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The Skin as a Route of Allergen Exposure: Part II. Allergens and Role of the Microbiome and Environmental Exposures

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Abstract

Purpose of Review—This second part of the article aims to highlight recent contributions in the literature that enhance our understanding of the cutaneous immune response to allergen.

Recent Findings—Several properties of allergens facilitate barrier disruption and cutaneous sensitization. There is a strong epidemiologic relationship between the microbiome, both the gut and skin, and atopic dermatitis (AD). The mechanisms connecting these two entities remain enigmatic; however, recent murine models show that commensal skin bacteria play an active role in supporting skin barrier homeostasis and defense against microbial penetration. Likewise, the association between the lack of colonization with *Staph* species and AD development suggests a potentially functional role for these organisms in regulating the skin barrier and response to environmental allergens. In undisrupted skin, evidence suggests that the cutaneous route may promote allergen tolerance.

Summary—Properties of environmental allergens and commensal bacteria add to the complex landscape of skin immunity. Further investigation is needed to elucidate how these properties regulate the cutaneous immune response to allergen.

Keywords

Atopic dermatitis; Epicutaneous allergy; Detergent; Cutaneous sensitization; Skin

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George Knaysi and Anna R. Smith contributed equally to this work.

Compliance with Ethical Standards

Conflict of Interest Drs. Knaysi, Smith, Wilson, and Wisniewski declare no conflicts of interest relevant to this manuscript.

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Introduction

Diverse immune processes occur through the skin. The quality of immune response to allergen encountered via the cutaneous route depends on the integrity of the skin barrier, qualities of the antigen itself, and characteristics of the cellular networks present in the skin at the time of allergen contact. See part I of this article for a discussion of the aspects of the skin barrier and immune cells in the skin that promote pathogenic Th2 responses to allergens. The objective of part II is to integrate recent knowledge on the properties of allergens and commensal bacteria that regulate skin barrier integrity and the immune response in allergen-exposed skin.

Properties of Skin Allergens and Allergen Particles

Proteolytic Properties of Allergens

Intrinsic enzymatic activity is a shared feature common to many allergens. Similar to the detrimental effects of dysregulated endogenous protease activity discussed previously in part I of this article, exogenous protease activity of house dust mite, insects, fungi, and pollen disrupts intercorneocyte connections, enhancing their behavior as skin allergens [1, 2]. Der f 1 (dust mite allergen), for example, disrupts epidermal tight junctions and induces inflammatory mediator release (IL-6, IL-8, GM-CSF) by keratinocytes [3]. Furthermore, itching and delayed skin barrier recovery from mite and cockroach allergen exposure is mediated by activation of protease-activated receptor-2 (PAR-2) expressed by keratinocytes and dermal unmyelinated nerve fibers [4]. PAR-2 expression is typically confined to keratinocytes residing in the lower one third of the epidermis; however, after ultraviolet (UV) radiation exposure, PAR-2 expression is increased in the superficial epidermis [5]. The UV enhancement in PAR-2 binding capacity for environmental allergens during different seasons may help explain seasonal trends toward atopic dermatitis (AD) and allergy development [6–8]. While peanut allergens do not exhibit inherent protease activity, a major source for cutaneous exposure to peanut and other food allergens is within house dust [9, 10].

Activation of Innate Immunity via Pattern Recognition Receptors

Another feature common to many allergens is an intrinsic capacity to activate pattern recognition receptors (PRRs) expressed on innate immune cells. Activation of PRRs relays danger signals about the microbe (or allergen) that are required to fully activate the adaptive arm of the immune system by allergen. Toll-like receptors (TLRs) are a major class of PRR widely expressed by skin keratinocytes and dendritic cells (DCs). Homology between the major dust mite allergen, Der p 2, and the TLR4 adapter protein MD-2 is well known. In a murine model of intranasal sensitization to dust mite, recombinant Der p 2 behaved similar to MD-2 and was shown to form disulfide-linked aggregates with TLR4 during lipopolysaccharide (LPS)-TLR4 signaling required for induction of Th2 effector functions [11]. For allergens not known to have any intrinsic activating properties or homology with common microbial pathogen-associated molecular patterns (PAMPs), contaminating TLR ligands and other PAMPs in allergen particles are thought to facilitate DC activation in a similar fashion. Many studies have examined the functional role of TLR adjuvants in

