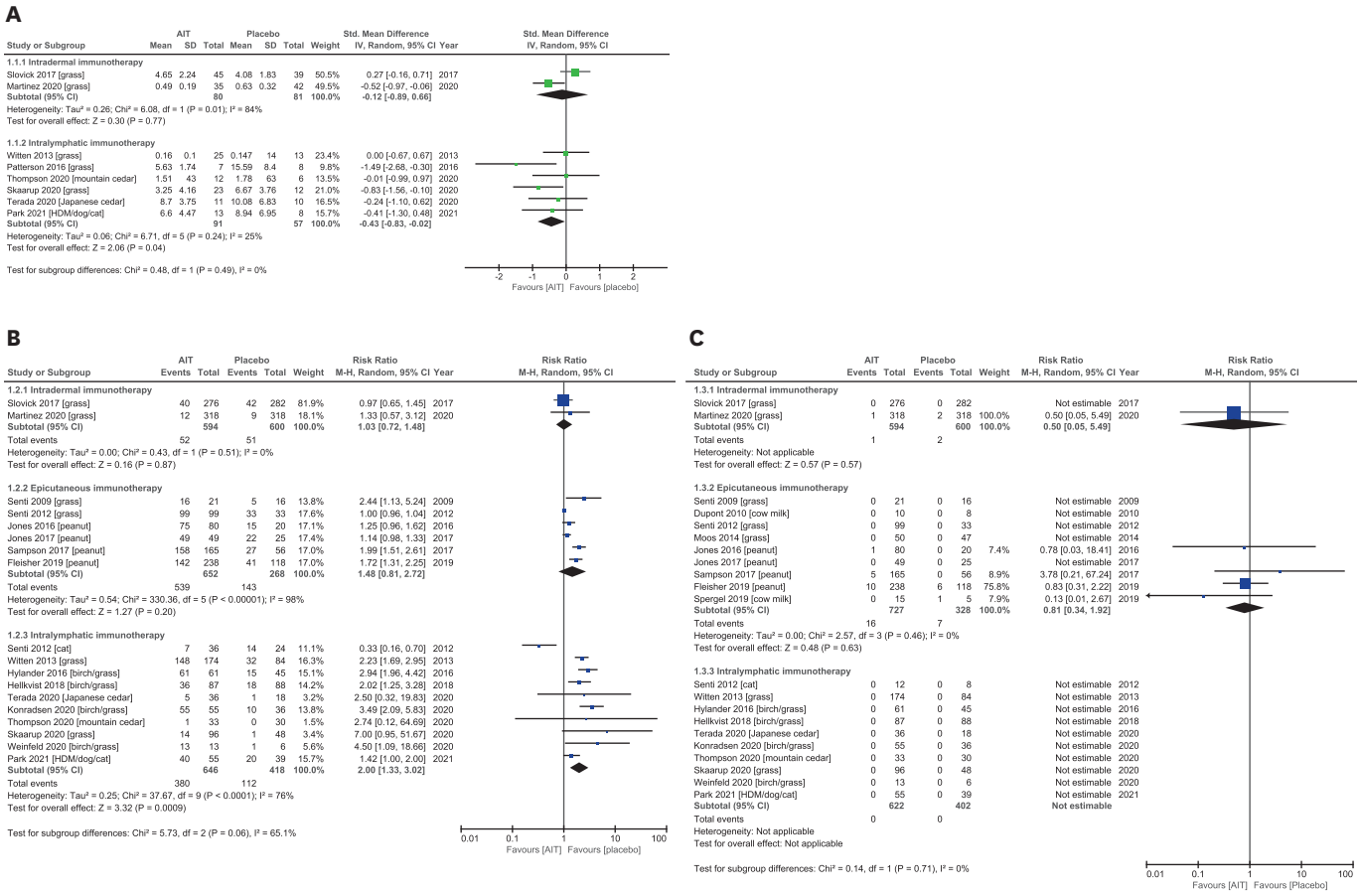

Figure 2. Therapeutic efficacy and safety of novel AITs with alternative routes of administration. (A) Comparison of CSMSs between IDIT- or ILIT-treated group and placebo group. (B) Comparison of TEAEs between IDIT-, EPIT-, or ILIT-treated group and placebo group. (C) Comparison of SAEs between IDIT-, EPIT-, or ILIT-treated group and placebo group.

provoked frequent local reactions (up to 63.5%) in addition to systemic reactions, which were rare. In a study of pediatric patients, Agostini et al. (35) performed EPIT on intact skin that had been hydrated by occlusive chambers; they reported that EPIT with 11.25 μg of Phl p 5 was effective for reducing symptom scores and rescuing medication scores. However, Senti and Agostini did not compare the therapeutic efficacy of EPIT with that of conventional AITs such as SCIT and SLIT, nor did they measure CSMSs, which are strongly recommended for evaluating the outcomes of AIT.

