

The cutaneous innate immune response in patients with atopic dermatitis

I-Hsin Kuo, MS,^{a,b} Takeshi Yoshida, PhD,^a Anna De Benedetto, MD,^a and Lisa A. Beck, MD^a Rochester, NY

INFORMATION FOR CATEGORY 1 CME CREDIT

Credit can now be obtained, free for a limited time, by reading the review articles in this issue. Please note the following instructions.

Method of Physician Participation in Learning Process: The core material for these activities can be read in this issue of the Journal or online at the JACI Web site: www.jacionline.org. The accompanying tests may only be submitted online at www.jacionline.org. Fax or other copies will not be accepted.

Date of Original Release: February 2013. Credit may be obtained for these courses until January 31, 2014.

Copyright Statement: Copyright © 2013-2014. All rights reserved.

Overall Purpose/Goal: To provide excellent reviews on key aspects of allergic disease to those who research, treat, or manage allergic disease.

Target Audience: Physicians and researchers within the field of allergic disease.

Accreditation/Provider Statements and Credit Designation: The American Academy of Allergy, Asthma & Immunology (AAAAI) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians. The AAAAI designates this journal-based CME activity for a maximum of 1 AMA PRA Category 1 Credit[™]. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

List of Design Committee Members: I-Hsin Kuo, MS, Takeshi Yoshida, PhD, Anna De Benedetto, MD, and Lisa A. Beck, MD

Activity Objectives

1. To recognize the innate immune receptors present in skin.
2. To recognize alterations in the innate immune response found in patients with atopic dermatitis.

Recognition of Commercial Support: This CME activity has not received external commercial support.

Disclosure of Significant Relationships with Relevant Commercial

Companies/Organizations: I-H. Kuo has received research and travel support from the Atopic Dermatitis Research Network. T. Yoshida has received research support from the Atopic Dermatitis Research Network. A. De Benedetto has received research support from the National Eczema Association and Dermatology Foundation and has received travel support from the Atopic Dermatitis Research Network. L. A. Beck has received research and travel support from the Atopic Dermatitis Research Network, has received research support from Regeneron and Genentech, is on the Society of Investigative Dermatology Board of Directors, and has received consultancy fees from Regeneron.

Orchestrating when and how the cutaneous innate immune system should respond to commensal or pathogenic microbes is a critical function of the epithelium. The cutaneous innate immune system is a key determinant of the physical, chemical, microbial, and immunologic barrier functions of the epidermis. A malfunction in this system can lead to an inadequate host response to a pathogen or a persistent inflammatory state. Atopic dermatitis is the most common inflammatory skin disorder and characterized by abnormalities in both skin barrier structures (stratum corneum and tight junctions), a robust T_H2 response to environmental antigens, defects in innate immunity, and an altered microbiome. Many of these abnormalities may occur as the consequence of epidermal dysfunction. The epidermis directly interfaces with the

environment and, not surprisingly, expresses many pattern recognition receptors that make it a key player in cutaneous innate immune responses to skin infections and injury. This review will discuss the role epidermal innate receptors play in regulation of skin barriers and, where possible, discuss the relevance of these findings for patients with atopic dermatitis. (J Allergy Clin Immunol 2013;131:266-78.)

Key words: Atopic dermatitis, tight junction, pattern recognition receptors, single nucleotide polymorphisms, innate immunity

From the Departments of ^aDermatology and ^bPathology and Laboratory Medicine, University of Rochester Medical Center.

Supported by the Atopic Dermatitis Research Network (contracts HHSN272201000020C and HHSN272201000017C to L.A.B.), DHHS/PHS/NIH 5 T32 AR007472-21 (to A.D.B.), a National Eczema Association research grant (to A.D.B. and L.A.B.), and a Dermatology Foundation research grant (to A.D.B.).

Received for publication November 20, 2012; accepted for publication December 13, 2012.

Corresponding author: Lisa A. Beck, MD, Department of Dermatology, University of Rochester Medical Center, Rochester, 601 Elmwood Ave, Box 697, Rochester, NY 14642. E-mail: lisa_beck@urmc.rochester.edu.

0091-6749/\$36.00

© 2013 American Academy of Allergy, Asthma & Immunology
<http://dx.doi.org/10.1016/j.jaci.2012.12.1563>

Abbreviations used

AD:	Atopic dermatitis
AMP:	Antimicrobial peptide
CARD:	Caspase activation and recruitment domain
CLR:	C-type lectin receptor
DC:	Dendritic cell
HBD:	Human β -defensin
KACL:	Keratinocyte-associated C-type lectin
LRR:	Leucine-rich repeat
LTA:	Lipoteichoic acid
MDA:	Melanoma differentiation-associated gene
MyD88:	Myeloid differentiation primary response gene-88
NF- κ B:	Nuclear factor κ B
NK:	Natural killer
NLR:	NOD-like receptor
NLRP:	NOD-like receptor protein
NOD:	Nucleotide-binding oligomerization domain-containing protein
PAMP:	Pathogen-associated molecular pattern
PGLYRP:	Peptidoglycan recognition protein
PGN:	Peptidoglycan
PRR:	Pattern recognition receptor
REG3 α :	Regenerating islet-derived protein 3 α
RIG-I:	Retinoic acid-inducible gene 1
RLR:	RIG-like receptor
SC:	Stratum corneum
SNP:	Single nucleotide polymorphism
TJ:	Tight junction
TLR:	Toll-like receptor
TSLP:	Thymic stromal lymphopoietin

many bacterial, fungal, and viral phyla and subphyla. These microorganisms play a key role in the initiation, prevention, or both of skin inflammation and immune responses and protect the host from pathogens such as *Staphylococcus aureus*. Finally, there is the immunologic barrier, which has an immediate but somewhat nonspecific arm (ie, innate immunity) and a highly specific and long-lasting arm (ie, adaptive immunity). Much of the character of the adaptive immune response (eg, antigen-specific tolerance or recall responses) is determined by the interplay of the innate immune system with the other epidermal elements. For example, a breach in the physical barrier would favor the entry and immunologic recognition of antigens, pathogens, or both, but the character and magnitude of this response will be modulated by the epidermal innate immune response, barrier repair response, and host-microbe micro-environment.

Atopic dermatitis (AD) is the most common inflammatory skin disease, affecting up to 15% to 25% of children and 3% of adults in the United States.^{1,2} Impaired skin barriers, reduced expression of epidermally derived antimicrobial products, T_H2 -skewed inflammation, a defect in innate receptor functions, and restricted cutaneous microbial diversity are critical biological features observed in the majority of patients with AD. For many years, AD was considered primarily an immunologic disease, but more recently, epithelial barrier dysfunction has emerged as another key feature.^{3,4} There is consensus that the leaky epithelial barrier promotes allergen sensitization and susceptibility to microbial colonization and even infections.⁵⁻⁷ The controversy emerges when one considers which of these defects initiate the disease. In other words, do barrier defects predate the immune abnormalities or *vice versa*?

Both possibilities can be supported by a number of observational and mechanistic studies. For example, T_H2 cytokines found in nonlesional AD skin adversely affect skin barrier protein expression and function and are strongly associated with risk of *S aureus* colonization or eczema herpeticum.^{3,8-11} Mice lacking the SC protein filaggrin (*fl/fl*) are more susceptible to epicutaneous allergen sensitization, but somewhat unexpectedly, the $CD4^+$ T-cell response is mixed ($T_H17 > T_H2 = T_H1$).¹² One wonders whether the relative contribution of these 2 abnormalities will ultimately help explain some of the heterogeneity observed in human subjects with this disease. Possibly this might help us predict the magnitude of allergen sensitization, age of onset, natural history, comorbidities, treatment responses, and susceptibility to cutaneous pathogens to name just a few unexplained variables noted in patients with AD.^{8-10,13-15}

The innate immune system is a defense strategy used by plants, fungi, invertebrates, and vertebrates that has evolved over millions of years. It provides a rapid and first-line response to pathogens before the host initiates and/or reactivates the adaptive response, which is more specific and has long-lasting memory. Keratinocytes express a number of innate immune receptors collectively referred to as pattern recognition receptors (PRRs) that enable them to respond to microbes or tissue damage by releasing a broad range of inflammatory mediators (eg, cytokines, chemokines, and AMPs). These PRRs can also affect TJ integrity. For example, Toll-like receptor (TLR) 2 agonists enhance the tightness of TJs and arguably limit penetration of surface proteins/microbes.^{16,17} Therefore a defect in this innate immune-mediated epidermal barrier repair process might lead to chronic inflammation from PRR signaling, as seen in patients with AD who are colonized with *S aureus*. The innate immune response is also shaped by the skin microbiome and *vice versa*. The balance between symbiotic and pathogenic microbial populations is determined by the local ecosystem which varies in different anatomical locations for reasons we do not yet understand.¹⁸ The epidermal contributions to this ecosystem that determine the local microbiome are areas of active research that hold much promise.

An effective innate immune system plays a critical role in orchestrating skin barrier functions, eliciting the proper adaptive immune response, and determining the character of the skin microbiome. Abnormal epithelial homeostasis, reduced AMP production, skewed adaptive immune responses, and changes in the local microbiome, which are known features of AD, could all be due in part to a diminished innate immune response (Fig 1). This review will highlight the importance of epidermal PRRs in AD pathogenesis. We will review what is known about epidermal PRR expression, function, and localization in the skin and how this can affect the 4 other epidermal barriers (the physical barrier, chemical barrier, microbiome, and immunologic barrier).

EXPRESSION AND FUNCTION OF PRRs IN HUMAN KERATINOCYTES

PRRs have been divided into 4 subclasses: TLRs, NOD-like receptors (NLRs), retinoic acid-inducible gene (RIG)-like receptors (RLRs), and C-type lectin receptors (CLRs).¹⁹ Although not included in this classification, peptidoglycan recognition proteins (PGLYRPs) are also recognized as PRRs and are thought to be important for bacterial infections.

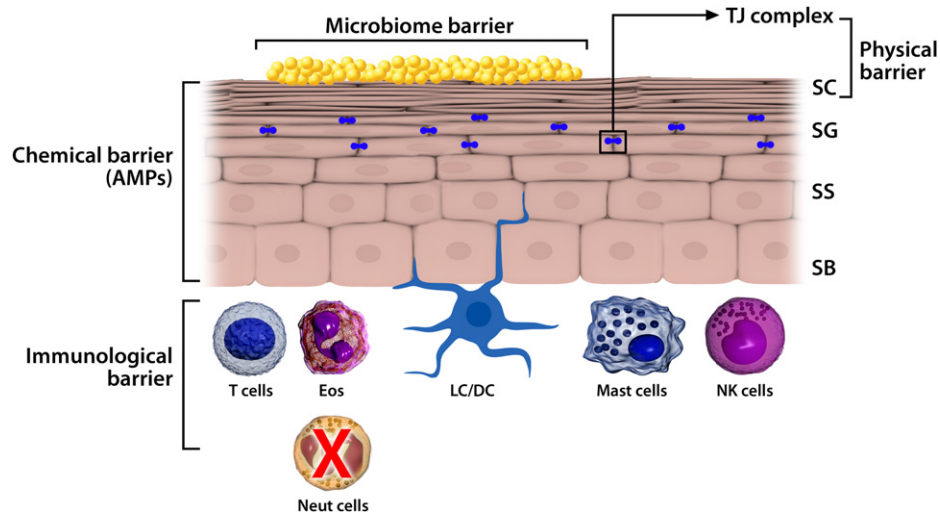


FIG 1. The skin as an impenetrable shield. The SC is the first physical barrier protecting the skin from the environment. Once this barrier has been breached, TJs found at the level of the stratum granulosum (SG) provide an additional barrier. Disruption of both physical barriers is thought to enable the uptake of allergens, irritants, and microbes by Langerhans cells (LC)/DCs. Keratinocytes produce AMPs as a chemical barrier in response to pathogen colonization/infection and tissue wounding to maintain skin homeostasis. The skin surface is colonized with a diverse array of microorganisms (microbiome barrier), which is thought to regulate local immune responses and to inhibit pathogenic microbes. The itch-scratch cycle and cutaneous microbes first promote immune responses from keratinocytes and, only if needed, from a number of hematopoietic cells, including T cells, neutrophils (*Neut cells*), eosinophils (*Eos*), LCs/DCs, NK cells, and mast cells. Collectively, these cells constitute the cutaneous immunologic barrier. PRRs regulate the function of all of these barriers (physical, chemical, microbiome, and immunologic). *SB*, Stratum basale; *SG*, stratum granulosum; *SS*, stratum spinosum. The figure is modified from Swindle EJ, Collins JE, Davies DE. Breakdown in epithelia barrier function in patients with asthma: identification of novel therapeutic approaches. *J Allergy Clin Immunol* 2009;124:23-36.

