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# Pattern Recognition Receptors in Immune Disorders Affecting the Skin

Heleen D. de Koning<sup>a–e</sup> Anna Simon<sup>b, d, e</sup> Patrick L.J.M. Zeeuwen<sup>a, c, d</sup>  
Joost Schalkwijk<sup>a, c–e</sup>

Departments of <sup>a</sup>Dermatology and <sup>b</sup>Medicine, Radboud University Nijmegen Medical Centre, <sup>c</sup>Nijmegen Centre for Molecular Life Sciences (NCMLS), <sup>d</sup>Nijmegen Institute for Infection, Inflammation and Immunity (N4i), and <sup>e</sup>N4i Centre for Immunodeficiency and Autoinflammation, Nijmegen, The Netherlands

## Key Words

Autoimmunity · Epithelium · Host defense · Immune response · Pathogen-associated molecular patterns · Pattern recognition receptors · Toll-like receptor · Skin

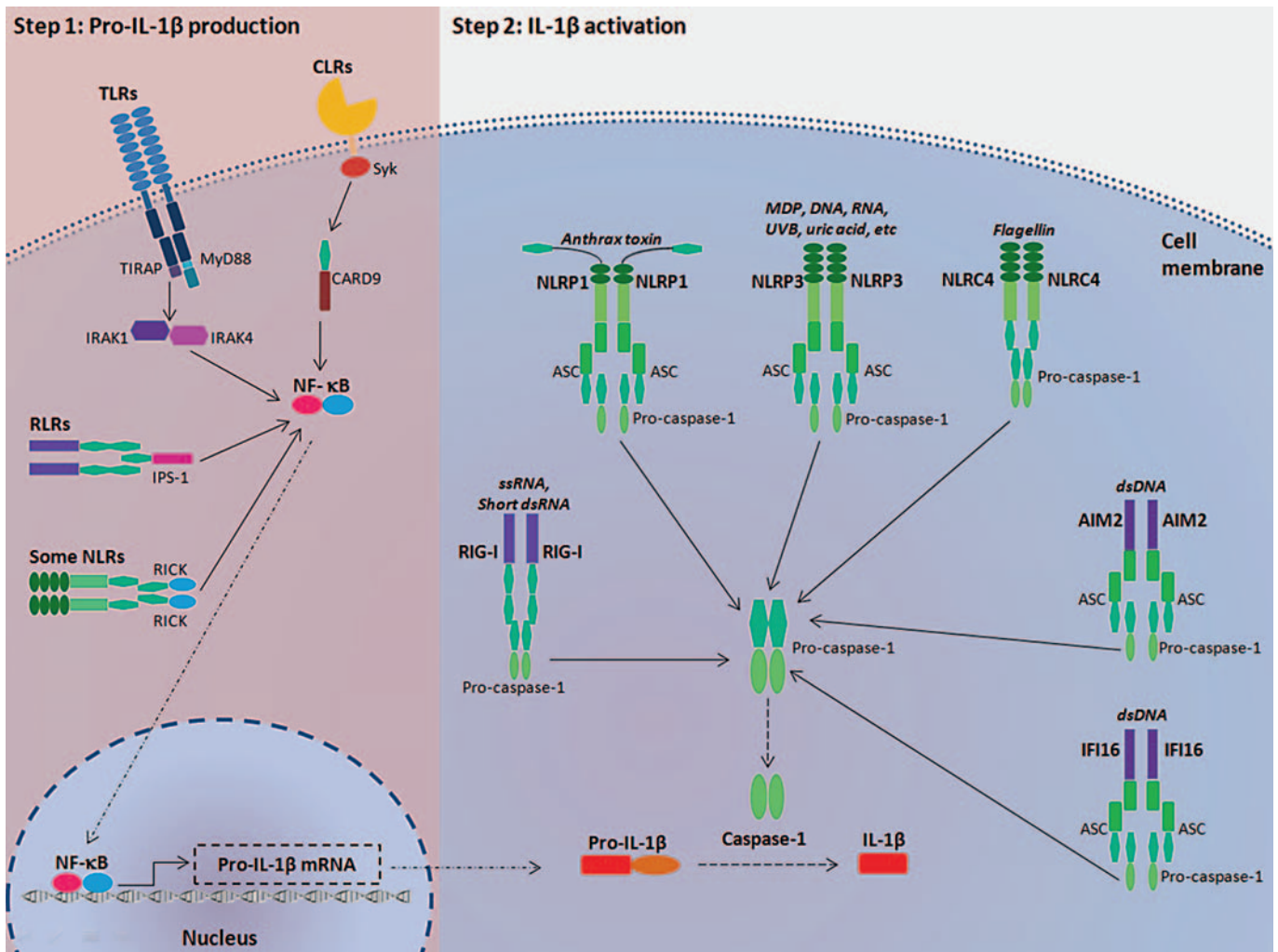
## Abstract

Pattern recognition receptors (PRRs) evolved to protect organisms against pathogens, but excessive signaling can induce immune responses that are harmful to the host. Putative PRR dysfunction is associated with numerous immune disorders that affect the skin, such as systemic lupus erythematosus, cryopyrin-associated periodic syndrome, and primary inflammatory skin diseases including psoriasis and atopic dermatitis. As yet, the evidence is often confined to genetic association studies without additional proof of a causal relationship. However, insight into the role of PRRs in the pathophysiology of some disorders has already resulted in new therapeutic approaches based on immunomodulation of PRRs.

