



## Invited Review Article

# Recent advancement to prevent the development of allergy and allergic diseases and therapeutic strategy in the perspective of barrier dysfunction

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AD, Atopic Dermatitis; BEAT, Beating Egg Allergy Trial; CI, confidence interval; EAACI, European Academy of Allergy and Clinical Immunology; EAT, Enquiring about Tolerance; HEAP, Hen's Egg Allergy Prevention; JSPACI, Japanese Society of Pediatric Allergy and Clinical Immunology; LEAP, Learning Early about Peanut Allergy; PETIT, Prevention of Egg allergy with Tiny amount Intake; RCT, randomized clinical trial; STAR, Solids Timing for Allergy Reduction; STEP, Starting Time of Egg Protein

## ABSTRACT

Therapeutic strategy in late 20th century to prevent allergic diseases was derived from a conceptual framework of allergens elimination which was as same as that of coping with them after their onset. Manifold trials were implemented; however, most of them failed to verify the effectiveness of their preventive measures. Recent advancement of epidemiological studies and cutaneous biology revealed epidermal barrier dysfunction plays a major role of allergen sensitization and development of atopic dermatitis which ignites the inception of allergy march. For this decade, therapeutic strategy to prevent the development of food allergy has been confronted with a paradigm shift from avoidance and delayed introduction of allergenic foods based on the theoretical concept to early introduction of them based on the clinical and epidemiological evidences. Especially, prevention of peanut allergy and egg allergy has been established with the highest evidence verified by randomized controlled trials, although application in clinical practice should be done with attention. This paradigm shift concerning food allergy was also due to the discovery of cutaneous sensitization risk of food allergens for an infant with eczema revealed by prospective studies. Here we have recognized the increased importance of prevention of eczema/atopic dermatitis in infancy. Two randomized controlled trials using emollients showed successful results in prevention of atopic dermatitis in infancy; however, longer term safety and prognosis including allergy march should be pursued. To establish more fundamental strategy for prevention of the development of allergy, further studies clarifying the mechanisms of interaction between barrier dysfunction and microbial milieu are needed with macroscope to understand the relationship between allergic diseases and a diversity of environmental influences.

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## Conceptual foundations for prevention of allergy and allergic diseases in dawning age

Hay fever was described as an unusual disease in the early 19th and had become commonplace by the end of same century in Europe and North America where industrial revolution was experienced.<sup>1</sup> In the fourth quarter of 20th century, hay fever prevailed as post industrial revolution epidemic in western countries,<sup>1,2</sup> and

cedar pollen allergy did in Japan along with asthma and atopic dermatitis thereafter.

Discovery of IgE by Profs. Ishizaka in 1966<sup>3,4</sup> was timely and an epoch-making milestone to contribute elucidating the mechanisms of allergic diseases.<sup>5</sup> Commercially available allergen specific IgE measurement kits and allergens liquid for skin test were developed after the discovery and have been applied for patients to diagnose their sensitized allergens.

Therapeutic strategy to cope with allergic diseases in the early stage of the first allergy epidemic was generated from the conceptual theory of avoidance of sensitized allergens and symptomatic therapy. Preventive strategy to allergic diseases also stood on the same conceptual framework based on the allergens avoidance.

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### *Preventive strategy to allergy and allergic diseases based on allergens elimination*

Concerning to therapeutic strategy after the onset of allergic diseases, elimination of sensitized allergens has been a fundamental approach and its effectiveness was proven not only in mono sensitized allergy but also poly-sensitized allergy such as asthma. An RCT enrolled 937 children with atopic asthma (age, 5–11 years) in seven major U.S. cities lasted one year of intervention including education and remediation for exposure to both allergens and environmental tobacco smoke, resulting in fewer days with symptoms of the intervention group than those of the control group both during the intervention year (3.39 vs. 4.20 days,  $P < 0.001$ ) and the year afterward (2.62 vs. 3.21 days,  $P < 0.001$ ), as well as greater declines in the levels of allergens at home such as HDM and cockroach.<sup>6</sup>

