Anthony A. Gaspari
Stephen K. Tyring
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Editors

Clinical and Basic Immunodermatology

Second Edition

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Preface

Building on Our Foundation

In 2008, we published the first edition of *Clinical and Basic Immunodermatology*. Over the past 9 years, much has changed in the basic science and clinical arenas, stimulating Steve Tyring and me to update our textbook. We have the good fortune that Dr. Daniel H. Kaplan, a highly accomplished immunodermatologist, joined our editorial team, which has augmented our knowledge in the field and expanded our network of experts to address the ever-broadening horizons of cutaneous immunology.

There continues to be a tremendous number of advances in the fields of cellular, molecular, innate, and adaptive immunity, as well as immunopharmacology, which have been translated to a better understanding and treatment of a number of dermatologic diseases. There are also a number of new therapeutic agents that are targeted therapies, or have an immune mechanism. All of these developments have occurred in the backdrop of the information age. Our goals of this textbook remain the same as with the first edition of *Clinical and Basic Immunodermatology*. We have recruited national and international experts to author chapters on their respective areas of expertise. Hence, our approach for this important endeavor is that of a multiauthored collection of chapters that would be integrated into this book. Our goal is to present the latest information related to fundamentals of the skin immune system, as well as a disease-focused textbook in the same concise, readable, and easily digested format that was initially developed by Dr. Dahl with his original *Clinical Immunology* textbook in 1981. We have recruited new experts to provide information summarized in their chapters. We have added new subject matter such as the expanded role of innate lymphocytes in the immune system and their role in dermatologic disease, a section on antimicrobial peptides, a chapter focused on auto-inflammatory diseases, as well as a chapter on the role of cell death in skin homeostasis and dermatologic diseases.

We thank the authors for their outstanding contributions. We remain grateful to Dr. Dahl for his vision and his original book, which has had profound influence on generations of dermatologists. We have strived to enhance the teaching of cutaneous immunology, particularly as related to skin disease, to the next generations of young dermatologists who will be caring for patients afflicted with immune-based skin diseases. We would be delighted if our textbook triggered the kind of interest in immunology that was stimulated in us, the editors, during our training.

Over the next few years, we look forward to watching the progress unfold in the field of immunodermatology that will lead to the third edition of our textbook *Clinical and Basic Immunodermatology*.

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Innate and Adaptive Components of the Cutaneous Immune Barrier: The Central Role of Dendritic Cells

1

Georg Stingl, Marie-Charlotte Brüggen, and Mariana Vázquez-Strauss

Abstract

Immune responses initiated in the skin can be extremely powerful at both a local and systemic level. One of the milestones in elucidating the mechanisms underlying this phenomenon was the discovery of the T cell response-inducing function of Langerhans cells (LC). In the meantime, we know that the family of dendritic antigen-presenting cells in the skin is much bigger and, in addition to LC, includes dermal dendritic cells (DDC), CD141+DC, CD14+DC, inflammatory DC and plasmacytoid DC. Depending on the cellular and molecular milieu, these cells can function as either sensitizing or tolerizing elements. Signals transmitted from (innate) receptors recognizing damage- or pathogen-associated patterns are involved in directing these different functions in DC. Toll-like pathogen recognition receptors (TLR) have been particularly well investigated in this regard. The distinct distribution of TLR on LC and other DC subsets allows the immune system to elegantly orchestrate the regulatory and pro-inflammatory functions of these cells. Intriguingly, TLR signaling in DC/LC not only allows to initiate adaptive immune responses, but also directly induces innate effector functions. This is demonstrated by our findings on the mechanisms underlying basal cell carcinoma (BCC) regression upon treatment with the pharmacological TLR7 agonist imiquimod. We observed that in imiquimod-treated BCC, plasmacytoid DC directly kill tumor cells via the apoptosis-inducing molecule TRAIL. Melanoma cells can also become TRAIL-susceptible, but the magnitude of this phenomenon varies from patient to patient. Our recent findings show that TRAIL susceptibility of melanoma cell lines can be increased upon exposure to the anti-inflammatory compound diclofenac.

Taken together, we begin to understand the exact position of LC and DC in the highly complex circuits of the immune system in the skin and how these cells could be manipulated for therapeutic purposes.

Keywords

Dendritic cells • Antigen-presenting cell • APC • BCC • Basal cell carcinoma • Contact hypersensitivity • Dermal dendritic cell • DC • DDC • Lipopolysaccharides • Lipotechoic acid • Adaptive immunity • TLR-transmitted

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Abbreviations

APC	Antigen-presenting cell
BCC	Basal cell carcinoma
CHS	Contact hypersensitivity
DC	Dendritic cell
DDC	Dermal dendritic cell
dsRNA	Double-strain RNA
LC	Langerhans cell
LPS	Lipopolysaccharides
LTA	Lipotechoic acid
MHC	Majory histocompatibility complex
PAMP	Pathogen-associated molecular pattern
pDC	Plasmacytoid dendritic cell
PRR	Pattern recognition receptor
S.	Staphylococcus
ssRNA	Single-stranded RNA
TLR	Toll-like receptor
TRAIL	Tumor necrosis factor related apoptosis inducing ligand
UV	Ultraviolet
poly I:C	Polyinosinic:polycytidylic acid

Introduction

One of the first documented examples revealing the potency of immune responses initiated in the skin is the successful smallpox vaccination by Edward Jenner [1]. In this heroic experiment performed on the son of his gardener, Jenner introduced scraping material obtained from an infectious cowpox pustule (of a dairymaid) into the skin. A few weeks later, upon re-inoculation with material from a fresh smallpox lesion, the boy was protected from the disease.

It took quite a while until it was realized that immunization via the skin can result in a stronger, longer lasting immune response than immunization via other routes. This is impressively illustrated by the induction of cancer immunity, which succeeded following the repeated intracutaneous, but not extracutaneous application of cancer (murine sarcoma) homogenates [2]. During the following decades, considerable efforts were made to unravel the mechanisms underlying this phenomenon.

The enigma seemed resolved when it was discovered that epidermal dendritic cells (namely Langerhans cells (LC)) can evoke very robust proliferative responses in naive and sensitized T cells [3, 4], which were greater in magnitude than those induced by mononuclear phagocytes (see also Chap. 9, Dendritic cells). Streilein et al. could show in a murine model of allergic contact dermatitis termed contact hypersensitivity (see Chap. 22) that the epicutaneous application of a hapten only led to sensitization when the skin of the application site contained LC but not when it was devoid of these cells [5]. Based on these findings, the idea evolved that an antigenic encounter in the skin/epidermis invariably results in LC-mediated T cell activation and thereby sensitization. Were this concept true, one would expect an army of hetero- or even autoreactive T cells to constantly populate, attack and injure the skin. Luckily, this is not the case. The possibility that the mere presence of LC is not necessarily predictive of the occurrence of productive T cell responses came from the observation that LC evoked robust T cell stimulation only upon receipt of activating stimuli [6]. These stimuli include the disruption of skin homeostasis and the exposure to danger signals (e.g. immunogenic haptens, microorganisms) that results in the release of proinflammatory cytokines and other mediators. By contrast, resting LC [7], LC from corticosteroid-treated patients [8] or LC from ultraviolet (UV) irradiated skin (via keratinocytic RANKL expression, [9]) lead to an expansion of CD4+/CD25+/GITR+/FoxP3+ regulatory T cells (Treg) capable of down-modulating proliferative and cytotoxic T cell responses. The exclusive role of LC in the initiation of T cell responses via the skin was further questioned by the discovery of a second DC population in normal human skin, namely CD1+ dermal dendritic cells (DDC). DDC are equally potent as LC with regard to their immunostimulatory capacity *in vitro* but exhibit certain phenotypic features that allow distinguishing them from LC (cf. Fig. 1.1a). The relative contribution of LC and DDC in the elicitation of sensitizing and tolerizing skin-derived immune responses is a matter of conjecture and heavy debate. On the one hand, the positive correlation between epidermal LC density and the success rate of epicutaneous sensitization (e.g., contact hypersensitivity (CHS), epicutaneous vaccination) clearly argue in favor of an important role of LC in this process. On the other hand, elimination of epidermal LC in mice by genetic manipulation results in an enhanced CHS response as compared to wild-type mice [10].

From a conceptual viewpoint, it makes perfect sense to assume that danger signals not reaching beyond the epidermis, i.e. the site of LC, will not mobilize all the armed forces the immune system is capable of activating. On the other hand, virulent microorganisms that breach the dermo-epidermal junction and thereby can reach DDC should trigger a massive host defense response that can successfully eliminate the pathogen. From this perspective, one would expect that LC mainly act as regulatory antigen-presenting cells (APC) inducing a state of antigen-specific non-responsiveness. Following this reasoning, DDC should remain inert under homoeostatic conditions and mature into potent sensitizing DC upon receiving appropriate activation signals. The “black and white” picture of the roles of LC vs. DC is probably not tenable under all circumstances. As an example, in response to an overwhelming microbial insult, LC can engage themselves

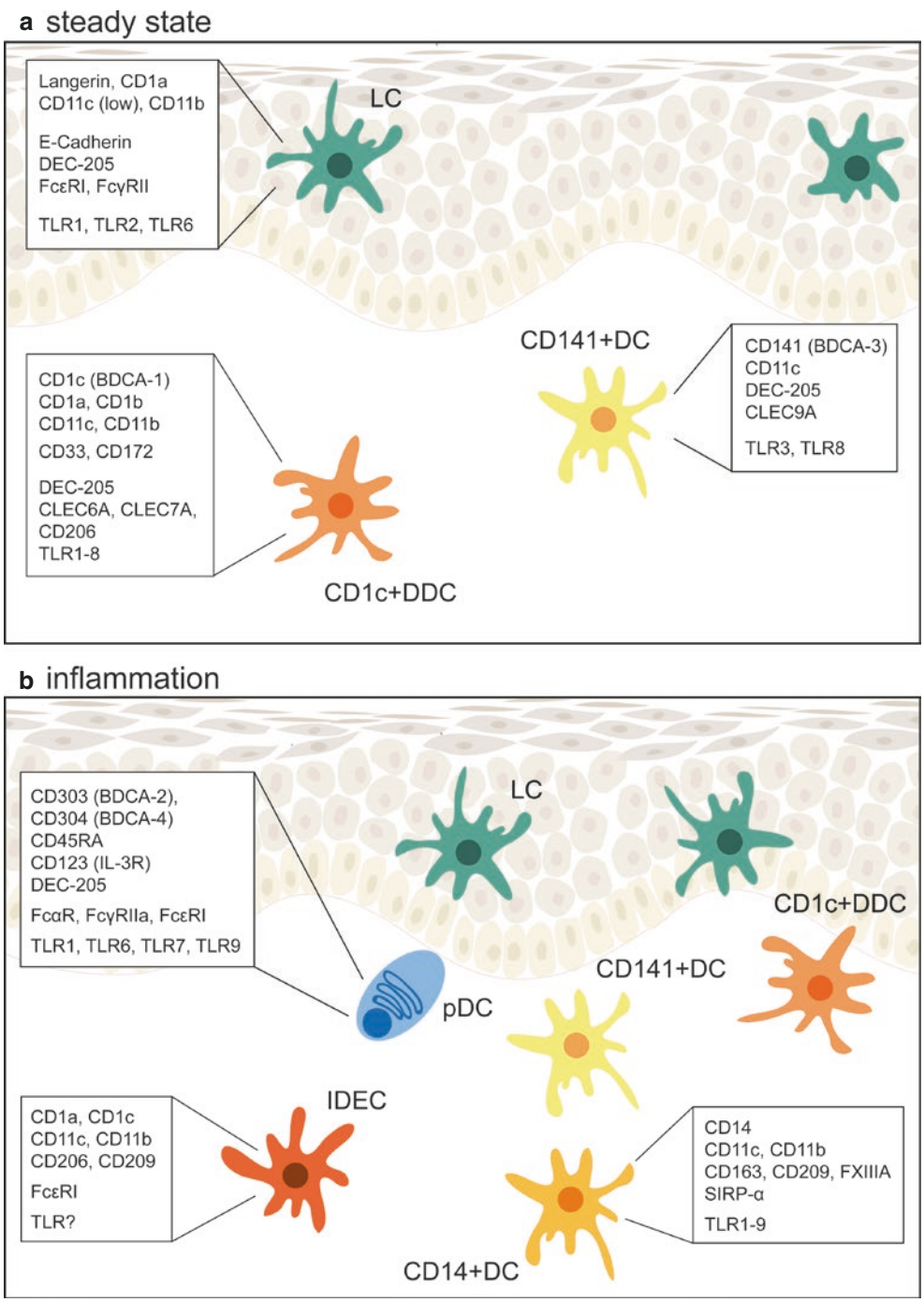

Fig. 1.1 LC and DC populations in human skin. (**a**) LC and DC residing in human skin in the steady state and (**b**) infiltrating the skin during inflammation. The main suface markers and pattern recognition receptors of the respective subpopulations are depicted