allergen response pathways in murine models. In many models, antigen tolerance or immune nonresponsiveness is established by blocking TLR stimulation or downstream TLR signaling at the time of antigen exposure [12, 13]. In contrast, low-dose LPS mixed with otherwise inert proteins, like ovalbumin, has been shown to induce Th2 responses, but high-dose LPS exposure blocks subsequent allergic responses on challenge [14]. A number of murine models have demonstrated the protective effects of exogenous TLR ligands in allergen responses through the skin: adjuvant stimulation with TLR2 [15], TLR4 [15, 16], and TLR9 [17] all favored protective Th1 vs Th2 immunity in mice. Based on this model, commonly referred to as the hygiene hypothesis, the protective mediator against childhood allergic disease is possibly the early exposure to microbes and TLR-mediated induction of Th1 responses to allergens. However, in subjects with AD, where impaired TLR expression [17–19] and defective innate signaling are well established, these favorable outcomes may be subverted. For example, among children who later developed allergy, activation of TLRs (TLR2, 4, 6) and to a lesser extent endosomal TLRs (TLR3, 7/8, 9) in cord blood mononuclear cells did not translate to the expected Th1 phenotype based on animal models discussed above [20]. Rather, TLR2/6-induced TNF- α correlated strongly with Th2 (IL-13+) responses to allergen, suggesting that higher responses to TLR ligands at birth drive a Th2, rather than Th1, phenotype [20].

Given the putative critical role of skin and TLR-mediated responses in shaping the allergic response early in life, it is important to understand the landscape of innate immunity in infant skin. In a series of experiments performed in the epidermis from embryonic, child, and adult skin specimens without known allergic classification, Iram et al. showed significantly higher mRNA expression of TLR1–5 and the TLR4 co-factor MD2 in embryonic and fetal skin compared to adults. Despite great differences compared to adult skin, the magnitude of expression of TLRs was mostly comparable between embryonic and fetal skin. TLR3 was the dominant TLR in the skin and localized to keratinocytes within the stratum granulosum of neonatal skin. Interestingly, despite identification of CD1c+ dermal DCs and CD207+ Langerhans cells (LCs) in prenatal skin, neither subset constitutively expressed TLR3 or 6. In keratinocytes cultured with TLR ligands, enhanced TNF- α responses were observed in infants compared to adults for multiple TLR ligands, and this was most notable for TLR3/poly I:C, mimicking enhanced innate viral immunity in infant skin [21]. Few studies have examined the functional ontogeny of TLR-mediated responses to allergen in skin. In adult keratinocytes, TLR activation was shown to enhance the skin barrier defenses against allergens, as demonstrated by observations of increased transepithelial resistance (TER) 3 h after stimulation of human keratinocytes cultured with ligands for TLR1, 2, 3, 4, 5, 6, and 9 [22]. Based on additional experiments using TLR2, the authors propose that TLR2 activation on stratum granulosum keratinocytes serves to protect the host against further paracellular diffusion of small molecules when the stratum corneum is damaged [22]. The expression of TLR2, TLR3, TLR4, and TLR9 within the epidermis of human skin has also been postulated to modify host responses to allergen via regulation of antimicrobial peptide production, tight junction (TJ) barrier integrity, and cytokine production [23] including thymic stromal lymphopoietin (TSLP) [24].

C-type lectins are another class of PRRs that recognize conserved polysaccharide moieties on microbes and certain glycosylated allergens. Dermal DCs, but not epidermal LCs,

uniquely express the C-type lectin DC-specific ICAM-grabbing nonintegrin (DC-SIGN) (CD209) [25]. DC-SIGN, on human monocyte-derived DCs (MDDCs), mediated Th2 responses to peanut allergen through direct binding and enhanced uptake of the major peanut allergen, Ara h 1, dependent on Ara h 1 being glycosylated [26]. The DC-SIGN pathway in MDDCs was also implicated for uptake and activation by Bermuda grass pollen [27]. Other glycosylated allergens that bind DC-SIGN including soy, tree nuts, egg, and milk have been identified [28], but the functional relevance of DC-SIGN binding has not been studied.