As to preventive strategy to allergen sensitization, asthma and the other allergic diseases, enormous effort has been done by many clinicians and researchers using measures based on allergen avoidance. A successful randomized controlled trial (RCT) to prevent allergic diseases by allergen avoidance measures was published in the *Lancet* in 1992. The Results seemed to be promising showing that one or more allergic diseases except food allergy had developed in 40% control children and 14% intervention (allergen avoidance) children by 12 months of age,<sup>7</sup> although significance between both groups was disappeared by 24 months of age.<sup>8</sup> A larger scale multinational RCT, The Study of Prevention of Allergy in Children in Europe (SPACE), was carried out by research teams including the first author of the *Lancet* to evaluate the effect of simple house dust mite (HDM) avoidance measures.<sup>9</sup> Although analysis of the 12 months follow-up of the newborn cohort showed a trend for a reduced prevalence of sensitization to HDM in the intervention group compared with the control group, HDM avoidance did not show a protective effect on the development of sensitization to HDM or symptomatic allergy in children at age 24 months.<sup>10</sup> Another environmental allergens elimination trial of which intervention started from prenatal period was reported from UK in 2004. Their stringent environmental control comprising allergen-impermeable covers for maternal and child's bed, a high-filtration cleaner and vinyl flooring in child's room was associated with increased risk of mite sensitization but better results for some measurement of lung function in high-risk children at the age of 3 years.<sup>11</sup>

Strong association between infants with eczema (atopic dermatitis) and food allergen sensitization has been observed and the latter had been thought to be a cause of eczema by some researches of pediatric allergy. Based on this hypothesis, maternal antigen avoidance trials during pregnancy and lactation were carried out and a systematic review verified those food allergen avoidance measures were useless not only to prevent offspring's atopic dermatitis but also to prevent food allergen sensitization.<sup>12</sup>

Although allergens avoidance measures may suppress some extents of asthma symptoms, prevention of development of allergen sensitization and allergic diseases could not be achieved in most of RCTs.

### **Paradigm shift from allergen avoidance to tolerance induction by intake**

#### *Findings from observational studies and guidelines*

The strategy for infants to avoid allergenic foods for preventing allergic diseases was a mainstream in the 20th century. At this time, the cause of sensitization to foods was thought to be an immature intestine in infants who intake allergenic foods. Therefore, the American Academy of Pediatrics issued a guideline that

recommended avoiding solid foods until 6 months of age. This guideline also recommended that dairy products be delayed until 1 year, eggs until 2 years, and peanuts, nuts, and fish until 3 years of age in high-risk infants.<sup>13</sup> However, conclusive studies were not yet available to permit definitive recommendations.

A meta-analysis then showed that maternal dietary antigen avoidance during pregnancy or lactation did not prevent allergic diseases of children.<sup>12</sup> With regard to introduction of foods in children, a cohort study showed that an increased diversity of food within the first year of life might have a protective effect on food allergy.<sup>14</sup> Some observational studies have reported allergenic foods, such as hen's egg and cow's milk. A population-based cross sectional study ( $n = 2589$ ) recruited infants at 11–15 months old reported a higher risk of developing hen's egg allergy with introduction of hen's egg after 12 months old (odds ratio: 3.4, 95% confidence interval [CI]: 1.8–6.5) compared with introduction at 4–6 months old.<sup>15</sup> With regard to cow's milk allergy, a birth cohort study ( $n = 13,019$ ), which recruited the general population, showed that the odds ratio was 19.3 (95% CI: 6.0–62.1) for developing cow's milk allergy in infants with exposure to cow's milk protein at 15 days old or older compared with those with earlier exposure.<sup>16</sup> With regard to fish, regular fish consumption before 1 year old is associated with a reduced risk of onset of asthma, atopic dermatitis, and allergic rhinitis during the first 4 years of life compared with avoidance (adjusted odds ratio, 0.76; 95% CI: 0.61–0.94).<sup>17</sup>

After 2008, the guidelines for food allergies world widely<sup>18–21</sup> stated their recommendation not to delay introducing any foods based on these observational studies. However, the guideline for primary prevention of food allergy from the European Academy of Allergy and Clinical Immunology (EAACI)<sup>22</sup> described in 2014 that there was insufficient evidence to recommend either withholding or encouraging exposure to potentially allergenic foods, such as hen's egg, cow's milk, and peanuts during infancy.