in the promotion of a T effector cell response [7]. Although we have gained more insight into the LC and DC network in the skin, we are still far from understanding it in all its complexity. This is illustrated by the recent finding of another (although quantitatively minor) DC subset residing in steady-state skin, i.e. CD141+ DC (Fig. 1.1a) [11]. These DC seem to be mainly engaged in antigen cross-presentation [12].

In an inflammatory setting, skin-resident LC, DDC and CD141+ DC undergo phenotypic and functional changes. In addition, various other types of DC are entering the stage (cf. Fig. 1.1b). These blood-derived DC include the so-called plasmacytoid DC (pDC), various DC with an inflammatory phenotype (inflammatory DC, IDEC) and CD14+ (monocyte-derived) DC. All of them exhibit distinct features with regard to their antigen presentation properties and interaction with other immune cells (for review, cf. [13, 14]). They play major roles in the pathogenesis of various skin conditions such as atopic dermatitis (Chap. 22) and psoriasis (Chap. 21). Their involvement

in these diseases will be discussed in the respective chapters of this book.

Toll-Like Receptors: Bridging Innate and Adaptive Immunity

Pattern Recognition Receptors: Sensing the Danger

Until quite recently, it was essentially unknown by which mechanisms danger signals (such as immunogenic haptens and microorganisms) trigger the activation and maturation of LC and other DC in the skin and ultimately initiate an adaptive immune response. A series of discoveries (for review, cf. [15, 16]) has shed new light on this issue by revealing that DC function and development are essentially modulated by innate immune receptors recognizing damage- or pathogen-associated molecular patterns (DAMP and PAMP; listed in Table 1.1) (see Chap. 2). Among this growing family of pattern recognition receptors (PRR), the so-called toll-like pathogen recognition receptors (TLR) have been particularly well investigated. Ten TLR have been described in humans so far (listed in Table 1.2). TLR can be broadly divided into two groups (extra- vs. intracellular). Extracellular TLR (TLR1, 2, 4, 5, 6) essentially recognize bacterial and fungal products. Briefly, TLR2 combined with TLR1 or TLR6 mostly recognizes motifs of gram-positive bacteria (e.g. lipoproteins, lipotechoic acid (LTA)), while TLR4 senses gram-negative bacteria-associated lipopolysaccharides (LPS). Bacterial flagellin is recognized by TLR5. The intracellular receptors TLR3 and TLR7-9 recognize mostly virus-derived nucleic acids, i.e. double-stranded RNA (dsRNA; TLR3), single-stranded RNA (ssRNA) (TLR7-8) and CpG oligodeoxynucleotides (TLR9).

The potency of TLR-mediated danger signals in triggering immune responses cannot be reduced to their impact on DC and other cells of hematopoietic origin. In fact, keratinocytes [17] express a series of TLR (at the mRNA level: TLR1-6 and 9-10; functionally: TLR3, 4, 5 and 9, [17, 18]). Engagement of their respective ligands can trigger (as illustrated in the following paragraphs) both innate and adaptive responses.

As far as LC and DC are concerned, studies investigating their TLR expression have yielded partially divergent results [18–21], probably due to differences in the experimental setting, e.g. culture conditions. It seems clear that LC and the various DC subsets do not share the same TLR expression patterns (cf. Fig. 1.1a, b) and, in consequence, exhibit differ-

Table 1.1 Pattern recognition receptors (PRR) and their principal ligands

A) Principal PRR families

Group of PRR	Examples of PRR	Principal PAMP/DAMP(s)
Nucleotide-binding oligomerization domain (NOD)-like receptors (NLR)[a]	NOD1 (CARD4) NLRP1B (NALP1) NLRP3 (NALP3)	iE-DAP, GM-tripeptide Anthrax letal toxin MDP, DNA, RNA, toxins
Retinoic acid inducible gene I (Rig1)-like receptors (RLR)	DDX58 (RIG-1) DHX9, DHX36	Short ds-RNA, ss-RNA DNA
C-type lectin receptors (CLR)	CD207 (langerin), CD209 (DC-SIGN), CLEC6A	Fucose, mannose High mannose High mannose
Toll-like receptors (TLR)	(cf. below)	

[a]For details, cf. [16, 55]

B) TLR

Localization	TLR subtype	Principal PAMP(s)	Mostly expressed on
Extracellular	TLR1/TLR2[a]	Lipoproteins	Gram-positive bacteria, mycobacteria
	TLR2	Lipoproteins, peptidoglycan (PGN)	Gram-positive bacteria
	TLR4	Lipoproteins, lipopolysaccharides (LPS)	Gram-negative bacteria
	TLR6/TLR2[a]	e.g. lipoteichoic acid (LTA)	Gram-positive bacteria, mycoplasma
	TLR10	Not known	
	TLR5	Flagellin	Flagellated bacteria
Intracellular	TLR7	Single-stranded RNA (ssRNA)	Viruses
	TLR8	ssRNA	Viruses
	TLR9	CpG oligodeoxynucleotide	Bacteria; DNA viruses
	TLR3	Double-strain RNA (dsRNA)	Viruses

[a]Heterodimerized; for review, cf. [15]

ent reactions to microbial or other immunogenic stimuli. This distinct distribution of TLR on DC allows the immune system to elegantly orchestrate innate and adaptive responses, which is why growing efforts have been put into the development of vaccine formulations making use of these mechanisms.

TLR as Gatekeepers of Tolerance Towards Bacteria?

According to the idea that LC are responsible for maintaining tolerance and DDC for initiating immune reactions, one would expect that LC do not react to the epidermal invasion of harmless, gram-positive bacteria belonging to the commensal skin flora. This theory seems to be supported by the finding that DDC abundantly secrete IL-6 and TNF-α when exposed to bacterial components (such as Pam3CSK, a synthetic TLR1/2 ligand, LPS and PGN [21]), while LC secrete IL-6, -8 and -10 only upon exposure to PGN [18, 21]. PGN-induced IL-10 could, via its inhibitory effect on the antigen presentation function of LC [22], contribute to LC-modulated tolerance towards commensal bacteria. The concept that TLR-mediated signals can contribute to maintaining tolerance is further strengthened by evidence from keratinocyte studies. The latter have shown that in keratinocytes engagement of LTA belonging to the commensal bacterium *Staphylococcus (S.) epidermidis*, but not to *S. aureus* induces an inhibitory effect on TLR3-triggered IL-6 and TNF-α expression [23] and even promotes the expression of antimicrobial peptides [24].

Orchestration of TLR-Transmitted Signals in Viral Infections

As far as viral infections are concerned, it was even before the discovery of TLR that the so-called pDC were identified as a rich source of the type I interferon IFN-alpha (IFN-α) in response to viruses (review in: [25]). IFN-α is a potent tool in the antiviral defense and acts against viruses both indirectly (by enhancing adaptive immune functions) and directly. Later, it was found that the abundant IFN-α production in pDC is triggered by signals from TLR recognizing virus components, i.e. ssRNA (TLR7) and CpG oligonucleotide (TLR9). In contrast, DDC as well as freshly isolated LC do not seem to undergo phenotypic or functional changes in response to direct exposure to these TLR ligands [18]. In the presence of CpG-stimulated keratinocytes however (which abundantly produce IL-1α, TNF-α and GM-CSF), LC up-regulate major histocompatibility complex (MHC) class II and the costimulatory molecule CD86 [26]. The complexity of TLR-transmitted "danger signals" is illustrated by the finding that dsRNA, the virus-associated ligand for TLR3, does not elicit any response in pDC (for review, cf. [25]) but instead enhances different functions in LC and DDC. In LC, the synthetic TLR3 ligand polyinosinic:polycytidylic acid (poly I:C) induces changes that promote an adaptive antiviral response. These include maturation, IL-6 production and upregulation of CD70 (a potent promoter of $CD8^+$ T cell responses) [27]. Meanwhile, exposure of CD141+ DC to poly I:C results in IFN-γ production in CD141+ DC [11] and enhances (in a skin explant model) maturation and migration [20]. Keratinocytes respond to poly I:C by up-regulating surface molecules such as MHCII, CD40 and the Fas receptor [17] and by abundantly secreting TNF-α and IL-6 [23].

TLR-Transmitted Danger Signaling Beyond Skin Infections

A role of TLR danger signals has been demonstrated in various skin conditions beyond infections including acne vulgaris (Chap. 24), roseacea, skin cancers and psoriasis (Chap. 21) (for overview, cf. [28]). In the case of CHS, it had long been known that immunogenic haptens induce the secretion of proinflammatory cytokines in keratinocytes, LC [29] and DC and that skin inflammation is required for the development of sensitization to a hapten. The molecular events behind this remained obscure. An involvement of certain TLR in CHS was indicated by studies revealing that TLR2/TLR4 double-deficient mice are completely resistant to CHS development (see also Chap. 23). The finding that germ-free mice still develop CHS pointed towards a role of endogenous (and not necessarily microbial) ligands in eliciting inflammation during the sensitization phase. In mice, some allergens (such as 2,4,6-trinitro-1-chlorobenzene, oxazolone, and fluorescein isothiocyanate) seem to indirectly activate TLR [28]. Meanwhile, Goebeler et al. were able to demonstrate in elegant experiments that Ni^{2+} ions directly bind to the human TLR4 and, by doing so, initiate a signaling cascade resulting in the generation of proinflammatory signals [30]. The respective role of keratinocytes, LC and DC in TLR-mediated inflammation during the sensitization phase of CHS remains to be elucidated. The lack of TLR expression on LC for instance did not dampen CHS development in a mouse model [31].

In atopic dermatitis (see also Chap. 22), patients exhibit reduced expression of TLR2 on keratinocytes and monocytes/macrophages [32, 33]. TLR2 recognizes *S. aureus*-associated patterns and enhances the expression of certain tight junction molecules [34]. The deficiency of TLR2 in atopic dermatitis patients could thereby not only contribute to their susceptibility to *S. aureus* infections but also reinforce barrier dysfunction, a major feature of the disease.