TLR family

TLRs are the most extensively studied innate receptors, with 10 identified to date in human subjects. TLRs are transmembrane proteins with a leucine-rich extracellular domain and a highly conserved intracellular signaling domain called the Toll-IL-1 receptor domain. They recognize a wide range of pathogen-associated molecular patterns (PAMPs), including LPS, lipoproteins, flagellin, and lipoteichoic acid (LTA) from bacteria and dsRNA, dsDNA, and unmethylated CpG DNA from bacteria and viruses (Table I).^{16,19-44} In addition to exogenous ligands, TLRs also recognize endogenous ligands released in response to tissue damage, which are collectively referred to as danger-associated molecular patterns (DAMPs). For example, fragmented hyaluronic acid and heat shock proteins can trigger TLR2 signaling,⁴⁵ and self-RNA can signal through TLR3.⁴⁶ Most TLRs signal through a myeloid differentiation primary response gene-88 (MyD88)-dependent pathway, activating nuclear factor κ B (NF- κ B) and resulting in the production of proinflammatory cytokines. Exceptions are TLR3 and TLR4, which use a MyD88-independent pathway that activates interferon regulatory factor 3 and results in IFN- β gene expression.¹⁹ Human keratinocytes express functional TLR1, TLR2, TLR3, TLR5, and TLR6,⁴⁷⁻⁵³ whereas there is some controversy about the expression of TLR4,^{52,54,55} TLR7, and TLR9.^{21,22,47,54} TLR10 expression has been reported in human keratinocytes, but its function is unclear.⁵⁶

Genetic variants in several TLRs have been associated with AD and disease severity, but only TLR2 has been studied in different ethnic populations (Table I).^{23,24,57} TLR2 was a major focus because it recognizes a number of *S aureus* cell-wall products, and patients with AD have a remarkable susceptibility for

cutaneous colonization (up to 90% compared with only 10% in healthy subjects⁵⁸) and infection with *S aureus*. Several groups have shown reduced TLR2 expression in circulating monocytes and keratinocytes from patients with AD.^{59,60} One group identified a *TLR2* missense mutation (R753Q) in AD subjects with a history of *S aureus* infections and increased total serum IgE levels.²⁵⁻²⁷ The fact that the R753Q single nucleotide polymorphism (SNP) localizes to the C-terminal end (cytoplasmic Toll-IL-1 receptor domain) of *TLR2* suggests that this variant might prevent the interaction of MyD88 and NF- κ B activation, which would severely inhibit the host response to bacterial colonization/infection. In line with this observation, the monocytes from patients with AD with the *TLR2* R753Q mutation have reduced expression of interferon-inducible protein 10, IL-8, IL-1 β , and TNF- α .²⁶ Recently, an SNP in the *TLR2* promoter region (A-16934T), which inhibits *TLR2* transcription, was identified that associates with disease severity, asthma, and family history of atopy in a subgroup of adult Japanese patients with AD with high total serum IgE levels.²⁸ In a German AD population the A allele in position -16934 was significantly associated with severe AD (scoring atopic dermatitis [SCORAD], >50).²⁹ In summary, these findings suggest that mutations in *TLR2* might play a role in patients with AD or certain AD subsets, but further studies are needed to clarify what the operative mutations are and whether they can be validated in more than 1 AD populations.

We observed reduced epidermal TLR2 immunoreactivity in skin biopsy specimens from patients with AD, which was more pronounced in lesional compared with nonlesional samples.¹⁷ As part of the Atopic Dermatitis Research Network, we examined 11 haplotype-tagging SNPs in the *TLR2* gene, including 1 associated

TABLE I. Summary of epidermal PRR functions and their relevance for AD

PRRs	Ligands	Keratinocyte expression	Function	SNPs in AD
TLRs	TLR2	Lipoprotein from bacteria, viruses, parasites (triacyl lipoprotein: TLR1/2; diacyl lipoprotein: TLR2/6) PGN from bacteria Endogenous ligands (Low molecular weight [<200 kDa] Hyaluronic acid; HSP; HBD3: TLR1/2; HMGB1)	✓ Skin: membrane staining (from SB to SG); cytosolic staining (SC)	↑ AMPs production ³⁸ ↑ TJ barrier ¹⁶ R753Q (intracellular signaling domain) ^{23,25-27} A-16934 T (promoter region) ^{28,29}
	TLR3	dsRNA from virus Endogenous ligands (self-RNA)	✓ Skin: cytosolic staining (from SB to SG)	↑ TNF- α production in wounded skin ³⁹ —
	TLR4	LPS from bacteria Envelope proteins of RSV Endogenous ligands (HBD2, HSP, S100A8/A9, fibrinogen)	X	↑ AMP, cytokine, and chemokine production ⁴¹ D299G (extracellular domain) ²³
	TLR9	CpG DNA from bacteria or virus	X	↑ Type I interferons ²² ↑ wound healing ⁴² C-1237T (promoter region) ²⁴
NLRs	NOD1	PGN from gram-negative bacteria	✓	↑ Cytokines ³¹ CARD domain, NOD domain, and LRR domain ³²
	NOD2	PGN from gram-positive bacteria	✓	↑ AMPs ³⁰ G2722C (LRR recognition domain) ³³
	NLRP1	MDP, anthrax lethal toxin	✓	↑ IL-1 β ³⁵ Promoter region ³⁴
	NLRP3	Uric acid crystal, silica, zymosan, ATP, asbestos	✓	↑ IL-1 β , IL-18 ^{36,43} —
RLRs	RIG-I	Short dsRNA (<300 bp polyI:C), 5' triphosphate dsRNA	✓	↑ IL-8 and IFN- β ²¹ —
	MDA5	Long dsRNA	✓	↑ IL-8 and IFN- β ²¹ —
CLRs	Dectin-1	β -glucan from fungi	✓	↑ IL-6, IL-8, IL-1 α ³⁷ —
PGLYRPs	PGLYRP3, 4	PGN from bacteria	✓	↑ AMPs ⁴¹ Bactericidal activities ⁴⁴ —

ATP, Adenosine triphosphate; HMGB1, high-mobility group box 1 protein; HSP, heat shock protein; MDP, muramyl dipeptide; polyI:C, polyinosinic-polycytidylic acid; RSV, respiratory syncytial virus; SB, stratum basale; SG, stratum granulosum.

✓, Expressed in keratinocytes; X, keratinocyte expression unclear.

with AD (R753Q), and observed no associations with AD in 2 American cohorts (European American and African American, unpublished data). Collectively, these findings suggest that local inflammatory mediators present in the skin of patients with AD play a role in the reduced epidermal TLR2 expression that we have observed. This contrasts with psoriasis, a T_H1/T_H17 -polarized disease, in which there is greater epidermal TLR2 staining.⁵³ Recently, pathogens have been shown to induce epigenetic changes in epithelial cells, and several microRNAs that affect TLR2 expression or function have been identified.^{61,62} For example, silencing of microRNA-146a in Langerhans cells enhances TLR2-dependent NF- κ B signaling.⁶³ Similarly, blocking the translation of microRNA-105 in human oral keratinocytes prevents TLR2 translation.⁶⁴ To date, none of these microRNAs have been investigated in patients with AD. In summary, these findings strongly suggest that genetic, acquired/epigenetic, or both TLR2 defects can contribute to the increased susceptibility of patients with AD to *S aureus* colonization/infection. Restoring TLR2 expression, function, or both might be a worthy therapeutic goal, but more studies need to be done to understand the basis for this defect.

NLR family

Nucleotide-binding oligomerization domain-containing protein. Nucleotide-binding oligomerization

domain-containing protein (NOD) 1 (or caspase activation and recruitment domain [CARD] 4) and NOD2 (or CARD15) are intracellular receptors. They recognize bacterial products through the C-terminal leucine-rich repeat (LRR) region and initiate downstream signaling cascades through their N-terminal CARDs that primarily involve the NF- κ B pathway.⁶⁵ Both NOD1 and NOD2 sense bacterial cell degradation peptidoglycan (PGN) fragments. However, NOD1 responds selectively to gram-negative bacteria, whereas NOD2 recognizes a motif common to all bacteria, making it more relevant for AD (Table I).⁶⁶ NOD2 also uses a PGN-independent mechanism to respond to both parasites and RNA viruses.⁶⁷ Human keratinocytes express NOD1 and NOD2, suggesting that they can recognize and elicit defense responses to intracellular bacterial products.^{30,31}

NOD1 is located on chromosome 7p14-p15, a region linked to atopy, whereas *NOD2* is located on chromosome 16q12, a locus associated with several autoimmune diseases.⁶⁸ *NOD2* SNPs have been associated with susceptibility to Crohn disease, a T_H1/T_H17 -driven inflammatory bowel disease.⁶⁹⁻⁷¹ The relevance of the NOD family in patients with T_H2 -driven inflammatory diseases, such as AD, has been recently suggested. In a German AD population-based and parent-offspring trio cohort, *NOD1* SNPs were associated with increased IgE levels, and more weakly with AD.³² In a similar population, a polymorphic *NOD2* allele was associated with an almost 2-fold risk of AD and modestly associated with increased IgE levels.³³ Lastly, a

NOD2 variant, R702W, and a rare *NOD1* haplotype were observed more frequently in patients with AD than in control subjects in a German population.³⁴ The functional consequences of these SNPs have not been investigated, but the fact that several of these SNPs are located within the LRR region where bacterial ligands are recognized, suggests that these SNPs may affect NOD function. Interestingly, *NOD2*-deficient mice have impaired clearance of *S aureus* after subcutaneous or intraperitoneal infection,^{72,73} suggesting that *NOD2* plays a critical role in clearance of *S aureus* infections in these locations. Whether *NOD2* SNPs are correlated with increased susceptibility to epicutaneous *S aureus* infections in patients with AD will need to be addressed. We have found that epidermal mRNA expression of *NOD2* in AD nonlesional skin is similar to that seen in control subjects,¹⁷ whereas a recent publication shows a slight increase in *NOD2* mRNA expression in keratinocytes isolated from AD nonlesional and lesional skin.⁷⁴

NOD-like receptor protein. NOD-like receptor proteins (NLRs) belong to a recently discovered intracellular PRR family, members of which respond to a broad array of ligands, including a number of DAMPs (eg, ATP and urate crystals) and exogenous agents (eg, nanoparticles, asbestos, and silica), through their C-terminal LRR motif.⁷⁵ Some NLRs (eg, NLRP1, NLRP3, and NLR family apoptosis inhibitory protein) form a multiprotein complex called the inflammasome.⁷⁵ Inflammasome activation leads to the production of IL-1 β and IL-18 through the actions of caspase-1. Gain-in-function mutations in the *NLRP3* gene and mutations in other inflammasome proteins that collectively result in an enhancement in IL-1 processing are the cause of a number of the autoinflammatory syndromes.^{75,76} Increased epidermal expression of the inflammasome-derived cytokine IL-1 β has been observed in patients with AD with *FLG* null mutations.⁷⁷

To date, 14 NLRs have been found in human subjects, but their function has been most extensively studied in myeloid cells. Primary human keratinocytes express NLRs 1 to 8, 10, 11, 13, and 14, whereas NLRP12 has only been detected in the human keratinocyte cell line HaCaT.^{78,79} The role of these NLRs in cutaneous innate immune system is still unclear. In primary human keratinocytes NLRP1 protein expression is enhanced by UVB exposure, resulting in the induction of its target gene, *IL-1 β* .³⁵ Recent studies of this gene in a German population identified a promoter polymorphism in *NLRP1* that may affect expression of other NLR genes, suggesting that these genes might be important collectively in AD pathogenesis.³⁴ In 2010, Grigoryev et al⁸⁰ found that *NLRP1* expression in the skin was inversely correlated with AD severity (based on Eczema Area and Severity Index scores), suggesting that local inflammation might inhibit NLRP1 expression or conversely that reduced expression of this protein promotes skin inflammation.

