—Review—

Dual Factors May Be Necessary for Development of Atopic March in Early Infancy

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The incidence of atopic diseases, including atopic dermatitis (AD), food allergies, allergic rhinitis, and asthma, has increased in recent decades, and currently affects approximately 20% of the population. Atopic march is the development of AD in infancy and subsequent food allergies, allergic rhinitis, and asthma in later childhood. Patients with infantile eczema may develop typical symptoms of AD, allergic rhinitis, and asthma at certain ages. Some patients' symptoms persist for several years, whereas others may have resolution with aging. Development of these diseases is strongly influenced by the following two factors: skin dysfunction caused by filaggrin mutations and development of colonization of microflora in early infancy. Filaggrin mutations predisposing to asthma, allergic rhinitis, and allergic sensitization, only in the presence of AD, strongly support the role of filaggrin in the pathogenesis of AD and in subsequent progression of the atopic march. Several studies have shown that development of colonization of microflora in early infancy might affect development of allergic disease or food desensitization. Therefore, massive allergen exposure to genetic skin dysfunction in early infancy and an imbalance of microflora might be necessary for development of atopic march.

(J Nippon Med Sch 2018; 85: 2–10)

Key words: atopic march, skin dysfunction, filaggrin mutation, microflora, infantile eczema

Introduction

The incidence of atopic diseases, including atopic dermatitis (AD), food allergies, allergic rhinitis, and asthma, has increased in recent decades. Currently, atopic diseases affect approximately 20% of the population. Allergic march is defined as development of AD in infancy and subsequent food allergies, allergic rhinitis, and asthma in later childhood[1]. Patients with infantile eczema may develop typical symptoms of AD, allergic rhinitis, and asthma at certain ages. Some patients' symptoms persist for several years, whereas others may experience resolution with aging[2]. Development of these diseases is strongly influenced by several factors, including genetic and environmental factors[3,4]. This review will address the cause of development of allergic march from the perspective of two factors: skin dysfunction and microflora in early infancy. We focused on these two factors because they strongly affect both a decreased Th1/Th2 pattern and decreased regulatory T cells in early infancy.

Development of Allergic March and Skin Dysfuntion

Filaggrin Mutations and Atopic March

Epidermal differentiation complex is caused by independent loss-of-function genetic variants (R510X and 2282del4) in the gene encoding filaggrin, which is a key structural protein in the outermost layer of the epidermis in up to 50% of patients with AD[5]. Recent genetic studies have shown a major role of filaggrin in the pathogenesis of AD and in subsequent progression of atopic march[6]. Filaggrin mutations are currently considered as a major risk factor for AD, particularly in patients who have onset of AD at 2 years or younger[7]. A recent study showed a significant association of two filaggrin gene mutations with asthma and allergic rhinitis[8]. However, this associa-

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tion was only found in subjects with co-existing AD and the association was not obvious without co-existing AD[8]. Additionally, the human bronchial epithelium does not express filaggrin protein[9,10]. Therefore, filaggrin mutations do not appear to exert effects in the upper airway. The association of filaggrin mutations with other atopic disorders appears to be due to the common feature of allergen sensitization through the skin. The fact that asthma is only found in a population of filaggrin mutation carriers with AD supports the hypothesis that asthma follows allergic sensitization that occurs after impairment of the epidermal skin barrier. Filaggrin mutations are likely to play a role in chronicity of the disease and immunoglobulin E (IgE) sensitization in patients with AD. Recent studies have shown that patients with early-onset AD and filaggrin mutations are likely to have persistent allergic disease into adulthood[11]. Patients with AD and filaggrin mutations are significantly associated with the extrinsic form of the disease (IgE-mediated sensitization to inhalants or food allergens) and development of allergic rhinitis and asthma[12–14].