TLR-Driven Innate Effector Functions of DC

Imiquimod: A Pharmaceutic TLR Ligand

In the early 1990s, it was reported that incubation of peripheral blood leukocytes with certain imidazoquinolines (e.g. imiquimod, resiquimod) results in the production of IFN-α by these cells. Soon, it became clear that imiquimod acts as an artificial ligand of TLR7 [35], single-strand sensing receptor important in triggering IFN-α in pDC [36].

Given the crucial role of IFN-α as a first line defense against viral infections, imiquimod has been developed into a topical cream compound (Aldara®) for the treatment of viral acanthomas such as genital warts [37]. In the years to come, Aldara® cream was also proven to be efficacious in superficial basal cell carcinomas (BCC), lentigo maligna and actinic keratoses (review in: [38]).

pDC as Effector Cells in Imiquimod-Induced Tumor Regression

We as well as other investigators set out to unravel the mode of action of topical imiquimod. In a first series of experiments, we observed that application of Aldara® cream to murine ear skin for only a few days causes massive infiltration of neutrophils, macrophages and, particularly noticeable, pDC. In subsequent experiments we transplanted a (murine) melanoma cell line into the skin of mice. After several weeks, melanomas had appeared and were then treated with either Aldara® cream or vehicle. Aldara® but not the vehicle regularly induced resolution of tumors not exceeding a volume of 130 mm^3. Again, pDC were conspicuously present around and within the regressing melanoma cell islands [39]. All these findings led us to hypothesize that pDC were, in one way or the other, involved in Aldara®-induced tumor regression. In a subsequent study, we treated sporadic superficial BCC from seven patients with topical imiquimod for a total of 6 weeks and examined the clinicopathologic features of the tumor during the course of therapy [40]. After 2 weeks of treatment, BCC lesions showed signs of severe inflammation that quickly resolved after termination of therapy and left behind an area of normal-appearing skin histopathologically free of cancer cell nests (Fig. 1.2a). Immunohistological analysis of lesional skin after 2 weeks of imiquimod treatment revealed changes similar to those seen in our murine model. This was evidenced by a considerable number of apoptotic cancer cells and tumor cell islands surrounded and/or infiltrated by a dense inflammatory infiltrate that contained considerable numbers of inflammatory DC of both the myeloid and the plasmacytoid type (Fig. 1.2b, c). When we evaluated by immunohistochemistry the expression of lytic molecules, we surprisingly found granzyme B and perforin mainly on myeloid DC and TRAIL (tumor necrosis factor related apoptosis inducing ligand) mainly on pDC. Strikingly, the apoptosis-inducing TRAIL-receptor 1 was expressed on BCC (Fig. 1.2c). These in vivo data received experimental support by *in vitro* studies demonstrating the capacity of imiquimod to induce TRAIL on peripheral blood pDC in a strictly IFN-α-dependent manner. TRAIL-expressing, but not unstimulated pDC were perfectly capable of lysing MHCI – bearing tumor cell targets [40, 41] implying that TRAIL-positive pDC in BCC are directly responsible for the killing of the cancer cells. The presence of the pro-apoptotic TRAIL receptor 1 on BCC cells supports this notion [40] as do studies in melanoma-bearing mice treated with imiquimod [42].

Melanoma

In the case of human melanoma, the situation is more complex. We have recently reported that pDC that had been rendered TRAIL-positive by imiquimod stimulation were capable of lysing certain melanoma cell lines, but not others [41]. Further investigations revealed that these differences in TRAIL sensitivity are due to distinct expression patterns of pro-apoptotic TRAIL receptors on different melanoma cell lines and, more importantly, of pro- and anti-apoptotic effector molecules within these cell lines (Fig. 1.3a, b) [43, 44]. When searching for ways to increase the TRAIL susceptibility of resistant cell lines, we found in accordance with previous reports [45] that the anti-inflammatory compound diclofenac was able to do so (Vazquez-Strauss et al., in preparation). In fact, diclofenac led to an enhanced expression of pro-apoptotic TRAIL receptors on melanoma cells as well as to an upregulation of pro-apoptotic and, vice versa, a downregulation of anti-apoptotic molecules within the cancer cells [46]. It will be interesting to explore whether the beneficial effect of diclofenac in the treatment of certain cancers is, at least partly, due to this phenomenon and, if so, whether ways can be found to maximize this tumoricidal effector mechanism.

Conclusions and Outlook

As a result of intensive and increasingly sophisticated research, we begin to understand the cellular and molecular pathways operative in immune responses starting and terminating in the skin. It has become apparent that a highly complex interplay between the innate and adaptive immune system is required to maintain skin homeostasis and initiate host defense. LC and other DC play a central role in this complex network due to their multifaceted roles under physiologic and pathologic conditions.

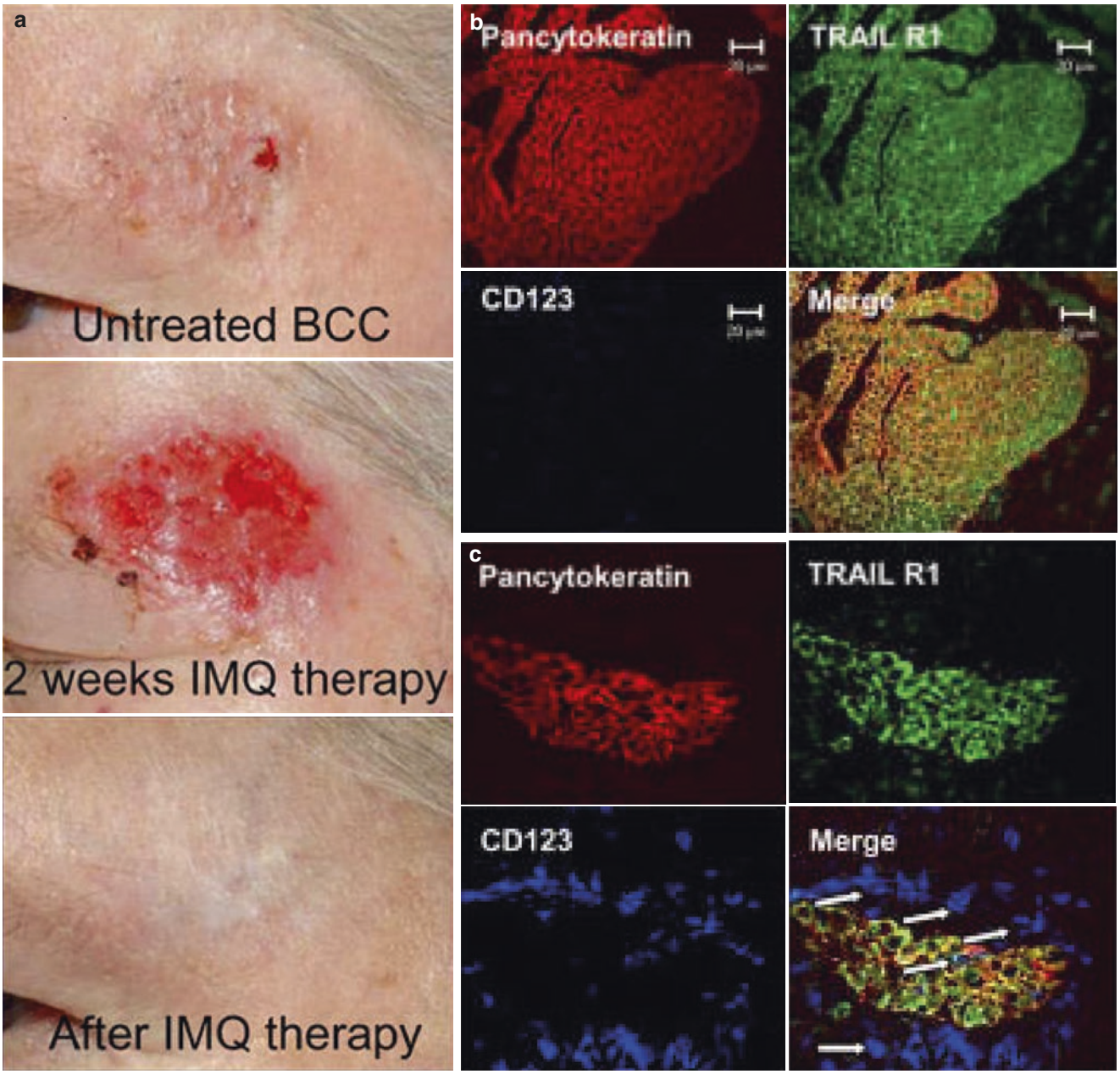

Fig. 1.2 Effects of imiquimod on BCC. (**a**) Imiquimod topically applied to superficial BCC five times a week for a period of 6 weeks led to a local inflammatory response, which resulted in a complete clinical and histopathological tumor clearance in all patients treated. The clinical pictures are representative for all patients (n = 7) treated with imiquimod. Immunofluorescence triple labeling of (**b**) untreated and (**c**) imiquimod-treated BCC with anti-pancytokeratin (TRITC), anti-TRAIL-R1 (A488) and anti-CD123 (Cy5) reveals TRAIL-R1+ BCC cells surrounded by CD123+ cells (*arrows* in **c**) (© 2007 Stary et al. [40])

In the recent past, methods have been developed to exploit the functional diversity of LC and other DC subpopulations for therapeutic purposes. This is exemplified by the development of new and better intradermally delivered vaccines (for review, [47]). In this setting, instead of simply injecting an antigen, the latter is selectively "addressed" to LC and/or certain DC subpopulations. Depending on both the type of ligand to which the antigen is coupled and the nature of the target structure, this allows to direct DC antigen-presenting function in one or the other direction. A good example is the engagement of C-type lectin receptors on DC surfaces such as DEC-205/CD205, langerin/CD207, DC-SIGN/CD209, Dectin, Clec9A, DCIR 1 and 2. These receptors facilitate antigen uptake and sometimes (e.g. TLR3 and TLR7,8 agonists, CD40) induce maturational events in these cells. Clinically, this results in robust humoral and cellular CD4+

