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CORRESPONDENCE

Differential DNA methylation in allergen-specific immunotherapy of asthma

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Asthma is a prevalent chronic disease that occurs due to airway inflammation. Exposure to allergic (e.g., house dust mites, pollens, and mold) or nonallergic (e.g., viral infection, tobacco smoke, and cold air) stimuli can trigger asthma. Allergic asthma is a T helper 2 (Th2) cell-mediated chronic inflammatory disease that is associated with genetic predisposition and environmental factors.[1] Th2 cells secrete cytokines that induce immunoglobulin class switching to IgE antibodies, eosinophil activation, mucus hyperproduction, and the secretion of histamines and other mediators by mast cells, thereby contributing to airway hyperresponsiveness and bronchoconstriction.[1] In recent years, epigenome-wide association studies (EWAS) have revealed an interaction between epigenetic signatures and allergic diseases, including pediatric asthma.[1,2]

Epigenetic modulations include DNA methylation, histone modifications, and noncoding RNA modifications. Small noncoding RNAs such as microRNAs (miRNAs) can be involved in the posttranscriptional regulation of gene expression, and the analysis of miRNAs has been used in the diagnosis of allergic diseases.[3] For example, miR-665 and miR-26a are reported to be disease-specific miRNAs related to asthma.[3] Histone modification plays a role in the development of asthma. For example, histone 3 acetylation (a transcriptional activation marker) in the *IL13* locus of $CD4^+$ T cells is higher in children with asthma than in healthy children and corresponds to higher protein levels in serum.[4] Many other studies have also disclosed the roles of histone modification in immune cells, airway smooth muscle cells, lung epithelial cells, and fibroblasts in allergic diseases.[4] According to our knowledge and our previous investigations, we now focus on DNA methylation in allergies and asthma, especially its correlation with T-cell immune responses.

DNA methylation is involved in the differentiation and functions of T cells. The intergenic regions of both the *IL4* and *IFNG* genes are methylated in resting $CD4^+$ T cells. The demethylation of the *IFNG* promoter region and methylation of the *IL4* promoter region are associated with Th1 cell differentiation, whereas demethylation of the promoter region in the *IL4* and *IL13* genes is correlated with the differentiation of Th2 cells. The DNA methylation patterns and gene expression of IL-4 and IFN-γ are different between patients with asthma and healthy controls. In peripheral blood mononuclear cells of children with asthma, hypomethylation of the *IL4* gene is associated with increased gene expression. Foxp3 expression is controlled by the proximal promoter and intronic regulatory elements designated conserved noncoding sequences (CNS1–3). The CNS2 region of the *FOXP3* gene is a Treg cell-specific demethylated region (TSDR) that plays a critical role in the stability of Foxp3 expression and thymic-derived Treg cell differentiation. In contrast to thymic-derived Treg cells, peripheral Treg cells are affected by TGF-β through the binding of SMAD3 to the CNS1 region in the *FOXP3* gene. Many studies have suggested that there is a relationship between CpG methylation of the *FOXP3* gene and allergic diseases. *FOXP3* demethylation and active Treg cells appear to be involved in the protective effects of farm milk against allergies.[1] Children with active IgE-mediated cow's milk allergy (CMA) showed significantly less *FOXP3* TSDR demethylation than those who outgrew CMA or healthy controls, thereby suggesting that *FOXP3* TSDR demethylation could serve as a biomarker for oral tolerance.[2]

Allergen-specific immunotherapy (AIT) represents the only curative treatment in which an allergic patient is incrementally exposed to increasing quantities of a specific antigen, such as pollen, fungi, house dust mite allergens, or food allergens. The mechanisms of tolerogenic responses to allergens induced by AIT include the upregulation of specific blocking of IgG antibody levels, suppression of allergen-specific Th1 and Th2 cells, and induction of T-cell anergy or Treg cells.[5] Subcutaneous (SCIT) or epicutaneous (EPIT) immunotherapy includes administration via the skin, whereas sublingual (SLIT) or oral (OIT) immunotherapy includes administration via the mucosal route. Cochrane systematic reviews and meta-analyses have demonstrated that the frequencies of rescue medication use and clinical symptoms are reduced in patients with allergic asthma and allergic rhinitis after SLIT and SCIT.[6] SLIT had a lower incidence of systemic reactions but a higher incidence of local site reactions than SCIT and is therefore strongly recommended in the treatment of allergic rhinitis.[6] The $CD4^+CD25^+Foxp3^+$ Treg cells induced by tolerogenic dendritic cells (DCs) in the oral mucosa mediate immune tolerance, thus improving the clinical efficacy after SLIT.[7] SLIT has no risk of severe systemic reactions, which is considered an advantage over SCIT, because allergens are mostly captured by tolerogenic DCs before they reach the mast cells during SLIT.[6] It has been suggested that SCIT contributes to the phenotypic deviation of Th2 cell responses to a protective Th1 profile, which enables SCIT to manifest earlier IFN-γ secretion than SLIT.[7] A recent study indicated that *Dermatophagoides pteronyssinus* (Der p)-specific IgG4 levels in asthma treated with SCIT were almost 30 times higher than those in asthma treated with SLIT.[7]