Other Allergen Promoting Factors

There are a number of other features that are common to allergens and allergen-containing particles. Fragrances in skin care products are a known source of hapten allergen exposure [29]. The antimicrobial endocrine-disrupting compound (EDC), triclosan (a phenoxyphenol antimicrobial), and parabens found in common skin care products have also been linked to risk for aeroallergen sensitization as well as food sensitization among male subjects [30]. Whether elevated triclosan is merely a biomarker for disrupted skin barrier function, or contributes to cutaneous allergen sensitization directly, remains unclear. There are also reports of contact allergy from certain “bioreactive” washing powders that add proteases from *Bacillus licheniformis* or *Bacillus subtilis*, in order to help treat stains [31]. *B. subtilis* is a known sensitizing agent linked to occupational asthma [32]. A number of protein enzymes in laundry detergents (protease, cellulase, lipase, amylase) and skin care products [33, 34] may also play a role in cutaneous sensitization directly or indirectly, but more data is needed to support this view.

Aside from the intrinsic immunostimulatory activity of allergens we have already discussed, there are many other PAMPs in the environment and within allergen-carrying particles that can exert adjuvant-like effects and thereby modify the immune response to allergen. For example, intrinsic NADPH oxidase activity and stimulation of the NALP3 inflammasome by ragweed pollen extract has been implicated in Th2 responses to ragweed [35]. Tobacco smoke, climatic factors, and various pollutants [36], which have been reviewed recently in the context of AD, fit into this category of potential cutaneous adjuvants of allergy. The role of PAMP-allergen interactions in the skin remains poorly understood at this time; however, given that skin DCs and keratinocytes are decorated in diverse PRRs, understanding their function in the skin is pivotal to understanding the development cutaneous sensitization.

The Microbiome and Skin Barrier Function

Skin Microbiome and Antimicrobial Immunity

The skin is inhabited by many microorganisms (e.g., bacteria, fungi, mites, and viruses) [37]. In parallel, keratinocytes produce two main categories of antimicrobial peptides (AMPs; β -defensins and cathelicidins) which provide innate broad-spectrum activity against pathogens but are often deficient in AD skin [38]. Despite the general view that washing skin with soap removes harmful pathogens, removal of natural AMPs and elevation in skin surface pH from the use of alkaline soap create an unfavorable environment for healthy skin microflora [39]. This is particularly relevant in newborn skin where the incidence of pathogens colonizing the skin of healthy infants is quite low [40]. The use of soap in

newborns not only removes natural vernix [41] but also potentially removes antimicrobial peptides, including lactoferrin and lysozyme, that protect the skin from fungal and bacterial pathogens [42, 43].

Based on work in mice, immune activation by commensal skin organisms is likely a critical step in programming the cutaneous immune system during the neonatal period [44]. LCs [45] and the induction of skin T regulatory (Treg) cells [44] have been implicated in the development of immunity to commensal bacteria in the skin. In healthy skin, commensals form a physical and chemical barrier against other pathogenic organisms without triggering allergic responses [46, 47]. In AD skin, commensal bacteria are less diverse and ineffectively prevent colonization and sensitization with pathogenic organisms, most notably *Staphylococcus aureus* [48]. In AD patients colonized with *S. aureus*, Staph-associated δ -toxin stimulates mast cell (MC) degranulation and release of Th2-promoting mediators [49]. Enhanced colonization with *S. aureus* is observed in filaggrin-deficient and cathelicidin-deficient mice, mimicking the landscape of AD skin [50]. Entry of *S. aureus* below the epidermis increases proinflammatory cytokines (IL-4, IL-13, and TSLP) [50], thus enhancing environmental allergen exposure through barrier disruption. These effects were abrogated by the application of skin emollient, which decreased the penetration of *S. aureus* to the subdermal tissues [50]. These findings add to a growing body of evidence that skin emollients mitigate clinically apparent cutaneous inflammatory responses [51, 52] and enhance multiple skin barrier mechanisms in AD skin [53]. Investigators developed an assay for CGN-culture gram-negative bacteria (CGN) from healthy subjects and showed that when live isolates were applied to epidermis-derived keratinocytes, CGN from healthy subjects enhanced the production of defensin β 4A, cathelicidin, and modulators of vitamin D metabolism, compared to cultures from AD subject. They further showed that application of CGN from healthy but not AD skin to mouse skin resulted in significantly improved transepidermal water loss, a measure of skin barrier integrity [54]. Based on these rather novel observations, commensal bacterial are key players in supporting skin barrier function and may serve as potential therapeutic targets.

