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Role of the gut-skin axis in IgE-mediated food allergy and atopic diseases

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Abbreviations

AD: Atopic Dermatitis

AR: Allergic Rhinitis

CTLA-4: Cytotoxic T-Lymphocyte Antigen 4

EPIT: Epicutaneous Immunotherapy

FA: Food Allergy

GWAS: Genome Wide Association Studies

IL: Interleukin

LAP: Latency Associated Peptide

OIT: Oral Immunotherapy

OVA: Ovalbumin

RCTs: Randomized Controlled Trials

SCFAs: Short Chain Fatty Acids

SCORAD: SCORing Atopic Dermatitis

SLIT: Sublingual Immunotherapy

TEWL: Transepidermal Water Loss

Th2: Type 2 T helper

Tregs: Regulatory T cells

TSLP: Thymic Stromal Lymphopoietin

Abstract

Purpose of review

In recent years, landmark clinical trials investigating the role of early oral exposure to food antigens for food allergy (FA) prevention have highlighted the importance of immunoregulatory pathways in the "gut-skin axis". This review highlights recent literature on the mechanisms of the immune system and microbiome involved in the gut-skin axis, contributing to the development of atopic dermatitis (AD), food allergy (FA), allergic rhinitis (AR) and asthma. Therapeutic interventions harnessing the gut-skin axis are also discussed.

Recent findings

Epicutaneous sensitization in the presence of AD is capable of inducing Th2 allergic inflammation in the intestinal tract and lower respiratory airways, predisposing one to the development of allergic rhinitis (AR) and asthma. Probiotics have demonstrated positive effects in preventing and treating AD, though there is no evident relationship of its beneficial effects on other allergic diseases. Prophylactic skin emollients use has not shown consistent protection against AD, while there is some evidence for the role of dietary changes in alleviating AD and airway inflammation. More RCTs are needed to clarify the potential of epicutaneous immunotherapy as a therapeutic strategy for patients with FA.

Summary

The growing understanding of the gut-skin interactions on allergic disease pathogenesis present novel avenues for therapeutic interventions which target modulation of the gut and/or skin.

Keywords: gut-skin axis; microbiome; allergic diseases; probiotics, EPIT

Introduction

As researchers gain a better understanding of multi-omic interactions in disease pathogenesis as well as the immunomodulatory effects of the host microbiome on distant organ sites, interdisciplinary fields in systems biology such as the gut-lung, gut-skin and gut-brain axes in health and disease are also growing [1]. Recent advancements in sequencing techniques, supercomputing capabilities and bioinformatics have also propelled research on the human microbiome and its role in human health and disease. There is now a rich body of evidence demonstrating the role of the gut-skin axis in the pathogenesis and treatment of allergic diseases. Cross-sectional studies have described differences in microbiota patterns in diseased vs healthy states [2*, 3**]; and others have observed longitudinal divergence of microbiota maturation in early life in relation to subsequent allergic disease outcomes [4**].

This review therefore aims to summarize the latest evidence on immunological and microbiota interactions between the gastrointestinal system and the skin in the pathogenesis of atopic diseases (atopic dermatitis (AD), food allergy (FA), allergic rhinitis (AR) and asthma), and will discuss strategies which harness the gut-skin axis for preventative and therapeutic purposes.

Gut-skin axis in pathogenesis of allergic diseases

Herein, we present two mechanisms associated with the gut-skin axis in the development of allergic diseases – i) the immunological crosstalk between the gut and skin and ii) the gut-skin microbiome. An illustration of the mechanisms involved are depicted in Figure 1 (Image created with BioRender).

Gut-skin immunological crosstalk

Defects in the skin barrier contributing to AD development are thought to be crucial in inducing epicutaneous sensitisation to food allergens. This represents the first cascade in

paving the way to development of other allergic diseases such as asthma and AR in a phenomenon known as the 'atopic march'. The mechanisms through which antigen sensitization in the skin can influence allergic inflammation in the gut and airways are still unclear. However, thymic stromal lymphopoietin (TSLP), interleukin (IL)-33 and IL-25 are some of the key epithelial-derived cytokines influencing the cascade of immunological events in eliciting such allergic responses [5, 6].