Increasing evidence has revealed that AIT can modify epigenetic mechanisms to induce the tolerance of allergens.[2] Timothy grass and dust mite dual SLIT increases allergen-specific $Foxp3^+$ memory Treg cells with reduced DNA methylation of CpG sites within the *FOXP3* locus.[1] In a mouse model, significant

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hypermethylation of the Gata3 promoter restricted to Th2 cells and hypomethylation of the *Foxp3* promoter in $CD62L^+$ Treg cells were found after the use of EPIT containing peanut proteins.[8] In patients with peanut allergies, treatment with peanut OIT results in the hypomethylation of *FOXP3* in antigen-induced Treg cells and upregulates the suppressive function of Treg cells.[2] However, an animal model demonstrated that peanut OIT only caused the demethylation of *Foxp3* but not the methylation of *Gata3*.[8] In contrast, milk EPIT increased the methylation of the *Gata3* promoter region, and the adoptive transfer of milk EPIT Treg cells could prevent sensitization to peanuts and peanut-induced anaphylaxis.[9] Our previous investigation indicated that after treatment with SCIT containing Der p, children with allergic asthma showed reduced IL-4, IL-5, and IL-2 production by peripheral blood mononuclear cells with increased DNA methylation at the *IL4* promoter, suggesting a role of SCIT in suppressing Th2 cell-mediated allergic asthma.[10] Compared with SLIT or OIT, in both patients and mice with allergies, SCIT did not reduce the DNA methylation level of the *FOXP3* gene. Several EWAS found differentially methylated regions of Th2 cell-associated genes, such as IL-4, in patients with asthma.[1,2] SCIT containing Der p appears to reduce allergen-specific Th2 cell cytokines with increased *IL4* DNA methylation but does not alter *FOXP3* DNA methylation in children with allergic asthma.[10]

In summary, AIT induced similar Treg cell changes with the use of both the skin route and oral route, but there were some differences. Different routes encounter different antigen-presenting cells, and allergens are captured by tolerogenic DCs in SLIT or OIT. AIT via the oral route may induce Treg cells by hypomethylation of *FOXP3*, and AIT via the skin route may change T helper cells by hypermethylation of the Th2 cytokine gene. Nevertheless, there is an increased need for more research to investigate epigenetic modifications during AIT.

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ADDITIONAL INFORMATION

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REFERENCES

1. Potaczek, D. P. et al. Epigenetics and allergy: from basic mechanisms to clinical applications. *Epigenomics* **9**, 539–571 (2017).
2. Kabesch, M. & Tost, J. Recent findings in the genetics and epigenetics of asthma and allergy. *Semin. Immunopathol.* **42**, 43–60 (2020).
3. Pattarayan, D., Thimmulappa, R. K., Ravikumar, V. & Rajasekaran, S. Diagnostic potential of extracellular microRNA in respiratory diseases. *Clin. Rev. Allergy Immunol.* **54**, 480–492 (2018).
4. Alaskhar Alhamwe, B. et al. Histone modifications and their role in epigenetics of atopy and allergic diseases. *Allergy Asthma Clin. Immunol.* **14**, 39 (2018).
5. Wambre, E. Effect of allergen-specific immunotherapy on CD4+ T cells. *Curr. Opin. Allergy Clin. Immunol.* **15**, 581–587 (2015).
6. James, C. & Bernstein, D. I. Allergen immunotherapy: an updated review of safety. *Curr. Opin. Allergy Clin. Immunol.* **17**, 55–59 (2017).
7. Xian, M. et al. Changes in CD4+CD25+FoxP3+ regulatory T cells and serum cytokines in sublingual and subcutaneous immunotherapy in allergic rhinitis with or without asthma. *Int. Arch. Allergy Immunol.* **181**, 71–80 (2020).
8. Mondoulet, L. et al. Gata3 hypermethylation and Foxp3 hypomethylation are associated with sustained protection and bystander effect following epicutaneous immunotherapy in peanut-sensitized mice. *Allergy* **74**, 152–164 (2019).
9. Mondoulet, L. et al. Specific epicutaneous immunotherapy prevents sensitization to new allergens in a murine model. *J. Allergy Clin. Immunol.* **135**, 1546–1557 e1544 (2015).
10. Wang, C. M., Chang, C. B., Chan, M. W., Wen, Z. H. & Wu, S. F. Dust mite allergen-specific immunotherapy increases IL4 DNA methylation and induces Der p-specific T cell tolerance in children with allergic asthma. *Cell Mol. Immunol.* **15**, 963–972 (2018).