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## Introduction

Pattern recognition receptors (PRRs) are a vital part of innate host defense. In several diseases, however, PRR signaling can be harmful to the host. Tissue damage can be inflicted by excessive pathogen-induced PRR signaling. Yet during the last decade several diseases were identified in which profuse PRR signaling was caused by endogenous factors. The cryopyrin-associated periodic syndrome (CAPS) is an example of genetically predisposed NLRP3 [nucleotide oligomerization domain (NOD), leucine-rich repeat- and pyrin domain-containing 3 protein] overactivation, resulting in mild to debilitating systemic inflammation [1, 2]. Multiple complex disorders have been linked to NLRP3 dysfunction, including gout, type 2 diabetes mellitus and atherosclerosis [1, 3, 4]. Although NLRP3 is the best-characterized inflammasome-related PRR, and it was shown to respond to an impressive array of endogenous and exogenous stimuli ranging from ATP to reactive oxygen species, the exact ligand sensing mechanism of NLRP3 remains unknown [5]. NLRP3 is part of an inflammasome. Inflammasomes are multiprotein complexes that upon ligand binding by certain PRRs activate caspase-1, which in turn activates the potent pro-



**Fig. 1.** The inflammasomes. Inflammasomes are protein complexes that activate caspase-1, which in turn activates the pro-inflammatory cytokine IL-1 $\beta$ . IL-1 $\beta$  production requires two signals. First, activation of multiple PRRs may lead to NF- $\kappa$ B activation, resulting in the production of pro-IL-1 $\beta$ , the precursor of IL-1 $\beta$ . Second, activation of an inflammasome results in activation of caspase-1, which in turn cleaves pro-IL-1 $\beta$  into the active cytokine. The PRRs that associate with inflammasomes are NLRP1, NLRP3, NLRC4, AIM2, IFI16 and RIG-I, each recognizing particular PAMPs and DAMPs. The adaptor protein ASC is

required in all inflammasome complexes to bridge the interaction between upstream PRRs and inflammatory caspases through its amino-terminal pyrin domain (PYD) and carboxy-terminal CARD, respectively. The major ligands of the PRRs are depicted in bold. NLRP1 = Nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family PYD-containing protein 1. Adaptor and signaling proteins: IRAK = IL-1R-associated kinase; SYK = spleen tyrosine kinase; ASC = apoptosis-associated speck-like protein containing a CARD; ssRNA = single-stranded RNA; MDP = muramyl dipeptide; UVB = ultraviolet B radiation.

inflammatory cytokines IL-1 $\beta$ , IL-18 and IL-33 (fig. 1). Since IL-1 $\beta$  is an extremely potent inflammatory cytokine, its activation is strictly regulated and requires more than inflammasome activation. The two-step model of IL-1 $\beta$  activation holds that prior to inflammasome activation the precursor of IL-1 $\beta$  (pro-IL-1 $\beta$ ) needs to be transcribed, for which priming with TLR ligands or cytokine tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is required

(fig. 1) [5, 6]. This is one of the many cases in which PRR cooperation is imperative. The PRRs that associate with inflammasomes are the NLRs NLRP1, NLRP3, NLRC4 [NOD-, leucine-rich repeat- and caspase-associated recruitment domain (CARD)-containing 4] [7], the DNA sensors AIM2 (absent in melanoma 2) [8–10] and IFI16 (interferon, gamma-inducible protein 16) [11], and RIG-I (retinoic-acid-inducible gene 1) [12].

The levels of evidence for associations between diseases and PRRs differ greatly. In some, there is a clear causal relationship between a mutation in a PRR gene and excessive inflammation, whereas in other diseases, only differences in expression levels of certain PRRs or associations with single nucleotide polymorphisms (SNPs) in genome-wide association studies were reported. This review summarizes the detrimental roles PRRs can play in primary inflammatory skin diseases and systemic diseases with skin manifestations.

### PRRs as Villains in Primary Skin Disorders

Despite the fact that these disorders principally affect the skin, several of them can also give systemic symptoms, and can display abnormalities in circulating immune cells or inflammatory mediators. The inflammatory skin disorders that are associated with PRRs are listed in table 1.

#### *Primary Skin Disorders in Which a Role of PRRs Is Suspected*

##### Psoriasis

Psoriasis is a chronic inflammatory skin disease characterized by erythematous squamous plaques. It is associated with skin barrier abnormalities and T helper cell 1 (Th1) and Th17 immune responses [13, 14]. More recently, the innate immune system was found to play a role too. The expression of multiple antimicrobial proteins (AMPs), including human  $\beta$ -defensin-2 (hBD-2), is strongly increased in psoriatic plaques [13], which could well be a downstream effect of PRR signaling. Several groups investigated PRR expression in psoriatic lesions. Lesional TLR2 mRNA and protein expression levels are similar to those in normal skin [15, 16], although two older studies suggested differently [17, 18]. Transforming growth factor alpha, which is induced in psoriasis, was shown to increase TLR5 and TLR9 expression and function in keratinocytes. TLR2, TLR5 and TLR9 ligands induce the expression of human  $\beta$ -defensins in primary keratinocytes [15, 19]. Also, TLR2, TLR3 and TLR4 ligands were shown to induce the psoriasis-associated cytokine TNF- $\alpha$  in human keratinocytes [18]. It is, however, controversial whether TLR4 is expressed in primary human keratinocytes, because several authors did not find TLR4 expression or effects of lipopolysaccharide (LPS) stimulation in these cells [15, 20].