The dual allergen exposure hypothesis posits that children with defective skin barrier function and/or AD are more likely to become sensitized to food allergens and are also more likely to become clinically food allergic; early oral allergen exposure (4 to 6 months) prior to acquiring sensitisation is therefore crucial for tolerance induction [7**, 8]. An RCT carried out in Japanese new-borns showed a protective effect against cow's milk allergy with daily ingestion of cow's milk formula between 1 to 2 months of age compared to the avoidance group (breastfeeding supplemented with soy formula). Participants, however, may have had some exposure to cow's milk formula prior to 1 month to supplement breastfeeding. Conversely, another RCT also carried out in Japan showed avoidance of cow's milk formula for at least the first 3 days of life to be protective against cow's milk sensitization in infants with a family history of atopy [9**]. These studies suggest that the timing of introduction of cow's milk formula supplementation may be critical for these high-risk infants. Clinical studies have also shown that continued skin exposure to food allergens such as peanut oil-containing emollients result in a higher risk of peanut allergy [10]. Food sensitization through the skin is possible through a disrupted skin barrier following the activation of mast cells, type 2 innate lymphoid cells, and IL-33 signalling. TSLP, an epithelial cytokine, is mainly expressed by epithelial cells of the skin, lungs, and intestine [11*] and mediates type 2 inflammation. Increased TSLP-elicited basophils in the skin were detected in an ovalbumin (OVA)/peanut sensitized AD murine model subjected to intragastric antigen challenge. It is

thought that Th2-dependent cytokine responses were activated, increasing serum IgE levels, leading to the accumulation of mast cells in the intestine and resulting in IgE-mediated FA [12].

In contrast, gut mucosal exposure to food antigens induce oral tolerance through differentiation of naive T cells into α4β7+ gut homing T cells, mediated by antigen presenting cells in the gut mucosa [13]. This is followed by elaboration of Foxp3+ Tregs, IL-10 and TGF-β, which inhibit Th2 dependent allergic responses, mast cell degranulation and increase IgG4 production[14]. A loss of the gut-skin immunological equipoise thus occurs in the face of AD skin inflammation, skewing the balance towards food sensitization and clinical FA, particularly if timely oral tolerance does not occur. These mechanisms are targeted for FA prevention through early introduction of allergenic foods and will be discussed in a later section.

Epicutaneous sensitization on barrier-disrupted skin is postulated to be able to trigger systemic Th2 allergic inflammation in distant sites such as the intestinal tract and lower respiratory airways[15]. Overexpression of TSLP, a key mediator by which epicutaneous sensitization can trigger and maintain chronic lung inflammation as well as intestinal FA [16], elicits allergic sensitization and bronchial hyper-responsiveness [15]. On the contrary, reduced TSLP levels attenuate Th2 responses and airway inflammation [15]. Mice epicutaneously exposed to the OVA antigen in the presence of TSLP have been shown to develop severe airway inflammation upon airway challenge [17]. IL-33 is also a crucial cytokine in the gut-skin-lung cross talk involved in asthma pathogenesis. Both IL-33 and ST2/IL1RL1 (IL-33 receptor through which IL-33 induce its signalling) genes have been identified as asthma susceptibility loci [18]. Mice exposed to recombinant IL-33 and OVA through the skin showed involvement of IL-33 in development of allergen sensitization leading to subsequent

157 pulmonary reactions as indicated by an increase in bronchial alveolar lavage cellularity and
158 IgE levels[6].

159 **Involvement of the gut and skin microbiome**

160 Gut and skin microbiota also play a key role in the gut-skin immunological pathways
161 implicated in the pathogenesis of allergic disorders. Gut dysbiosis in early life has been
162 identified as a potential marker of AD, FA and allergic airway disease.