#### *Trials to prevent peanut allergy*

The first RCT to prevent peanut allergy named the Learning Early about Peanut Allergy (LEAP) study was published in 2015.<sup>23</sup> This study randomly assigned infants from 4 to 11 months old with atopic dermatitis or egg allergy into the avoidance group or consumption group. In the consumption group, infants took 2 g of peanut protein three times a week. The prevalence of peanut allergy at 5 years old was significantly higher (13.7%) in the avoidance group than in the consumption group (1.9%,  $p < 0.001$ ). The EAT study<sup>24</sup> recruited 1303 infants of 3 months old from general population and examined whether early introduction of 6 weaning foods including peanut could have superior effects to prevent food allergy at 1 and 3 years of age compared to the standard introduction according to the WHO statement which recommends exclusive breast feeding without starting weaning foods until 6 months of age. Although the result of intention-to-treat analysis did not show a significant difference between the two groups, the prevalence of peanut allergy was 2.5% (13/525) in the standard introduction group and 0% (0/310) in the early-introduction group in per-protocol analysis ( $p = 0.003$ ). A meta-analysis combined these two RCTs showed a preventive effect of early introduction of peanut in infancy.<sup>25</sup> The National Institute of Allergy and Infectious Diseases recommended early introduction of peanuts in an addendum guideline<sup>26</sup> based on these RCTs. A total of 13% (6/47) of the participants with 1–4 mm of skin prick test in the LEAP study had an immediate allergic reaction at first consumption. Therefore, this addendum guideline divided infants into three groups for being introduced peanuts. First, infants without eczema or any food allergies were recommended to be introduced peanuts at an appropriate age and in accordance with cultural practices. Second, infants

with mild-to-moderate eczema were recommended to be introduced peanuts at approximately 6 months old. Third, infants with severe eczema or egg allergy were recommended to be evaluated by serum-specific-IgE measurement or the skin prick test, followed by introduction of peanuts at 4–6 months old.

### Trials to prevent egg allergy

The first RCT that investigated the preventive effect of early introduction of hen's egg on egg allergy was the Solids Timing for Allergy Reduction (STAR) study<sup>27</sup> in 2013 and the second was EAT study<sup>24</sup> in 2016. Additionally, the other RCTs, including the Starting Time of Egg Protein (STEP) study,<sup>28</sup> the Beating Egg Allergy Trial (BEAT) study,<sup>29</sup> the Hen's Egg Allergy Prevention (HEAP) study,<sup>30</sup> and the Prevention of Egg allergy with Tiny Amount Intake (PETIT) study,<sup>31</sup> were published in 2017. A meta-analysis of these studies,<sup>25</sup> except the STEP study, reported that early introduction of hen's egg at 4–6 months was associated with reduced incidence of egg allergy (risk ratio: 0.56, 95% CI: 0.36–0.87). We calculated the risk ratio of the above-mentioned 6 study, including the STEP study, by using Review Manager version 5.3 (Fig. 1). The risk ratio of egg introduction at 4–6 months was 0.59 (95% CI: 0.45–0.77) compared with later egg introduction. An overview of these studies is shown in Table 1.

The EAT study,<sup>24</sup> STEP study,<sup>28</sup> BEAT study,<sup>29</sup> and HEAP study<sup>30</sup> did not show significant preventative effects on the onset of egg allergy by early introduction of egg. One of the reasons for their failure to show the significance might have been attributable to the smaller sample size caused by the inclusion criteria which included infants without eczema. The risk ratio, which we calculated, for developing hen's egg allergy in these four studies was 0.68 (95% CI: 0.49–0.94, Fig. 2). Birth cohort studies showed that eczema in infants was a risk factor of developing food allergy<sup>32–34</sup> and the severity of eczema was related to the strength of allergens sensitization.<sup>35</sup> The STAR study<sup>27</sup> and the PETIT study<sup>31</sup> recruited infants with eczema. The STAR study<sup>27</sup> paused recruitment during the study because 31% (15/49) of participants experienced allergic reactions to the study powder, which was pasteurized raw whole egg powder. Therefore, the STAR study showed only a tendency of a preventative effect. The PETIT study<sup>31</sup> was the first RCT showed a preventative effect on egg allergy by applying infants with low-dose heated whole egg powder, without adverse events caused by either egg nor placebo. The incidence of egg allergy at the 12 months of age in the PETIT study was significantly lower in the egg group than in the placebo group (8%, 5/60 vs 38%, 23/61;  $p = 0.0001$ ). These results indicate that early introduction of hen's egg is effective for preventing hen's egg allergy in infants with eczema.