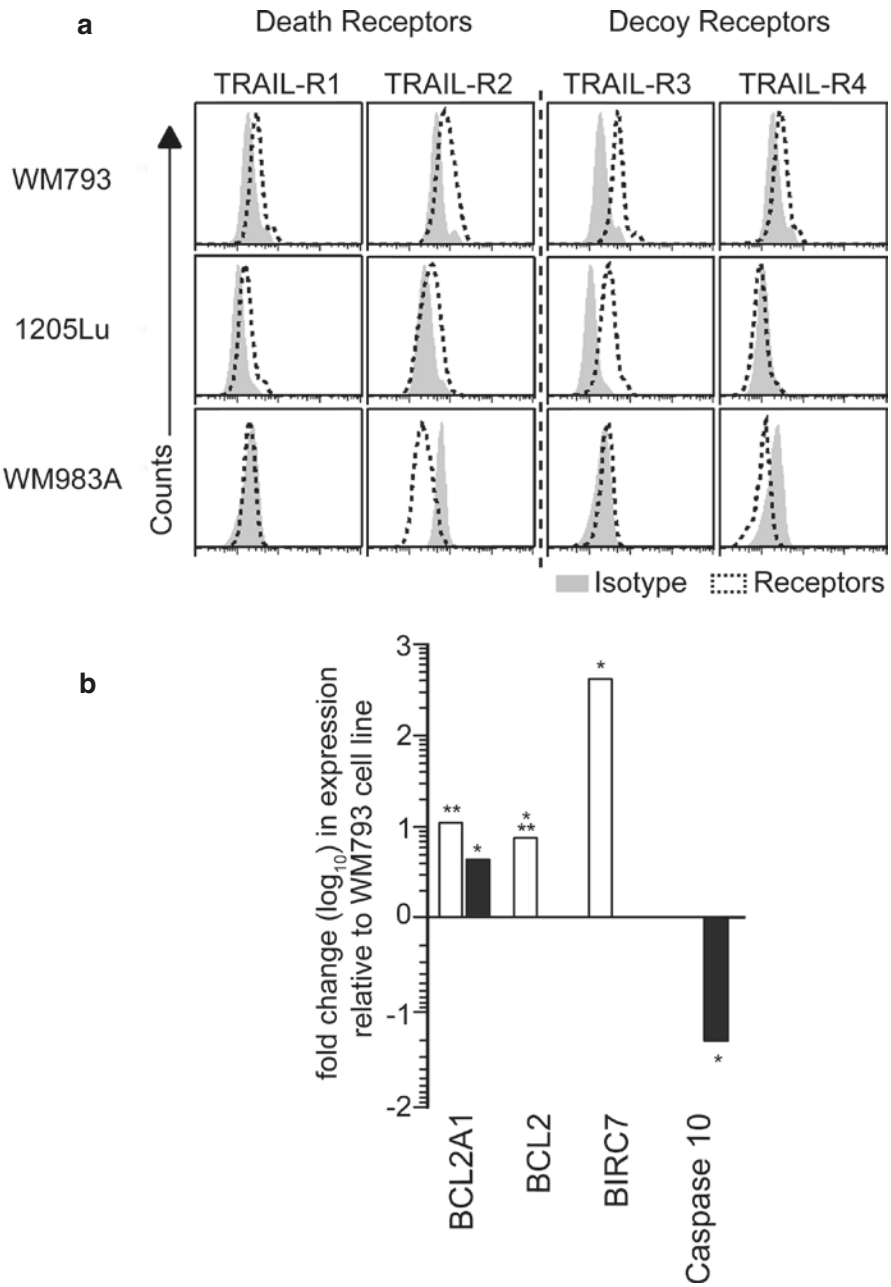

Fig. 1.3 Expression of TRAIL receptors and apoptosis-related genes in TRAIL-resistant and -susceptible melanoma cell lines. (**a**) The expression of TRAIL receptors was assesed by flow cytometry in in two resistant melanoma cell lines (WM983A and 1205Lu) and one susceptible melanoma cell line (WM793). Death receptors (TRAIL-R1, TRAIL-R2) and decoy receptors (TRAIL-R3, TRAIL-R4) for TRAIL were analyzed. Histograms representative for three experiments are shown. (**b**) Using qPCR array technology, the expression of 91 apoptosis-related genes was screened in the same three melanoma cell lines (TRAIL resistant: WM983A and 1205Lu; TRAIL-susceptible: WM793). Three biological replicates were performed. Data represent the mean fold (log_{10}) change in mRNA expression of the depicted molecules in the resistant cell lines as compared to the susceptible WM793 cell line. Some of the most significantly up- and down-regulated genes (>4-fold change in expression) are displayed. *p<0.05; **p<0.01; ***p<0.001

and CD8+ T cell responses. In the absence of adjuvants, however, targeting DEC-205+ DC *in vivo* can induce tolerance [48]. Activation of Clec9A promotes potent antibody responses and facilitates cross presentation [49]. Particularly efficient in this latter regard is the CD40 receptor, probably because of its relatively poor uptake and intraendosomal degradation [50]. By contrast, targeting the lectin-like receptor DC-asialoglycoprotein favors the generation of IL-10-producing CD4+ suppressor cells [51]. Other approaches resulting in either sensitization or tolerization include the use of nanoparticles [52, 53] as well as of cholera toxin [54]. On the other hand we could show that the use of TLR7, 8 agonists can drive innate effector functions in certain DC, i.e. their transformation into killer cells. Thus, the prospect to an efficacious DC-based immunotherapy, tailored to the needs of the individual patients, is realistic, yet challenging.

Questions

1. Which one of the following statements on LC in the skin is correct?
   A. UV-irradiation and the treatment with corticosteroids enhance the ability of LCs to induce cytotoxic T cell responses

B. LC are exclusive stimulators of Th1 cells
C. Under certain conditions, LC can induce the expansion of Tregs and down-regulate proliferative and cytotoxic T cell responses
D. In contrast to keratinocytes, LC cannot produce any inflammatory cytokines
E. LC are found in the dermis but not in the epidermis

2. Which statement regarding TLR is true?
   A. TLR are exclusively expressed on cells of hematopoietic origin
   B. While TLR signaling is a major modulator of innate immune responses, it does not have any effect on adaptive immune responses
   C. TLR recognizing lipids are located on the outer cell membrane while those recognizing proteins are found intracellularly
   D. The different DC subsets in skin express the same TLR repertoire
   E. TLR belong to the PRR family that includes receptors recognizing damage- and pathogen-associated molecular patterns

3. Which statement does not describe parts of the mechanism underlying the imiquimod-induced clinical regression of BCC?
   A. Imiquimod acts as an artificial TLR7-ligand
   B. Imiquimod induces pDC to kill BCC cells in a mostly TRAIL-mediated fashion
   C. Imiquimod induces the killer molecule TRAIL on peripheral blood pDC in an IFN-α-dependent manner
   D. Imiquimod-treated BCC become selectively infiltrated by NK cells
   E. Imiquimod application leads to the apoptosis of BCC cancer cells

Answers

1. C
2. E
3. D

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Toll-Like Receptors

2

Jessica Shiu and Anthony A. Gaspari

Abstract

There are two major arms of the immune system: the innate immune response and the adaptive immune response. Innate immunity is the first line of defense against microbes and serves to limit infection within the early hours after exposure to a pathogen. It is classically associated with the recognition of pathogens by phagocytic cells via specific receptor recognition molecules or through complement fixation. Essential components of the innate immune response include neutrophils, natural killer cells, natural killer T cells, mast cells, complement, and antimicrobial peptides. Innate immune activation via pattern recognition receptors results in a specific expression of co-stimulatory molecules and cytokines. This inflammatory milieu shapes the subsequent adaptive response, which involves B cell activation and T cell-mediated recognition of foreign antigens presented on major compatibility complexes (MHC) I and II on the cell surface of antigen-presenting cells (APCs). Activated B and T lymphocytes then undergo clonal expansion to provide an antigen-specific immune response.

Keywords

Dermatitis • Inflammation • Proteins • Toll • Keratinocyte

There are two major arms of the immune system: the innate immune response and the adaptive immune response. Innate immunity is the first line of defense against microbes and serves to limit infection within the early hours after exposure to a pathogen [1]. It is classically associated with the recognition of pathogens by phagocytic cells via specific receptor recognition molecules or through complement fixation [1–3]. Essential components of the innate immune response include neutrophils, natural killer cells, natural killer T cells, mast cells, complement, and antimicrobial peptides. Innate immune activation via pattern recognition receptors results in a specific expression of co-stimulatory molecules and cytokines. This inflammatory milieu shapes the subsequent adaptive response, which involves B cell activation and T cell-mediated recognition of foreign antigens presented on major compatibility complexes (MHC) I and II on the cell surface of antigen-presenting cells (APCs) [3–5]. Activated B and T lymphocytes then undergo clonal expansion to provide an antigen-specific immune response.

The discrimination between innate and adaptive immunity has long been recognized but the mechanisms that linked the two major arms of immunity were largely unknown until Charles Janeway first proposed the theory of pattern recognition in 1989 [2]. He suggested that highly conserved microbial molecular constituents called pathogen associated molecular patterns (PAMPs) activate germline-encoded receptors on innate cells coined 'pattern recognition receptors' (PRRs). Janeway's pattern recognition theory was later confirmed by the discovery of the toll-like receptor (TLR) family as well as other PRRs such as NOD1 and the family of NOD-like receptors (NLRs) [6–8]. TLRs represent

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Key Points

- Toll-like receptors (TLRs) represent a key receptor family of the innate immune response that recognize pathogen associated molecular patterns as well as damage associated molecular patterns
- TLRs play essential roles in shaping both innate and adaptive immune responses
- TLRs work through two pathways:
  - Adaptor protein myeloid differentiation factor 88 (MyD88) to activate transcription factor NF-κB and MAP kinases (used by all TLRs except TLR3)
  - Adaptor protein TIR domain-containing adaptor protein inducing interferon-beta (TRIF) dependent pathway used by TLR3 and TLR4 that results in type I interferon expression
- TLRs play diverse roles in multiple dermatologic diseases and mutations in TLR signaling pathways have been mapped in human patients, some examples include:
  - TLR2, TLR9 and TOLLIP polymorphisms have been identified in atopic dermatitis patients
  - Activation of TLR4 by nickel, cobalt and palladium in allergic contact dermatitis
  - LL-37, an antimicrobial peptide, complexes with self DNA and activates plasmacytoid dendritic cells to create a DAMP, and drive psoriatic inflammation
- Studies in modulating TLRs for treatment strategies have yielded promising results in a variety of dermatological diseases including treatment of psoriasis, melanoma etc.

a key component of the innate immune system involved in sensing danger. Depending on the particular stimulatory antigen involved, specific downstream components of the signaling pathway are activated, which leads to the generation of an inflammatory response that shapes the subsequent adaptive immune response. Thus, TLRs play an essential role in bridging the gap between innate and adaptive immunity. In support of this notion, studies have implicated TLRs in a variety of human diseases – TLR5 mutations have been linked to an increased susceptibility to Legionnaire's disease [9] while TLR3 deficiency has been associated with herpes simplex encephalitis [10]. In the skin, TLRs have been shown to impact a variety of skin diseases and some widely used dermatologic drugs may possibly exert their therapeutic effects through TLR signaling (Table 2.1) [76]. This chapter will review recent evidence that demonstrates how TLRs affect a variety of skin diseases and infections.

Discovery of TLRs in Humans and Its Expanding Role in Immunity

After Janeway proposed the theory of pattern recognition, based on what was then known about other innate immune receptors, his group was in search for cell-surface receptors expressed on APCs that resulted in NF-κB activation [77]. Lemaitre et al. first identified the antifungal function of Drosophila Toll and demonstrated that it plays a key role in regulating antibacterial gene expression through the NF-κB-like signaling pathway [78]. This seminal discovery paved the path for the discovery of its human counterpart in which Janeway et al. [79] demonstrated that the mammalian Toll homolog induced expression of genes encoding B7 and cytokines that affect the adaptive immune response, providing confirmation for the theory of pattern recognition. Researchers began a fervent search for the ligand of human Toll (now known as TLR4). The first clue came when researchers found that C3H/HeJ mice were unresponsive to bacterial lipopolysaccharide (LPS) and mapped the genetic locus required for LPS responsiveness to *TLR4* [80, 81]. Subsequent studies that attempted to clarify this ligand-receptor interaction proved to be difficult until the other protein in the receptor complex, MD2, was discovered [77, 82]. Since then, studies by many groups have identified multiple other members in the TLR family and elucidated many of their ligands [83]. For their efforts in discovering the toll receptors in Drosophila, Bruce Beutler and Jules Hoffmann won the Nobel Prize in Physiology or Medicine in 2011. TLRs are now the most well characterized PRRs and it is established that different TLR members recognize a variety of PAMPs. Up to 13 TLRs have been identified in mice but only 10 are present in humans as TLR11, 12 and 13 have been lost from the human genome [84]. In contrast, the C-terminal of TLR10 in mice is disrupted by a retrovirus insertion and is nonfunctional. For a detailed look at the history of TLRs, see Table 2.2.