Interestingly, topical application of imiquimod, a ligand for both TLR7 and TLR8, has been described as

aggravating psoriatic lesions, but also inducing de novo psoriasis [21–24]. TLR7 and TLR8 signaling leads to a type I interferon (IFN) response. IFN- $\alpha$ -producing plasmacytoid dendritic cells (pDCs) are thought to be important in the induction of psoriasis [25]. Moreover, psoriasis was reported to be induced or exacerbated by treatment with IFN- $\alpha$  or IFN- $\beta$  for various indications (hepatitis C [26], chronic myeloid leukemia [27] or multiple myeloma [28]). In a mouse model, daily application of imiquimod induced psoriasis-like lesions with increased epidermal expression of IL-23, IL-17A and IL-17F, and an increase in splenic Th17 cells. However, this phenotype was prevented in mice deficient in the IL-23 or IL-17 receptor. This study provided a link between TLR7 or TLR8 signaling and the IL-23/IL-17 axis, which is important in the pathogenesis of psoriasis [29]. The role of other type I IFN-inducing PRRs is unclear, since in one study RIG-I and MDA5 (melanoma differentiation-associated gene 5) were slightly upregulated [30], while others did not find any difference in RLR (RIG-like helicase receptor family) expression levels [15].

A meta-analysis demonstrated a lack of association between *NOD2* polymorphisms and psoriasis and psoriatic arthritis, suggesting that this NLR is not a susceptibility gene for psoriasis [31].

Compared to normal skin, the CLR dectin-1 is increased in the epidermis in psoriatic plaques at both the mRNA and protein level. MRC1 (macrophage mannose receptor 1) mRNA expression was also upregulated [15]. In monocytes and macrophages, dectin-1 signaling induces IL-23, which in turn promotes differentiation of Th17 cells [32]. As a sensor of fungal  $\beta$ -glucan, dectin-1 signaling stimulates immune cells to produce antifungal AMPs, which are highly expressed in psoriasis lesions. Stimulation of primary human keratinocytes with  $\beta$ -glucan or heat-killed *Candida*, however, did not induce proinflammatory cytokines or AMPs, either with or without TLR2, TLR5 or MRC1 costimulation [15]. Possibly, dectin-1 expression levels were too low for proper functioning, or costimulation with other ligands is required.

LL37, one of the upregulated AMPs in psoriasis, forms aggregates with extracellular self-DNA that can enter pDCs, activate TLR9 and trigger type I IFN production [33]. Recently, LL37 was reported to neutralize cytosolic DNA in keratinocytes and block activation of the AIM2 inflammasome. The authors found upregulation of AIM2 in psoriatic lesional skin and a 3-fold induction of IL-1 $\beta$  secretion by keratinocytes upon stimulation with double-stranded DNA (dsDNA) [34].

**Table 1.** PRRs implicated in immune disorders affecting the skin

Disease	Genetic association	Endogenous expression in affected human tissues	PRR-mediated effect on immune response to ligands in human cells	Functional association in mice
<b>Primary skin disorders</b>				
<i>Primary skin disorders in which a role of PRRs is suspected</i>				
Psoriasis		AIM2: increased Dectin-1: increased MRC1: increased	<i>In keratinocytes:</i> TLR2: induction of hBD-2 and TNF- $\alpha$ TLR3: induction of TNF- $\alpha$ TLR4: induction of TNF- $\alpha$ or no effect TLR5: induction of hBD-2 TLR9: induction of hBD-2 Dectin-1: no effect of $\beta$ -glucan +/- TLR2 or TLR5 or MRC1 ligands AIM2: IL-1 $\beta$ activation <i>In monocytes:</i> Dectin-1: induction of IL-23	TLR7/TLR8: imiquimod induced psoriasis-like disease
AD	<i>TLR2</i> A-16934T promotor SNP: disease severity <i>TLR2</i> R753Q SNP: disease severity and increased colonization with <i>S. aureus</i> <i>TLR9</i> C-1237T promotor SNP: association in subgroup <i>NOD1</i> SNPs: association in some	Dectin-1: increased MRC1: increased TLR1: increased TLR2: normal or decreased	<i>In keratinocytes:</i> TLR2/TLR6: induction of TSLP	
ACD			MyD88 RNAi: inhibited nickel-induced inflammation IRAK1 RNAi: reduced nickel-induced inflammation TLR4 RNAi: inhibited nickel-induced inflammation Caspase-1: mediated IL-1 $\beta$ activation upon TNCB and SDS	<i>Tlr2</i> <sup>-/-</sup> / <i>Tlr4</i> <sup>-/-</sup> : impaired TNCB-induced ACD <i>Tlr4</i> <sup>-/-</sup> : no effect on TNCB-induced ACD <i>hTLR4</i> , not <i>mTLR4</i> : mediated nickel-induced ACD <i>Asc</i> <sup>-/-</sup> : reduced TNCB-induced ACD <i>Nlrp3</i> <sup>-/-</sup> : reduced TNCB-induced ACD <i>Nlrp12</i> <sup>-/-</sup> : reduced oxazolone or FITC ACD
<i>Primary skin disorders in which a role of PRRs is speculative</i>				
Rosacea		TLR2: increased	TLR2: induction of kallikrein 5	
Vitiligo	<i>NLRP1</i> SNPs: association with generalized vitiligo			
Stevens-Johnson syndrome	<i>TLR3</i> SNPs: association			
<b>Systemic immune disorders affecting the skin</b>				
<i>Monogenic disorders directly linked to a mutation in a PRR gene</i>				
CAPS	<i>NLRP3</i> mutations: autosomal dominant, disease-causing		<i>NLRP3</i> mutants: excessive endogenous and PAMP-induced IL-1 $\beta$ activation	
NLRP12AD	<i>NLRP12</i> mutations: autosomal dominant (?), disease-causing		<i>NLRP12</i> mutants: impaired inhibition of NF- $\kappa$ B signaling in some; increased IL-1 $\beta$ and ROS activation in others	
Blau syndrome	<i>NOD2</i> mutations: disease-causing		<i>NOD2</i> mutants: constitutive activation of NF- $\kappa$ B	