163 ***Food allergy and atopic dermatitis***

164 An altered gut microbiota composition has been associated with FA. For example,
165 antibiotic usage reduces gut microbiota diversity and has been associated with the
166 development of FA[19].

167 In AD, studies have also shown that gut dysbiosis appears to precede AD onset, as
168 distinct differences have been observed in the early life gut microbiome of those who develop
169 AD and those who do not. Gut microbiota of infants with AD had higher abundance of
170 *Enterobacteriaceae (E. coli and K. pneumoniae)* and lower abundance of *Bacteroidaceae*
171 *family (B. fragilis)* at 3 weeks of life [20]. Mechanistically, this may occur through the 'leaky
172 gut' / 'epithelial barrier' hypothesis, whereby the disrupted epithelial barrier increases
173 increased epithelial permeability, enhancing translocation of gut microbiota into the
174 bloodstream and skin, leading to systemic inflammation [21, 22*].

175 ***Asthma and AR***

176 Studies have also linked gut dysbiosis in early life to an increased asthma risk later in
177 life. Lower gut microbiome diversity (*Rothia*, *Faecalibacterium*, *Lachnospira*,
178 and *Veillonella)* in the first month of life was observed in children who developed asthma at
179 school age compared to non-asthmatic children [23]. Correspondingly, OVA/alum-sensitized
180 adult offspring of mice colonized with *Rothia*, *Faecalibacterium*, *Lachnospira*,

and *Veillonella* also showed attenuated allergic airway inflammation, evident by the lower number of immune cells detectable in the bronchoalveolar lavage fluid [23, 24].

Adults with AR were also reported to have a markedly reduced microbial diversity and alteration in the abundance of certain microbes (*Clostridium hylemonae, Ruminococcus gnavus,* and *Acidaminococcus intestini* species) compared to controls [25**]. *Ruminococcus gnavus* has previously been associated with respiratory allergies - *Ruminococcus gnavus*-infected mice displayed increased airway hyperresponsiveness and histologic airway inflammation after sensitization and challenge with OVA [26]. In mice, a significantly increased abundance of *Ruminococcus gnavus* in the AR group compared to controls was observed. Taken together, there is increasing evidence that there is an interplay between gut and lung for maintenance of airway immune homeostasis [27].

Gut-skin axis in therapeutic interventions

Therapeutic strategies which harness the immunological interactions between the gut and skin are now being developed for prevention and management of AD, FA and allergic airway diseases. A summary of the interventions is provided in Table 1.

Prevention strategies

The use of probiotics, diet and skin care interventions have been suggested to have beneficial effects in preventing AD and food allergy.

Probiotics

Probiotics are live microorganisms thought to provide a health benefit by modulating the composition of the gut microbiota, exerting systemic immunomodulatory effects in other organ systems beyond the gut [28]. Randomized controlled trials (RCTs) have demonstrated a role for probiotics and/or prebiotics in AD prevention [29*]. In a systematic review of 21 studies, the most effective approach to reduce AD risk was suggested to be a combination of

maternal probiotic supplementation during pregnancy as well as lactation, alongside concurrent supplementation duration of between 6 months to 2 years in their offspring [30]. However, the efficacy of probiotics in randomized clinical trials in FA prevention remains contentious [31**]. Several systematic reviews and meta-analyses of randomized control studies on probiotic formulations or specific bacterial strains for FA prevention were mostly inconclusive due to methodological flaws and imprecise estimated effects inherent in these studies which preclude definitive recommendations at this time [31**, 32]. Recent evidence however has shown maternal carriage of *Prevotella copri*, responsible for producing short chain fatty acids (SCFAs) and endotoxins, to be associated with a reduction in FA risk [33**]. This suggests a possible role of antenatal maternal gut microbiota modification to ameliorate FA in the offspring.