As our understanding of TLRs has expanded in the past couple of decades, increasing evidence has indicated that TLRs are not limited to recognizing PAMPs but can also bind to signals released from damaged tissues, a notion first pioneered by Polly Matzinger who proposed the danger theory as an alternative to the mechanism of immunity initiation [92]. Non-pathogen associated material that leads to tissue injury and other endogenous ligands released during cellular injury such as chromatin bound high mobility group 1 and heat shock proteins also bind and activate TLR signaling [93–97]. Thus, in addition to being the first line of defense against pathogens, TLRs also survey the expression of danger-associated molecular patterns (DAMPs) seen in tissue injury (Fig. 2.1). TLR activation by DAMPs results in sterile inflammation that may play a role in chronic skin

Table 2.1 Toll-like receptors (TLRs) in dermatological disease

TLR	Disease	Comments
1	Tuberculoid	TLR1 favors Th1 phenotype [11]
	Leprosy	*TLR1* I602S mutation protects from *M. leprae* [12]
	Psoriasis	TLR1 expression increased in keratinocytes [13]
	Lyme disease	*TLR1* polymorphism associated with severe disease [14, 15]
	Syphilis	Increased neurosyphilis risk in *TLR1* polymorphisms [16]
2	Acne vulgaris	*P. acnes* stimulates TLR2 and causes hypercornification of sebaceous glands [17]
		Retinoids exert anti-inflammatory effects via TLR2 [18–20]
	Atopic dermatitis	*TLR2* R753Q mutation associated with severe disease [21–23]
		TLR2 signaling necessary for skin barrier repair [24–26]
		TLR2 skews cytokine profile towards a Th2 phenotype [27–30]
	Psoriasis	Increased TLR2 expression in keratinocytes [13]
	Staphylococcus aureus infection	TLR2 deficiency led to increased susceptibility [31, 32]
	Lepromatous leprosy	Associated with $Arg^{677}Trp$ mutation in Korean population [33]
		$Arg^{677}Trp$ mutation: decreased cytokine production [34]
	Syphilis	Lipoproteins stimulate TLR2 [35]
		Increased neurosyphilis risk in *TLR2* polymorphisms [16]
	Lyme disease	Outer surface proteins stimulate TLR2 [36]
		Patients with $Arg^{753}Gln$ mutation secreted less proinflammatory cytokines [37]
	Candidiasis	Phospholipomannans and glycans stimulate TLR2 [38, 39]
	HSV	Glycoproteins stimulate TLR2 [40, 41]
		$TLR2^{-/-}$ animals are more susceptible to HSV encephalitis [42]
3	Psoriasis	Mutation in *AP1S3*, gene required for TLR3 trafficking, associated with pustular psoriasis [43]
	HSV	$TLR3^{-/-}$ astrocytes fail to produce type I IFN [44]
		Humans with TLR3 deficiencies are more susceptible to HSV encephalitis [10]
4	Acne vulgaris	*P. acnes* LPS stimulates TLR4 [45]
	Allergic contact dermatitis	Nickel, cobalt and palladium binds and activates TLR4 signaling [46–48]
	Psoriasis	Increased HSPs that can activate TLR4 signaling [49, 50]
	Syphilis	Lipoproteins stimulate TLR4 [35]
	Candidiasis	Polysaccharides activate TLR4 [38, 39]
		Important for neutrophil recruitment [51]
	UV exposure	TLR4 hyporesponsiveness leads to impaired TNFα production [52]
		TLR4-MyD88 axis deficiencies led to increased cell survival and upregulation of necroptic markers [53]
		TLR4 deficiency led to increased nucleotide excision repair [54]
6	Syphilis	Increased neurosyphilis risk in *TLR6* polymorphisms [16]

(continued)

Table 2.1 (continued)

TLR	Disease	Comments
7	Psoriasis	Imiquimod, TLR7 agonist, drives psoriasis formation [55, 56]
	Systematic lupus erythematous (SLE)	pDCs bind self nucleic acids to stimulate IFN production via TLR7 and 9 [57]
		Small nuclear RNA binds and activates TLR7 and 8 [58]
		Gene duplications of TLR7 increases autoantibody production [59]
		Chronic TLR7 and 9 stimulation leads to glucocorticoid resistance [60]
		Dual TLR7 and TLR9 inhibitor led to decreased autoantibody production in animals and being tested in humans [61, 62]
	Melanoma	Imiquimod and 852A, TLR7 agonist, has been shown to have antitumor effects [63, 64]
	Mycosis fungoides	Imiquimod shown to have clinical responses [65]
	UV exposure	Imiquimod enhances DNA repair and decreased DNA damage [66]
8	SLE	Small nuclear RNA binds and activates TLR7 and 8 [58]
9	Atopic dermatitis	Polymorphisms associated with disease [67]
	Psoriasis	DNA complex with LL-37 stimulates TLR9 to drive IFNα-mediated inflammation [68]
	SLE	pDCs bind self nucleic acids to stimulate IFN production via TLR7 and 9 [57]
		Paradoxical role as TLR9 deficient mice promoted SLE development [69, 70]
		Chronic TLR7 and 9 stimulation leads to glucocorticoid resistance [60]
		Dual TLR7 and TLR9 inhibitor led to decreased autoantibody production in animals and being tested in humans [61, 62]
	Melanoma	PF-3512676, TLR9 agonist, currently being tested in melanoma patients with other modes of therapy [71–73]
	Mycosis fungoides	TLR9 agonist demonstrated to have antitumor activity [74, 75]

Table 2.2 Historical timeline: discovery of Toll-like receptors

	Discovery
1979	Identification of the *dorsal* mutation [85]
1984	Characterization of *toll* mutation and other dorsoventral mutations
1989	Janeway proposes the theory of pattern recognition [2]
1993	Demonstration that NF-κB is required for Drosophila antimicrobial resistance 209
1996	Drosophila *Toll* identified; found to be required for resistance to fungal infections [78]
1997	Human homologue of *Drosophila* Toll, signals activation of adaptive immunity [79]
1998	TLR4 is lipopolysaccharide receptor [80, 86]
1999	MD2 identified as coreceptor for TLR4-LPS interaction [82]
2000	TLR9 recognizes bacterial DNA [87]
2000	TLR2 can pair with TLR6 to recognize bacterial proteins [88]
2000	TLR2 can also associate with TLR1 [88]
2001	TLR3 mediates response to viral double-standed RNA [89]
2001	TLR5 detects flagellate protein in whiplike tails of bacteria [90]
2004	TLR8 (humans), TLR 7 (mice) recognize single-stranded RNA [91]
2011	Bruce Beutler and Jules Hoffmann awarded the Nobel Prize in Medicine for their role in the identification of TLRs

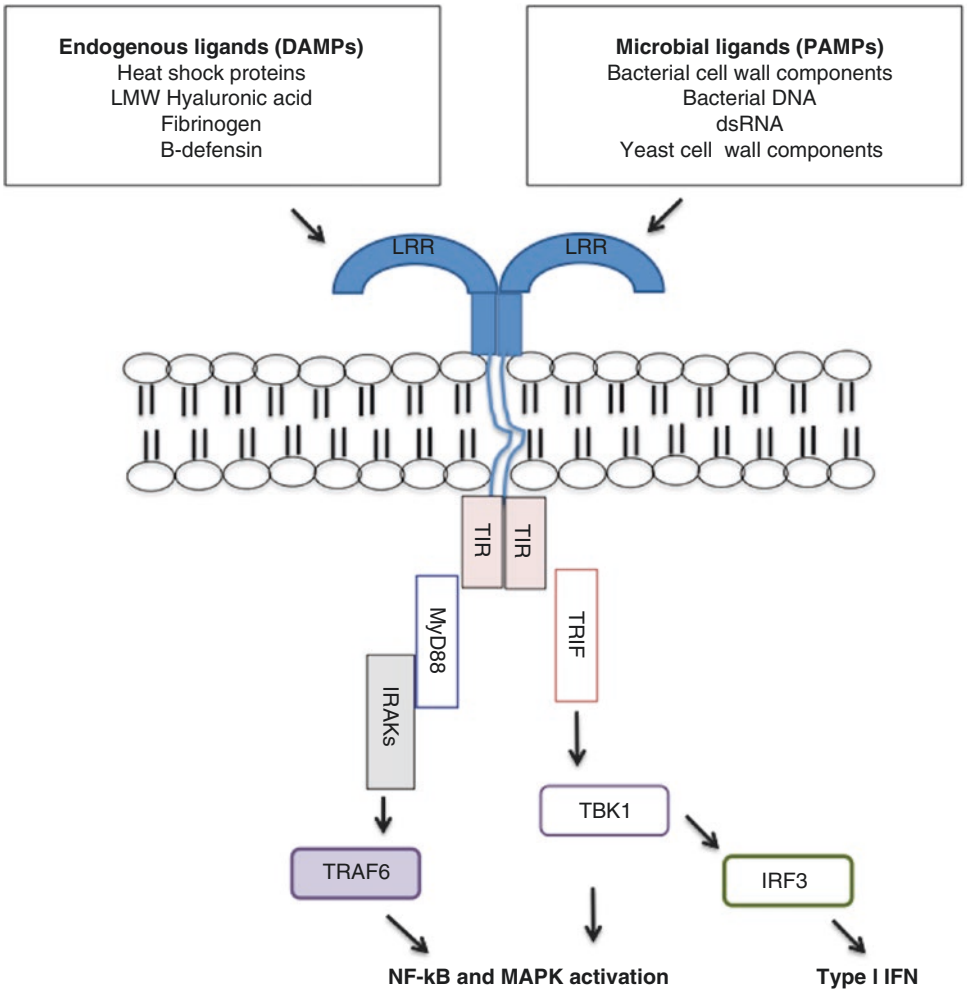

Fig. 2.1 Schematic diagram of TLR activation by various established endogenous and exogenous ligands

diseases such as psoriasis (Fig. 2.2) [99]. For a detailed look at PAMPs and DAMPs that activate specific TLRs, please see Table 2.3.

Toll-Like Receptors in Innate and Adaptive Immunity

As mentioned previously, the pattern recognition theory and identification of TLRs provided the missing link between innate and adaptive immune responses. It is now established that specific ligands activate distinct TLRs and other PRRs, which result in the expression of molecules that shape and fine-tune the adaptive immune response depending on the stimulus involved. On the innate immunity side, activation of TLRs leads to the release of antimicrobial peptides and chemokines that recruit phagocytic cells to the site of infection [120]. TLR activation also induces maturation of dendritic cells to potent APCs via the upregulation of surface expression of MHCII and costimulation markers such as CD80 and CD86 [121].