Attention to avoid allergic reactions of infants should be required in introducing hen's egg to them. The amount of egg to introduce for the first time and heated or not were important factors not only for success of egg allergy prevention but also safety introduction. The STAR study,<sup>27</sup> STEP study,<sup>28</sup> BEAT study,<sup>29</sup> and

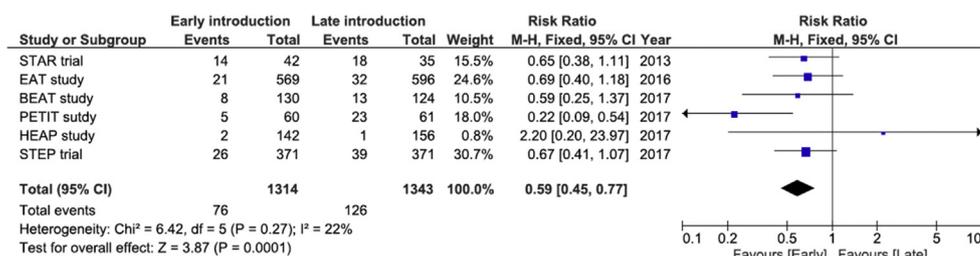
HEAP study<sup>30</sup> used raw egg powder for intervention groups and the incidence of their adverse events was 31%, 6.1%, 8.5%, and 9.2%, respectively, which was significantly higher than that of placebo groups. On the other hands, the EAT study<sup>24</sup> and PETIT study<sup>31</sup> used heated egg showed no significant difference in adverse events between the placebo group and the early consumption group. All participants in the egg group of the PETIT study took the study powder without any allergic reaction through all intervention period, although half of the participants were sensitized to hen's egg at the first introduction of egg at the 6 months of age. The study powder applied for 3 months from 6 months of age contained egg protein equivalent to 0.2 g boiled whole egg and that applied from 9 months of age contained egg protein equivalent to 1.1 g boiled whole egg. These findings indicate small amount of heated egg was generally safe to be introduced to infants and two step approach was effective to introduce oral tolerance of egg.

The Japanese Society of Pediatric Allergy and Clinical Immunology (JSPACI) published a statement concerning prevention of hen's egg allergy in June 2017 (only in Japanese).<sup>36</sup> This statement recommends that infants with eczema should be introduced hen's egg from 6 months old and infants without eczema should start it according to the Support Guidelines of Breast feeding and Complementary feeding 2007 in Japan. This statement also emphasized to treat eczema and induce remission before starting introduction of hen's egg.

### Prevention of cow's milk allergy

A meta-analysis of 2 RCTs did not show any significant preventative effect of early consumption of cow's milk protein for preventing cow's milk allergy (risk ratio: 0.76, 95% CI: 0.32–1.78).<sup>25</sup> The reason for not showing significant finding might be shortage of power. Lowe *et al.*<sup>37</sup> recruited 620 infants with a high risk with a family history of allergic disease and less than a half of the participants had eczema. The prevalence of cow's milk allergy within first 2 years was 4.2% (8/191) in the soy formula (avoidance) group and was 3.1% (6/193) in the conventional cow's milk formula group. Adherence rate of allocated formula were about 63% by 6 months of age and 76% by 12 months of age. Low adherence might also cause non-significant difference. The EAT study<sup>24</sup> recruited infants in the general population. The prevalence of cow's milk allergy was 0.67% (4/597) in the standard introduction group and 0.53% (3/569) in the early introduction group. This study defined that intake of less than 300 ml of formula milk as avoidance of cow's milk. Therefore, early introduction of cow's milk protein may be introduced in both groups in the EAT study.

Katz *et al.*<sup>16</sup> recruited a large number of participants ( $n = 13,019$ ) in a birth cohort study consisting of general population. They reported that a significantly higher proportion of infants among those who were exposed to cow's milk protein at 15 days old or older developed cow's milk allergy (odds ratio: 19.3, 95% CI: 6.0–62.1).



**Fig. 1.** Summary of the effect of early introduction of egg on preventing egg allergy. Data shown are the mean risk ratio (for immediate egg allergy diagnosed with an oral food challenge) with 95% confidence intervals for early introduction of egg compared with avoidance (control). STAR, Solids Timing for Allergy Reduction; EAT, Enquiring about Tolerance; BEAT, Beating Egg Allergy Trial; PETIT, Prevention of Egg allergy with Tiny amount Intake; HEAP, Hen's Egg Allergy Prevention; STEP, Starting Time of Egg Protein.