NLRP3 polymorphisms have been linked to food-induced anaphylaxis and aspirin-induced asthma in human subjects⁸¹ and contact hypersensitivity to trinitrochlorobenzene in mice.⁷⁸ Importantly, a number of AD triggers such as *S aureus*-derived hemolysins and lipoproteins^{82,83} as well as protease-activated house dust mite allergens³⁶ have been shown to activate NLRP3. Collectively, this suggests that activation of the NLRP3 inflammasome might play a role in AD pathogenesis.

NLRP12, in contrast to many other NLRs, inhibits activation of noncanonical NF- κ B, and as such, its function is still quite elusive.⁸⁴ An intronic SNP in *NLRP12* was weakly associated

with AD (eg, insignificant after Bonferroni correction).³⁴ Mice deficient in NLRP12 exhibit attenuated inflammatory responses in 2 contact dermatitis models but it had little effect in an OVA-induced asthma model.^{85,86} The neutrophils and dendritic cells (DCs) from these mice have reduced migratory capacity. These findings suggest that NLRP12 is a unique NLR that might attenuate inflammation and therefore loss-of-function mutations would lead to chronic inflammation as is observed in patients with AD. In summary, although there are suggestions that NOD and NLR proteins might be relevant for AD pathogenesis, much more work needs to be done to validate these preliminary observations.

RLR family

RIG-I protein and melanoma differentiation-associated gene (MDA) 5 are the best characterized members of the RLR family. These are intracellular innate receptors, containing a C-terminal helicase domain that recognizes viral genomic RNA and signals through an N-terminal CARD domain.²⁰ Activation of these receptors is crucial for eliciting antiviral responses, including induction of type I interferon gene expression. Human keratinocytes constitutively express RIG-I and MDA5, which are inducible on stimulation with synthetic dsRNA (polyinosinic-polycytidylic acid).^{21,87} The expression of RIG-I and MDA5 is significantly increased in the skin of psoriasis subjects,^{88,89} suggesting that these patients might have enhanced sensitivity to viruses. However, to the best of our knowledge, no study has evaluated whether RLRs play a role in AD pathogenesis.

CLR family

DC-associated C-type lectin 1 (dectin-1). CLR, as the name implies, contain 1 or more C-type lectin-like domains and recognize a broad array of structurally unrelated molecules from viruses, bacteria, and fungi. Most of the CLRs are expressed on hematopoietic cells, but dectin-1 and a member of the CLEC2 (C-type lectin domain family 2) family of CLRs called keratinocyte-associated C-type lectin (KACL) are expressed on human keratinocytes.^{37,48,90} The transmembrane receptor dectin-1 is a receptor for β -glucans⁹¹ found on fungal cell walls. Dectin-1 activates Src and Syk kinases, which leads to activation of NF- κ B and secretion of proinflammatory cytokines.⁹² Histamine enhances dectin-1-induced cytokine and chemokine production.^{37,50} Interestingly, mast cells isolated from patients with AD had less upregulation of dectin-1 mRNA in response to the yeast *Malassezia sympodialis* than those from healthy control subjects.⁹³ T_H1/T_H17 cytokines (IFN- γ , IFN- α , and IL17A) enhance the expression of dectin-1 on keratinocytes.⁴⁸ This is consistent with the observation that epidermal dectin-1 is highly expressed in skin biopsy specimens from chronic psoriatic lesions.⁴⁸ Costimulation of dectin-1 and TLR5 ligands on keratinocytes induces the production of IL-23, suggesting that this PRR might help initiate the immune cascade observed in patients with psoriasis.⁴⁸ The expression and function of dectin-1 in patients with AD have not been evaluated.

KACL. KACL belongs to the CLEC2 family, which is a subfamily of CLRs encoded by the human orphan gene *CLEC2A*.⁹⁴ KACL expression is largely limited to the skin where, and as the name implies, it is primarily expressed by the keratinocyte. KACL binds to NKp65, triggering natural killer (NK) cell cytolytic activity and cytokine secretion.

Interestingly, in both patients with psoriasis and those with AD, circulating NK cell numbers appear to be reduced, but only patients with AD have a defect in NK natural cytotoxicity.⁹⁵ These observations suggest that this newly identified innate receptor-ligand pair may contribute to NK cell-mediated skin immunosurveillance and that this pathway might be defective in patients with AD.

PGLYRPs

PGLYRPs are secreted proteins that function as bacterial PGN scavengers. Human subjects have 4 PGLYRPs (1-4), some of which have direct bactericidal activity mediated by their interaction with PGN on bacterial cell walls or by scavenging dissociated PGN. One function of bacterial PGN is to counteract the high osmotic pressure of the bacterial protoplast. Consequently, binding of a PGLYRP to the bacteria results in osmotic lysis. PGLYRP-1 is expressed primarily in the granules of polymorphonuclear leukocytes. PGLYRP-2 is an amidase that hydrolyses bacterial PGN and reduces its proinflammatory activity. PGLYRP-2 is expressed in keratinocytes in response to bacteria (eg, *Bacillus subtilis* and *Enterobacter cloacae*) or cytokines (eg, IL-1 β and TNF- α).⁹⁶ PGLYRP-3 and PGLYRP-4 are mainly expressed in tissues that are directly exposed to the environment, such as epithelial surfaces (eg, eyes, oral cavity, intestinal tract, and skin).^{97,98} We have found that *S aureus*-derived LTA enhances the expression of PGLYRP-3 in human keratinocytes.⁹⁹ Interestingly, *PGLYRP3* and *PGLYRP4* are located in the epidermal differentiation complex gene cluster on chromosome 1q21, where linkage has been observed for both psoriasis (*PSOR4*) and atopic dermatitis (*ATOD2*).¹⁰⁰ Several SNPs in *PGLYRP3* and *PGLYRP4* have been associated with psoriasis in a small family-based analysis, but the function of these SNPs is unknown.¹⁰⁰

Both PGLYRP3 and PGLYRP4 knockout (*pglyrp3*^{-/-} and *pglyrp4*^{-/-}) mice have a more dramatic response to oxazolone-induced contact dermatitis.¹⁰¹ In this model the knockout mice had reduced regulatory T-cell recruitment, enhanced T_H17 inflammation, and histology suggestive of greater keratinocyte proliferation.¹⁰¹ The mechanisms responsible for these observations are unclear. We have found that patients with AD have similar epidermal *PGLYRP3* and *PGLYRP4* expression compared with control subjects.¹⁷ Clearly much more work needs to be done to understand the importance of *PGLYRP3* and *PGLYRP4* in patients with AD.

PRRs REGULATE THE OTHER BARRIER ELEMENTS

PRRs and the physical barrier

The epidermal physical barrier is made up of the SC and TJs. It is assumed but still not proved that these 2 barrier structures interact dynamically. Whether these interactions will be compensatory is speculative because this would seem to confer an evolutionary advantage.¹⁰² A number of SC defects have been observed in patients with AD, and they occur on both an acquired and genetic basis.^{103,104} Our group recently found that patients with AD also have a defect in TJ barrier function and composition.^{105,106} The role that these barrier defects play in AD subphenotypes has been addressed in part for the SC protein filaggrin. For example, patients with AD with an *FLG* mutation have more severe and persistent disease,¹⁰⁷ greater susceptibility to skin infections with herpes simplex virus,¹⁰⁸ and higher

likelihood of having comorbid asthma.¹⁰⁹ For further information, please see the comprehensive overview of filaggrin and AD within this same *Journal of Allergy and Clinical Immunology* series by McAleer and Irvine.¹¹⁰

A critical feature of an effective innate immune response is the maintenance and repair of epithelial barriers. Very recently, it has been recognized that this might be mediated by the activations of PRRs expressed on epithelial cells. This was first shown in 2004, when Cario et al^{111,112} demonstrated that TLR2 expressed on intestinal epithelial cells was critical for the maintenance of the TJ barrier in a murine model of irritant-induced colitis. Several groups have confirmed this in human full-thickness epidermis (*ex vivo*) and in primary human keratinocytes (*in vitro*).^{16,17} This does not appear to be generalizable to all TLRs expressed in all epithelial beds, as TLR3 ligands disrupt TJ function in lung epithelium.¹¹³ Cutaneous wound repair is impaired in *MyD88*^{-/-}, *Tlr3*^{-/-}, and *Tlr9*^{-/-} mice.¹¹⁴⁻¹¹⁷ Our group observed that *Tlr2*^{-/-} mice have delayed and incomplete skin barrier recovery after tape stripping, a mechanical injury that mimics the itch-scratch cycle commonly observed in patients with AD.¹⁷ Importantly, a number of endogenous PRR ligands, including fragmented hyaluronic acid and heat shock proteins, are released in wound models such as tape stripping.¹¹⁸ The relative importance of wound-induced danger-associated molecular patterns versus the PAMPs from skin microbiota in these models is still unclear. Nevertheless, these findings suggest a dynamic crosstalk between the epidermal innate immune system and the physical barrier.

These studies raise a number of questions. Will all PRRs expressed on epithelial cells have an effect on barrier function and in what direction? Do PRRs affect barrier function only under pathologic conditions (eg, wound or pathogen), or do they also have a role in barrier homeostasis? What effect do these PRRs have on SC function? Will the barrier effects differ in different epithelium? Are PRR-mediated changes in barrier function a direct outcome of PRR signaling or secondary to downstream paracrine actions of innate immune mediators?

An innate immune response must be tightly controlled to achieve the right balance between the immediate response while limiting chronic inflammation, which is often more harmful than beneficial. Epithelial cells have developed several strategies to address these issues. One strategy is to control the subcellular and epithelial localization of PRRs within mature epithelium and thus determine where and when innate immune signals are initiated. Additionally, epithelial cells become tolerant in response to repeated or continuous exposure to TLR ligands. Intestinal epithelial cells exposed to either TLR2 or TLR4 ligands become hyporesponsive to both TLR2 and TLR4 ligands.¹¹⁹ This functional change was associated with decreased surface expression, with no change in total cellular expression. The internalization of innate receptors typically found on the cell membrane (eg, TLR1, 2, 4, 5 and 6) is another way to limit their activation. For example, TLR2 and TLR4 are primarily expressed in the cytoplasm of keratinocytes grown in culture and only migrate to the membrane after TNF- α and IFN- γ stimulation.⁵³ Similar findings were observed in lung and intestinal epithelial cells.^{120,121} The cellular localization of TLRs is also tightly controlled in mature epithelial structures. In human intestinal epithelium TLR5 is present on the basolateral membrane below the TJs, and consequently, it is activated

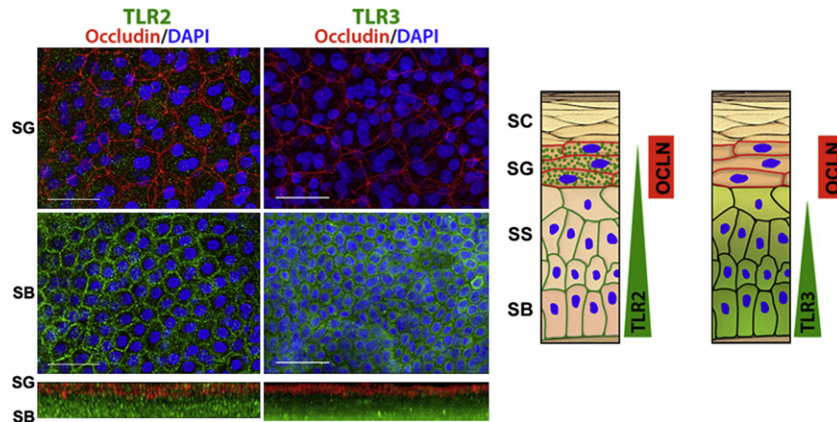


FIG 2. TLR2 and TLR3 are expressed below the TJs. Photomicrographs demonstrating the spatial relationship between TJs denoted by the presence of immunoreactivity for occludin (red) and 2 innate immune receptors highly relevant for AD (ie, TLR2 [left panel, green] and TLR3 [right panel, green]) are shown. Nuclei (4'-6-diamidino-2-penylindole dihydrochloride) are denoted by blue staining in these whole mounts of adult human epidermis (bars = 40 μ m). In the bottom panel Z-stack images through the stratum granulosum (SG) to the stratum basale (SB) are rotated 90° (thickness = 15 μ m). The image to the right summarizes the staining patterns and their intensity. Cell membrane expression of TLR2 is only observed below the TJs at the level of the stratum spinosum (SS) and SB. Faint cytosolic TLR2 immunoreactivity is observed in SG keratinocytes. The biologic significance of this intracellular staining is unclear. Similarly, the intracellular innate immune receptor TLR3 is found only in the cytoplasm of keratinocytes below the TJs (at the level of the SS and SB).