A meta-analysis of 17 RCTs carried out in 5264 children did not demonstrate sufficient evidence that probiotics are able to prevent the development of AR [34]. Nonetheless, a probiotic mixture of *B. longum* IM55 and *L. plantarum* IM76, appeared to alleviate AR symptoms in both human and mice studies. In a double-blind, randomized clinical trial, total nasal symptom scores and rhinitis control assessment scores in subjects with perennial AR improved following administration of probiotics over four weeks [35].

Less is known about the role of probiotics in asthma prevention. A meta-analysis of 14 studies showed no significant association between probiotic supplementation and asthma prevention [34]. While there was some evidence that administration of *Lactobacillus rhamnosus* in the pre/postnatal period protected against asthma compared to placebo, this effect did not remain significant in high-risk groups [34]. Larger RCTs are thus still required to demonstrate the efficacy of these interventions in humans.

Dietary patterns

Apart from probiotics, dietary factors may also play a role in modulating gut microbiota composition in relation to AD risk. Dietary patterns may alter the natural transition patterns of the infant gut microbiome and impact on allergic disease risk. The gut microbiota composition transitions gradually after birth from a predominant *Enterobacteriaceae* and *Staphylococcus* to *Bifidobacterium* and some lactic acid bacteria in early infancy and stabilizes after solid food introduction, eventually resulting in an adult-like composition consisting of bacteria in the genera *Bacteroides, Prevotella, Ruminococcus, Clostridium, and Veillonella* [36]. Thereafter, the microbiota is largely determined by dietary intake [37]. Reduced consumption of fruit[38] and vegetables [39], alongside high consumption of fatty acids, have been linked to AD risk [40*, 41].

SCFAs are also thought to protect against the development of allergic airway inflammation [42]. Observational studies have shown lower stool SCFA levels along with butyrate producing microbiota were associated with increased asthma risk [43] while lower stool acetate levels were associated with wheeze [23]. Dietary interventions to increase SCFA levels may therefore be a promising approach in preventing allergic diseases.

Skin care interventions

Epicutaneous sensitization through the defective skin barrier in AD is postulated to be the major pathway through which FA develops. Therefore, it was proposed that barrier restoration interventions, such as prophylactic emollient applications in high-risk infants from birth might prevent AD and subsequent FA development. However, recent RCTs (Preventing Atopic Dermatitis and ALLergies - PreventADALL and Barrier Enhancement for Eczema Prevention -BEEP) did not support the use of emollients as a preventative measure [44*, 45*]. A recent meta-analysis on 33 clinical trials also showed no significant effect of different skin interventions, which included the use of bathing practices, oils as well as moisturizers/emollients, during the first year of life in healthy infants on reducing AD risk by

1-3 years. However, the analysis was not restricted to emollients alone, and AD outcomes were measured across a wide age range [46**]. Pooled data on food sensitization and FA outcomes are not yet available as several of these trials are still ongoing. There thus remains a gap in the understanding of how skin emollients alone may impact epicutaneous sensitization, immunological pathways and gastrointestinal responses to food allergens, and future studies and meta-analyses could be designed to address this specific question.

Treatment strategies

Apart from complete allergen avoidance, epicutaneous immunotherapy (EPIT) have been proposed as an alternative treatment approach in FA. The potential of probiotics as a preventive strategy have also prompted clinical trials to explore its potential as a standalone and/or add-on intervention in treating AD.

Epicutaneous immunotherapy

EPIT involves the administration of small amount of the allergen incorporated into patches which are placed on the skin for 8 to 48 hours daily[47] to elicit desensitization, and to raise the threshold for allergic responses, in the allergic patient. In a recent phase 3 EPIT trial, children with peanut allergy aged 4 to 11 years randomized to a patch containing 250μg of peanut protein over a 12-month treatment period, experienced higher reaction thresholds compared to children with placebo, although the study did not meet its primary efficacy outcome [48]. EPIT was generally well tolerated apart from moderate local skin reactions [49*]. Sustained unresponsiveness to peanut has also been demonstrated following two to three years of EPIT[50**].