**Table 1** (continued)

Disease	Genetic association	Endogenous expression in affected human tissues	PRR-mediated effect on immune response to ligands in human cells	Functional association in mice
<i>Disorders caused by overstimulation of PRR by endogenous ligands</i>				
Gout			NLRP3: excessive uric-acid-induced IL-1 $\beta$ activation, provided LPS is present	<i>Nlrp3</i> <sup>-/-</sup> : no gout upon urate injection <i>Asc</i> <sup>-/-</sup> : no gout upon urate injection
Pseudogout			NLRP3: excessive calcium pyrophosphate dihydrate-induced IL-1 $\beta$ activation, provided LPS is present	
<i>Systemic immune disorders in which a role of PRRs is suspected</i>				
BD	TLR4 SNP: more prevalent in HLA-B51+ patients TIRAP SNP: associated in one population			
SLE	<i>TLR5</i> SNP: protective <i>TIRAP</i> SNP: decreased susceptibility <i>HSP70</i> SNPs: association in some, but controversial	TLR2, TLR7, TLR9, AIM2 and IFI16: mRNA increased in PBMCs	TLR2, TLR4 and/or TLR9: in serum increased HMGB1 (ligand) protein and anti-HMGB1 antibodies TLR7: overexpression induced autoimmunity	<i>Tlr2</i> <sup>-/-</sup> : impaired autoantibody production induced by nucleosomes containing HMGB1 Tlr7 overexpression: induced autoimmunity <i>TLR9</i> <sup>-/-</sup> : decreased or increased disease in SLE mouse model
GVHD	<i>NOD2</i> SNPs: increased susceptibility			Recipient <i>Tlr9</i> <sup>-/-</sup> : reduced GVHD Donor <i>Tlr9</i> <sup>-/-</sup> : no effect Tlr9 blocking by iODN: reduced GVHD Recipient Myd88: no effect Recipient TRIF: no effect Recipient <i>TLR2</i> <sup>-/-</sup> : no effect Recipient <i>TLR4</i> <sup>-/-</sup> : no effect or more severe GVHD Donor <i>TLR4</i> <sup>-/-</sup> : no effect or reduced GVHD Recipient <i>NOD2</i> <sup>-/-</sup> : more severe GVHD Donor <i>NOD2</i> <sup>-/-</sup> : no effect
<i>Systemic immune disorders in which a role of PRRs is speculative</i>				
Sarcoidosis	<i>NOD2</i> SNPs: association with severe pulmonary disease, not in general			
Schnitzler syndrome	<i>NLRP3</i> V198M SNP in a single patient, to date not in others		Inflammasome: increased IL-1 $\beta$ activation upon stimulation with LPS	

### Atopic Dermatitis

Atopic dermatitis (AD) is a common chronic inflammatory skin disease that mainly affects children and is characterized by pruritus, eczematous lesions and xerosis. AD is associated with skin barrier abnormalities and a Th2 immune response [13]. AD lesions display lower levels of AMPs than psoriatic plaques [35, 36], and are

often infected with *Staphylococcus aureus*, which is associated with AD flares and severity [37, 38]. Herpes simplex virus (HSV) infections can also exacerbate AD and *Candida* species often colonizes atopic skin [37]. Defects in the innate immune system were hypothesized to predispose to AD development and to colonization with these pathogens. Hence, differences in PRR expression

and function became research targets. *S. aureus*-diacylated lipoproteins were shown to induce expression of thymic stromal lymphopoietin (TSLP), which is highly expressed by keratinocytes in skin lesions of patients with AD. This process required signaling of the TLR2/TLR6 heterodimer and was enhanced by Th2 cytokines [39]. The role of TLR2 in AD is controversial, as is the case in other diseases. One study reported the association of the TLR2 A-16934T promoter polymorphism with severe AD, which did not affect TLR2 mRNA expression and resulted in decreased TLR2-induced IL-6, but not TNF- $\alpha$  production [40]. In one population the TLR2 R753Q polymorphism was associated with AD disease severity and increased colonization with *S. aureus* [41]. Previously, this polymorphism had been implicated in *S. aureus* infections [42]. The TLR2 R753Q polymorphism led to decreased cell surface expression of TLR2 in CD3/CD28-activated CD4<sup>+</sup> T cells, and impaired TLR2-mediated IL-8 secretion by monocytes [43]. In other populations, no associations between TLR1, TLR2, TLR4 and TLR6 polymorphisms and AD were found [44, 45]. The data on TLR2 protein expression levels in AD lesions are also conflicting, since one study reported no difference with normal skin [16], whereas another study reported decreased TLR2 and increased TLR1 expression in AD lesions [46]. In this study, TLR4 and TLR9 protein expression levels were similar in AD lesions and normal skin [46]. The C-1237T polymorphism in the TLR9 promoter was significantly associated with AD in a subgroup of patients, and resulted in significantly higher promoter activity. This association was not seen in a case-control cohort in the same study, so it may only apply to some cases of AD [47]. One genetic study revealed an association of *NOD1* polymorphisms with AD [48], but in other studies, genetic associations of AD with *NOD1*, *NOD2* or *NLRP12* were only slightly significant, which renders the pathophysiological implications questionable [49, 50].

A comprehensive study on epidermal PRR mRNA expression showed that expression of the majority of PRRs was similar in psoriasis, AD and normal skin. In AD, dectin-1 was upregulated 6-fold and MRC1 10-fold [15]. Progenitor-derived mast cells from AD patients were shown to display lower dectin-1 expression, but the implications are yet to be determined [51].