The finding of filaggrin mutations predisposing to asthma, allergic rhinitis, and allergic sensitization only in the presence of AD strongly supports the role of filaggrin in the pathogenesis of AD and subsequent progression of atopic march. Expression of the filaggrin gene is downregulated in AD skin by Th2 cytokines (interleukin [IL]-4 and IL-13)[15] and in normal human keratinocytes by sphingosylphosphorylcholine, a proinflammatory cytokine in AD[16,17]. These findings indicate that filaggrin defects can develop as an acquired and/or genetic defect. However, almost 40% of carriers of filaggrin mutations do not have AD[18]. Additionally, patients with the inherited skin disorder ichthyosis vulgaris, who have filaggrin mutations, do not have AD-like skin inflammation or infection[19]. Therefore, additional factors may affect the pathogenesis of AD.

Infantile Eczema and Its Association with Development of Atopic March

Ekbäck et al[20] showed that infantile eczema with high scoring atopic dermatitis (SCORAD) points (scoring of AD severity) was associated with an increased risk of asthma at 10 years of age. Children with eczema and wheezing episodes during infancy are more likely to develop asthma than infants with eczema alone. Eczema in infancy, combined with an early onset of allergic rhinoconjunctivitis, appears to indicate a more severe allergic disease, which often leads to progression of asthma. Progression from eczema in infancy, to allergic rhinoconjunctivitis at an early age and asthma later in childhood, supports the relevance of the term "atopic march", at least in more severe allergic disease. The first unselected birth cohort study showed that eczema in the first year of life was associated with asthma at 6 years old in girls and boys[21]. Since this study report, multiple birth cohort studies have reported the march from eczema to other allergic diseases. The Tucson Children's Respiratory Study of an unselected birth cohort showed that eczema at the age of 1.5 years was associated with persistent wheezing at 6 years (odds ratio [OR] 2.4; 95% confidence interval [CI] 1.3–4.6)[22]. Similar to this previous study, a whole population-based birth cohort study from the Isle of Wight reported that eczema at 4 years increased the risk of asthma at 10 years (OR 2.15; 95% CI 1.24–3.73)[23]. While these studies provide evidence supporting the concept of atopic march, the German Multi-Centre Allergy Study (MAS)[24], published almost a decade after the Tucson Children's Respiratory Study, provided conflicting results.

The MAS followed a birth cohort, which comprised children with a family history of allergies. The MAS showed that eczema in the first 2 years of life was related to an increased risk of current wheeze at 7 years (OR 1.93; 95% CI 1.22–3.06). However, Illi et al[24] found no evidence for an association between eczema and asthma at 7 years old once they adjusted for early wheeze and early-life sensitization (OR 1.46; 95% CI 0.73–2.90). The authors argued that eczema was not an independent risk factor for subsequent asthma, and attributed the association to confounding by early wheeze and sensitization that co-manifested with early-life eczema.

A study of children with a family history of allergies, the Melbourne Atopic Cohort Study (MACS)[25], showed a similar pattern when analyzed in the same manner as the MAS[24]. In the MACS, eczema in the first 2 years of life was associated with an increased risk of asthma at 6–7 years old (OR 2.02; 95% CI 1.33–3.07), but after adjustment for early-life wheeze and sensitization, this association was not significant (OR 1.45; 95% CI 0.92–2.31). However, further analysis showed that early-life eczema remained associated with an increased risk of childhood asthma, when adjusted only for early-life wheeze (OR 1.83; 95% CI 1.17–2.85). This finding suggested that the major cause of confounding was sensitization. This study also showed that, independent of wheeze and sensitization, AD within the first 2 years of life was associated with an increased risk of childhood asthma in boys (OR 2.45 95% CI, 1.31–4.46), but not girls.

Whether sensitization should be considered a confounder between early-life eczema and the risk of other forms of allergic disease remains controversial. Eczema may precede and increase the risk of subsequent sensitization, which in turn increases the risk of asthma, as discussed above. Therefore, sensitization that follows eczema is likely to be a step in the biological pathway between eczema and asthma, but not a confounder. Adjusting for early-life sensitization without considering the temporal sequence of sensitization and eczema will underestimate the effect of eczema on asthma. The interest raised by the "manifestation" argument by the MAS prompted a systematic review and meta-analysis of the evidence on atopic march[26]. This review included four population-based cohort studies[23,27–30] that achieved an 80% follow-up. This review showed that young children with AD have an elevated risk of developing asthma (pooled OR 2.14; 95% CI 1.76–2.75).