The mechanisms of desensitization for EPIT differ from other approaches such as oral (OIT) and sublingual (SLIT) immunotherapy (Table 2). While FoxP3+ Tregs remain central to all immunotherapy regimens, the subset of Tregs activated in EPIT differs from OIT and

SLIT[51]. Levels of latency associated peptide (LAP) LAP+ Tregs, a late-stage Treg activation marker [52], are elevated in EPIT and OIT while IL-10+ is induced in SLIT. EPIT-induced Tregs also require the expression of cytotoxic T-lymphocyte antigen 4 (CTLA4), a surface marker expression, involved in the natural desensitization pathway of the skin[53]. OIT requires both IL-10 and CTLA-4 while SLIT acts through IL-10 alone[54]. Although OIT has consistently been shown to be more efficacious, it is inundated with issues of tolerability and adverse side effects compared to EPIT[53, 54]. Conversely, SLIT has fewer adverse effects but is less efficacious than OIT and EPIT. Further RCTs in EPIT are required to ascertain whether the differences in mechanisms of desensitization may be key in bringing about a more sustained effect against further sensitizations. If so, highlighting EPIT as a novel treatment alternative that is safe and convenient in treatment of food allergies.

Probiotics

An oral probiotic mixture comprising of *B. animalis subsp. lactis CECT 8145*, *B. longum CECT 7347*, and *L. casei CECT 9104*, have successfully been shown to be effective in AD treatment through the modulation of the gut microbiome in humans [55*]. While there were no observable significant changes in microbial diversity, changes in several microbial species in the probiotic group have been associated with AD severity (SCORAD). In particular, a reduction in *Faecalibacterium* concentrations was observed with a corresponding decrease in SCORAD scores.

Extending these results, a systematic review on intervention studies of emollients supplemented with probiotic strains showed considerable improvement in clinical measures such as SCORAD and transepidermal water loss (TEWL), with none reporting any serious adverse events[56**]. However, the authors cautioned that a risk of bias assessment showed methodological concerns in several studies which may weaken the overall evidence base. Notwithstanding, current evidence on probiotics as a standalone or a supplement to existing

treatment measures of AD have shown beneficial effects and a good safety profile which holds promise as future therapeutic adjuncts.

Conclusion

Numerous studies have shown that early life is a critical window during which gut microbiota dysbiosis and subsequent immunodysregulation may lead to the development of many allergic and/or respiratory diseases[57]. This therefore represents a window of opportunity for preventative interventions. While promising beneficial effects of microbiome-related interventions have been demonstrated in allergic disease prevention, carefully designed clinical trials are still required to assess effectiveness of these targeted treatments in various patient groups. Continued research into mechanistic studies to enhance understanding of the underlying mechanisms are also crucial in aiding the development of new therapeutic targets.

Key points

- The gut-skin axis plays a role in the development of allergic diseases via the immunological and microbiome interaction between the gastrointestinal system and the skin.
- Epicutaneous sensitization in presence of atopic dermatitis is able to trigger systemic Th2 allergic inflammation in the intestinal tract and lower respiratory airways, thereby, potentially increasing risk of asthma and allergic rhinitis.
- Randomised controlled trials on probiotics as a standalone or add-on therapy have demonstrated promising beneficial effects as a preventive and treatment strategy for atopic dermatitis, but not for food allergy, asthma or allergic rhinitis.
- Current evidence supports epicutaneous immunotherapy as a safe and well-tolerated treatment for food allergy given its long-lasting sustained unresponsiveness effects compared to other immunotherapies and side effects limited to the local patch site.

- Further research into mechanistic studies to extend our knowledge and understanding of the underlying mechanisms of the gut-skin axis are necessary in the development of new therapeutic targets.

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This latest Cochrane systematic review showed that emollient use is unlikely to reduce risk of eczema and its effect on food allergy risk is also inconclusive.

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Meta-analyses from this systematic review shows promising evidence for use of epicutaneous immunotherapy in peanut allergy.

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This study showed promising results for the use of epicutaneous immunotherapy in treatment of food allergy. Sustained unresponsiveness was achieved in participants 2 to 3 years after epicutaenous immunotherapy.