#### Allergic Contact Dermatitis

Allergic contact dermatitis (ACD) is a type IV-delayed hypersensitivity reaction in the skin typically after sensitization by haptens. In mice, the concomitant absence of Tlr2 and Tlr4 prevented the induction of ACD to

2,4,6-trinitro-1-chlorobenzene (TNCB). Also, in Tlr4/IL-12R $\beta$ 2 double knockout mice, dendritic cell (DC)-mediated sensitization, generation of effector T cells, and the subsequent contact hypersensitivity response to TNCB, oxazolone, and fluorescein isothiocyanate were absent. This was not the case in TLR4 or IL-12 single knockout mice [52]. Epicutaneous immunization with protein antigen is applied as desensitization because it induces suppression of subsequent T cell-dependent contact hypersensitivity reactions after active immunization. Ptak et al. [53] found that this suppression can be reversed by crude bacterial components and purified TLR2, TLR3, TLR4, and TLR9 ligands. Also, the effect of TLR4 ligand LPS was not observed in Tlr4 mutant C3H/HeJ mice, which indicates that this effect was dependent upon intact TLR4 signaling. The inflammatory response in nickel ACD was shown to be TLR4 dependent. Interestingly, mouse Tlr4 could not generate this response, but transgenic expression of human TLR4 in TLR4-deficient mice allowed efficient sensitization to nickel and elicitation of ACD [54].

Watanabe et al. [55] showed involvement of the inflammasome in ACD. In primary human keratinocytes, TNCB and SDS induced IL-1 $\beta$  activation in a caspase-1-dependent manner. In Asc and Nlrp3 knockout mice, TNCB-induced ACD was reduced.

NLRP12 is one of the latest identified NLRs. In two models of ACD (oxazolone and FITC), *Nlrp12*-deficient mice exhibited attenuated inflammatory responses. *Nlrp12* knockout DCs were less capable of migrating to draining lymph nodes, and both *Nlrp12* knockout DCs and neutrophils failed to respond to chemokines in vitro [56].

#### *Primary Skin Disorders with Speculative Associations with PRRs*

In other skin disorders, the evidence for a role of PRRs is still more speculative, although it may have important implications. We will mention three examples of these.

#### Rosacea

Rosacea is a common skin disease that is characterized by facial inflammation, abnormal vascular dilatation and proliferation, and formation of granulomas. Symptoms are exacerbated by external triggers, such as UV light, heat, and a variety of microbes. TLR2 stimulation induced expression of kallikrein 5, a critical protease involved in the pathogenesis of rosacea, since it processes cathelicidin [16]. The increased TLR2 expression in rosacea may cause increased susceptibility to pathogen- and

damage-associated molecular patterns (PAMPs and DAMPs) that trigger disease [16].

### Vitiligo

Vitiligo is an autoimmune disease characterized by the destruction of melanocytes in the epidermis, resulting in depigmented maculae. It is associated with several other autoimmune disorders including autoimmune thyroid disease, rheumatoid arthritis, systemic lupus erythematosus (SLE), and diabetes [57]. *NLRP1* genetic variants have been associated with vitiligo [57–59], which is interesting from a pathophysiological point of view, since NLRP1 is part of an inflammasome that activates IL-1 $\beta$  and can result in apoptosis. The functional aspects require further investigation.

### Stevens-Johnson Syndrome

Stevens-Johnson syndrome and the related toxic epidermal necrolysis are severe acute-onset mucocutaneous disorders that can be induced by drugs or pathogens. In Japanese patients, *TLR3* polymorphisms were associated with Stevens-Johnson syndrome and toxic epidermal necrolysis [60], while in another population *TLR9* polymorphisms were not [61].

## PRRs in Systemic Inflammatory Disorders with Skin Manifestations

PRRs are implicated in many systemic disorders, such as the autoinflammatory syndromes, which are characterized by a predisposition towards excessive innate immune activation, often affecting the skin [62]. Several autoinflammatory diseases somehow affect PRR signaling pathways, but in this review we chose to discuss those that are directly linked to PRRs. We will also discuss examples of multifactorial diseases in which PRRs were implicated, as listed in table 1.

### *Monogenic Disorders Directly Linked to a Mutation in a PRR Gene*

#### Cryopyrin-Associated Periodic Syndrome

CAPS refers to a spectrum of autoinflammatory diseases, previously known as familial cold associated periodic syndrome, Muckle-Wells syndrome, and the debilitating chronic infantile neurologic, cutaneous, articular syndrome. The latter was also known as neonatal-onset multisystem inflammatory disease [1, 2]. CAPS is clinically characterized by urticarial-like skin rashes which may be cold-induced, recurrent fevers, arthralgia

or arthritis, ocular symptoms, sometimes amyloidosis, and, in severely affected patients, severe neurological symptoms [63]. These diseases were collectively classified as CAPS upon recognition that all three were caused by heterozygous mutations in *NLRP3*, previously referred to as *NALP3* or cryopyrin [64, 65]. These are regarded as gain of function mutations, resulting in a hyperactive or constitutively active inflammasome, leading to systemic IL-1 $\beta$ -induced inflammation. Monocytes and macrophages from Muckle-Wells syndrome patients display a basal secretion of mature IL-1 $\beta$  in the absence of any external stimulus [66]. Together, these data formed the rationale for trials with IL-1 blocking therapies. Indeed, the IL-1 receptor antagonist anakinra, the IL-1 receptor-Fc fusion protein riloncept, and the human IgG1 anti-IL-1 $\beta$  monoclonal antibody canakinumab are all successful in preventing inflammation in CAPS [2, 67–69].