**Table 1**  
Summary of randomized, clinical trials on preventing egg allergy.

Study name (Country)	Subjects	Period of intervention	Amount and frequency of intake as intervention	Timing of the primary outcome	Results (Prevalence of immediate egg allergy)	Adverse events (% in the early-introduction group)
EAT study <sup>24</sup> (United Kingdom)	General population	3 to 6 months old	Equivalent to approximately half of a heated egg, twice a week	3 years old	No difference (significant difference in per-protocol analysis)	No difference
BEAT study <sup>29</sup> (Australia)	Family history of allergic disease, but the infants had no sensitization to egg	4 to 8 months old	Equivalent to approximately 1/16 of a raw egg, every day	1 year old	No difference	Significant difference (8.5%)
HEAP study <sup>30</sup> (German)	General population without sensitization to egg	4–6 to 12 months old	Equivalent to approximately one third of a raw egg, three times a week	1 year old	No difference	Significant difference (9.20%)
STEP study <sup>28</sup> (Australia)	Maternal history of allergic disease, but the infants had no eczema	4–6 to 8 months of age	Equivalent to approximately 1/14 of a raw egg, every day	1 year old	No difference	Significant difference (6.10%)
STAR trial <sup>27</sup> (Australia)	Atopic dermatitis	4 to 8 months old	Equivalent to approximately one sixth of a raw egg, every day	1 year old	No difference	Significant difference (31%)
PETT study <sup>31</sup> (Japan)	Atopic dermatitis	6 to 12 months of age	Equivalent to approximately 0.2 g of a boiled egg between 6 and 9 months of age, 1.1 g of a boiled egg between 9 and 12 months old, every day	1 year old	Significant difference	No difference

EAT, Enquiring about Tolerance; BEAT, Beating Egg Allergy Trial; HEAP, Hen's Egg Allergy Prevention; STEP, Starting Time of Egg Protein; STAR, Solids Timing for Allergy Reduction; PETT, Prevention of Egg allergy with Tiny amount Intake.

In 2006, Osborn *et al.*<sup>38</sup> reported that there is limited evidence that prolonged feeding with a hydrolyzed formula reduces infant and childhood allergy in high-risk infants who are unable to be completely breast fed compared with a cow's milk formula by meta-analysis. Boyle *et al.*<sup>39</sup> reported meta-analysis showing that there was no preventive effect of a hydrolyzed formula during infancy for developing allergic diseases, including cow's milk allergy. The guidelines of the EAACI and the American Academy of Allergy Asthma and Immunology<sup>22,40</sup> recommended hydrolyzed milk formula for high-risk infants whose breast feeding was insufficient for the first 4 months. Meanwhile, the guidelines of the JSPACI<sup>21,41</sup> did not positively recommend hydrolyzed milk formula for preventing allergic diseases. Further studies need to be performed to determine whether hydrolyzed formula is recommended.

### Food allergy and skin barrier dysfunction of atopic dermatitis

As described above, varied epidemiological studies demonstrated eczema/atopic dermatitis is a strong risk factor for the development of food allergens sensitization and onset of food allergies in childhood. Mice model studies showed disrupted skin barrier and inflammation enhanced allergen sensitization and subsequent onset of food allergy and/or asthma.<sup>42,43</sup> Analysis of interview data from participants in a birth cohort study in England showed a significant independent relation of peanut allergy with the use of skin preparations containing peanut oil (odds ratio 6.8; 95 CI: 1.4 to 32.9) suggesting that sensitization to peanut protein may occur in children through the application of peanut oil to inflamed skin.<sup>44</sup> As in mouse skin,<sup>45,46</sup> activated Langerhans cells (LCs) penetrate TJs in human skin and the number of LCs with TJ penetration increased approximately 5-fold in erythematous lesional skin of patients with AD but not in nonlesional skin of patients with AD or lesions of patients with ichthyosis vulgaris or psoriasis.<sup>47</sup> This behavior of LCs in inflamed skin well explains the mechanisms of percutaneous sensitization of food allergens exposed to patients with AD.

Atopic dermatitis (AD) in human is also demonstrating defects of skin barrier function, culminating in a clinical phenotype of itchy skin lesions with type 2 allergic inflammation. Skin barrier comprises mainly epidermis consisting of basal layer, prickle cell layer, granular layer and horny layer. Epidermal skin barrier is composing from various substances such as natural moisturizing factors (NMF) in a corneocytes, intercellular lipid in horny layer, corneodesmosomes combining corneocytes and tight junctions between granular cells. NMF are derived from pro-filaggrin, a mix of hygroscopic compounds, which help maintain skin hydration. NMF are catalyzed by bleomycin hydrolase of which function was suppressed in patients with atopic dermatitis.<sup>48,49</sup> The loss of-function mutations within the FLG gene encoding profilaggrin, precursor for the structural protein, filaggrin predisposed to atopic dermatitis was first identified in 2006.<sup>50</sup> FLG mutations are associated with eczema, dry skin and high Trans epidermal loss (TEWL) at 3 months of age.<sup>51</sup> FLG mutations in Japanese was also reported but different from those in Europeans.<sup>52</sup> Majority patients with atopic dermatitis have not FLG mutations, however, their epidermal inflammation suppresses filaggrin expression resulting in the lack of NMF.<sup>53,54</sup> Patients with AD with FLG mutations is associated with an increased SC IL-1 cytokine profile and these levels were inversely correlated with NMF levels, which were also inversely correlated with skin surface pH.<sup>55</sup> Increased skin surface pH enhanced serine proteases activity and accelerates degradation of corneodesmosomal components<sup>56</sup> resulting in epidermal dysfunction. Levels of ceramide composing normally half of intercellular lipid substances are decreased in SC of AD and especially lesser in lesions than no-lesions.<sup>57,58</sup>