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*55. Climent E, Martinez-Blanch JF, Llobregat L, et al. Changes in Gut Microbiota Correlates with Response to Treatment with Probiotics in Patients with Atopic Dermatitis. A Post Hoc Analysis of a Clinical Trial. Microorganisms. 2021;9(4).

This study analyses the results from a recent clinical trial on a probiotics mixture to examine the changes in gut microbiome in the treatment group. The probiotic mixture showed improvements in SCORAD accompanied by a reduction in *Faecalibacterium* levels.

**56. Ambrozej D, Kunkiel K, Dumycz K, Feleszko W. The use of probiotics and bacteria-derived preparations in topical treatment of atopic dermatitis-A systematic review. J Allergy Clin Immunol Pract. 2021;9(1):570-5 e2.

This is the first systematic review to review the evidence for the use of emollients in conjunction with probiotics as a treatment for atopic dermatitis. Current evidence on 7 studies showed positive effects of the intervention, although its safety is yet to be determined.

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530 **Table 1 Summary of the gut-skin axis and its potential therapeutic strategies in allergic diseases.**

Gut skin immunology	Refer to Figure 1
Gut skin microbiome	**AD**: ↑ abundance of *Enterobacteriaceae (E. coli and K. pneumoniae)*, ↓ abundance of *Bacteroidaceae family (B. fragilis)* in AD infants. **FA**: ↓ in gut microbiota diversity associated with ↑ food allergy. **Asthma:** ↓ gut microbiome diversity (*Rothia*, *Faecalibacterium*, *Lachnospira*, and *Veillonella*) in children with asthma. **AR:** ↓ microbial diversity and changes in abundance of certain microbes (*Clostridium hylemonae*, *Ruminococcus gnavus*, and *Acidaminococcus intestini* species) in adults with AR.
Prevention strategies	
Probiotics	**AD:** Combination of maternal probiotic supplementation during pregnancy and lactation, alongside concurrent infant supplementation ↓ AD risk. **FA:** Human studies mostly inconclusive due to methodological differences/flaws. Maternal carriage of *Prevotella copri during pregnancy associated with* ↓ infant FA. **Asthma:** No evidence of a significant association between supplementation and asthma. **AR:** Insufficient evidence of a beneficial effect in preventing development of allergic rhinitis.

Dietary patterns	**AD:** Microbiota changes from birth up till when solid food is introduced, thereafter diet plays a bigger role. Reduced consumption of fruit and vegetables, alongside high consumption of fatty acids, have been linked to AD risk. **Asthma:** Short chain fatty acids protect against development of allergic airway inflammation. Butyrate producing microbiota associated with ↑ asthma risk.
Skin care emollients	**AD:** No significant effect of emollients and different skin interventions on reducing risk of atopic dermatitis. **FA:** Lack of evidence on pooled data of food sensitization and food allergy outcomes.
Treatment strategies	
Epicutaneous immunotherapy	**FA:** Insufficient RCTs to conclude on potential of EPIT as superior to other immunotherapies. Treatment well tolerated apart from moderate local skin reactions.
Probiotics	**AD:** Probiotic mixture comprising of *B. animalis subsp. lactis CECT 8145*, *B. longum CECT 7347*, and *L. casei CECT 9104*, effective in AD treatment. ↓ in *Faecalibacterium* concentrations with corresponding ↓ in SCORAD scores. **AD:** Emollients supplemented with probiotic strains improved SCORAD and TEWL.