Epidermal barrier dysfunction and the keratinocyte-derived cytokine thymic stromal lymphopoietin may also play critical roles in the onset of atopic march followed by development of asthma[30].

Prevention of Atopic March

Primary prevention of allergic disease has been studied for many years. However, studies of avoidance of food allergens, aeroallergens, or both, have generally produced disappointing results[31]. Horimukai et al first reported that daily application of moisturizer to neonates at high risk for AD may prevent development of AD in a cohort randomized, controlled study[32]. They also reported that the presence of skin lesions (including AD) is a risk factor for allergic sensitization. Development of atopic march may explain dysfunction of the skin barrier in early infancy by genetic and environmental backgrounds. Preventing allergic sensitization and development of AD by skin care and steroid ointment may be possible in early infancy.

Cutaneous Microbiota and Skin Dysfunction

A recent study showed that the skin microbiota plays a crucial role in skin function and also a loss in diversity of the microbiota, resulting in chronic inflammation on and in the skin[33]. Water is crucial to growth of the microbiota on the skin and for maintaining diversity of the skin microbiota. Bacteria on the skin are from four main bacterial phyla: actinobacteria, firmicutes, proteobacteria, and bacteroidetes. The three most common genera are corynebacteria, propionibacteria, and staphylococci[34].

Water activity varies from 0 (no free water available) to 1.0 (all molecules of water are free)[35]. Water activity strongly affects growth of microorganisms and differs considerably between the main environments of human skin[36]. *Staphylococcus aureus* is able to grow until the aw is 0.83, *Staphylococcus epidermidis* is less resistant (unable to grow below an aw of 0.87), and *Pseudomonas fluorescens* is unable to grow below an aw of 0.97. Therefore, dry skin favors growth of potentially invasive staphylococci and inhibits the growth of commensal organisms. Consequently, moisturizers play two important roles in barrier function of the skin: 1) preservation of the physical barrier; and 2) maintenance of the normal composition of the skin microbiota. The composition of bacterial communities depends on skin characteristics, such as sebaceous gland concentrations, moisture content, and temperature, as well as on host genetics and exogenous environmental factors[37]. An example of this situation is that defects in the skin structural barrier permit penetration of the epidermis by chemical, allergic, and/or infectious agents. This may result in chronic inflammation and a loss of microbial diversity with an associated increase in staphylococci, including *S. aureus*[38].

Therefore, in early infancy, a loss of skin microbiota caused by a loss of water with genetic and environmental skin dysfunction may easily induce allergenic inflammation. This could be followed by sensitization, resulting in development of atopic march.

Microflora and Atopic March

Development of Microflora

Development of a method of measuring 16S RNA in bacteria led to the finding that microflora play a crucial role in maintaining a healthy condition and preventing onset of disease. At birth, neonates acquire bacteria, especially from bifidobacterial strains in the vagina and fecal stool that passes through the birth canal[39]. At the same time, microorganisms first invade the intestines. Twenty-four hours after birth, these microorganisms spread out in the large intestines. Within 1 week, gut microbiota can be colonized among which anaerobic bacteria are predominant. These colonized microorganisms can affect immunological factors in the host defense. According to Tsuji et al[40], bifidobacteria predominantly colonize by 3 months of age in infants with breast-feeding and gradually increase at 6 months of age. The percentage of these bacteria is greater than 90%. With introduction of baby food, the numbers of *Escherichia coli*, streptococci, clostridium, bacteroides, and anaerobic bacteria have increased. There is no difference in the number of these bacteria between breast feeding and artificial feeding.

Surprisingly, a large cohort study showed shared features of functional maturation of the gut microbiome during the first 3 years of life in healthy children and adults from the Amazonas of Venezuela, rural Malawi, and US metropolitan areas[41].