TLR-mediated effects on the adaptive immune response can be shaped via APCs or T cells directly. It is well known that physical interaction between APCs and T cells requires two signals with signal 1 being the antigen specific signal via MHCII and signal 2 being the expression of costimulation molecules on dendritic cells [122]. TLR stimulation in dendritic cells results in increased expression of MHCII, CD80 and CD86 and is instrumental in promoting both signals required for robust antigen-specific T cell responses [5, 76]. TLR activation on dendritic cells also influences cytokine production, which provides key signals for helper T cell differentiation into different phenotypes with distinct effector functions [123]. For example, TLR-activated dendritic cells produce IFNγ in response to *E.coli* LPS stimulation which is associated with T helper cell 1 (Th1) differentiation while *P. gingivalis* LPS induces expression of IL-5, IL-13 and IL-10, cytokines classically associated with Th2 differentiation [124]. Stimulation of APCs with TLR ligands also leads to interleukin-6 (IL-6) secretion, which can result in the loss of suppressor activity by regulatory T cells, allowing for a

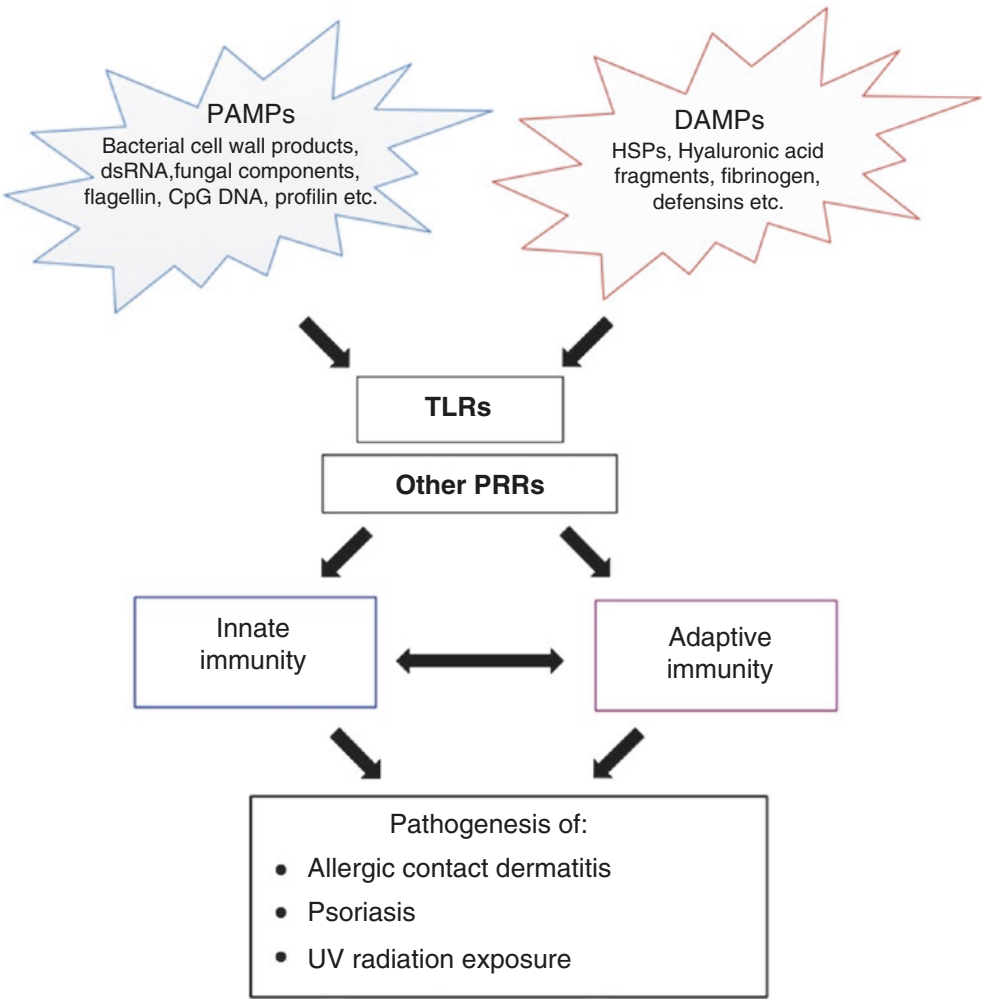

Fig. 2.2 The interplay of PAMPs and DAMPs in the activation of TLRs as well as other PRRs. Activation of these receptors influences both arms of immunity and dysregulation of these pathways can lead to inflammation and the development of a variety of dermatological diseases [98]

more effective immune response [125]. Alternatively, TLRs are also expressed in T lymphocytes and TLR ligands can modulate T cell function directly [126]. Direct TLR2 stimulation of T lymphocytes in the absence of APCs has been shown to induce proliferation of regulatory T cells [127]. Intrinsic B cell TLR activation mediates B-cell proliferation and antibody production to T-dependent antigens and similar results were seen in human B cells [128, 129]. Thus, while TLRs are traditionally associated with the innate immune response, they also play key roles in shaping the adaptive immune response and can directly affect the functions of both T and B lymphocytes.

Expression of Human TLRs in Skin

Based on their cellular localization, TLRs can be broadly classified into two groups [84]. TLRs 1, 2, 4, 5 and 6 are expressed on the cell membrane and recognize predominantly microbial membrane components. TLRs 3, 7, 8 and 9, on the other hand, are expressed in intracellular components such as the endoplasmic reticulum, endosomes and lysosomes and primarily recognize microbial nucleic acids. As the primary physical barrier against the environment, it is not surprising that many cell types residing in the skin express a variety of TLRs to survey for pathogens as well as tissue damage signals.

In the epidermis, keratinocytes constitutively express messenger RNA (mRNA) for TLRs 1–6, 9 and 10 [13, 130]. With the exception of TLR10, many studies have demonstrated that keratinocyte TLRs are functional and respond to their respective ligands [130, 131]. Langerhans cells (LCs) express TLRs 1–10 but are most responsive to TLRs 2, 3, 7 and 8 ligands [132, 133]. In the dermis, stimulation of skin/muscle fibroblasts with ligands to TLRs 2, 3, 4, 5 and 9 led to production of specific chemokines [134, 135]. Expression of human TLRs has also been detected on skin resident and trafficking immune cells such as neutrophils, macrophages, dendritic cells, dermal endothelial cells, mucosal epithelial cells, B cells, and T cells (Table 2.4) [133, 145].

Table 2.3 TLRs: exogenous ligands (PAMPs) vs. endogenous ligands (DAMPs)

TLR	Exogenous ligands	Endogenous ligands	Signaling pathway	
1	Triacyl lipoproteins (w/TLR2)	hBD3	Heterodimerizes with TLR2; MyD88-dependent signaling	[100, 101]
2	Triacyl lipoproteins (w/TLR1) Diacyl lipoproteins lipoteichoic acid, zymosan (w/TLR6)	HMGB1, HSPs, Hyaluronan, Biglycan, Versican, Antiphospholipid antibodies	Heterodimerizes with TLR1 or TLR6; MyD88-dependent signaling	[93, 97, 102–106]
3	dsRNA	Endogenous mRNA from tissue necrosis	TRIP dependent signaling to induce antiviral genes	[100, 107, 108]
4	LPS, viral envelope proteins	HMGB1, HSPs, Hyaluronan, Biglycan, Heparan sulphate, hBD2, fibronectin, s100 proteins Fibronectin extra domain A	MyD88 and TRIF/TRAM dependent signaling	[93, 97, 102–104, 109–113]
5	Flagellin	None identified	MyD88-dependent signaling	[100, 114]
6	Diacyl lipoproteins Zymosan Lipoteichoic acids	HMGB1, HSPs, ECM (with TLR2)	Heterodimerizes with TLR2; MyD88-dependent signaling	[100, 106]
7	ssRNA	Antiphospholipid antibodies ssRNA	MyD88-dependent signaling	[58, 115, 116]
8	ssRNA	Antiphospholipid antibodies ssRNA	MyD88-dependent signaling	[58, 115, 116]
9	CpG-DNA	DNA released from acetaminophen-induced hepatoxicity Mitochondrial DNA Immune complexes	MyD88-dependent signaling	[93, 117, 118]
10	Unknown	Unknown	MyD88-dependent signaling	[119]

HMGB1 high mobility group box 1, HSPs heat shock proteins, double stranded RNA (*dsRNA*), *LPS* lipopolysaccharide, *hBD3* human β-defensin 3, *hBD2* human β-defensin 2, *ECM* extracellular matrix

Table 2.4 TLR expression in different cell types

Cell type	TLR1	2	3	4	5	6	7	8	9	10
Keratinocytes [13, 130]	+	+	+	+	+	+			+	+
Melanocytes [136, 137]		+	+	+	+		+		+	+
LC [132, 133]	+	+	+	+	+	+	+	+	+	+
Skin endothelial cells [138]	+	+	+	++	+	+	+	+	+	+
FB [134, 135]		+	+	+	+				+	
Adipocytes [139, 140]	+	+	+	+		+				
MC [141]	+	+	+	+	+	+	+		+	
mDC[a] [142]	+	+	+	+	+	+		+		+
pDC [142]	+/–					+/–	+		+	+/–
MΦ[b] [143]	+	+	+	+	+	+	+	+	+	+
N [144]	+	+		+	+	+	+	+	+	+
B cell [143]	+	+	+	+	+	+	+	+	++	++
T cell [133, 143]	+	+	+	+	+	+	+	+	+	+

++ strong expression, + expressed, +/– low level expression, *LC* Langerhans cell, *MC* mast cell, *FB* fibroblasts, *mDC* myeloid dendritic cell, *pDC* plasmacytoid dendritic cell, *MΦ* macrophage, *N* neutrophil

[a]Representative of all myeloid DCs, TLR expression varies within myeloid DC subsets

[b]TLR1-10 transcripts are detected but predominantly express 1, 2, 4, 5 and 8

Toll-Like Receptor Signaling

All members of the TLR family are type I transmembrane proteins and contain: (1) extracellular leucine-rich repeats that mediate the recognition of PAMPs, (2) a transmembrane domain and (3) an intracellular tail that contains the Toll/IL-1R (TIR) domain, which bears homology to the IL-1 receptor [84, 146]. Activating ligands lead to homo- or

heterodimerization of one TLR with another TLR and result in the dimerization of TIR domains, which serve as the scaffold for downstream adaptor proteins. Important adaptor proteins in TLR signaling include myeloid differentiation factor 88 (MyD88), TIR domain-containing adaptor protein inducing interferon-beta (TRIF) and TRIF-related adaptor molecule (TRAM). MyD88 and TRIF represent distinct signaling pathways that TLRs utilize that result in activation of specific gene programs in response to different activating stimuli.

MyD88 is an adaptor protein that is used by most TLRs with the exception of TLR3 for the initiation of downstream signaling. It should be noted that TLR4 is unique in that its activation results in both MyD88-dependent and TRIF-dependent pathways. In the MyD88 dependent pathway, MyD88 activation results in the recruitment of interleukin-1 receptor-associated kinases 1 (IRAK1) and IRAK4 [147]. IRAK4 then activates IRAK1, leading to IRAK1 autophosphorylation and the dissociation of both members from MyD88 and downstream interaction with tumor necrosis factor receptor–associated factor 6 (TRAF6), an E3 ubiquitin ligase [146]. This signaling complex results in the activation of NF-κB and mitogen-activated protein kinases (MAPKs) and the production of inflammatory cytokines (Fig. 2.1) [84]. Although all TLRs utilize MyD88 as an adaptor protein, it is important to recognize that each TLR utilizes different combinations of adaptor proteins and kinases to generate an immune response that is appropriate for the initial activating stimuli. For instance, activation of TLR2 by lipoproteins leads to TNFα expression while CpG stimulation of TLR9 results in the expression of IFN-α and TNFα [148].

The TRIF-dependent signaling pathway is mainly utilized by TLR3 and TLR4. TLR3 activation results in TRIF recruitment and subsequent activation of TANK-binding kinase 1 (TBK1) and interferon regulatory factor 3 (IRF3), a transcription factor required for induction of type I IFNs [148]. TLR4 requires an additional adaptor protein TRAM to stabilize its interaction with TRIF. The discovery of TRIF provided the first molecular explanation for why only TLR3 and TLR4, but not TLR2, can induce IFN-β secretion. Indeed, TRIF-deficient mice were incapable of secreting IFNβ upon stimulation by TLR3 and TLR4 ligands [149]. The TRIF-dependent pathway also results in the activation of NF- κB and MAPKs.

