Introduction

Now it has been over 100 years since the first publication on allergen-specific immunotherapy by Leonard Noon in 1911. Numerous evidence has demonstrated that immunotherapy is highly effective in the treatment of allergic diseases such as allergic rhinitis, allergic conjunctivitis, allergic asthma, and sting insect hypersensitivity. There is recent clinical evidence suggesting its potential efficacy for atopic dermatitis, oral allergy syndrome, or food allergy. In addition, its benefits may go beyond the current clinical responsiveness, and extend further toward the prevention of new allergy or toward the persistent disease-modifying effects. Regarding the mechanism of action, it is accepted that the key factor is the induction of peripheral tolerance. However, the details of immunologic mechanisms are still less clear compared than its evident clinical efficacy. The present presentation will review historical and recent evidence for the mechanism of successful allergen immunotherapy.

Modulation of allergen-specific antibody

In the earlier years, the clinical response was assessed using allergen provocation (target organ), allergen-skin reactivity, or specific IgE antibody measurements since its discovery. However, the levels of allergen-specific IgE antibody did not correlate well with clinical improvements despite gradual decrease of allergen-specific IgE levels were observed over time. Rather, allergen-specific IgG antibody, particularly IgG4 subclass, has been the subject of attention. Cooke et al. were the first to describe the concept of serum blocking antibodies. They demonstrated that heat-stable, non-reaginic antibodies were able to block allergic serum-mediated skin reactions when incubated with the allergen extract before injection.

The increase in IgG4 is a feature observed during successful immunotherapy. IgG4 is a non-inflammatory
isotype protecting from the development of allergic inflammation. IgG4 does not fix complement,\textsuperscript{12} has the property to capture the allergen before reaching the effector cell-bound IgE, and thus can prevent the activation of mast cells and basophils. However, the quantity of IgG4 antibody did not reflect the clinical efficacy consistently or closely.\textsuperscript{13} Rather, the inhibitory function of IgG4 is being investigated to be promising marker, using flow cytometry—the IgE-facilitated allergen binding (IgE-FAB) assay.\textsuperscript{14} This antibody subclass can block the interaction of allergen-IgE binding to low affinity IgE receptors, which prevents IgE-facilitated antigen presentation and subsequent activation of effector Th2 cells.\textsuperscript{13} CD23 is the molecule expressed on antigen presenting cells such as activated B-cells,\textsuperscript{15} and the formation of allergen-IgE-CD23 complexes is an important step for allergen to be internalized into endosomes.\textsuperscript{16} The functional assay of IgG4 would provide better understanding of immunological changes which occur during the immunotherapy.

**Induction of regulatory T cells**

The role of antigen-specific suppressor T cells in immunotherapy was first identified by Rocklin et al.\textsuperscript{17} The clinical effects of immunotherapy had been thought to be mainly associated with a shift from Th2 to Th1 cytokines, systemically or locally.\textsuperscript{18-20} However, now, rather than Th1/Th2 imbalance, the induction of immune regulatory T cells (Treg) are considered to be more critical factor in its mechanisms of action. Treg was first identified by Sakaguchi et al. in 1995,\textsuperscript{21} as expressing CD4 and CD25, the IL-2 receptor $\alpha$-chain. Forkhead box P3 (FOXP3) is a master control gene to program Treg cell development and function,\textsuperscript{22} and now Treg is classified into the naturally occurring, thymus-selected CD4+CD25+FOXP3+Treg cells, the inducible type 1 IL-10-secreting Treg (Tr1) cells, and transforming growth factor $\beta$ (TGF-$\beta$)-secreting Th3 cells. The critical roles of Treg and IL-10 have been introduced by Akdis et al.\textsuperscript{23} and Yamanaka et al.\textsuperscript{24}

In clinical studies, grass pollen immunotherapy induced the increase of local CD4+CD25+FOXP3+Treg cells in the nasal mucosa of rhinitis patients and their increase was well correlated with clinical efficacy.\textsuperscript{25} Moreover, in a tracking study for antigen-specific T cells with allergen class II tetramers, induction of clinical tolerance was associated with loss of IL-4-producing T cells and the appearance of IL-10-producing and FOXP3+T cells.\textsuperscript{26} Circulating CD4+CD25+Treg cells in non-allergic healthy subjects were related with prevention of allergen-specific proliferation of effects cells upon stimulation.\textsuperscript{27}

Allergen-specific Tr1 cells also play a key role in allergen responses. Studies have shown that three different allergen-specific T cell subsets (Th1, Th2, and Tr1) co-exist in an individual at different proportions, and Th2 cells were observed more frequent among allergic individuals, while Tr1 was the dominant subset in healthy individuals.\textsuperscript{28} Immunotherapy for grass pollen increased Tr1 numbers in allergic patients.\textsuperscript{29} In non-allergic healthy beekeepers, repeated exposure to bee venom allergens resulted in switching of bee venom-specific Th1 and Th2 cells into IL-10-secreting Tr1 cells.\textsuperscript{30}

IL-10 can downregulate major histocompatibility complex (MHC) class II expression on dendritic cells (DC)
and tyrosine phosphorylation of CD28 in T cells, and also inhibits Th1, Th2 and Th17 cells in vitro and leads them into anergy.\textsuperscript{31} The induction of IL-10 during immunotherapy can influence immunoglobulin isotype class switching, and the co-presence of IL-4 and IL-10 potentiates a preferential class switch favoring IgG4.\textsuperscript{32,33} Additionally, IL-10 reduces IL-5 production by Th0 and Th2.\textsuperscript{34}