Change in Microflora during Growth According to Environmental Factors

Immediately after virginal birth, the newborn intestine is colonized by microflora, which are from vaginal flora and microflora in the mother[39]. In healthy infants, species of lactobacillus or bifidobacterium are predominantly found. In breast-fed infants, the number of bifidobacteria is 10 times greater than that in formula-fed infants at 1 month of age[42]. Renz-Polster et al reported that cesarean section may be associated with an increased risk of developing allergic rhinitis (AR), but not AD, bronchial asthma (BA), and food allergies (FA), in childhood[43]. Penders et al[44] demonstrated that delivery by cesarean section strongly affects the microbiota of neonates, especially by decreasing colonization rates of bacteroides and increasing the prevalence of clostridia. This effect was still notable at the age of 7 months postpartum. Another recent prospective study on the effect of birth mode on development of the microbiota in early life showed delayed bacteroides colonization[45]. This study also showed reduced microbial diversity and reduced Th1 responses in children born by cesarean section. Penders et al[46] also found that the colonization patterns of firstborns, similar to colonization patterns of neonates born by cesarean section, were characterized by a higher colonization rate of *Clostridium difficile* and other clostridia. They also found lower rates of lactobacilli, bifidobacteria, and *E. coli*. Additionally, they found that the effects of birth mode and birth order (having an older sibling) appeared to be independent. Briefly, there was a low relative abundance (partly explained by low colonization rates) of bacteroides in neonates born by cesarean section, which largely disappeared by the age of 7 months in children with siblings; however, this was only partly true for children without siblings[45]. Therefore, several environmental factors affect development of colonization of microflora in neonates after growth.

Allergic Disease May Be Affected by Changes in Microflora during Growth

An Asian at-risk cohort study reported that longitudinal analysis of fecal microbiota composition at 3 days, 1 month, 3 months, and 1 year of life showed a higher abundance of enterobacteriaceae (coefficient [B]: 1.081, 95% CI: 0.229−1.933, adj p=0.014) and *Clostridium perfringens* [coefficient [B]: 0.521, 95% CI: 0.556−0.988, adj p= 0.03) in those who developed eczema in the first year of life. This study also showed that a lower abundance of bifidobacterium was observed in those with eczema at 5 years old (coefficient [B]: −27.635, 95% CI: −50.040 to −5.231, adj p=0.018)[47].

Azad et al also reported that food sensitization was affected by infant gut microflola[48]. They concluded that low gut microbiota richness and an elevated enterobacteriaceae/bacteroidaceae ratio in early infancy are associated with subsequent food sensitization. This suggests that early gut colonization contributes to development of atopic disease, including food allergies.

In a small study, Björkstén et al[49] reported that neonates who developed allergies were less often colonized with enterococci during the first month of life and with bifidobacteria during the first year of life compared with healthy neonates. They suggested that differences in the composition of gut flora between infants who will develop and those who will not develop allergies are present before development of any clinical manifestations of atopic dermatitis. Therefore, colonization of microflora in early infancy may affect development of allergic disease or food desensitization.

Effect of Development of a Th1/Th2 Pattern in Infancy on Atopic March

Atopic disease, including AD, BA, FA and AR, may be caused by production of antigen-specific immunoglobulin production (IgE) associated with failure of T cell immunotolerance. In fetuses and neonates, the immunological status shows a Th2 pattern rather than Th1. Subsequently, the normal immunoresponse moves to the Th1 pattern. If the pattern of Th2 does not move to that of Th1, atopic march associated with production of IgE antibody begins in early infancy[50]. IL-12, which changes naive T cells to Th1 cells, is produced by antigen-presenting cells stimulated by lipopolysaccharide through Toll-like receptor 4. In childhood, the capacity of production of IL-12 is lower than that in adults[50]. Therefore, this phenomenon may be one of the causative factors for easily shifting from the pattern of Th1 to that of Th2.