Despite their role in established AD, how commensal organisms contribute to the development of AD and/or cutaneous sensitization to other allergens is less clear. This question was recently addressed in a single study that performed bacterial 16S ribosomal RNA sequencing on skin samples procured at birth 2 and 6 months of life from 10 infants with AD compared to 10 infants without AD among a birth cohort of 50 randomly selected children. Investigators found that higher commensal staphylococci at 2 months of life was the determining feature of infants who were unaffected by AD by 1 year. The most prevalent species associated with protection from development of AD were *Staphylococcus epidermidis* and *Staphylococcus cohnii*. Most interesting among infants who did develop AD was that their development of skin disease was preceded by a lower abundance of staphylococci colonization overall and not the presence of *S. aureus* [55]. The study by Kennedy et al. highlights the point that skin pathogens are common among children with ongoing skin inflammation like AD [56–59], but pathogens colonizing the skin of healthy infants are rare [40]. Further, the study showed that the birthing method and the feeding method had little effect on infant skin microbiota [55]. Additionally, recent work found that exposure to antibiotics in the first year of life, but not prenatally, increases the risk of

childhood AD diagnosis [60]. Together, these findings confirm an important role for skin microbiota in the development of cutaneous tolerance and maintaining the skin barrier against allergens. The mechanisms that underlie this protection remain an active area of investigation.

The Gut Microbiome as It Relates to Skin Health

Epidemiology has linked skin barrier health to digestive and dietary factors in the mother, growing fetus, and child. Earlier studies that have examined fecal microflora in AD and healthy subjects found that patients with AD had lower counts of *Bifidobacterium* than healthy controls and higher counts of *Staphylococcus* [61] and that low intestinal microbial diversity during the first month of life was a predictor of AD [62, 63]. One way that diet and digestive health are thought to regulate allergen sensitization is through epigenetic modification of skin barrier-associated genes [64]. The microbiota of the maternal and infant gastrointestinal tract also likely modifies the proportion of individuals who develop allergic skin disease [65]. Breast-feeding for over 1 month of life is thought to help populate the baby's skin and gut with healthy flora [66] and is associated with lower risk for AD and sensitization [67, 68]. With the exception of the LEAP study [69], data on food quality, timing, and dosing of complementary foods and supplements remain equivocal [70–72]. The many theories on dietary mechanisms in relation to cutaneous allergen responses continue to be actively investigated [73–75].

Mechanisms Contributing to Cutaneous Tolerance

The specific mechanisms that govern tolerance to food allergens remain elusive; however, the route of exposure is thought to be important [76]. In our current paradigm of tolerance to food allergens, there is a requirement for oral exposure to food antigens to prevent cutaneous sensitization. Pre-existing immunity from primary oral or gut priming of naive allergen-specific T cells is thought to contribute to tolerance, including through the skin [77]. However, if the primary route of exposure is via inflamed skin, the outcome will favor allergy [69, 78].