only when its ligand is applied basally and not when it is applied to the apical surface.¹²² It is believed that TJ integrity plays a key role in determining when TLR5 is activated by luminal contents. TLR2 and TLR4 are predominately expressed in the intestinal crypts, where the stem cells are located, and their expression is decreased at the apical surface of mature intestinal epithelium, where the contact with luminal bacterial is greater.¹²³

In the skin we and others have found that TLR1, TLR2, TLR3, and TLR5 are mainly expressed in basal or undifferentiated keratinocytes (eg, well below the SC and even below TJs).¹⁷ To more accurately visualize the subcellular localization of TLRs in mature human epidermis obtained from healthy donors, we examined Z-stack images taken with a 2-dimensional confocal microscope. We observed intense epidermal TLR1 (data not shown) and TLR2 (green) membrane immunoreactivity only in the epidermal layers beneath the TJs as indicated by occludin (red) staining (Cover Image and Fig 2). In addition, TLR3 (stained green), which is an intracellular TLR, was also only observed below the TJs and in fact was mainly detected in the basal layer (Fig 2). This observation indicates that TLR1, TLR2, and TLR3 in keratinocytes are primarily restricted to the stratum spinosum and stratum basale in healthy human epidermis, suggesting that activation of these innate immune pathways is prevented by an intact physical barrier. Studies are underway to look at the spatial relationship of other PRRs and TJs in healthy and diseased skin samples. Consequently, innate immune responses would only develop when there is a breach in both epidermal physical barriers (ie, SC and TJs) that would allow PAMPs to reach the PRRs. Patients with AD who have a chronic disruption of their physical barriers (SC and TJs) and who also have an impairment in the TLR2-mediated barrier repair process, would likely suffer from persistent low level inflammation that may in part explain the chronic inflammation observed in this disorder.

PRRs and the epidermal chemical barrier

In addition to establishing a formidable physical barrier, keratinocytes are the major producers of AMPs. This topic has been reviewed recently, and therefore we will limit our discussion to only those AMPs, which have been shown to affect epithelial functions.^{119,124}

AMPs serve as a chemical defense against cutaneous pathogens and are increasingly recognized for their effects on wound repair. The classical human AMPs are LL-37 (a cathelicidin) and the β -defensin family. A number of other proteins produced by keratinocytes, including ribonucleases (RNases), S100 family proteins (eg, S100A7, S100A8, and S100A9), dermcidin, and regenerating islet-derived protein 3 α (REG3 α), are also recognized for their antimicrobial actions.^{119,125} Human keratinocytes constitutively express human β -defensin (HBD) 1, whereas HBD2, HBD3, and LL-37 are produced in response to inflammatory cytokines or PRR signaling.^{30,126,127} For example, TLR2 signaling induced by microbes (*S aureus*,¹²⁸ *Staphylococcus epidermidis*,³⁸ and *Malassezia furfur*¹²⁹) or skin injury results in epidermal production of HBD2, HBD3, and LL-37.¹³⁰ RNase7 has broad antimicrobial activity and is constitutively expressed by human keratinocytes, but this is further enhanced by inflammation or bacterial exposure.¹³¹ Psoriasin (S100A7) is produced by differentiated keratinocytes and is most highly expressed around hair follicles and sebaceous units. Its expression is enhanced by IL-1, TNF- α , IL-17A, and IL-22 and repressed by IL-4 and histamine.^{132,133} Keratinocytes also express S100A8 and S100A9 that can exist as monomers or heterodimers (calprotectin).¹³⁴ Calprotectin is induced by inflammation (TNF- α and IFN- γ), as well as exposure to *S aureus* or *S epidermidis*.¹³⁵⁻¹³⁹ Dermcidin is constitutively produced by eccrine glands with broad antimicrobial activity.¹³⁹ It has proinflammatory actions inducing the epidermal production of cytokines and chemokines.¹⁴⁰ REG3 α is produced in response to wounding or IL-17A exposure

and not surprisingly is highly expressed in psoriatic skin lesions.¹²⁵ Lastly, filaggrin is proteolytically cleaved into the hygroscopic amino acids urocanic acid and pyrrolidone carboxylic acid, which are referred to as natural moisturizing factors. These filaggrin breakdown products slow the growth of *S aureus* and reduce its expression of immune evasion proteins.¹⁴¹ Strictly speaking, these breakdown products are not “antimicrobial.” Nevertheless, up to 40% of Northern Europeans with AD carry a *FLG* mutation and would therefore have reduced urocanic acid and pyrrolidone carboxylic acid levels, making them more susceptible to chronic *S aureus* colonization. This is an example in which an alteration in the physical barrier directly affects the chemical barrier.

In summary, there is a growing list of keratinocyte-derived antimicrobial proteins, only some of which are classical AMPs. This apparent redundancy might reflect subtle but important differences in epidermal expression patterns, antimicrobial specificity, and mechanism of action. Additionally, these classical and nonclassical AMPs have a number of nonantimicrobial actions, thus a more suitable name for them would be “host defense peptides.”¹¹⁹

Patients with AD are more susceptible to cutaneous fungal, viral, and bacterial pathogens. This observation has led to the speculation that patients with AD might have reduced epidermal expression of host defense peptides. Indeed, patients with AD have reduced expression of dermcidin in their sweat compared with control subjects, and this observation was most pronounced in patients with AD with a history of cutaneous viral and bacterial infections.¹⁴² Several groups have found reduced epidermal levels of LL-37, HBD2, and HBD3 in the lesional skin of patients with AD as compared to those with psoriasis.^{126,143,144} When comparing patients with AD with nonatopic healthy control subjects, the levels of many of these AMPs are in fact increased,^{132,144,145} which is consistent with the theory that AMPs are induced by infection, inflammation, and wounding. The differences observed between patients with AD and those with psoriasis are believed to be due to the inhibitory effects of T_H2 cytokines on the epidermal expression of a number of AMPs, including S100 family members, and the robust enhancement of these proteins by T_H17 cytokines found more commonly in the skin of psoriatics.^{15,133,146} Additionally, we have found that T_H2 cytokines inhibit TLR2-induced HBD2 and HBD3 expression in cultured keratinocytes (unpublished data).

The relatively low AMP levels observed in patients with AD is hypothesized to increase the risk of both *S aureus* and viral infections, such as the widespread herpes simplex infection called eczema herpeticum or eczema vaccinatum that develops as the consequence of exposure to the smallpox vaccine.¹⁴⁷⁻¹⁴⁹ In support of this theory, skin samples from patients with AD with eczema herpeticum express less HBD2, HBD3, and LL-37 than those of patients with AD who have never experienced this infection.^{148,150} Mice deficient in CRAMP (the murine equivalent of LL-37) developed significantly more skin pox lesions in response to vaccinia inoculation than wild-type mice,¹⁵¹ highlighting the importance of cathelicidins in combating cutaneous viral infections.¹⁵² Collectively, patients with AD have diminished epidermal production of host defense peptides compared with that seen in other inflammatory skin conditions, and these levels inversely correlate with the risk of skin infection. This reduced expression is probably due to

the combinatorial effects of reductions in epidermal PRR expression, signaling, or both; T_H2 cytokines in the tissue; and *FLG* null mutations.

In addition to their antimicrobial activities, AMPs have been found to play a role in physical barrier repair. This is not too surprising because these host defense peptides are commonly expressed in wound settings.^{128,153} Overexpression of HBD3 in skin wounds from Yorkshire pigs shortened wound closure times in an *S aureus*-infected diabetic wound model.¹⁵⁴ Similarly, neutralizing antibodies directed against LL-37 inhibited re-epithelialization in *in vitro* skin equivalents.¹⁵⁵ Mice deficient in the mouse orthologue of HBD3 (mBD14) have delayed skin barrier repair after tape stripping, as measured based on transepidermal water loss.¹⁵⁶ The novel AMP REG3 α enhances wound repair at least in part by inducing keratinocyte proliferation and differentiation.¹²⁵ Not surprisingly, a number of PRRs are induced in response to wounding. For example, the expression of CD14 and TLR2 are increased in the wound edge after skin injury.¹⁵⁷ This expression is dependent on the enzyme CYP27B1, which converts 25-hydroxyvitamin D to the active 1,25 dihydroxyvitamin D form. This highlighted an unexpected role for vitamin D in innate immune responses observed at sites of wounding, and suggests that therapeutic approaches that increase vitamin D levels might enhance the host innate immune response and help repair wounds. In support of this notion, patients with AD given oral vitamin D3 (cholecalciferol, 4000 IU/d for 21 days) expressed more LL-37 in their skin than patients who did not take vitamin D3.¹⁵⁸

In summary, innate immune receptors regulate the chemical barrier. Defects in epidermal PRR expression, signaling, or both have been observed in patients with AD. These defects compromise the antimicrobial and wound repair capabilities of the skin.

PRRs and the skin microbiome (microbial barrier)

Understanding the interactions between the diverse community of resident microbes and the host immune response is the charge of the National Institutes of Health-sponsored Human Microbiome Project, established in 2007. Through use of culture-independent methods, such as DNA pyrosequencing of the conserved 16S rRNA gene, which allows for phylogenetic analysis, a number of key observations have been made. The first observation is that the skin microbiome dwarfs the human genome in size, reflecting the trillions of bacteria, fungi, viruses, and small arthropods colonizing the skin surface. The second observation is that the diversity of the microbial flora in healthy human subjects varies based on anatomic site and stage of sexual maturation.^{157,159} The third observation is that microbes are not passive inhabitants but actively interact with epithelial cells and other host cells and affect the character of the host immune response both directly and indirectly.¹⁶⁰ In a seminal article Kong et al¹⁶¹ observed low bacterial diversity with enrichment of *S aureus* from an active AD lesion, which normalized during disease remission. After treatment, the diversity of the skin microbiome in patients with AD closely approximated that observed in the skin of healthy subjects. Interestingly, levels of the cutaneous commensal bacterium *S epidermidis* were also increased during AD flares, suggesting that this might be a compensatory mechanism to control the pathogenic microbe *S aureus*. In

support of this hypothesis, *S epidermidis* produces 2 AMPs called phenol-soluble modulin γ and δ .^{162,163} These peptides have pore-forming actions similar to those attributed to classical mammalian AMPs and are selective for several skin pathogens, including *S aureus*, group A *Streptococcus*, and *Escherichia coli* but with no effect on *S epidermidis*.^{163,164} Additionally, LTA released from *S epidermidis* inhibits skin inflammation during skin injury through a TLR2-dependent mechanism.³⁹ Lastly, a small molecule (<10 kDa) secreted by *S epidermidis* enhances HBD expression by human keratinocytes through TLR2 signaling.³⁸ These findings indicate that skin-resident microflora can inhibit the survival of skin pathogens while promoting the preservation of the normal skin flora. This skin homeostasis maintained by commensal bacteria is in part mediated by epithelial PRR and IL-1R signaling.¹⁶⁵ It has been hypothesized that the AD skin microbiome can be altered by exogenous (eg, topical cleaners, topical steroids, or topical/systemic antibiotics) or endogenous (eg, *FLG* or *LEKT1* mutations) factors and that this might alter the host responses to allergens or microbes or injury.