The potential therapeutic roles of EPIT in alleviating allergies to foods (e.g., peanuts and cow milk) were thoroughly investigated with a step-wise approach in large-scale double-blinded placebo-controlled trials sponsored by pharmaceutical companies to overcome the limitations of oral immunotherapy; such limitations included high risks of local and systemic hypersensitivity reactions and the failure of long-lasting immune tolerance after treatment cessation (36-41). In a meta-analysis of these studies, EPIT was reported to induce desensitization to the target food allergens of cow milk and peanuts (risk ratio, 2.34; 95% confidence interval [CI], 1.69–3.23; p<0.00001; I^2=0%) (42), but it did not provide permanent or long-lasting allergen tolerance. Furthermore, no study has compared the clinical outcomes of EPIT and conventional oral immunotherapy in patients with food allergies.

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ILIT

With the exception of studies by Witten et al. (23) and Park et al. (43), in which ILIT was associated with negative results, ILIT has been widely reported to decrease reactivity to allergens in allergy skin tests and nasal allergen provocation tests, alleviate allergic symptoms, and reduce rescue medication use. These therapeutic effects have been observed within 4 months after treatment initiation (17-20,22,24,25,44-49). Notably, meta-analyses have shown that ILIT significantly reduces CSMSs (**Fig. 2A**) (23,26,43,45,47,48,50,51). Regarding the negative results reported by Witten et al. (23), Kündig et al. (52), who first introduced ILIT for allergic diseases, suggested that a 2-week interval between intralymphatic injections may be insufficient. However, a recent study also reported that symptoms, quality of life, and CSMSs did not differ between ILIT-treated and placebo groups, despite three intralymphatic allergen injections at 4-week intervals (43). This result is presumably because the target allergens were not grass or birch pollen allergens; instead, they were indoor inhalant allergens (e.g., house dust mites or pet dander), which were evaluated in only a few previous studies (17,25,46,49). Furthermore, the L-tyrosine-adsorbed allergen preparations used for ILIT in the intralymphatic injection study had low allergenicity and immunogenicity (53).

In addition to the target allergens and their preparations, there are several unsolved issues involved in establishing the optimal treatment protocols in ILIT. For example, it has not been determined whether gradual escalation (17,25,43,45,46) or fixation (in most studies) (18,19,22-24,44,47,48) of allergen doses is more effective during repeated intralymphatic injections. There is a need for further investigation regarding whether multiple allergens should be injected as a mixture into the same LN or separately into different LNs (20,25,29,43,46). Regarding the LN injection site, most studies have injected allergens into inguinal LNs, while one study used cervical LNs. However, cervical LNs may be suboptimal injection sites for ILIT because a severe local reaction can directly obstruct a patient's airway (49). Recently, a single preseason ILIT booster injection was suggested to have a role in the maintenance of therapeutic efficacy in patients who had already received three injections of ILIT in the previous year; this requires verification by further studies (26-28,54). Finally, no study has compared the clinical efficacy of ILIT and conventional AIT (e.g., SCIT and SLIT) except the first evaluation of ILIT (44).

SAFETY OF AITS WITH ALTERNATIVE ROUTES OF ADMINISTRATION

Treatment-emergent adverse events (TEAEs) associated with IDIT, EPIT, and ILIT are shown in **Fig. 2B** (11,17,19,20,23,26,27,30,31,33,34,36-43,47,48,50,51,55). Compared with the placebo group, the risk of TEAEs did not increase in the IDIT group (risk ratio, 1.03; 95% CI, 0.72–1.48; p=0.87; I^2=0%) or EPIT group (risk ratio, 1.48; 95% CI, 0.81–2.72; p=0.20; I^2=98%). However, the risk of TEAEs was greater for ILIT (risk ratio, 2.00; 95% CI, 1.33–3.02; p<0.0009; I^2=76%). Compared with the placebo group, the incidence rates of serious adverse events (SAEs) did not differ for the IDIT group (risk ratio, 0.50; 95% CI, 0.05–5.49; p=0.57) or the EPIT group (risk ratio, 0.81; 95% CI, 0.34–1.92; p=0.63; I^2=0%) (**Fig. 2C**). No SAEs were observed in double-blinded placebo-controlled trials regarding ILIT. However, three open-label studies reported moderate-to-severe systemic reactions that required intramuscular injections of epinephrine to manage anaphylaxis or inhalations of short-acting beta2 agonist to manage bronchospasm (22,24,25).