Oral Immunotolerance and Microflora

Oral immunotolerance is one of the immunoresponses characterized by intestinal immune phenomena. After the intake of allergen, the same allergen was administrated parentally, and this response was markedly suppressed compared with no intake of allergen. In the germfree mouse, inducing immunotolerance is difficult[51]. One of the representative microflora, *Bifidobacterium infantis*, in-

duces immunotolerance for colonization in germfree mouse intestine[51]. Hori S et al[52] showed that natural regulatory T (Treg) cells play a crucial role in immunological self-tolerance and homeostasis in humans. Furusawa et al[53] found that in a mouse model, clostridium species increased the number and percentage of Treg cells that were mediated by transforming growth factor-β. After colonization of this species in early life, the production of IgE was subsequently suppressed. They also reported that short chain fatty acids produced by these species can cause differentiation of Treg from naive T cells, which is mediated through histone acetylation of FOX P genes. Therefore, some microflora may be significantly related to induction of oral immunotolerance.

Development of Treg Subsets and Dysregulation of Treg Populations Are Associated with Development of Allergic Disorders during Growth

Treg cells play a crucial role in development of allergic disease and the onset of atopic march. However, a few studies[54] on atopic disease in children reported that Treg cells display substantial deficiencies in atopic children, especially in children with multiorgan involvement, compared with patients with single organ manifestations. There is also an association between Treg cells and serum IgE serum concentrations. Better identification and characterization of Treg cells in allergies are required because they limit responses to foreign antigens, thereby minimizing T cell-mediated immunopathology in allergic diseases[54].

Another study reported development of Treg cell subsets during the first postnatal year. In healthy children, in the first year of age, Treg cells gradually increase from 6 months to 1 year old, and do not change in the adult[55].

Effect of Probiotics on Allergic Disease

Development of atopic disease is observed in early infancy. In atopic march, administration of probiotics may be expected to be useful. Administration of probiotics in early life may be an effective method for preventing AD, but little is known about its long-time preventive effect. A meta-analysis was conducted to evaluate the long-term effect of early-life supplementation with probiotics on preventing AD[56]. Six trials and a total of 1,955 patients were included in this meta-analysis. The combined risk ratio of the meta-analysis that compared probiotics with placebo to investigate the long-term preventive effect of AD was 0.86 (95% CI 0.77−0.96). This finding demonstrates that probiotics are likely to cause long-term prevention of AD. Another study suggested that probiotics have shown more promise, albeit limited, in the primary prevention of allergic disease than in the treatment of established disease[57].

Effect of Probiotics for Immunotherapy

Most children who develop food allergies in infancy outgrow these food allergies before school age. Approximately 50% of children can tolerate allergic food by 5 years old, increasing to 75% by their early teenage years[58]. Nevertheless, some children experience persistent allergic reactions[59,60]. Oral immunotherapy (OIT) is regularly used for young children with food allergies and has been shown to be effective[61–70]. However, adverse effects frequently occur during OIT (especially during the escalation phase), and use of parenteral epinephrine is frequent.

Therefore, several trials on hypoallergic food, changes in administration routes, and combination of administration of anti-IgE monoclonal antibodies or probiotics and prebiotics, have been performed to decrease adverse events during OIT.

Tang[71] first reported a randomized, placebo-controlled trial that evaluated novel co-administration of a probiotic using *Lactobacillus rhamnosus* CGMCC 1.3724 and peanut OIT, and assessed sustained unresponsiveness in children with a peanut allergy. This trial showed that possible sustained unresponsiveness was achieved in 82.1% of children who received probiotics and peanut oral immunotherapy (PPOIT) and in 3.6% who received placebo ($p < 0.001$). Additionally, among the subjects, 89.7% who received PPOIT and 7.1% who received placebo were desensitized ($p<0.001$). They concluded that PPOIT was effective in inducing possible sustained unresponsiveness and immune changes that suggested modulation of the peanut-specific immune response. Shi et al[72] reported that specific immunotherapy (SIT) in combination with *Clostridium butyricum* inhibited allergic inflammation in the mouse intestine. Briefly, in their study, an ovalbumin (OVA)-specific allergic inflammation mouse model was created. The mice were treated with SIT or/and *C. butyricum*. They showed that intestinal allergic inflammation was only moderately alleviated by SIT, which was significantly enhanced by a combination with *C. butyricum*. Treatment with *C. butyricum* alone did not show much inhibitory efficacy. An increase in frequency of IL-10-producing OVA-specific B cells was observed in mice in parallel to the inhibitory effect on the intestinal allergic inflammation. They concluded that administration with *C. butyricum* enhances the inhibitory effect of SIT on allergic inflammation in the mouse intestine.