PRRs and the immunologic barrier

Keratinocytes produce a broad range of mediators both constitutively and in response to the environment.¹⁶⁶⁻¹⁶⁸ Many of these help maintain a formidable physical barrier, establish an effective chemical barrier, help balance the harmony between commensal and pathogenic skin flora, and both recruit and activate immune cells to shape local and systemic immune responses. We can no longer consider the epidermis a passive responder to the signals coming from infiltrating hematopoietic cells, but must now consider its importance in determining when, how, and in what manner to initiate an adaptive immune response. AD is considered a T_H2 -skewed disease, but until recently, the primordial events that led to this T helper polarization pattern were unclear. The complementary and sometimes synergistic actions of the keratinocyte-derived pro- T_H2 cytokines, including thymic stromal lymphopoietin (TSLP), IL-25, and IL-33, drive the immune response to surface antigens toward a T_H2 profile. For example, TSLP acts as an activator of $CD11c^+$ DCs, promoting their capacity to induce T_H2 memory cells and enhancing their production of T_H2 -promoting chemokines.¹⁶⁶ TSLP is highly expressed in acute and chronic AD epidermis, whereas its expression level is not detectable in nonlesional skin of patients with AD or healthy control subjects.¹⁶⁹ Genetic variants in *TSLP* associate with the presence of AD and even more significantly in patients with AD who have a history of eczema herpeticum.¹⁷⁰ A number of murine models have demonstrated the importance of epidermally derived TSLP in AD pathogenesis and have been reviewed elsewhere.¹⁷¹ In addition to TSLP, IL-25 and the IL-33 receptor (ST2) are also overexpressed in AD skin.^{172,173} Infusion of IL-25 (or IL-17E) results in the production of the T_H2 cytokines IL-4, IL-5, and IL-13; eosinophilia; and increased serum IgE levels in mice.¹⁷⁴ There is equally strong evidence that IL-33 plays a critical role in activating T_H2 cells.^{169,175}

Innate immune activation is a major pathway responsible for the production of these pro- T_H2 cytokines. For example, the simple act of disrupting the skin barrier by means of tape stripping leads to epidermal TSLP and IL-33 production.^{118,176,177} Whether IL-25 production is similarly induced by barrier

disruption or tissue injury has not been addressed in the literature. Keratinocytes stimulated with the TLR2/6-dependent ligand, *S aureus*-derived diacylated lipopeptide or flagellin, the TLR5-specific ligand, induce the expression of TSLP.^{49,178} The TLR3 agonist polyinosinic-polycytidylic acid also induces TSLP production, and this effect is augmented by T_H2 cytokines (IL-4 and IL-13) but reduced by T_H1 (IFN- γ), regulatory T (TGF- β), or T_H17 (IL-17A) cytokines.¹⁷⁹ The epidermal expression of TSLP is induced by allergen-derived proteases, as well as endogenous proteases, such as kallikrein 5, which is overexpressed in patients with AD. This TSLP production is mediated by protease-activated receptor 2.^{180,181} IL-33 and its receptor, ST2, are enhanced in murine epidermis after epicutaneous exposure to staphylococcal enterotoxin B and allergens (ovalbumin).¹⁷³ Lastly, reductions in the SC barrier protein filaggrin are associated with increased epidermal production of TSLP and IL-33.^{173,182} DCs are probably the major source of IL-25 in the skin, whereas keratinocytes express low levels of *IL25* mRNA.¹⁸³ To our knowledge, there is no study examining the induction and regulation of IL-25 in skin. Collectively, these findings demonstrate that the activation of the epidermal innate immune system by pathogens, allergens, injurious stimuli, or barrier disruption can initiate a cascade that would favor a T_H2 response, as seen in patients with AD.

Interestingly, recent findings suggest that these pro- T_H2 cytokines might feedback on the innate immune system, affecting the expression, function, or both of the PRRs. For example, activation of the IL-33 receptor inhibits TLR4 signaling in macrophages by sequestering the adaptor proteins MyD88 and Mal.¹⁸⁴ ST2 is also a negative regulator of TLR2 signaling.¹⁸⁵ The production of HBD2 and filaggrin by human keratinocytes is inhibited by IL-33¹⁸⁶ and IL-25,¹⁸³ respectively. However, treatment with these pro- T_H2 cytokines did not significantly affect epidermal TJ, suggesting that they do not globally affect keratinocyte biology but specifically affect differentiation or AMP production. In summary, these pro- T_H2 cytokines are considered "alarmins" released in response to skin injury and infection which is at least in part mediated by PRR pathways. Dysregulated PRR signaling might also interfere with the production of these keratinocyte-derived pro- T_H2 cytokines.

CONCLUSION

Epithelial innate immune responses are not simply "inflammatory" with the sole purpose of pathogen clearance. Recent evidence indicates that this immediate response system might also enhance cutaneous physical, chemical, microbial, and immunologic barriers. In patients with AD, each of these elements is compromised, but what is primary and what is secondary remains unclear. More work needs to be done to fully understand how innate immune signaling pathways mediate some of these effects and how the innate immune system is altered by the character of local T-cell inflammation. Although the leading hypotheses to explain AD pathogenesis incorporate a role for both barrier and adaptive immune defects, it is becoming increasingly clear that defects in epidermal PRR function might deserve a seat at this table. As we clarify the relative hierarchy of these abnormalities we will likely see the development of new therapeutic strategies for this common inflammatory disorder.

What do we know?

- There are 4 epidermal elements that help maintain skin homeostasis. They are physical, chemical, microbial, and immunologic barriers, and most of these have been shown to be defective in patients with AD.
- Epidermal PRR modulate the skin barrier and maintain the balance between inflammation and host defenses.
- A number of acquired or genetic defects in PRR have been observed in patients with AD and some of these strongly associate with specific disease subphenotypes and susceptibility to microbial colonization/infection.

What is still unknown?

- The mechanisms by which PRRs regulate TJ barrier integrity the diversity of the microbiome and immunologic barriers are still unknown.
- How innate immune responses to microbes differ in patients with AD compared with healthy control subjects.
- A better understanding of the mechanisms responsible for the reduced expression and function of PRRs is needed and will pave the way for new treatment approaches for patients with AD.

REFERENCES

- Laughter D, Istvan JA, Tofte SJ, Hanifin JM. The prevalence of atopic dermatitis in Oregon schoolchildren. *J Am Acad Dermatol* 2000;43:649-55.
- Odhambo JA, Williams HC, Clayton TO, Robertson CF, Asher MI. Global variations in prevalence of eczema symptoms in children from ISAAC Phase Three. *J Allergy Clin Immunol* 2009;124:1251-8.e23.
- Boguniewicz M, Leung DY. Atopic dermatitis: a disease of altered skin barrier and immune dysregulation. *Immunol Rev* 2011;242:233-46.
- Holgate ST. Epithelium dysfunction in asthma. *J Allergy Clin Immunol* 2007;120:1233-46.
- Elias PM, Steinhoff M. "Outside-to-inside" (and now back to "outside") pathogenic mechanisms in atopic dermatitis. *J Invest Dermatol* 2008;128:1067-70.
- De Benedetto A, Kubo A, Beck LA. Skin barrier disruption: a requirement for allergen sensitization? *J Invest Dermatol* 2012;132:949-63.
- Kubo A, Nagao K, Amagai M. Epidermal barrier dysfunction and cutaneous sensitization in atopic diseases. *J Clin Invest* 2012;122:440-7.
- Howell MD, Kim BE, Gao P, Grant AV, Boguniewicz M, DeBenedetto A, et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. *J Allergy Clin Immunol* 2009;124:R7-12.
- Kim BE, Leung DY, Boguniewicz M, Howell MD. Loricrin and involucrin expression is down-regulated by Th2 cytokines through STAT-6. *Clin Immunol* 2008;126:332-7.
- Sehra S, Yao Y, Howell MD, Nguyen ET, Kansas GS, Leung DY, et al. IL-4 regulates skin homeostasis and the predisposition toward allergic skin inflammation. *J Immunol* 2010;184:3186-90.
- Beck LA, Boguniewicz M, Hata T, Schneider LC, Hanifin J, Gallo R, et al. Phenotype of atopic dermatitis subjects with a history of eczema herpeticum. *J Allergy Clin Immunol* 2009;124:260-9, e1-7.
- Oyoshi MK, Murphy GF, Geha RS. Filaggrin-deficient mice exhibit TH17-dominated skin inflammation and permissiveness to epicutaneous sensitization with protein antigen. *J Allergy Clin Immunol* 2009;124:485-93.e1.
- Howell MD, Fairchild HR, Kim BE, Bin L, Boguniewicz M, Redzic JS, et al. Th2 cytokines act on S100/A11 to downregulate keratinocyte differentiation. *J Invest Dermatol* 2008;128:2248-58.
- Cornelissen C, Marquardt Y, Czaja K, Wenzel J, Frank J, Luscher-Firzlaff J, et al. IL-31 regulates differentiation and filaggrin expression in human organotypic skin models. *J Allergy Clin Immunol* 2012;129:426-33, e1-8.
- Howell MD, Boguniewicz M, Pastore S, Novak N, Bieber T, Girolomoni G, et al. Mechanism of HBD-3 deficiency in atopic dermatitis. *Clin Immunol* 2006;121:332-8.
- Yuki T, Yoshida H, Akazawa Y, Komiya A, Sugiyama Y, Inoue S. Activation of TLR2 enhances tight junction barrier in epidermal keratinocytes. *J Immunol* 2011;187:3230-7.
- Kuo IH, Carpenter-Mendini A, Yoshida T, McGirt LY, Ivanov AI, Barnes KC, et al. Activation of epidermal Toll-like receptor 2 enhances tight junction function: implications for atopic dermatitis and skin barrier repair. *J Invest Dermatol* 2012 [Epub ahead of print].
- Grice EA, Segre JA. The skin microbiome. *Nat Rev Microbiol* 2011;9:244-53.
- Kumagai Y, Akira S. Identification and functions of pattern-recognition receptors. *J Allergy Clin Immunol* 2010;125:985-92.
- Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell* 2010;140:805-20.
- Kalali BN, Kollisch G, Mages J, Muller T, Bauer S, Wagner H, et al. Double-stranded RNA induces an antiviral defense status in epidermal keratinocytes through TLR3-, PKR-, and MDA5/RIG-I-mediated differential signaling. *J Immunol* 2008;181:2694-704.
- Morizane S, Yamasaki K, Muhleisen B, Kotol PF, Murakami M, Aoyama Y, et al. Cathelicidin antimicrobial peptide LL-37 in psoriasis enables keratinocyte reactivity against TLR9 ligands. *J Invest Dermatol* 2012;132:135-43.
- Salpietro C, Rigoli L, Miraglia Del Giudice M, Cuppari C, Di Bella C, Salpietro A, et al. TLR2 and TLR4 gene polymorphisms and atopic dermatitis in Italian children: a multicenter study. *Int J Immunopathol Pharmacol* 2011;24:33-40.
- Novak N, Yu CF, Bussmann C, Maintz L, Peng WM, Hart J, et al. Putative association of a TLR9 promoter polymorphism with atopic eczema. *Allergy* 2007;62:766-72.
- Niebuhr M, Langnickel J, Sigel S, Werfel T. Dysregulation of CD36 upon TLR-2 stimulation in monocytes from patients with atopic dermatitis and the TLR2 R753Q polymorphism. *Exp Dermatol* 2010;19:e296-8.
- Mrabet-Dahbi S, Dalpke AH, Niebuhr M, Frey M, Draing C, Brand S, et al. The Toll-like receptor 2 R753Q mutation modifies cytokine production and Toll-like receptor expression in atopic dermatitis. *J Allergy Clin Immunol* 2008;121:1013-9.
- Ahmad-Nejad P, Mrabet-Dahbi S, Breuer K, Klotz M, Werfel T, Herz U, et al. The Toll-like receptor 2 R753Q polymorphism defines a subgroup of patients with atopic dermatitis having severe phenotype. *J Allergy Clin Immunol* 2004;113:565-7.
- Oh DY, Schumann RR, Hamann L, Neumann K, Worm M, Heine G. Association of the toll-like receptor 2 A-16934T promoter polymorphism with severe atopic dermatitis. *Allergy* 2009;64:1608-15.
- Potaczek DP, Nastalek M, Okumura K, Wojas-Pelc A, Undas A, Nishiyama C. An association of TLR2-16934A >T polymorphism and severity/phenotype of atopic dermatitis. *J Eur Acad Dermatol Venereol* 2011;25:715-21.
- Voss E, Wehkamp J, Wehkamp K, Stange EF, Schroder JM, Harder J. NOD2/CARD15 mediates induction of the antimicrobial peptide human beta-defensin-2. *J Biol Chem* 2006;281:2005-11.
- Harder J, Nunez G. Functional expression of the intracellular pattern recognition receptor NOD1 in human keratinocytes. *J Invest Dermatol* 2009;129:1299-302.
- Weidinger S, Klopp N, Rummel L, Wagenpfeil S, Novak N, Baurecht HJ, et al. Association of NOD1 polymorphisms with atopic eczema and related phenotypes. *J Allergy Clin Immunol* 2005;116:177-84.
- Kabesch M, Peters W, Carr D, Leupold W, Weiland SK, von Mutius E. Association between polymorphisms in caspase recruitment domain containing protein 15 and allergy in two German populations. *J Allergy Clin Immunol* 2003;111:813-7.
- Macaluso F, Nothnagel M, Parwez Q, Petrasch-Parwez E, Bechara FG, Epplen JT, et al. Polymorphisms in NACHT-LRR (NLR) genes in atopic dermatitis. *Exp Dermatol* 2007;16:692-8.
- Feldmeyer L, Keller M, Niklaus G, Hohl D, Werner S, Beer HD. The inflammasome mediates UVB-induced activation and secretion of interleukin-1beta by keratinocytes. *Curr Biol* 2007;17:1140-5.
- Dai X, Sayama K, Tohyama M, Shirakata Y, Hanakawa Y, Tokumaru S, et al. Mite allergen is a danger signal for the skin via activation of inflammasome in keratinocytes. *J Allergy Clin Immunol* 2011;127:806-14, e1-4.
- Hau CS, Tada Y, Shibata S, Uratsuji H, Asano Y, Sugaya M, et al. High calcium, ATP, and poly(I: C) augment the immune response to beta-glucan in normal human epidermal keratinocytes. *J Invest Dermatol* 2011;131:2255-62.
- Lai Y, Cogen AL, Radek KA, Park HJ, Macleod DT, Leightle A, et al. Activation of TLR2 by a small molecule produced by *Staphylococcus epidermidis* increases antimicrobial defense against bacterial skin infections. *J Invest Dermatol* 2010;130:2211-21.
- Lai Y, Di Nardo A, Nakatsuji T, Leightle A, Yang Y, Cogen AL, et al. Commensal bacteria regulate Toll-like receptor 3-dependent inflammation after skin injury. *Nat Med* 2009;15:1377-82.
- Jin H, Kumar L, Mathias C, Zurakowski D, Oettgen H, Gorelik L, et al. Toll-like receptor 2 is important for the T(H)1 response to cutaneous sensitization. *J Allergy Clin Immunol* 2009;123:875-82.e1.