↑: increased/high; ↓: reduction/lower AD: Atopic Dermatitis; AR: Allergic Rhinitis; EPIT: Epicutaneous Immunotherapy; FA: Food Allery; SCORAD: SCORing Atopic Dermatitis; RCTs: Randomised controlled trials; TEWL: Transepidermal Water Loss

Table 2 Comparisons between EPIT, OIT and SLIT

	Epicutaneous Immunotherapy	**Oral immunotherapy**	**Sublingual immunotherapy**
Site	Skin	Oral	Sublingual
Mechanistic pathway	↑↑ Foxp3+, ↑ LAP+ Tregs Requires CTLA-4	↑ Foxp3+, ↑ LAP+ Tregs Requires IL-10 and CTLA-4	↑ Foxp3+ ↑ IL-10^{+} cells Requires IL-10
Efficacy	Moderate efficacy [53]	Most effective [52]	Less effective [54]
Safety profile	Fewer AEs than OIT and SLIT	Highest rates of AEs	Fewer AEs than OIT

AE: adverse events; CTLA-4: cytotoxic T-lymphocyte antigen 4; EPIT: epicutaneous immunotherapy; IL: interleukin; LAP: latency associated peptide; OIT: oral immunotherapy; SLIT: sublingual immunotherapy

Figure Legend

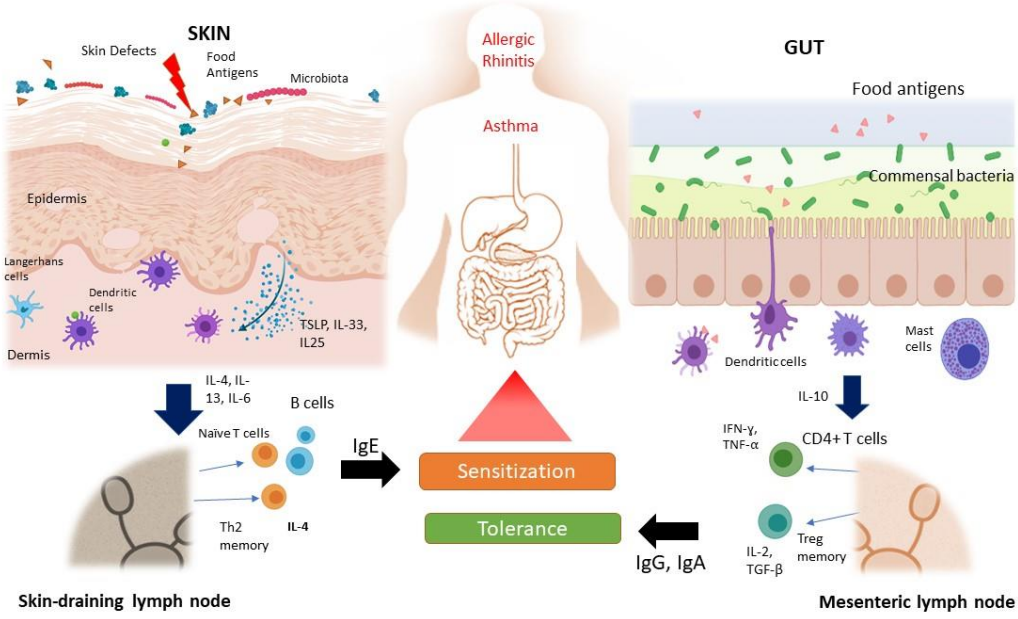

Figure 1 The role of skin-gut axis in sensitization and oral tolerance: Defects in the skin lead to the introduction of food allergens to antigen-presenting cells (APCs), assisted by skin microbiome and bacterial toxins. This leads to induction of B cells that produce TSLP and other IL-33 and IL-25 that stimulates generation of mast cells as well as type 2 T helper (Th2) dendritic cells (DCs) that secrete IL-4, IL-13 and IL-6 in order to stimulate the generation of Th2 skewed effector T cells. Th2 CD4+ T cells secrete IL-4, inducing IgE production by B cells. In the gastrointestinal tract, macrophages, CX3CR1+ APCs, or CD103+ DCs ensure maintenance of tolerance through the development of IL-10–producing Tregs and IgA-secreting B cells. In the induction of oral tolerance, food antigen, and gut microbiota stimulate DCs to produce IL-10 and to induce regulatory T cells. Oral tolerance has been stipulated to play a protective role in allergic diseases such as asthma and allergic rhinitis. Image created with BioRender.