#### NLRP12-Associated Periodic Syndrome

A very rare hereditary periodic fever syndrome results from mutations in the *NLRP12* gene, and manifests with mainly cold-induced recurrent fevers, arthralgia, and in some cases urticarial rashes or abdominal pain [70–72]. The pathophysiology is not completely clear yet. *NLRP12* was previously shown to inhibit nuclear factor- $\kappa$ B (NF- $\kappa$ B) signaling [73], and in some of the *NLRP12* mutations a clear reduction of these inhibitory properties could be found, in keeping with a loss of function [70], but this was not true in all cases [74]. In another family, monocytes produced more IL-1 $\beta$  and reactive oxygen species upon stimulation with PAMPs [71]. Altogether, *NLRP12* mutations cause an autoinflammatory syndrome through increased NF- $\kappa$ B and/or IL-1 $\beta$  signaling.

#### Blau Syndrome

Blau syndrome, also known as early-onset sarcoidosis, is a rare autosomal dominant disorder which is characterized by granulomatous arthritis, uveitis and skin rash [75]. Various mutations in the *NOD2* gene were found in patients with Blau syndrome, and all mutations were associated with constitutive activation of the transcription factor NF- $\kappa$ B [75]. These mutations confer a gain of function to *NOD2*, while Crohn's disease-associated *NOD2* variants impair NF- $\kappa$ B activation [76, 77].

### *Systemic Immune Disorders in Which a Role of PRRs Is Suspected*

#### Gout and Pseudogout

Monosodium urate and calcium pyrophosphate dihydrate crystals were long known to cause arthritis in gout



and pseudogout, respectively. In the so-called tophi, uric acid depositions induce inflammation in the skin. Both monosodium urate and calcium pyrophosphate dihydrate crystals were shown to be able to activate the NLRP3 inflammasome in vitro, provided there was costimulation with LPS, resulting in excessive IL-1 $\beta$  production [78]. The authors showed that macrophages from mice deficient in various components of the inflammasome, such as pro-caspase-1, ASC or NLRP3, did not respond to injection of urate crystals. The excessive production of IL-1 $\beta$  provides a rationale for IL-1 $\beta$  blocking therapies. Indeed, anakinra and riloncept proved highly effective in clinical trials in gout patients [79, 80].

#### Behçet's Disease

Behçet's disease (BD) is a multisystem disease characterized by recurrent oral and genital ulcers, relapsing uveitis, and articular, neurologic, vascular, intestinal and pulmonary manifestations. Several groups studied *NOD2* polymorphisms in BD patients, but found no association [81–83]. SNP analyses of *TLR2*, *TLR4*, *CD14* and *TLR9* in BD patients was also negative [84–88]. However, the S180L polymorphism in *Toll/interleukin receptor 1 domain-containing adaptor protein (TIRAP)*, a protein involved in TLR2 and TLR4 signaling, was significantly associated with BD in UK, but not Middle Eastern, patients [86]. Also, a *TLR4* variant was significantly more prevalent in HLA-B51-positive, but not HLA-B51-negative BD patients, compared with healthy control participants, which suggests a synergistic increase in susceptibility of BD in this population [89]. Thus, most genetic studies on PRRs in BD showed no association.

#### Systemic Lupus Erythematosus

SLE is predominantly regarded as a disorder of the adaptive immune system, but recent studies point towards a concomitant role of innate immune responses [90]. In murine SLE models, autoantibodies bind DNA or chromatin released from dying cells, forming complexes that can stimulate IFN- $\alpha$  production by DCs via TLR9 [91–94]. In addition, TLR9/MyD88 (myeloid differentiation factor 88) signaling is required for class switching to pathogenic IgG2a and 2b autoantibodies in autoreactive B cells in SLE, resulting in a pathogenic loop [95]. The role of TLR9 is controversial, however, since others found that *TLR9* knockdown resulted in exacerbation of autoimmunity rather than reduction [96]. Moreover, TLR9 was found to regulate TLR7- and MyD88-dependent autoantibody production and disease in a murine SLE model [97]. Possibly, differences between mouse strains account

for these discrepancies. *TLR9* polymorphisms were associated with lupus nephritis in a Chinese Han population, but the functional consequences of the polymorphisms are unclear [98]. Patients with active SLE displayed an increased number of TLR9-positive B cells, which correlated with elevated titers of autoantibodies against dsDNA. In vitro, serum from patients with SLE upregulated expression of TLR9 on plasma cells [99]. Tian et al. [100] showed that B cells and pDCs can be activated by immune complexes that contain DNA and high mobility group box (HMGB)-1 in a TLR9- and MyD88-dependent manner involving *RAGE* (receptor for advanced glycation end products). HMGB1 is also recognized by TLR2 and TLR4 [101]. In the sera of SLE patients, HMGB1 and anti-HMGB1 antibodies are often found [102], as are HMGB1-containing nucleosomes [103]. These HMGB1-containing nucleosomes stimulated the production of proinflammatory cytokines in macrophages, and induced autoantibody production in BALB/c mice, both in a TLR2-dependent manner [103].

Activation of TLR7 and TLR8 by RNA and RNA containing immune complexes is also implicated in the pathogenesis of SLE [91, 92, 104, 105]. *TLR7* and *TLR8* are encoded on the X chromosome, which is intriguing since 90% of SLE cases occur in women. Hypothetically, a copy number increase of the *TLR7* or *TLR8* genes could account for this predominance. Indeed, overexpression of *TLR7* triggered autoimmune responses in mice and transgenic cell lines [106]. In PBMCs (the peripheral blood mononuclear cells) of patients with SLE, mRNA expression levels of TLR2, TLR7 and TLR9 were elevated, whereas TLR3, TLR4, TLR5 and TLR8 remained the same [107]. A *TLR5* stop codon polymorphism abrogating TLR5 function was found to be associated with resistance to SLE [108]. Polymorphisms of some, but not all investigated heat shock protein 70 (HSP70) genes were associated with SLE [109, 110]. Some studies suggest HSP70 is an endogenous TLR2 and TLR4 ligand [111, 112]. These associations are controversial, however, since some argue that contaminating PAMPs are responsible for the reported in vitro cytokine effects of HSPs, as highly purified HSPs do not show any cytokine effects [113].