**Fig. 2.** Summary of the effect of early introduction of egg on preventing egg allergy in low-risk infants. Data shown are the mean risk ratio (for immediate egg allergy diagnosed with an oral food challenge) with 95% confidence intervals for early introduction of egg compared with avoidance (control). Low-risk infants are defined as infants from the general population, with no sensitization to egg or no eczema. EAT, Enquiring about Tolerance; BEAT, Beating Egg Allergy Trial; STEP, Starting Time of Egg Protein; HEAP, Hen's Egg Allergy Prevention.

Skin microbiome is also associated with pathogenesis of AD. In AD, the proportion of *Staphylococcus aureus*, was greater during disease flares than at baseline or post-treatment, and correlated with worsened disease severity.<sup>59</sup> *S. aureus* strains isolated from the colonized skin lesions of patients with acute-phase AD were reported to produce various extracellular proteolytic enzymes<sup>60</sup> implying that *S. aureus* disrupts epidermal barrier by enhancing pyrolysis of corneodesmosomes. Unlike established AD, patients with infantile AD did not have noticeably dysbiotic communities before or with disease and were not colonized by *S. aureus*.<sup>61</sup> Infants who had affected skin at 12 months of age had statistically significant differences in bacterial communities on the antecubital fossa at months 2 compared with infants who were unaffected at month 12. In particular, commensal staphylococci were significantly less abundant in infants affected at month 12.<sup>61</sup> Although these findings may suggest that microbiome flora in early infants predict onset of future AD, emollient treatment improved the clinical symptoms of AD in 72% of the study population and microbial communities associated with affected skin of these treatment responders more closely resembled unaffected skin after treatment as indicated by increased overall diversity and a decrease in the abundance of *Staphylococcus* species.<sup>62</sup>

### Prevention of atopic dermatitis by skin barrier enhancement with emollients

Both ecological perspective<sup>63,64</sup> and basic cutaneous biology<sup>65–67</sup> have suggested that epidermal barrier dysfunction is a key initiator to the development of AD and have prompted a non-controlled trial based on the hypothesis that improving the properties of skin barrier early in life by applying emollients on neonate from birth might protect against the onset of AD in infants and early childhood.<sup>68</sup> To verify the effectiveness, two RCTs were carried out for the first time by two independent study groups<sup>69,70</sup>: one was a dermatologists group working in the United States (USA) and United Kingdom (UK) and the other was a pediatric allergists and dermatologists group in Japan.

The study design of the US and UK group was a multicenter, multinational, assessor-blind RCT of high risk infants having a parent or sibling who had physician-diagnosed AD, asthma, or allergic rhinitis.<sup>69</sup> Parents in the intervention group were offered a choice of three emollients of different viscosities. Parents were asked to apply the emollient to the baby's entire body surface, with the exception of the scalp, starting as soon as possible after birth and continuing until 6 months of age. Both the intervention and control groups were given an infant skin care advice booklet, which reflected current European guidelines.<sup>71</sup>

The research nurse contacted parents by telephone at 10 days and 6 weeks, with a face-to-face visit at 12 weeks and a further telephone call at 18 weeks, and the final contact was a clinic visit at 24 weeks for assessment of the skin. Of the 295 eligible families, 124 (42%) accepted the initial invitation to participate. In the

intervention group, the cream/gel formulation was the preferred emollient (67.2%), followed by oil (23.4%) and then ointment (9.4%). Daily emollient use significantly reduced the cumulative incidence of AD at 6 months (43% in the control group vs. 22% in the emollient group). This corresponds to a relative risk reduction to 50% (relative risk, 0.50; 95% CI, 0.28–0.90; P 5.017). This trial is a feasible study of large-scale RCT named the BEEP (Barrier Enhancement for Eczema Prevention) study which already finished the recruitment of 1395 participants to be followed up to 2 years of age to find out whether skin care advice using emollients can prevent eczema in newborn babies compared with skin care advice alone.