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CONVENTIONAL ALLERGEN PREPARATIONS FOR AIT

To avoid IgE-mediated local or systemic hypersensitivity reactions (so-called allergenicity) and achieve therapeutic efficacy (i.e., immunogenicity) during AIT, allergen extracts for AIT have been adsorbed to alum hydroxide or tyrosine; alternatively, they have been denatured by treatment with formaldehyde or glutaraldehyde. These denatured allergens are known as *allergoids* and may reduce absorption rates into the body or the affinity of IgE binding, allowing the use of allergen doses that are sufficient to induce adaptive immune responses other than Th2 immunity (56-59). In some allergens for AIT, monophosphoryl lipid A (a TLR 4 agonist that stimulates the Th1 immune response) has been added as an adjuvant. AITs with these allergens are associated with reduced CSMSs, although subsequent studies with higher doses had negative results (60). However, the concept of modified natural allergen extracts was challenged by an in vitro experiment in which allergoids exhibited reduced allergenicity in basophil activation tests and reduced immunogenicity in T-cell stimulation assays although most allergoids have appeared to perform well in clinical studies and clinical practice (53). Recently, allergen delivery by vectors such as virus-like particles has also been evaluated for the modulation of allergic diseases (61-63).

ALTERNATIVE ALLERGEN PREPARATIONS FOR AIT

Alternative allergen preparations for SCIT, including recombinant allergens and allergens conjugated to immunostimulatory molecules, have been evaluated.

The DNA sequencing of major allergens has enabled the introduction of synthetic recombinant allergens in AIT. Recombinant allergens have multiple benefits: selective expression of the allergenic protein in fragments or as peptides; deletion or mutation of specific amino acids within the protein or peptides to reduce allergenicity; fusion with immunostimulatory molecules such as TLR, C-type lectin receptor (CLR), and Fc receptor to enhance immunogenicity; and production of nearly unlimited quantities of allergenic proteins (64).

A mixture of five recombinant grass pollen allergens was reported to reduce CSMSs; recombinant birch pollen allergen could also induce a clinical response that was equal to whole birch extracts in patients with seasonal allergic rhinitis (65,66). Subsequently, a hypoallergenic folding variant of recombinant birch allergen (rBet v 1-FV) was developed; however, the incidences of systemic reactions during AIT did not differ between this allergen and native birch pollen extracts (67). T-cell epitopes have been used in AIT to stimulate a pure T-cell response while completely avoiding IgE-mediated hypersensitivity reactions. However, this treatment also induced peptide-specific IgE production and provoked systemic reactions several hours after injection; later studies showed negative clinical responses (68-72).

Allergen conjugation with immunostimulatory molecules has also been explored to enhance the immunogenicity of AIT. In patients with allergic rhinitis, AITs that involved allergens conjugated with CpG DNA motif (a TLR 9 agonist that stimulates Th1 immunity) afforded significantly lower allergen reactivity, along with symptom alleviation, reduced medication use, and improved quality of life. However, subsequent clinical trials had negative results, and further work was abandoned (73-77). In recent years, allergens have been conjugated with non-oxidized mannan to enhance endocytosis into DCs through binding to CLR (**Fig. 3**) (78). Allergens have also been conjugated with Fcγ portions to inhibit allergic reactivity by binding