TLR3, which recognizes dsRNA, is absent in B cells, but its activation on glomerular mesangial cells and antigen-presenting cells was shown to aggravate lupus nephritis in a mouse model [114]. However, TLR3 costimulation did not influence TLR7-induced complex glomerulonephritis in this mouse model [104]. The S180L polymorphism in the TLR2 and TLR4 pathway adaptor

protein *TIRAP* was shown to decrease susceptibility to SLE in a Colombian population [115].

The majority of studies investigated the role of TLRs in SLE, but other PRRs may well be involved too. Kimkong et al. [116] found increased mRNA expression levels of IFI16 and AIM2 in PBMCs of SLE patients. Since these are cytosolic dsDNA receptors, they may well be involved in the immune reactions against host-derived DNA.

#### Graft-versus-Host Disease

Graft-versus-host disease (GVHD) occurs when donor cells from a bone marrow transplant attack healthy tissues of the host. The adaptive immune system is thought to be the main culprit, but recent findings implicate the host innate immune system as well. In the case of intestinal GVHD, microbiota may modulate innate immune response via PRRs [117]. For example, in murine experimental GVHD models, *Tlr9* deficiency in the host but not the donor reduced intestinal immunopathology and GVHD-related mortality. GVHD was also reduced in mice upon treatment with synthetic inhibitory oligodeoxynucleotide (iODN) that blocks TLR9 signaling. However, it is not clear whether TLR9 inhibition impairs the graft-versus-tumor response too, which would be highly undesirable [116]. No increased GVHD was seen in recipient mice deficient in the crucial TLR adaptor proteins *Myd88* (*myeloid differentiation factor 88*) or *Trif* (*Toll-interleukin 1 receptor-domain-containing adapter-inducing IFN- $\beta$* ). Recipient *Tlr2* deficiency did not affect GVHD outcome in mice. Recipient *Tlr4* deficiency did not affect GVHD in two murine studies, and more severe GVHD was seen in another. Donor *Tlr4* deficiency did not affect GVHD in one murine study, but decreased GVHD severity in two others, while graft-versus-leukemia activity was preserved in one [117]. *NOD2* polymorphisms were associated with GVHD in human transplant recipients [118, 119]. In mice models, *Nod2*-deficient transplant recipients developed more severe GVHD, which was suggested to be caused by an increased activation status of DCs. *NOD2* may therefore inhibit DC activation. *Nod2* knockout in donor mice did not affect GVHD [120].

#### Systemic Immune Disorders in Which a Role of PRRs Is Speculative

##### Sarcoidosis

Sarcoidosis is characterized by granulomas affecting multiple organs. In one study, severe pulmonary sarcoidosis was associated with *NOD2* polymorphisms [121], but no *NOD2* association was found in sarcoidosis in gen-

eral [122]. In a Japanese population, an association between *NOD1* gene polymorphisms and sarcoidosis susceptibility was found. The polymorphism was associated with reduced *NOD1* expression and impaired NF- $\kappa$ B activation upon infection with *Propionibacterium acnes* [123].

#### Schnitzler Syndrome

Schnitzler syndrome is an acquired syndrome characterized by chronic urticaria paraproteinemia with signs and symptoms of systemic inflammation, such as arthralgia and recurrent fever [124]. It has similarities to the hereditary syndrome CAPS (see above), and IL-1 $\beta$  has been shown to be a central mediator of this disorder as well [125, 126]. The exact pathophysiology is unknown. In 1 patient, the common variant V198M was found in the *NLRP3* gene [127], but not in others [128]. The success of IL-1 inhibition as treatment in Schnitzler syndrome supports the suspicion of a role of PRRs in the pathophysiology of this rare syndrome [126, 128, 129].

#### PRRs as Therapeutic Target in Immune Disorders

In view of their potential detrimental role in a multitude of immune disorders, inhibition of PRR pathways may be a promising therapeutic approach. Conversely, PRR triggering has become a prominent topic in research on the immunomodulation of tumors, which we will discuss separately below.

#### Immunomodulation of PRRs by Established Treatments in Immune Disorders

##### Calcineurin Inhibitors

Calcineurin inhibitors, such as cyclosporin A, tacrolimus and pimecrolimus, are used in the treatment of psoriasis and AD, and to prevent rejection in transplant patients. These agents suppress T cell-mediated immune responses, especially the production of proinflammatory cytokines in T cells, but recently evidence emerged that these calcineurin inhibitors also directly affect PRRs. In an immunohistochemical analysis of AD lesions, tacrolimus was found to reverse the increased TLR1 and decreased TLR2 expression levels [46]. In normal human epidermal keratinocytes, pimecrolimus enhanced TLR2/TLR6-induced expression of antimicrobial peptides. Interestingly, pimecrolimus also increased the functional capacity of keratinocytes to inhibit growth of *S. aureus*, which often colonizes the skin of AD patients and causes superinfections of AD lesions [130].

## Chloroquine

The antimalarial drugs chloroquine and hydroxychloroquine have been applied as therapeutic agents for SLE for many years, although the mechanism of action is unclear. A direct role of modulation of PRRs is suggested by studies which show that they inhibit stimulation of TLR3, TLR8 and TLR9, presumably by direct binding to nucleic acids, thereby masking the TLR-binding epitopes [131, 132].