Development of Atopic March and Immunoregulation by Microflora

Several factors related to the development for atopic march were discussed above. We highlighted two factors, including skin dysfunction by genetic and environmental factors and imbalance of gut microflora. In the fetus and neonate, the immunological pattern shows a decreased Th1/Th2 because of prevention of graft-versus-host-disease (GVHD). At birth, abundant bacteria, especially bifidobacterium species from the vagina and the mother's intestine, are colonized in the neonatal intestine in aseptic conditions. Björkstén et al[49] showed that the frequency of bifidobacteria in children who developed eczema at 1 year old was lower compared with that in those who did not develop eczema. The frequency of bifidobacteria in infancy with artificial milk or in delivery by cesarean section is low and these infants may have a high risk for allergic disease[44]. Supplementation of bifidobacterium can change naive T to Th1 lymphocytes in early infants[73] and may possibly potentiate to Treg lymphocytes. Several studies[56,57] have reported that mixed probiotics, including lactic acid bacteria and bifidobacterium species, can prevent and cure allergic disease, but the immunological mechanism still remains unclear. Lactic acid bacteria might produce lactic acid, resulting in a decreased pH in the small intestine. These changes alter environmental effects in the large intestine, and this induces an increase in bifidobacteria. An animal model and *in vitro* study[53] showed that clostridium species induce naive T cells to Treg cells by production of butyric acid and this process may require the 17 species of clostridium bacteria. The role of clostridium species in the human intestine still remains unknown. However, we speculate that increased bifidobacteria in early infancy affects the quantity and quality of clostridium species. This could result in an increased Th1/Th2 pattern and increase in the number and function of Treg cells. In early infancy, immunological changes in the quality and quantity of CD4 lymphocytes are observed[74]. Increased bifidobacteria associated with some other enterobacteria might compensate for a decreased Th1/Th2 pattern in the neonatal period. If these bacterial numbers are low, the Th1/Th2 pattern persists in early infancy to adults. This is because the composition of microflora in the intestines may be not be changeable after 3 years old[41]. Skin barrier dysfunction in early infancy, representing high transepidermal water loss, persists until 1year old[75]. Skin dysfunction might gradually improve before school age, similar to adult skin function[75]. Therefore, in early infancy, massive allergen exposure to the skin induces sensitization of allergens and also produces IL-4, followed by allergen-mediated IgE production. If a normal balance of microflora is present in the gut in infancy, it improves this Th2-dominant pattern in the fetus[76]. Th1 and Th2 lymphocytes are thought to be differentiated from naive T cells. Therefore, as early as possible, the Th1/Th2 pattern may easily change by a change in microflora. Several studies on administration of probiotics have been performed to prevent the onset of allergic disease. The effect of probiotics was observed when they were administrated in early infancy and late pregnacy[56,57]. The effect of probiotics may be explained by the fact that naive T cells may more easily change to Th1 instead of Th2. After complete colonization of microflora, maybe by 3 years old, there might be no effect of immunological change, with no changeable composition of microflora.

A recent study showed that fecal transplantation was curable in *C. difficile* infection-related diarrhea[77]. Although there are no data for using fecal transplantation in fetuses, this transplantation may be useful for curing allergic disease, such as food allergies, grass pollens, and allergic asthma.

Conclusion

Massive allergen exposure to skin genetic dysfunction in early infancy and an imbalance in microflora are necessary for development of atopic march. Therefore, application of skin moisturizer and use of steroid ointment, as well as administration of probiotics during pregnancy and early infancy, may be useful for preventing development of this march. However, further studies are required to evaluate the effects of these treatments.

Acknowledgments: We thank Ellen Knapp, PhD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

Conflict of Interest: The authors declare no conflict of interest to declare.

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(Received, July 27, 2017)
(Accepted, October 2, 2017)