A Preventive RCT from Japan<sup>70</sup> was also published at the same time of US and UK group RCT.<sup>69</sup> The study design of the Japanese study was an investigator-blinded, single-center RCT of which participants were born in the National Center for Child Health and Development in Tokyo, Japan. One hundred and eighteen neonates were enrolled and then randomly assigned to the intervention (n = 59) group or the control (n = 59) group. The intervention group started daily application of an emulsion-type emollient from the first week of life and continued for 32 weeks. Petroleum jelly was prescribed to all infants in both groups on request by the institutional review board because of the institutional treatment by default. All infants were examined by the same dermatologist blinded to the assignment of the groups and were recorded at scheduled visits of 4, 12, 24, and 32 weeks of life.

Adverse events caused by this emulsion-type emollient were not observed during this RCT. The mean daily amount of emulsion-type moisturizer used by the intervention group was  $7.86 \pm 4.34$  g and the mean daily amount of petroleum jelly applied to the control group was  $0.101 \pm 0.286$  g. During their first 32 weeks of life, 19 infants in the intervention group had AD/eczema compared with 28 infants in the control group. Calculation of cumulative incidence values for AD/eczema by using the Kaplan–Meier method showed that the intervention group maintained intact skin for a significantly longer period than the control group (P = 0.012, log-rank test). Cox regression analysis showed the risk of AD/eczema to be significantly lower in the intervention group (hazard ratio, 0.48; 95% CI, 0.27–0.86).

In analyses of secondary outcomes, the serum levels of anti-egg white and anti-ovomucoid IgE in infants at 32 weeks were evaluated by using the diamond-like carbon [DLC] chip with high sensitivity.<sup>72</sup> The proportions of infants who were sensitized by allergens were similar between the intervention and control groups, although the intervention group had significantly higher levels of stratum corneum hydration in the lower leg at weeks 12 and 24 compared with those seen in the control group. A greater proportion of infants with AD/eczema had allergic sensitization based on the serum levels of anti-egg white IgE than those without AD/eczema (P = 0.043).

Another pilot RCT was recently published to exam the effect of twice daily application of ceramide dominant emollient for the first six months of life on the incidence of AD and skin barrier function

in high risk infants up to 12 months of age.<sup>73</sup> Eighty infants with a family history of allergic disease were recruited and clinical follow-up of infants by a blinded assessor occurred at six weeks, six months and 12 months of age. Intention to treat analysis showed no significant effect of routine barrier lipid replacement in early life on AD or sensitization outcomes. However, there was a trend towards reduced risks of cumulative incidence of AD (RR = 0.60, 95% CI: 0.26–1.38) and food sensitization (RR = 0.56, 95% CI: 0.20–1.56) in the intervention group at six and 12 months of age. Per protocol analyses revealed a significant reduction in food sensitization at 12 months in the treatment group (0% [0/21] versus 19.4% [7/36],  $p = 0.04$ ). There were no differences between groups for biophysical properties of the skin. Among the intervention group, children who developed food sensitization had a later initiation of treatment. Twice daily prophylactic use of a ceramide dominant emollient may have stronger potency to prevent the development of food sensitization than once daily application of emollients used in the past trials.

### Probiotics and prevention of atopic dermatitis and atopic march

Inverse association between microbial exposure in early life and allergy was indicated by hygiene hypothesis<sup>2</sup> and a decade later the first RCT was carried out in Finland to show the effectiveness of the probiotics as potentially beneficial bacteria of healthy gut microflora to prevent allergic diseases.<sup>74</sup> The result of lactobacillus GG administration to mothers prenatally and infants postnatally for 6 months was successful to reduce AD at 2 years of age in intervention group (RR = 0.52, 95% CI: 0.32–0.84). A Japanese birth cohort study also showed protective effect of yogurt intake in infancy against AD and food allergy at 5 years of age.<sup>75</sup> Many studies of probiotics to prevent allergy and allergic conditions published so far has yielded conflicting results. However, results of meta-analysis in most of the systematic reviews have shown protective effects of probiotics administered both prenatally and postnatally against offspring's AD, while no protective effects against asthma/wheezing were shown.<sup>76–80</sup> A meta-analysis combined 2 preventive studies with synbiotics has not reached significance in prevention of AD (RR = 0.44, 95% CI: 0.11–1.83).<sup>81</sup>

Previous researches show that gut microbiome composition plays an important role in the development of eczema. A systematic review selected six studies (total 1990 participants) identified that the composition of gut microbiota specific to eczema could be influenced by the following environmental factors: length of gestation, mode of delivery, type of feeding, method of treatment, number of older siblings, and other life style factors.<sup>82</sup> There has been inconsistent empirical evidence as to the modulatory effects of gut microbiota on immunological functions in children with eczema.