### *Immunomodulation of PRRs in Experimental Models of Immune Disorders*

#### TLR9 Agonists

Not only inhibition, but also stimulation of PRRs might be of benefit in a therapeutic setting. Applications of TLR9 agonists, also referred to as CpG oligodeoxynucleotides (ODN), are investigated in mouse models of allergy, since the Th1-biased immune response upon TLR9 activation may improve desensitization strategies in allergy treatment. Indeed, CpG ODN inhibited the Th2 response in allergic mice, preventing inflammatory disease manifestations. In human clinical trials with a conjugate of CpG ODN and part of the ragweed allergen as an allergy vaccine, selective redirection of the allergic Th2 response towards a Th1 response, and reduced allergic symptoms were observed [131]. Even though these studies were mainly on murine asthma models, they could have implications for the treatment of allergic cutaneous diseases.

Of note, CpG ODN were also found to accelerate wound healing in mice and rhesus macaques, which could have therapeutic implications for chronic wounds in humans [133, 134].

#### Combined TLR7/TLR9 Inhibition

TLR7 signaling is involved in the pathophysiology of SLE, while results on the role of TLR9 are conflicting. A specific inhibitor of TLR7 and TLR9, immunoregulatory sequence (IRS) 954, was shown to inhibit the induction of IFN- $\alpha$  by human pDCs upon stimulation with DNA and RNA viruses and isolated immune complexes from SLE patients [91]. In SLE-prone mice, IRS 954 prevented progression of disease [135]. Recently, TLR7 and TLR9 signaling was shown to hamper glucocorticoid efficacy in SLE. Triggering of TLR7 and TLR9 by nucleic acid-containing immune complexes or by synthetic ligands enhanced survival of IFN- $\alpha$ -producing pDCs [136]. The role of TLR9 is controversial, however, since others found that TLR9 knockdown resulted in exacerbation of autoimmunity rather than reduction [96].

## PRRs as Therapeutic Target in Tumors

### *Antitumor Immunomodulation of PRRs by Established Treatments*

#### Imidazoquinolines

The prototype of a PRR-targeting therapy is imiquimod, an imidazoquinoline compound which is a synthetic agonist of TLR7 and to a lesser extent TLR8 [137, 138]. In contrast, imidazoquinolines were found to inhibit TLR3 and TLR9 signaling [132]. Imiquimod has potent antitumor and antiviral properties and is an approved topical therapy for superficial basal cell carcinoma, actinic keratosis and genital warts [139, 140]. There are multiple off-label indications for imiquimod, including HSV infections, verruca vulgaris, molluscum contagiosum, keloids, squamous cell carcinoma, Bowen's disease, lentigo maligna, cutaneous T cell lymphoma, Kaposi's sarcoma, and Paget's disease [141, 142]. Imiquimod induces the production of several proinflammatory cytokines, stimulates T cell responses, and instigates the migration of Langerhans cells and pDCs to the lymph nodes [138, 143–145]. Moreover, at higher concentrations imiquimod induced apoptosis in basal cell carcinomas and melanoma metastases, but it is not clear whether this is a direct or indirect effect on the tumor cells [138]. Resiquimod is a more potent TLR7- and TLR8-activating imidazoquinoline which was reported to be effective in the treatment of actinic keratosis [146] and genital HSV-2 infections [147, 148]. Because of its immunomodulating properties, imiquimod has been tested as an adjuvant in antitumor vaccines. In a murine model, topical imiquimod significantly enhanced the protective antitumor effects of a live, recombinant *Listeria* vaccine against melanoma [149]. In melanoma patients, the combination of a NY-ESO-1 vaccine with topical imiquimod elicited both humoral and cellular responses in a significant fraction of patients, but the additive effect of imiquimod was unclear since a vaccine-only control arm was lacking [150]. The downside of immunomodulation by TLR7 agonists is excessive immune responses. Indeed, imiquimod often induces local skin inflammation at the application site, but it was also reported to aggravate psoriatic lesions and even to induce de novo psoriasis [21–24].

### *Investigational Antitumor Immunomodulation of PRRs*

#### Loxoribine

Loxoribine, another TLR7 agonist, enhances the production of IFN, activates NK cells and B cells [151], and was recently found to induce maturation of human

monocyte-derived DCs and to stimulate their Th1- and Th17-polarizing capability [152]. Hence, loxoribine has been under investigation for antiviral and antitumor properties [153], although not as intensively as imiquimod.

### TLR9 Agonists

CpG ODN directly induce the activation and maturation of pDCs and enhance differentiation of B cells into antibody-secreting plasma cells [131]. As an adjuvant, CpG ODN were shown to induce strong CD4+ and CD8+ T cell responses and rapid production of antigen-specific antibodies to many types of antigen [154]. They are therefore considered as promising adjuvants in anti-cancer vaccines, e.g. in the treatment of melanoma [131, 155–157]. In mice with cutaneous melanoma, combination therapy of topical CpG ODN with systemic dacarbazine inhibited tumor growth significantly more than with monotherapy with either agent [156]. Importantly, TLR9 expression patterns differ between mice and humans, and CpG DNA are less stimulating in humans than in mice; therefore these results cannot automatically be extrapolated to humans [154]. In patient trials, monotherapy with the TLR9 agonist PF-3512676 induced immune responses, but for optimal clinical efficacy CpG ODN are currently under investigation as anti-melanoma vaccine adjuvants [155].

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### Conclusion

PRRs have been implicated in the pathophysiology of multiple immune disorders that affect the skin. Once evolved to protect us from pathogens, at which they are quite successful, they can become detrimental if signaling is excessive. Indications that PRRs are involved in the pathophysiology of multifactorial immune disorders are mainly based on genetic association studies and murine knockout models. Comprehensive endogenous expression analyses and functional studies are urgently needed to determine their actual contribution to the pathophysiology. This will also lead to more targeted therapies, since immunomodulation of PRRs seems a promising therapeutic approach to various immune disorders and malignancies.

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