Negative Regulators of TLR Signaling

TLR-mediated signaling plays a key role in the regulation of immunity and excessive TLR signaling has detrimental effects that contribute to autoimmune and inflammatory disease development [150, 151]. Not surprisingly, TLR signaling pathways are tightly controlled and multiple negative regulators of TLR signaling exist at various levels to ensure that immune homeostasis is maintained [100]. IRAK-M, Toll-interacting protein (Tollip) and Suppressor of Cytokine Signaling 1 (SOCS-1) are examples of well-described inhibitors of the TLR signaling pathway. IRAK-M, for instance, is thought to prevent the dissociation of IRAK4 and IRAK1 from MyD88 [152, 153]. Accordingly, IRAK-M$^{-/-}$ macrophages secrete higher levels of inflammatory cytokines and IRAK-M$^{-/-}$ animals are more vulnerable to inflammatory-mediated damage in lupus and lung infection models [154–156]. It is thought that specific genotypes of IRAK-M are associated with sepsis risks (see Table 2.5).

Another negative regulator in the TLR pathway is Toll-interacting protein (TOLLIP), which limits MyD88-dependent NF-κB activation at two different levels [181, 182]. First, overexpression of TOLLIP has been shown to inhibit TLR4– and TLR2–mediated NF-κB activation. TOLLIP also binds directly to IRAK1 to inhibit IRAK1 autophosphorylation and downstream recruitment of signaling proteins required for NF-κB activation [182, 183]. In contrast to IRAK-M$^{-/-}$ mice, TOLLIP deficient animals did not exhibit any overt inabilities to limit the inflammatory response [184]. However, TOLLIP$^{-/-}$ macrophages secreted lower levels of IL-6 and TNFα when stimulated with low doses of LPS, suggesting that TOLLIP is involved in fine-tuning inflammation in response to different levels of stimulation. Polymorphisms of TOLLIP have been associated with atopic dermatitis and inflammatory bowel diseases (see Table 2.5 for other negative TLRs and their association with human diseases). As the role of negative regulators in disease pathogenesis becomes increasingly clear, there is promise that specific targeting of these molecules may lead to the development of new therapeutics.

TLR and Dermatologic Diseases

Acne Vulgaris

Acne vulgaris, a common disorder involving the pilosebaceous unit, is one of the most prevalent conditions in dermatology (see also Chap. 24). It affects more than 45 million people in the United States and is characterized by the presence of inflammatory papules, pustules, nodules and noninflammatory comedones [76, 185]. The pathogenesis of acne is multifactorial but it is generally thought to involve increased sebum production, altered follicular keratinization and an inflammatory response to *Propionibacterium acnes*, a Gram-positive anaerobe that is a part of normal skin flora, a finding that has been confirmed by recent skin microbiome mapping projects [186, 187]. It is thought that the host immune response [188], and not *P. acnes* overgrowth, is the main determinant of disease as PBMCs from acne vulgaris patients produce higher levels of IFNγ, IL-12 and IL-8.

Table 2.5 Negative regulators of Toll-like receptors

Negative regulator	Mechanism of action	Role in human diseases	References
Protein regulators			
IRAK-M	Prevents IRAK1/IRAK4 dissociation Negatively regulates alternative NF-κB activation after TLR2 stimulation	G/G genotype associated with increased sepsis risk A/A genotype is protective against sepsis Possible role in IBD	[153, 157–160]
MyD88s	MyD88 antagonist	Upregulated in septic patients	[161–163]
TOLLIP	Autophosphorylates IRAK1	Polymorphisms mapped in Atopic Dermatitis IBD	[164, 165]
A20	De-ubiquitylates TRAF6	Polymorphisms and mutations associated with rheumatoid arthritis, psoriasis, Sjogren's Syndrome, SLE, lymphomas	[166, 167]
SOCS1	Suppresses IRAK by promoting their degradation	Decreased SOCS1 expression in SLE MS, RA	[168, 169]
SIGIRR	Orphan receptor that suppresses inflammation	No clear demonstrated role in human disease	[170, 171]
ABIN-1	Ubiquitin binding protein that inhibits TLR/C/EBPβ signaling	Protects again psoriasis	[172, 173]
MicroRNAs Targets 3′-untranslated regions to modulate gene expression			
miR-146	Inhibits IRAK1 and TRAF6	RA Psoriatic arthritis	[174–176]
miR-9	Blocks NF-κB	Leukemias Cancer	[177, 178]
miR-21	Blocks NF-κB and PCDC4	Cancer	[175, 179]
miR-155	Stimulates TNFα Blocks TAK1 activation	Cancer	[180]

IRAK-M IL-1R-associated kinase M, *MyD88s* myeloid differentiation factor 88 short, *TOLLIP* Toll-interacting protein, *SOCS1* suppressor of cytokine signaling 1, *ABIN*-1 A20 binding and inhibitor of NF-κB-1, *SIGIRR* single immunoglobulin IL-1 related receptor, *SLE* systemic lupus erythematous, *IBD* inflammatory bowel disease, *RA* rheumatoid arthritis, *MS* multiple sclerosis

However, the notion that the host immune response is the main contributor of disease has been challenged by a recent study that showed that acne vulgaris patients harbor different *P. acnes* strains compared to healthy controls [189].

Early studies demonstrated that soluble factors produced by *P. acnes* stimulated proinflammatory cytokine production but the exact mechanisms were poorly understood [190, 191]. After the discovery of TLRs, Kim et al. demonstrated that *P. acnes*-mediated induction of proinflammatory cytokines was dependent on TLR2 expression and that TLR2 was abundantly expressed on perifollicular macrophages [192]. It was thought that *P. acnes* possessed two potential cell wall components, LPS and peptidoglycan (PG), that can serve as ligands and activate TLR2 and TLR4 to mediate its downstream proinflammatory response [76]. Indeed, distinct strains of *P. acnes* with presumably varied modifications in their cell wall components differentially induced upregulation of hBD2, and IL-8 mRNA levels in keratinocytes in a TLR2- and TLR4-dependent manner [45]. Subsequent studies have also found that expression of TLR2 and TLR4 in keratinocytes increased in the epidermis of inflammatory acne lesions and *P. acnes* exposure led to an increase in TLR2 expression [192, 193]. Other than proinflammatory cytokine production, PAMP stimulation also caused hypercornification of sebaceous glands in a TLR2-dependent manner [17]. While the host immune response is an essential component of acne vulgaris pathogenesis, the molecular mechanisms that differentiate healthy controls and acne vulgaris patients remain poorly characterized. As mentioned earlier, recent studies have showed that different *P. acnes* strains are found in acne vulgaris patients and there is evidence that these strains can modulate cutaneous innate immunity differentially [189, 194]. Specifically, Jasson et al. demonstrated that only some strains have the capacity to recruit TLR2 receptors and trigger a downstream inflammatory response [194]. It will be interesting to see if the differential capacity of TLR2 recruitment by various *P. acnes* strains affects keratinocyte proliferation in pilosebaceous units and have clinical implications in acne vulgaris treatment strategies in the future.

Interestingly, retinoids, one of the treatments commonly used for acne vulgaris, have been shown to exert anti-inflammatory effects by decreasing local expression of TLR2

in vitro [18, 19]. These results were recently confirmed in human patients – systemic administration of isotretinoin in acne patients resulted in downregulation of TLR2 cell surface expression on monocytes and decreased levels of IL-1β, IL-6, IL-12 as well as IL-10 release [20]. Of note, systemic isotretinoin decreased TLR2 cell surface expression to levels comparable to those seen in healthy controls. A similar reduction in proinflammatory cytokines was also evident and this effect was sustained for 6 months after the cessation of therapy.

Atopic Dermatitis

Atopic dermatitis (AD) is a common chronic inflammatory skin condition that affects up to 3 % of adults and 15–25 % of children in the United States (see also Chap. 22) [195, 196]. Multiple defects have been identified in AD patients, including impaired skin barrier function, reduced expression of antimicrobial peptides, concomitant skin infections and Th2 skewing. Moreover, it has been demonstrated that up to 90 % of AD patients are colonized with *Staphyloccus aureus* in both lesional and nonlesional skin, whereas only 5 % of healthy controls exhibit colonization [197]. The molecular details underlying AD pathogenesis are currently under investigation but defects in the TLR signaling pathway have been identified in AD patients. AD patients have decreased TLR2 expression on their circulating monocytes and are impaired in their proinflammatory response to known TLR2 ligands [198, 199]. Werfel and colleagues further reported that a missense mutation in the *TLR2* gene (*R753Q*) is associated with AD patients with a more severe phenotype, higher serum levels of immunoglobulin E (IgE), and greater susceptibility to *S. aureus* colonization [21–23]. TLR9 and TOLLIP polymorphisms have also been shown to be associated with AD patients [67, 164].

TLRs also directly affect skin barrier function by modulating both physical and chemical properties of barrier function [195]. TLR2 signaling has been shown to increase the expression of tight junction proteins and enhance skin barrier repair [24, 25]. Accordingly, $TLR2^{-/-}$ mice demonstrated impaired repair responses to epidermal injury by tape-stripping, suggesting that TLR2 may contribute to a chronic itch-scratch cycle often seen in AD patients. Other than TLR2, TLR3 signaling in response to dsRNA stimulation from epidermal injury also stimulates the expression of genes involved in permeability barrier repair [26]. In addition, TLR signaling is necessary for the keratinocyte production of antimicrobial peptides (AMPs), a key component of cutaneous chemical barrier function. Previous studies demonstrated that human β-defensin-2 (hBD2) and cathelicidin LL-37 (two AMPs important in keratinocyte defense against *S. aureus*) were significantly decreased in acute and chronic lesions of AD when compared to controls and patients with psoriasis [200]. LL-37 and hBD2 production, in turn, is dependent on intact TLR2 signaling after *S. aureus*, *S. epidermis* and skin injury [201–203].

Consistent with their tendency towards a Th2 immune response, AD patients often suffer from other atopic diseases such as allergic rhinitis, asthma and seasonal allergies. Early lesions in AD have a Th2 cytokine profile, which has been shown in murine models to promote preferential binding to *S. aureus* [27]. In support of the key role Th2 cytokines (IL-4, IL-13 and TSLP) play in AD pathogenesis, patients with moderate to severe AD treated with dupilumab, an antibody that targets the Th2 cytokine IL-4, showed remarkable improvement in their symptoms [28]. Increasing evidence suggests that TLRs affect the balance between Th1 and Th2 cytokines in the skin. For example, TLR2 stimulation by purified *S. aureus*-derived diacylated lipopeptitde induces expression of Th2 cytokines like thymic stromal lymphopoietin (TSLP) by keratinocytes [29]. TLR2 ligands also play a role in exaggerating and prolonging Th2-mediated inflammation in AD [26]. TLR2 also has complex roles in modulating other arms of immunity and has been shown to affect mast cell degranulation as well as subsequent IgE antibody production by B cells [30]. Collectively, these data indicate that TLRs, especially TLR2, influence multiple aspects of AD pathogenesis, including barrier function, *S. aureus* colonization as well as skewing of the immune response towards a Th2 phenotype. Further dissection of how TLRs affect the various altered skin functions in AD will likely lead to development of new therapeutic strategies.