Epicutaneous immunotherapy (EPIT) is a novel experimental method of attempting to induce tolerance in allergic individuals by delivering low concentrations of food allergens through healthy skin, which challenges our current paradigms about oral tolerance and cutaneous sensitization. In milk-sensitized mice, EPIT was shown to induce milk-specific Treg cells that subsequently promoted tolerance to cutaneously administered peanut and house dust mite [79]. Similar to respiratory and sublingual routes of allergen exposure, where allergen tolerance is attributed to the induction of Treg cells and IL-10 [80], epicutaneous tolerization to antigen is likely to involve Treg-like cells [81]. Based on the localization of LCs below the stratum corneum and TJ within the stratum granulosum, it was largely believed that LCs could only be activated by antigens small enough (<500 Da) to surpass the stratum corneum (SC)/TJ barrier or else after disruption of the SC/TJ skin barrier integrity [82]. This view was challenged by video confocal laser microscopy showing that activated Langerhans cells in healthy human skin elongate their dendrites into undisrupted occludin+ TJs between adjacent granular keratinocytes [83•]. These observations combined with similar studies in murine skin [84] provide a novel view where uptake of antigens by

LCs that have *not yet* violated the epidermal barrier is possible. The interactions between cutaneous T cells and LCs in skin draining lymph nodes play a major role in ongoing immune surveillance in nonallergic individuals and possibly in the process of allergen tolerance, defined as the active process of attenuation of effector T cell responses mediated through the development of T regulatory cells [76]. Whereas early models supported a role for LCs in mediating contact hypersensitivity, later work revealed that LCs are dispensable for induction of contact hypersensitivity and more likely play a role in the tolerance to contact allergens. This view is supported by observations of increased contact sensitivity among patients with depleted skin LCs. Several LC-deficient murine models have revealed that T cell priming to hapten allergens can occur independently of LCs and suggest that LCs may attenuate the inflammatory response in an IL-10-dependent manner [85]. In another murine model, application of the innocuous hapten allergen, 2,4-dinitrothiocyanobenzene (DNTB), to uninflamed mouse skin provided further evidence that LCs can mediate tolerance through anergy of DNTB-primed CD8⁺ T cells and induction of ICOS⁺CD4⁺Foxp3⁺Treg cells [86]. LCs were also essential for inducing protective and neutralizing IgG1 antibodies against cutaneous application of ovalbumin and exposure to *S. aureus*-derived exfoliative endotoxin [84]. In the latter murine model, investigators confirmed that immunization occurred in the absence of antigen penetration of TJs [84]. Taken together, these studies support a novel model in which LCs may play an important role in tolerance (Treg-mediated) or protective humoral immunity (IgG1-mediated) to skin antigens that have not violated the epidermal barrier [87]. In summary, recent findings challenge the simple paradigm that oral exposure favors tolerance whereas cutaneous exposure favors sensitization. Further studies are needed to support whether similar tolerogenic mechanisms are operational in humans.

Conclusion

Our understanding of cutaneous immunity, specifically the factors that are involved in maintaining tolerance, has progressed in recent years with key contributions from genomics, insight into microbiota, and identification of novel immune pathways. However, despite these advances, further research is needed to clarify the specific mechanisms that govern tolerance induction in nondisrupted skin. This is all the more important when considering that atopic dermatitis and early skin sensitization is a strong risk factor for future development of asthma and food allergy. Putative avenues for further investigation include understanding of mechanisms of skin barrier regulation by commensal bacteria in infancy and how these mechanisms are influenced by topical skin care in newborns. Given that dynamic fluctuations in the skin microbiome accompany clinical disease exacerbations in patients with AD and factors secreted by commensal bacteria on non-AD skin may support skin barrier integrity, this is another area of investigation that could provide opportunities for development of novel strategies for allergy treatment and prevention through the cutaneous route.

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Abbreviations

AD	Atopic dermatitis
AMP	Antimicrobial peptide
CGN	Culture gram-negative bacteria
DC	Dendritic cell
DC-	Dendritic cell-specific ICAM-grabbing
SIGN	nonintegrin
TLR	Toll-like receptor
Treg	T regulatory cell
EDC	Endocrine-disrupting compound
EPIT	Epicutaneous immunotherapy
PRR	Pattern recognition receptor
PAR-2	Protease-activated receptor-2
DNTB	2,4-Dinitrothiocyanobenzene
LC	Langerhans cell
LPS	Lipopolysaccharide
MC	Mast cell
MDDC	Monocyte-derived DC
PAMP	Pathogen-associated molecular pattern
TER	Transepithelial resistance
UV	Ultraviolet

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