41. Lee HM, Shin DM, Choi DK, Lee ZW, Kim KH, Yuk JM, et al. Innate immune responses to *Mycobacterium ulcerans* via toll-like receptors and dectin-1 in human keratinocytes. *Cell Microbiol* 2009;11:678-92.
42. Yamamoto M, Sato T, Beren J, Verthelyi D, Klinman DM. The acceleration of wound healing in primates by the local administration of immunostimulatory CpG oligonucleotides. *Biomaterials* 2011;32:4238-42.
43. Cho KA, Suh JW, Lee KH, Kang JL, Woo SY. IL-17 and IL-22 enhance skin inflammation by stimulating the secretion of IL-1beta by keratinocytes via the ROS-NLRP3-caspase-1 pathway. *Int Immunol* 2012;24:147-58.
44. Royet J, Dziarski R. Peptidoglycan recognition proteins: pleiotropic sensors and effectors of antimicrobial defences. *Nat Rev Microbiol* 2007;5:264-77.
45. Jiang D, Liang J, Fan J, Yu S, Chen S, Luo Y, et al. Regulation of lung injury and repair by Toll-like receptors and hyaluronan. *Nat Med* 2005;11:1173-9.
46. Bernard JJ, Cowing-Zitron C, Nakatsuji T, Muehleisen B, Muto J, Borkowski AW, et al. Ultraviolet radiation damages self noncoding RNA and is detected by TLR3. *Nat Med* 2012 [Epub ahead of print].
47. Lebre MC, van der Aar AM, van Baarsen L, van Capel TM, Schuitmaker JH, Kapsenberg ML, et al. Human keratinocytes express functional Toll-like receptor 3, 4, 5, and 9. *J Invest Dermatol* 2007;127:331-41.
48. de Koning HD, Rodijk-Olthuis D, van Vlijmen-Willems IM, Joosten LA, Netea MG, Schalkwijk J, et al. A comprehensive analysis of pattern recognition receptors in normal and inflamed human epidermis: upregulation of dectin-1 in psoriasis. *J Invest Dermatol* 2010;130:2611-20.
49. Le TA, Takai T, Vu AT, Kinoshita H, Chen X, Ikeda S, et al. Flagellin induces the expression of thymic stromal lymphopoietin in human keratinocytes via toll-like receptor 5. *Int Arch Allergy Immunol* 2011;155:31-7.
50. Kobayashi M, Yoshiki R, Sakabe J, Kabashima K, Nakamura M, Tokura Y. Expression of toll-like receptor 2, NOD2 and dectin-1 and stimulatory effects of their ligands and histamine in normal human keratinocytes. *Br J Dermatol* 2009;160:297-304.
51. Pivarski A, Bodai L, Rethi B, Kenderessy-Szabo A, Koreck A, Szell M, et al. Expression and function of Toll-like receptors 2 and 4 in human keratinocytes. *Int Immunol* 2003;15:721-30.
52. Kawai K, Shimura H, Minagawa M, Ito A, Tomiyama K, Ito M. Expression of functional Toll-like receptor 2 on human epidermal keratinocytes. *J Dermatol Sci* 2002;30:185-94.
53. Begon E, Michel L, Flageul B, Beaudoin I, Jean-Louis F, Bachelez H, et al. Expression, subcellular localization and cytokine modulation of Toll-like receptors (TLRs) in normal human keratinocytes: TLR2 up-regulation in psoriatic skin. *Eur J Dermatol* 2007;17:497-506.
54. Mempel M, Voelcker V, Kollisch G, Plank C, Rad R, Gerhard M, et al. Toll-like receptor expression in human keratinocytes: nuclear factor kappaB controlled gene activation by *Staphylococcus aureus* is toll-like receptor 2 but not toll-like receptor 4 or platelet activating factor receptor dependent. *J Invest Dermatol* 2003;121:1389-96.
55. Song PI, Park YM, Abraham T, Harten B, Zivony A, Neparidze N, et al. Human keratinocytes express functional CD14 and toll-like receptor 4. *J Invest Dermatol* 2002;119:424-32.
56. Kollisch G, Kalali BN, Voelcker V, Wallich R, Behrendt H, Ring J, et al. Various members of the Toll-like receptor family contribute to the innate immune response of human epidermal keratinocytes. *Immunology* 2005;114:531-41.
57. Moller-Larsen S, Nyegaard M, Haagerup A, Vestbo J, Kruse TA, Borglum AD. Association analysis identifies TLR7 and TLR8 as novel risk genes in asthma and related disorders. *Thorax* 2008;63:1064-9.
58. Leyden JJ, Marples RR, Kligman AM. *Staphylococcus aureus* in the lesions of atopic dermatitis. *Br J Dermatol* 1974;90:525-30.
59. Hasannejad H, Takahashi R, Kimishima M, Hayakawa K, Shiohara T. Selective impairment of Toll-like receptor 2-mediated proinflammatory cytokine production by monocytes from patients with atopic dermatitis. *J Allergy Clin Immunol* 2007;120:69-75.
60. Niebuh M, Lutat C, Sigel S, Werfel T. Impaired TLR-2 expression and TLR-2-mediated cytokine secretion in macrophages from patients with atopic dermatitis. *Allergy* 2009;64:1580-7.
61. Sepulveda AR, Yao Y, Yan W, Park DI, Kim JJ, Gooding W, et al. CpG methylation and reduced expression of O6-methylguanine DNA methyltransferase is associated with *Helicobacter pylori* infection. *Gastroenterology* 2010;138:1836-44.
62. O'Neill LA, Sheedy FJ, McCoy CE. MicroRNAs: the fine-tuners of Toll-like receptor signalling. *Nat Rev Immunol* 2011;11:163-75.
63. Jurkin J, Schichl YM, Koeffel R, Bauer T, Richter S, Konradi S, et al. miR-146a is differentially expressed by myeloid dendritic cell subsets and desensitizes cells to TLR2-dependent activation. *J Immunol* 2010;184:4955-65.
64. Benakanakere MR, Li Q, Eskin MA, Singh AV, Zhao J, Galicia JC, et al. Modulation of TLR2 protein expression by miR-105 in human oral keratinocytes. *J Biol Chem* 2009;284:23107-15.
65. Strober W, Murray PJ, Kitani A, Watanabe T. Signalling pathways and molecular interactions of NOD1 and NOD2. *Nat Rev Immunol* 2006;6:9-20.
66. Girardin SE, Philpott DJ. Mini-review: the role of peptidoglycan recognition in innate immunity. *Eur J Immunol* 2004;34:1777-82.
67. Magalhaes JG, Sorbara MT, Girardin SE, Philpott DJ. What is new with Nods? *Curr Opin Immunol* 2011;23:29-34.
68. Laitinen T, Daly MJ, Rioux JD, Kauppi P, Laprise C, Petais T, et al. A susceptibility locus for asthma-related traits on chromosome 7 revealed by genome-wide scan in a founder population. *Nat Genet* 2001;28:87-91.
69. Hampe J, Cuthbert A, Croucher PJ, Mirza MM, Mascheretti S, Fisher S, et al. Association between insertion mutation in NOD2 gene and Crohn's disease in German and British populations. *Lancet* 2001;357:1925-8.
70. Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001;411:599-603.
71. Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, et al. A frame-shift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001;411:603-6.
72. Deshmukh HS, Hamburger JB, Ahn SH, McCafferty DG, Yang SR, Fowler VG Jr. Critical role of NOD2 in regulating the immune response to *Staphylococcus aureus*. *Infect Immun* 2009;77:1376-82.
73. Hruz P, Zinkernagel AS, Jenikova G, Botwin GJ, Hugot JP, Karin M, et al. NOD2 contributes to cutaneous defense against *Staphylococcus aureus* through alpha-toxin-dependent innate immune activation. *Proc Natl Acad Sci U S A* 2009;106:12873-8.
74. Rebane A, Zimmermann M, Aab A, Baurecht H, Koreck A, Karelson M, et al. Mechanisms of IFN-gamma-induced apoptosis of human skin keratinocytes in patients with atopic dermatitis. *J Allergy Clin Immunol* 2012;129:1297-306.
75. Davis BK, Wen H, Ting JP. The inflammasome NLRs in immunity, inflammation, and associated diseases. *Annu Rev Immunol* 2011;29:707-35.
76. Goldbach-Mansky R, Kastner DL. Autoinflammation: the prominent role of IL-1 in monogenic autoinflammatory diseases and implications for common illnesses. *J Allergy Clin Immunol* 2009;124:1141-51.
77. Kezic S, O'Regan GM, Lutter R, Jakasa I, Koster ES, Saunders S, et al. Filaggrin loss-of-function mutations are associated with enhanced expression of IL-1 cytokines in the stratum corneum of patients with atopic dermatitis and in a murine model of filaggrin deficiency. *J Allergy Clin Immunol* 2012;129:1031-9.e1.
78. Watanabe H, Gaide O, Petrilli V, Martinon F, Contassot E, Roques S, et al. Activation of the IL-1beta-processing inflammasome is involved in contact hypersensitivity. *J Invest Dermatol* 2007;127:1956-63.
79. Kummer JA, Broekhuizen R, Everett H, Agostini L, Kuijk L, Martinon F, et al. Inflammasome components NALP 1 and 3 show distinct but separate expression profiles in human tissues suggesting a site-specific role in the inflammatory response. *J Histochem Cytochem* 2007;55:443-52.
80. Grigoryev DN, Howell MD, Watkins TN, Chen YC, Cheadle C, Boguniewicz M, et al. Vaccinia virus-specific molecular signature in atopic dermatitis skin. *J Allergy Clin Immunol* 2010;125:153-9.e28.
81. Hitomi Y, Ebisawa M, Tomikawa M, Imai T, Komata T, Hirota T, et al. Associations of functional NLRP3 polymorphisms with susceptibility to food-induced anaphylaxis and aspirin-induced asthma. *J Allergy Clin Immunol* 2009;124:779-85.e6.
82. Craven RR, Gao X, Allen IC, Gris D, Bubeck Wardenburg J, McElvania-Tekippe E, et al. *Staphylococcus aureus* alpha-hemolysin activates the NLRP3-inflammasome in human and mouse monocytic cells. *PLoS One* 2009;4:e7446.
83. Munoz-Planillo R, Franchi L, Miller LS, Nunez G. A critical role for hemolysins and bacterial lipoproteins in *Staphylococcus aureus*-induced activation of the Nlrp3 inflammasome. *J Immunol* 2009;183:3942-8.
84. Lich JD, Williams KL, Moore CB, Arthur JC, Davis BK, Taxman DJ, et al. Monarch-1 suppresses non-canonical NF-kappaB activation and p52-dependent chemokine expression in monocytes. *J Immunol* 2007;178:1256-60.
85. Arthur JC, Lich JD, Ye Z, Allen IC, Gris D, Wilson JE, et al. Cutting edge: NLRP12 controls dendritic and myeloid cell migration to affect contact hypersensitivity. *J Immunol* 2010;185:4515-9.
86. Allen IC, Lich JD, Arthur JC, Jania CM, Roberts RA, Callaway JB, et al. Characterization of NLRP12 during the development of allergic airway disease in mice. *PLoS One* 2012;7:e30612.
87. Vu AT, Chen X, Xie Y, Kamijo S, Ushio H, Kawasaki J, et al. Extracellular double-stranded RNA induces TSLP via an endosomal acidification- and NF-kappaB-dependent pathway in human keratinocytes. *J Invest Dermatol* 2011;131:2205-12.
88. Prens EP, Kant M, van Dijk G, van der Wel LI, Mourits S, van der Fits L. IFN-alpha enhances poly-IC responses in human keratinocytes by inducing expression of cytosolic innate RNA receptors: relevance for psoriasis. *J Invest Dermatol* 2008;128:932-8.