As described above, both gut microbiome and skin microbiome have strong relationship with atopic dermatitis, although the mechanism connecting the two entities remains enigmatic. The birth cohort study implementing in Tokyo suggests antibiotic use within the first 2 years of life was a risk factor for current asthma, current atopic dermatitis, and current allergic rhinitis in 5-year-old children.<sup>83</sup> This report supports the findings of a systematic review reported that a pooled analysis of 10 longitudinal studies showed that postnatal antibiotic exposure in the first year of life increased the risk of eczema (OR 1.40, 95% CI 1.19–1.64).<sup>84</sup> Finding of the birth cohort study on the association of cephem and cephalosporin with asthma was similar to previous reports,<sup>85–87</sup> however, did not show an association of penicillin and macrolide with asthma,<sup>83</sup> which differed from results of previous studies. The differences in results could be due to differences in exposures to antibiotics, populations, and target sample size.

AD in infancy is associated with development of asthma and rhinitis later up to 5 years of follow up<sup>88</sup> and especially early onset persistent phenotype has stronger association with developing asthma than early transient phenotype.<sup>32</sup> Eczema/AD is one of the strongest risk factors of not only food allergy but also asthma and allergic rhinitis. Antibiotics use in infancy might have influence on the increase of asthma onset via alteration of microbiota directly and via onset of atopic dermatitis indirectly.

Meta-analyses with fixed- and random-effect models using 31,742 children from eight ongoing European birth cohorts indicated that exposure to visible mold and/or dampness during first 2 years of life was associated with an increased risk of asthma particularly in young children and allergic rhinitis symptoms in school-age children.<sup>89</sup> Urban life style living in a low ventilated house/building in crowded town increases the exposure to mold and/or dampness and enhances the risk of insulting commensal microbiota of human resulting in dysbiosis of skin, gut and respiratory tracts. One of the causes for inconsistent results of many trials with probiotics could be attributable to the exposure difference in mold species and doses in the populations and geographical lesions.

### Conclusions and therapeutic strategy in the perspective of barrier dysfunctions

Insults in microbiota and epithelial barrier dysfunction caused by numerous environmental changes occurred for these decades rapidly increased the incidence of allergy and non-communicable diseases such as allergic diseases would widely. Manifold hypothetical mechanisms by which microbiota influences allergy and onset of allergic diseases have been proposed.<sup>90,91</sup> Bacterial modulation of the innate lymphoid cells, their direct action on regulatory T cells through toll-like receptors and production of short chain fatty acid (SCFA) may protect us from intestinal inflammation.<sup>92</sup>

Recent development of new therapeutic measures for AD may provide not only better control of AD but also better secondary prevention of allergic diseases. Since AD is locating on the root of atopic march, the target for primary prevention of allergy should be AD. Barrier enhancement of bronchial tract is not easy at this moment, however, that of skin can be implemented by direct application of emollients on epidermal surface after elimination of allergens and stimulants attached on the skin. Next target organs may be nasal cavity and eyes, which can be coped with washing and application of nasal sprayers and eye drops.

Although allergen avoidance measures failed to prevent allergic disease, environmental control such as elimination of harmful molds and proteases generated from house dust mites was effective to suppress symptoms of asthma and wheezing. Cohabitation with healthy commensal microbiome may protect our epithelial barrier from environmental insults and lead to decrease incidence of allergic diseases. Application of emollients on infantile skin and probiotics may have some effect on protection from onset of AD, however, there are a lot of room to be improved and early intervention for early onset AD might be more practical in the real-world practice. In addition to those strategy, tolerance induction of food for infants without delayed introduction should be considered if they had eczema.

Urban life style in modern age has decreased exposure of natural soil and its components and has increased exposure of environmental chemicals such as phthalate, dioxin, paraben and etc. which penetrate infantile skin more than adult's skin and are suspected to have adverse health effects.<sup>93</sup> Rapid urbanization and life style changes might cause altered commensal microbiome of human body and inclined immunological balance to Th 2 type milieu resulting in increased onset of allergic diseases.

Our current therapeutic strategy to prevent allergy could be developed by composing safer skin treatment, oral tolerance induction and environmental controls including life style change.

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## Conflict of interest

YO received speaker's honoraria from Maruho. ON has no conflict of interest.

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