TGF-\(\beta\) induces the conversion of naïve CD4+CD25-T cells into CD4+CD25+T cells by inducing FOXP3,\textsuperscript{35} modulates the expression of cutaneous T-lymphocytes antigen (CTLA)-4 on Treg cells.\textsuperscript{36} TGF-\(\beta\) is also critical in maintaining the expansion and immunosuppressive capacity of CD4+CD25+T cells.\textsuperscript{37} In addition, TGF-\(\beta\) inhibits B-cell proliferation and differentiation, and decreases levels of immunoglobulin except mucosal IgA.\textsuperscript{18,38}

### Influence on antigen presenting cells

The primary regulator of T cell differentiation is the antigen presenting cell (APC) like DC. The maturation status of DC plays a pivotal role between sensitization and tolerance, and thus the alteration of DC by immunotherapy would be an important step in considering the mechanisms of the therapy. DC expresses innate immune receptors toll-like receptors (TLRs) and Fc ε RI, and the cross-regulation of TLR9 and Fc ε RI was demonstrated on immature plasmacytoid dendritic cells (pDC).\textsuperscript{39} Allergic subjects are known to have peripheral DCs with high levels of Fc ε RI expression but impaired IFN response to TLR9 stimulation.\textsuperscript{40} In a recent study, allergen immunotherapy successfully restored innate immune responses (IFN-\(\alpha\) production) to TLR9 stimulation.\textsuperscript{41} In another study on Hymenopter venom allergy patients, immunotherapy influenced the surface expression of function-associated molecules on DCs such as CD32, CD40 and TLR2. Taken together, the influence of immunotherapy on the phenotypes and functions of DCs may be another critical factor in the induction of peripheral tolerance.

### Modulation of effector cells

During successful immunotherapy, the thresholds for mast cell and basophil activation are altered, and the IgE-mediated histamine release is decreased. Treg cells and IL-10 are thought to play key roles in these modulations. Treg cells directly inhibit mast cell degranulation by OX40-OX40L interaction.\textsuperscript{42} IL-10 reduces pro-inflammatory cytokine release from mast cells,\textsuperscript{43} downregulates eosinophil activity, and decreases IL-5 production by Th0 and Th2 cells.\textsuperscript{30}

### Recent findings

The indoleamine 2,3-dioxygenase (IDO) is an intracellular enzyme that catalyzes the initial rate-limiting step
in the degradation of tryptophan.\textsuperscript{44} IDO is widely expressed in human tissues and cells for antimicrobial resistance, and is induced mainly by IFN-\(\gamma\).\textsuperscript{45} As it is induced by pro-inflammatory signals such as IFN-\(\gamma\), IFN-\(\alpha\), TGF-\(\beta\), TNF-\(\alpha\), or lipopolysaccharide,\textsuperscript{46} the original role of IDO is supposed to be preventive of hyperinflammation to pathogen invasion, that would be harmful to hosts.\textsuperscript{47} However, accumulating evidence suggests more immunoregulatory roles of IDO. Munn et al. demonstrated that tolerance to allogeneic fetus is regulated by IDO-expressing cells in the placenta, and the IDO inhibitor treatment (blocking tryptophan catabolism) caused the allograft fetal rejection.\textsuperscript{48} The evidence exists for its regulatory role in allergic inflammation; the crosslinking of Fc\(\varepsilon\)RI on ACPs induced tryptophan degradation pathway and IDO expression and thus resulted in impaired T cell proliferation in asymptomatic atopic individuals, in vitro and \textit{in vivo}.\textsuperscript{49,50} In autoimmune disease models, IDO could induce FOXP3+Tregs from mature peripheral CD4+CD25-T cells, and a positive feedback loop of Treg expansion by IDO has been suggested.\textsuperscript{51} In this regard, for allergen immunotherapy, IDO may play an important role in peripheral tolerance induction. It is reported that increased tryptophan degradation occurs during allergen immunotherapy in humans\textsuperscript{52} and the generation of tryptophan metabolites is a contributing factor to tolerance induction in a mouse model of allergen immunotherapy.\textsuperscript{53}

IL-35 is a recently identified anti-inflammatory cytokine. It is a heterodimeric protein with two subunits of IL-12A and Epstein-Barr virus induced 3 (EBI3) which is a downstream target of FOXP3.\textsuperscript{54,55} In humans, IL-35 is conditionally induced in response to inflammatory stimuli unlike its constitutive expression in mouse.\textsuperscript{56} IL-35 induces IL-10 production in CD4+CD25+Treg cells, and inhibits Th17 differentiation.\textsuperscript{57} It effectively suppressed IL-17-dependent lung inflammation in mouse asthma models.\textsuperscript{58} Taken together, the induction of IL-35 secreting Treg cells could be additional mechanism by which allergen immunotherapy exert its effects, which requires further investigations.

Conclusions

Allergen-specific immunotherapy is effective treatment for allergic diseases, but its mechanism of action involves the complex interactions of Tregs and inflammatory cells/mediators. Studying the mechanisms of peripheral tolerance induction by immunotherapy will provide us better understanding of the pathogenesis of allergic inflammation, and better controls of allergic diseases.

References


47. von Bubnoff D, Bieber T. The indoleamine 2,3-dioxygenase (IDO) pathway controls allergy. Allergy 2012.