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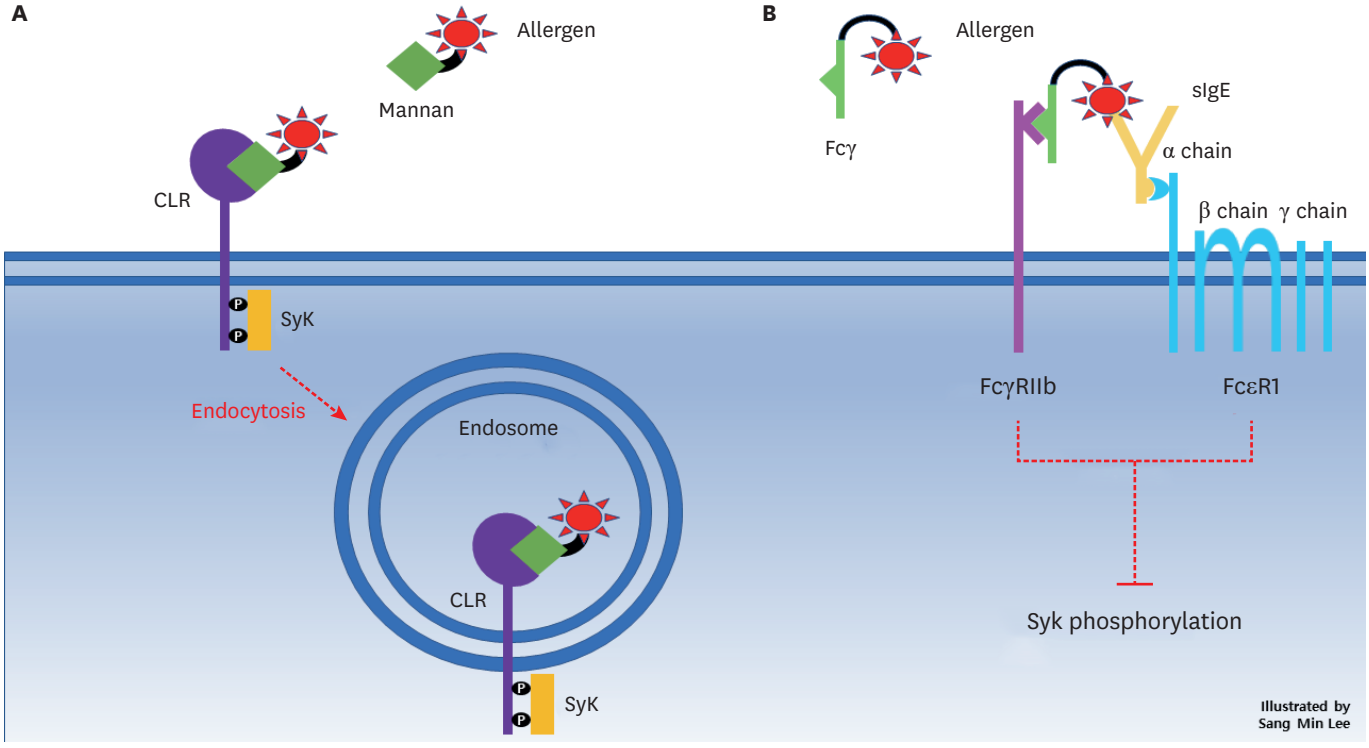

Figure 3. Alternative synthetic recombinant allergens conjugated with non-oxidized mannan (A) and Fcγ (B) for AIT. The binding of mannan-allergen complexes with CLR promotes their endocytosis by DCs. The binding of Fcγ-allergen complexes with both FcγRIIb and FcεR1 suppresses allergic reactivity through inhibition of Syk phosphorylation.
P, phosphorylation.

both Fcε receptor I and inhibitory Fcγ RIIb; this offers a potential alternative to synthetic allergen preparations for AITs (79). However, no clinical study has evaluated the usefulness of allergens conjugated to non-oxidized mannan or the Fcγ portion.

CONCLUDING REMARKS

To overcome the limitations of conventional AITs, novel AITs with alternative administration routes and synthetic recombinant allergen preparations have been evaluated. Among novel AITs with alternative administration routes, the therapeutic efficacies of IDIT and EPIT have not been established, although double-blinded placebo-controlled trials have shown that both are as safe as placebo treatments. In contrast, ILIT has been shown to reduce CSMSs, a major primary outcome of AITs in patients with allergic diseases; however, ILIT is associated with the occurrence of clinically significant TEAEs, including moderate-to-severe hypersensitivity reactions. Recent studies suggested therapeutic roles for synthetic recombinant allergen preparations in AITs; however, subsequent studies have failed to verify those results. In conclusion, further studies are needed to establish that novel AITs with alternative routes of administration or synthetic recombinant allergens are safer and more effective than conventional AITs with native allergen extracts or allergoids.

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