89. Kitamura H, Matsuzaki Y, Kimura K, Nakano H, Imaizumi T, Satoh K, et al. Cytokine modulation of retinoic acid-inducible gene-I (RIG-I) expression in human epidermal keratinocytes. *J Dermatol Sci* 2007;45:127-34.
90. Spreu J, Kuttruff S, Stejfova V, Dennehy KM, Schitteck B, Steinle A. Interaction of C-type lectin-like receptors NKp65 and KACL facilitates dedicated immune recognition of human keratinocytes. *Proc Natl Acad Sci U S A* 2010;107:5100-5.
91. Brown GD, Herre J, Williams DL, Willment JA, Marshall AS, Gordon S. Dectin-1 mediates the biological effects of beta-glucans. *J Exp Med* 2003;197:1119-24.
92. Reid DM, Gow NA, Brown GD. Pattern recognition: recent insights from Dectin-1. *Curr Opin Immunol* 2009;21:30-7.
93. Ribbing C, Engblom C, Lappalainen J, Lindstedt K, Kovanen PT, Karlsson MA, et al. Mast cells generated from patients with atopic eczema have enhanced levels of granule mediators and an impaired Dectin-1 expression. *Allergy* 2011;66:110-9.
94. Spreu J, Kienle EC, Schrage B, Steinle A. CLEC2A: a novel, alternatively spliced and skin-associated member of the NKC-encoded AICL-CD69-LLT1 family. *Immunogenetics* 2007;59:903-12.
95. Luci C, Gaudy-Marqueste C, Rouzaire P, Audonnet S, Cognet C, Hennino A, et al. Peripheral natural killer cells exhibit qualitative and quantitative changes in patients with psoriasis and atopic dermatitis. *Br J Dermatol* 2012;166:789-96.
96. Wang H, Gupta D, Li X, Dziarski R. Peptidoglycan recognition protein 2 (N-acetylmuramoyl-L-Ala amidase) is induced in keratinocytes by bacteria through the p38 kinase pathway. *Infect Immun* 2005;73:7216-25.
97. Lu X, Wang M, Qi J, Wang H, Li X, Gupta D, et al. Peptidoglycan recognition proteins are a new class of human bactericidal proteins. *J Biol Chem* 2006;281:5895-907.
98. Mathur P, Murray B, Crowell T, Gardner H, Allaire N, Hsu YM, et al. Murine peptidoglycan recognition proteins Pglyrp1alpha and Pglyrp1beta are encoded in the epidermal differentiation complex and are expressed in epidermal and hematopoietic tissues. *Genomics* 2004;83:1151-63.
99. McGirt LY, Brummet ME, Bankova LG, Beck LA. Keratinocyte expression of receptors relevant for the innate immune response to *Staphylococcus aureus* in subjects with atopic dermatitis [abstract]. *J Invest Dermatol* 2006;126:S116.
100. Sun C, Mathur P, Dupuis J, Tizad R, Ticho B, Crowell T, et al. Peptidoglycan recognition proteins Pglyrp3 and Pglyrp4 are encoded from the epidermal differentiation complex and are candidate genes for the Psors4 locus on chromosome 1q21. *Hum Genet* 2006;119:113-25.
101. Park SY, Gupta D, Kim CH, Dziarski R. Differential effects of peptidoglycan recognition proteins on experimental atopic and contact dermatitis mediated by Treg and Th17 cells. *PLoS One* 2011;6:e24961.
102. Gruber R, Elias PM, Crumrine D, Lin TK, Brandner JM, Hachem JP, et al. Filaggrin genotype in ichthyosis vulgaris predicts abnormalities in epidermal structure and function. *Am J Pathol* 2011;178:2252-63.
103. O'Regan GM, Sandilands A, McLean WH, Irvine AD. Filaggrin in atopic dermatitis. *J Allergy Clin Immunol* 2009;124:R2-6.
104. Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006;38:441-6.
105. De Benedetto A, Rafaels NM, McGirt LY, Ivanov AI, Georas SN, Cheadle C, et al. Tight junction defects in patients with atopic dermatitis. *J Allergy Clin Immunol* 2011;127:773-86, e1-7.
106. De Benedetto A, Slifka MK, Rafaels NM, Kuo IH, Georas SN, Boguniewicz M, et al. Reductions in claudin-1 may enhance susceptibility to herpes simplex virus 1 infections in atopic dermatitis. *J Allergy Clin Immunol* 2011;128:242-6, e5.
107. Henderson J, Northstone K, Lee SP, Liao H, Zhao Y, Pembrey M, et al. The burden of disease associated with filaggrin mutations: a population-based, longitudinal birth cohort study. *J Allergy Clin Immunol* 2008;121:872-7.e9.
108. Gao PS, Rafaels NM, Hand T, Murray T, Boguniewicz M, Hata T, et al. Filaggrin mutations that confer risk of atopic dermatitis confer greater risk for eczema herpeticum. *J Allergy Clin Immunol* 2009;124:507-13, e1-7.
109. Irvine AD. Fleshing out filaggrin phenotypes. *J Invest Dermatol* 2007;127:504-7.
110. McAleer MA, Irvine AD. The multifunctional role of filaggrin in allergic skin disease. *J Allergy Clin Immunol* 2013;131:280-91.
111. Cario E, Gerken G, Podolsky DK. Toll-like receptor 2 enhances ZO-1-associated intestinal epithelial barrier integrity via protein kinase C. *Gastroenterology* 2004;127:224-38.
112. Cario E, Gerken G, Podolsky DK. Toll-like receptor 2 controls mucosal inflammation by regulating epithelial barrier function. *Gastroenterology* 2007;132:1359-74.
113. Rezaee F, Meednu N, Emo JA, Saatian B, Chapman TJ, Naydenov NG, et al. Polyinosinic:polycytidylic acid induces protein kinase D-dependent disassembly of apical junctions and barrier dysfunction in airway epithelial cells. *J Allergy Clin Immunol* 2011;128:1216-24.e11.
114. Macedo L, Pinhal-Enfield G, Alshits V, Elson G, Cronstein BN, Leibovich SJ. Wound healing is impaired in MyD88-deficient mice: a role for MyD88 in the regulation of wound healing by adenosine A2A receptors. *Am J Pathol* 2007;171:1774-88.
115. Lin Q, Wang L, Lin Y, Liu X, Ren X, Wen S, et al. Toll-like receptor 3 ligand polyinosinic:polycytidylic acid promotes wound healing in human and murine skin. *J Invest Dermatol* 2012;132:2085-92.
116. Lin Q, Fang D, Fang J, Ren X, Yang X, Wen F, et al. Impaired wound healing with defective expression of chemokines and recruitment of myeloid cells in TLR3-deficient mice. *J Immunol* 2011;186:3710-7.
117. Sato T, Yamamoto M, Shimosato T, Klinman DM. Accelerated wound healing mediated by activation of Toll-like receptor 9. *Wound Repair Regen* 2010;18:586-93.
118. Dickel H, Gambichler T, Kamphowe J, Altmeyer P, Skrygan M. Standardized tape stripping prior to patch testing induces upregulation of Hsp90, Hsp70, IL-33, TNF-alpha and IL-8/CXCL8 mRNA: new insights into the involvement of 'alarmins'. *Contact Dermatitis* 2010;63:215-22.
119. Nakatsuji T, Gallo RL. Antimicrobial peptides: old molecules with new ideas. *J Invest Dermatol* 2012;132:887-95.
120. Guillot L, Medjane S, Le-Barillec K, Balloy V, Danel C, Chignard M, et al. Response of human pulmonary epithelial cells to lipopolysaccharide involves Toll-like receptor 4 (TLR4)-dependent signaling pathways: evidence for an intracellular compartmentalization of TLR4. *J Biol Chem* 2004;279:2712-8.
121. Otte JM, Cario E, Podolsky DK. Mechanisms of cross hyporesponsiveness to Toll-like receptor bacterial ligands in intestinal epithelial cells. *Gastroenterology* 2004;126:1054-70.
122. Rhee SH, Im E, Riegler M, Kokkotou E, O'Brien M, Pothoulakis C. Pathophysiological role of Toll-like receptor 5 engagement by bacterial flagellin in colonic inflammation. *Proc Natl Acad Sci U S A* 2005;102:13610-5.
123. Furrie E, Macfarlane S, Thomson G, Macfarlane GT. Toll-like receptors-2, -3 and -4 expression patterns on human colon and their regulation by mucosal-associated bacteria. *Immunology* 2005;115:565-74.
124. Schitteck B. The antimicrobial skin barrier in patients with atopic dermatitis. *Curr Probl Dermatol* 2011;41:54-67.
125. Lai Y, Li D, Li C, Muehleisen B, Radek KA, Park HJ, et al. The antimicrobial protein REG3A regulates keratinocyte proliferation and differentiation after skin injury. *Immunity* 2012;37:74-84.
126. Nomura I, Goleva E, Howell MD, Hamid QA, Ong PY, Hall CF, et al. Cytokine milieu of atopic dermatitis, as compared to psoriasis, skin prevents induction of innate immune response genes. *J Immunol* 2003;171:3262-9.
127. Harder J, Bartels J, Christophers E, Schroder JM. A peptide antibiotic from human skin. *Nature* 1997;387:861.
128. Sumikawa Y, Asada H, Hoshino K, Azukizawa H, Katayama I, Akira S, et al. Induction of beta-defensin 3 in keratinocytes stimulated by bacterial lipopeptides through toll-like receptor 2. *Microbes Infect* 2006;8:1513-21.
129. Baroni A, Orlando M, Donnarumma G, Farro P, Iovene MR, Tufano MA, et al. Toll-like receptor 2 (TLR2) mediates intracellular signalling in human keratinocytes in response to *Malassezia furfur*. *Arch Dermatol Res* 2006;297:280-8.
130. Gariboldi S, Palazzo M, Zanobbio L, Selleri S, Sommariva M, Sfondrini L, et al. Low molecular weight hyaluronic acid increases the self-defense of skin epithelium by induction of beta-defensin 2 via TLR2 and TLR4. *J Immunol* 2008;181:2103-10.
131. Harder J, Schroder JM. RNase 7, a novel innate immune defense antimicrobial protein of healthy human skin. *J Biol Chem* 2002;277:46779-84.
132. Glaser R, Meyer-Hoffert U, Harder J, Cordes J, Wittersheim M, Kobliakova J, et al. The antimicrobial protein psoriasin (S100A7) is upregulated in atopic dermatitis and after experimental skin barrier disruption. *J Invest Dermatol* 2009;129:641-9.
133. Kvarnhammar AM, Rydberg C, Jarnkrantz M, Eriksson M, Uddman R, Benson M, et al. Diminished levels of nasal S100A7 (psoriasin) in seasonal allergic rhinitis: an effect mediated by Th2 cytokines. *Respir Res* 2012;13:2.
134. Gallo RL, Hooper LV. Epithelial antimicrobial defence of the skin and intestine. *Nat Rev Immunol* 2012;12:503-16.
135. Brandtzaeg P, Gabrielsen TO, Dale I, Muller F, Steinbakk M, Fagerhol MK. The leucocyte protein L1 (calprotectin): a putative nonspecific defence factor at epithelial surfaces. *Adv Exp Med Biol* 1995;371A:201-6.
136. Nukui T, Ehama R, Sakaguchi M, Sonogawa H, Katagiri C, Hibino T, et al. S100A8/A9, a key mediator for positive feedback growth stimulation of normal human keratinocytes. *J Cell Biochem* 2008;104:453-64.
137. Abtin A, Eckhart L, Glaser R, Gmeiner R, Mildner M, Tschachler E. The antimicrobial heterodimer S100A8/S100A9 (calprotectin) is upregulated by bacterial flagellin in human epidermal keratinocytes. *J Invest Dermatol* 2010;130:2423-30.

138. Mork G, Schjerven H, Mangschau L, Soyland E, Brandtzaeg P. Proinflammatory cytokines upregulate expression of calprotectin (L1 protein, MRP-8/MRP-14) in cultured human keratinocytes. *Br J Dermatol* 2003;149:484-91.
139. Rieg S, Garbe C, Sauer B, Kalbacher H, Schitteck B. Dermcidin is constitutively produced by eccrine sweat glands and is not induced in epidermal cells under inflammatory skin conditions. *Br J Dermatol* 2004;151:534-9.
140. Niyonsaba F, Suzuki A, Ushio H, Nagaoka I, Ogawa H, Okumura K. The human antimicrobial peptide dermcidin activates normal human keratinocytes. *Br J Dermatol* 2009;160:243-9.
141. Miajlovic H, Fallon PG, Irvine AD, Foster TJ. Effect of filaggrin breakdown products on growth of and protein expression by *Staphylococcus aureus*. *J Allergy Clin Immunol* 2010;126:1184-90, e3.
142. Rieg S, Steffen H, Seeber S, Humeny A, Kalbacher H, Dietz K, et al. Deficiency of dermcidin-derived antimicrobial peptides in sweat of patients with atopic dermatitis correlates with an impaired innate defense of human skin in vivo. *J Immunol* 2005;174:8003-10.
143. Ong PY, Ohtake T, Brandt C, Strickland I, Boguniewicz M, Ganz T, et al. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N Engl J Med* 2002;347:1151-60.
144. Gambichler T, Skrygan M, Tomi NS, Othlinghaus N, Brockmeyer NH, Altmeyer P, et al. Differential mRNA expression of antimicrobial peptides and proteins in atopic dermatitis as compared to psoriasis vulgaris and healthy skin. *Int Arch Allergy Immunol* 2008;147:17-24.
145. Asano S, Ichikawa Y, Kumagai T, Kawashima M, Imokawa G. Microanalysis of an antimicrobial peptide, beta-defensin-2, in the stratum corneum from patients with atopic dermatitis. *Br J Dermatol* 2008;159:97-104.
146. Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, et al. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med* 2006;203:2271-9.
147. Howell MD, Gallo RL, Boguniewicz M, Jones JF, Wong C, Streib JE, et al. Cytokine milieu of atopic dermatitis skin subverts the innate immune response to vaccinia virus. *Immunity* 2006;24:341-8.
148. Howell MD, Wollenberg A, Gallo RL, Flaig M, Streib JE, Wong C, et al. Cathelicidin deficiency predisposes to eczema herpeticum. *J Allergy Clin Immunol* 2006;117:836-41.
149. Wollenberg A, Wetzel S, Burgdorf WH, Haas J. Viral infections in atopic dermatitis: pathogenic aspects and clinical management. *J Allergy Clin Immunol* 2003;112:667-74.
150. Hata TR, Kotol P, Boguniewicz M, Taylor P, Paik A, Jackson M, et al. History of eczema herpeticum is associated with the inability to induce human beta-defensin (HBD)-2, HBD-3 and cathelicidin in the skin of patients with atopic dermatitis. *Br J Dermatol* 2010;163:659-61.
151. Howell MD, Jones JF, Kisich KO, Streib JE, Gallo RL, Leung DY. Selective killing of vaccinia virus by LL-37: implications for eczema vaccinatum. *J Immunol* 2004;172:1763-7.
152. Howell MD, Streib JE, Leung DY. Antiviral activity of human beta-defensin 3 against vaccinia virus. *J Allergy Clin Immunol* 2007;119:1022-5.
153. Lee KC, Eckert RL. S100A7 (Psoriasin)—mechanism of antibacterial action in wounds. *J Invest Dermatol* 2007;127:945-57.
154. Hirsch T, Spielmann M, Zuhaili B, Fossum M, Metzger M, Koehler T, et al. Human beta-defensin-3 promotes wound healing in infected diabetic wounds. *J Gene Med* 2009;11:220-8.
155. Heilborn JD, Nilsson MF, Kratz G, Weber G, Sorensen O, Borregaard N, et al. The cathelicidin anti-microbial peptide LL-37 is involved in re-epithelialization of human skin wounds and is lacking in chronic ulcer epithelium. *J Invest Dermatol* 2003;120:379-89.
156. Graumann N, Neumann C, Jürgen H, Walker C, Müller W, Proksch E. Deficiency in mouse beta-defensin 14 leads to a delay in skin permeability barrier repair [abstract]. *J Invest Dermatol* 2012;132:S110.
157. Grice EA, Kong HH, Renaud G, Young AC, Bouffard GG, Blakesley RW, et al. A diversity profile of the human skin microbiota. *Genome Res* 2008;18:1043-50.
158. Hata TR, Kotol P, Jackson M, Nguyen M, Paik A, Udall D, et al. Administration of oral vitamin D induces cathelicidin production in atopic individuals. *J Allergy Clin Immunol* 2008;122:829-31.
159. Oh J, Conlan S, Polley E, Segre JA, Kong HH. Shifts in human skin and nares microbiota of healthy children and adults. *Genome Med* 2012;4:77.
160. Cogen AL, Nizet V, Gallo RL. Skin microbiota: a source of disease or defence? *Br J Dermatol* 2008;158:442-55.
161. Kong HH, Oh J, Deming C, Conlan S, Grice EA, Beatson MA, et al. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res* 2012;22:850-9.
162. Hajjar AM, O'Mahony DS, Ozinsky A, Underhill DM, Aderem A, Klebanoff SJ, et al. Cutting edge: functional interactions between toll-like receptor (TLR) 2 and TLR1 or TLR6 in response to phenol-soluble modulin. *J Immunol* 2001;166:15-9.
163. Cogen AL, Yamasaki K, Sanchez KM, Dorschner RA, Lai Y, MacLeod DT, et al. Selective antimicrobial action is provided by phenol-soluble modulins derived from *Staphylococcus epidermidis*, a normal resident of the skin. *J Invest Dermatol* 2010;130:192-200.
164. Cogen AL, Yamasaki K, Muto J, Sanchez KM, Crotty Alexander L, Tanios J, et al. *Staphylococcus epidermidis* antimicrobial delta-toxin (phenol-soluble modulin-gamma) cooperates with host antimicrobial peptides to kill group A *Streptococcus*. *PLoS One* 2010;5:e8557.
165. Naik S, Bouladoux N, Wilhelm C, Molloy MJ, Salcedo R, Kastenmuller W, et al. Compartmentalized control of skin immunity by resident commensals. *Science* 2012;337:1115-9.
166. Carmi-Levy I, Homey B, Soumelis V. A modular view of cytokine networks in atopic dermatitis. *Clin Rev Allergy Immunol* 2011;41:245-53.
167. Albanesi C. Keratinocytes in allergic skin diseases. *Curr Opin Allergy Clin Immunol* 2010;10:452-6.
168. Esche C, de Benedetto A, Beck LA. Keratinocytes in atopic dermatitis: inflammatory signals. *Curr Allergy Asthma Rep* 2004;4:276-84.
169. Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002;3:673-80.
170. Gao PS, Rafaels NM, Mu D, Hand T, Murray T, Boguniewicz M, et al. Genetic variants in thymic stromal lymphopoietin are associated with atopic dermatitis and eczema herpeticum. *J Allergy Clin Immunol* 2010;125:1403-7.e4.
171. Ziegler SF. Thymic stromal lymphopoietin and allergic disease. *J Allergy Clin Immunol* 2012;130:845-52.
172. Wang YH, Angkasekwinai P, Lu N, Voo KS, Arima K, Hanabuchi S, et al. IL-25 augments type 2 immune responses by enhancing the expansion and functions of TSLP-DC-activated Th2 memory cells. *J Exp Med* 2007;204:1837-47.
173. Savinko T, Matikainen S, Saarialho-Kere U, Lehto M, Wang G, Lehtimäki S, et al. IL-33 and ST2 in atopic dermatitis: expression profiles and modulation by triggering factors. *J Invest Dermatol* 2012;132:1392-400.
174. Fort MM, Cheung J, Yen D, Li J, Zurawski SM, Lo S, et al. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. *Immunity* 2001;15:985-95.
175. Humphreys NE, Xu D, Hepworth MR, Liew FY, Grecis RK. IL-33, a potent inducer of adaptive immunity to intestinal nematodes. *J Immunol* 2008;180:2443-9.
176. Oyoshi MK, Larson RP, Ziegler SF, Geha RS. Mechanical injury polarizes skin dendritic cells to elicit a Th2 response by inducing cutaneous thymic stromal lymphopoietin expression. *J Allergy Clin Immunol* 2010;126:976-84, e1-5.
177. Angelova-Fischer I, Fernandez IM, Donnadieu MH, Bulfone-Paus S, Zillikens D, Fischer TW, et al. Injury to the stratum corneum induces in vivo expression of human thymic stromal lymphopoietin in the epidermis. *J Invest Dermatol* 2010;130:2505-7.
178. Vu AT, Baba T, Chen X, Le TA, Kinoshita H, Xie Y, et al. *Staphylococcus aureus* membrane and diacylated lipopeptide induce thymic stromal lymphopoietin in keratinocytes through the Toll-like receptor 2-Toll-like receptor 6 pathway. *J Allergy Clin Immunol* 2010;126:985-93, e1-3.
179. Kinoshita H, Takai T, Le TA, Kamijo S, Wang XL, Ushio H, et al. Cytokine milieu modulates release of thymic stromal lymphopoietin from human keratinocytes stimulated with double-stranded RNA. *J Allergy Clin Immunol* 2009;123:179-86.
180. Briot A, Deraison C, Lacroix M, Bonnart C, Robin A, Besson C, et al. Kallikrein 5 induces atopic dermatitis-like lesions through PAR2-mediated thymic stromal lymphopoietin expression in Netherton syndrome. *J Exp Med* 2009;206:1135-47.
181. Kouzaki H, O'Grady SM, Lawrence CB, Kita H. Proteases induce production of thymic stromal lymphopoietin by airway epithelial cells through protease-activated receptor-2. *J Immunol* 2009;183:1427-34.
182. Lee KH, Cho KA, Kim JY, Baek JH, Woo SY, Kim JW. Filaggrin knockdown and Toll-like receptor 3 (TLR3) stimulation enhanced the production of thymic stromal lymphopoietin (TSLP) from epidermal layers. *Exp Dermatol* 2011;20:149-51.
183. Hvid M, Vestergaard C, Kemp K, Christensen GB, Deleuran B, Deleuran M. IL-25 in atopic dermatitis: a possible link between inflammation and skin barrier dysfunction? *J Invest Dermatol* 2011;131:150-7.
184. Brint EK, Xu D, Liu H, Dunne A, McKenzie AN, O'Neill LA, et al. ST2 is an inhibitor of interleukin 1 receptor and Toll-like receptor 4 signaling and maintains endotoxin tolerance. *Nat Immunol* 2004;5:373-9.
185. Liu J, Buckley JM, Redmond HP, Wang JH. ST2 negatively regulates TLR2 signaling, but is not required for bacterial lipoprotein-induced tolerance. *J Immunol* 2010;184:5802-8.
186. Alase A, Seltmann J, Werfel T, Wittmann M. Interleukin-33 modulates the expression of human beta-defensin 2 in human primary keratinocytes and may influence the susceptibility to bacterial superinfection in acute atopic dermatitis. *Br J Dermatol* 2012;167:1386-9.