Allergic Contact Dermatitis

Allergic contact dermatitis (ACD) is a common skin disorder caused by type IV delayed hypersensitivity reactions to skin-exposed chemical allergens (see also Chap. 23) [204]. In the clinically silent phase of sensitization, dendritic cells migrate to skin-draining lymph nodes and present contact allergens to naïve T lymphocytes, which may take weeks to months of repeated exposures to low molecular weight compounds. Upon re-exposure to the contact allergen, effector T cells are recruited back to the skin to mediate the type IV delayed hypersensitivity reaction (known as the 'elicitation phase') seen in ACD. It is estimated that more than 3000 contact allergens have been described; some of the common contact allergens include nickel, fragrances and hair dyes [98]. Martin et al. [205] first demonstrated a role for TLRs in ACD by showing that mice lacking both TLR2 and TLR4 failed to develop contact hypersensitivity (CHS), the experimental model used to study ACD. Importantly, CHS development was dependent on IL-12 expression that was stimulated by either TLR2 or TLR4 activation of dendritic cells as dendritic

cells from TLR2$^{-/-}$ TLR4$^{-/-}$ double knockout animals were resistant to CHS stimulation in wild type animals. Interestingly, CHS developed normally in germ free animals, suggesting that TLR2 and TLR4 activating signals were most likely derived from endogenous ligands such as DAMPs rather than microbial ligands. Further analyses revealed that contact allergens lead to reactive oxygen species (ROS) production, which stimulates the degradation of high molecular weight hyaluronic acid (HA) to low molecular weight HA products [206]. Low molecular weight HA, in turn, can serve as endogenous ligands for TLR2 and 4 signaling and potentiate an inflammatory cascade [207, 208]. A recent study by Gallo and colleagues [209], however, has challenged this notion that HA alone can cause ACD. The group overexpressed hyaluronidase, an enzyme involved in the generation of low molecular weight HA in mice, and showed that small HA fragments alone did not lead to spontaneous cutaneous inflammation resembling CHS. However, the addition of antigen along with small HA fragments accelerated allergic sensitization in a TLR4-dependent manner. Thus, rather than acting as the inflammatory stimuli for ACD, low molecular weight HA controls the antigen presentation capacity of the skin.

Other than DAMP-mediated activation of TLRs, nickel, cobalt and palladium have all been shown to bind and activate human TLR4 [46–48]. Specifically, binding of human TLR4 to nickel was mediated by histidine residues missing in murine TLR4 and provided molecular evidence for why mice are naturally resistant to nickel-induced CHS [48]. Whether nickel alone is sufficient in driving CHS remains unknown although the natural resistance to nicked-induced CHS seen in mice can be overcome by the addition of LPS [210], suggesting that microbial ligands that activate TLR4 may help to amplify the stimulus to promote sensitization to contact allergens [98]. Together, these studies provide evidence that contact allergens like nickel, DAMPS such as low molecular weight HA and PAMPs are all capable of activating TLRs in ACD. However, the relative contribution of each in either the sensitization phase or elicitation phase remains unknown and whether different TLR-expressing skin cells maybe involved in specific phases present exciting future research opportunities for learning more about ACD pathogenesis.

Psoriasis

Psoriasis is a chronic, recurrent, inflammatory disease characterized by dry, scaly, circumscribed erythematous plaques predominantly located in the scalp, nails, extensor surfaces of the limbs, umbilical region, and sacrum (see also Chap. 21). The pathogenesis of psoriasis, which is characterized by the predominance of Th1/Th17 cytokine profiles, involves hyperproliferation and parakeratosis of keratinocytes, which ultimately leads to thickening of the epidermis [99]. Many advances have been made in understanding the mechanisms involved in psoriasis and developments of new immunosuppressive and biologic treatments. Not surprisingly, TLRs have also been found to play a role in the pathogenesis of psoriasis. A study demonstrated that TLR1 and TLR2 expression was increased in the suprabasal layer of keratinocytes in psoriasis patients compared to skin isolated from normal controls [13]. In contrast, TLR5 expression in basal keratinocytes from psoriatic patients was decreased compared to healthy controls. Other studies have found increased TLR1, 2, 4, 5 and 9 expression in keratinocytes isolated from psoriatic lesions [211]. A recent study also identified mutations in the gene *AP1S3*, a protein involved in TLR3 trafficking, that are associated with pustular psoriasis [43]. Furthermore, application of imiquimod, a known TLR7 agonist, is known to trigger psoriasis in both humans and animal models [55, 56]. It is thought that imiquimod activates TLR7 signaling on DCs to drive psoriatic plaque formation by activating the production of IL-17 and IL-22 by innate lymphoctyes. ABIN-1, a negative regulator of TLR signaling, protects against psoriasis development by preventing exaggerated NF-κB and MAPK signaling in response to TLR7 agonists [172]. Therefore, TLR expression on various cell types in the skin may drive psoriatic pathogenesis and it is plausible that different cell types maybe involved in different phases of disease progression.

In contrast to AD patients who are more susceptible to *S. aureus* infections (see above), it is generally accepted that psoriatic plaques are relatively resistant to *S. aureus* infection [212]. It is thought that increased AMP production such as hBD2 and syndecans seen in psoriatic plaques is partially responsible for this phenotype [213, 214]. Keratinocyte growth factor, TGFα, has been found at high levels in psoriatic lesions and is responsible for increased TLR5 and TLR9 expression as well as TLR-dependent release of AMPs and proinflammatory cytokines [215]. While the increased production of AMPs is beneficial against pathogenic microorganisms, it has been postulated that they may also contribute to inflammation by modulating host immune receptors such as TLRs [185]. For example, LL-37 has been shown to complex with self DNA to create a novel DAMP and activate plasmacytoid dendritic cells (pDCs) via the TLR9 pathway and drive inflammation in psoriatic skin by stimulating IFNα production [68]. A recent study showed that LL-37 and an alternatively processed cathelicidin peptide KS-30 also stimulate keratinocytes to produce more type I IFNs but this was not dependent on its complexed DNA that was important for pDC activation [216].

Other than AMPs, heat shock protein (HSP) expression is also thought to contribute to TLR-mediated inflammation. HSP is induced by exposure to microbial pathogens and

other stressful stimuli [49]. Heat shock protein 27, 60, 70 and 90 have been shown to be overexpressed in psoriasis [49, 50] and can trigger an innate immune response through TLR4 on APCs, resulting in the secretion of TNFα, IL-12, and other Th1 cytokines. They also may act on the adaptive immune response by serving as autoantigens for self-reactive T cells that migrate into psoriatic lesions.

These discoveries are opening doors for novel treatments in psoriasis (see Chaps. 43). It is thought that systemic and topical retinoids used in the treatment of psoriasis may control inflammation through their inhibitory effects via TLR2 [76]. Monomethylfumarate (MMF), a bioactive metabolite of fumaric acid ester, is an immunotherapy for psoriasis that causes decreased production of Th1 cytokines and lymphocytopenia [217]. Monomethylfumarate was shown to decrease DC response to LPS and decreased IL-12p70 and IL-10 production. Etanercept, a TNFα inhibitor that has been successful in psoriasis treatment, has been shown to be associated with decreased LL-37 expression, which may dampen TLR9 activation and further suppress the chronic inflammatory response in psoriasis [218]. Thus, TLR dysregulation appears to play a role in psoriasis pathogenesis although whether a predominant TLR is involved remains unclear. Continued research in these areas will yield interesting findings that will impact treatment options for psoriasis patients.

Bacterial Infections

Bacterial cell wall components were the original ligands shown to stimulate TLR signaling [80, 81]. Accordingly, TLRs have been implicated in the pathogenesis of multiple bacterial diseases.

S. aureus Infections

S. aureus, a gram-positive extracellular bacteria, is the causative agent of a variety of skin infections, including impetigo, folliculitis and cellulitis (see Chap. 16) [219]. It is estimated that 20% of the population is persistently colonized, harboring *S. aureus* on the skin and the nares, while 50% are intermittent carriers [185]. *S. aureus* lipoproteins, peptidoglycan and lipoteichoic acid signal through TLR2/6 and TLR2/2 dimers [220, 221]. Accordingly, TLR2 deficient mice were more susceptible to *S. aureus* infection and harbored higher bacterial loads in blood compared to wild type controls [31, 32]. Animals deficient in MyD88, the key adaptor protein required for all TLR signaling with the exception of TLR3, were also more susceptible to *S. aureus* infection and demonstrated a neutrophil recruitment defect that was not seen in TLR2$^{-/-}$ mice. In corroboration of these animal studies, MyD88-deficient and IRAK4-deficient patients are more susceptible to *S. aureus* infections [222]. Mutations in the IRAK4 kinase that led to premature stop codons have been shown to increase susceptibility to pyogenic infections caused by *S. aureus* as well as *Streptococcus pneumonia* [223]. Cells from patients with this disease did not respond to any known ligands from TLRs 1 to 6 and 9. Consistent with an immune deficient phenotype, these patients suffered recurrent pyogenic infections with minimal febrile or inflammatory responses.

Leprosy

Leprosy, or Hansen's disease, caused by *Mycobacterium leprae*, is a chronic, debilitating disease that encompasses a spectrum of clinical manifestations [76]. At one end, tuberculoid leprosy (TL) presents in patients with a strong cell-mediated immune response, resulting in high resistance to *M. leprae* and few, localized, paucibacillary lesions. At the other end of the spectrum, lepromatous leprosy (LL) patients have a weak immune response, resulting in disseminated, multibacillary disease, including cutaneous and nerve involvement [224]. Other forms of the disease with unstable resistance include borderline tuberculoid, borderline, and borderline lepromatous. The former is Th1 mediated (e.g., IFNγ, IL-12, IL-18, and granulocyte-macrophage colony-stimulating factor), whereas the latter is Th2 driven (e.g., IL-4 and IL-10). There is accumulating evidence to suggest that whether a patient develops one response over the other may be in part due to variations in the TLR signaling pathway.

In 1999, it was discovered that mycobacteria activated macrophages through TLR2, resulting in production of TNFα, a proinflammatory cytokine [225]. An introduction of a dominant negative mutation in TLR2 rendered the receptor unresponsive to *M. tuberculosis*. Furthermore, a mutation in Arg^{677}Trp in TLR2 has been associated with LL in the Korean population [33]. A separate study confirmed that this mutation halts the ability of TLR2 to respond to both *M. leprae* and *M. tuberculosis*, confirming the clinical importance of this polymorphism [224].

Upon stimulation with *M. leprae*, patients with the Arg^{677}Trp TLR2 mutation were found to have decreased production of IL-2, IL-12, IFNγ, and TNFα, and increased IL-10 (an anti-inflammatory cytokine) when compared to those with the wild-type TLR2 [34]. Thus, the mutated TLR2 favored a Th2 phenotype, which is consistent with the observed LL phenotype. Based on these findings, TLR2 appears to play a critical role in the alteration of cytokine profiles and determination of the type of leprosy that develops.

M. leprae products were shown to activate both TLR2 homodimers as well as TLR1-TLR2 heterodimers [11]. Interestingly, TL lesions had higher TLR1 and TLR2

expression compared to LL lesions, suggesting that the expression of TLR2 and TLR1 contributes to the host response. Moreover, this study demonstrated that type 1 cytokines enhance TLR1 and TLR2 activation, whereas the Th2 cytokines inhibited activation. Therefore, not only does innate TLR signaling affect the adaptive immune response, but also the adaptive immune response, through cytokine release, may also influence the innate response. Further evidence that TLRs play a role in *M. leprae* pathogenesis was shown in a recent genetic study. Wong et al. showed that individuals homozygous for the *TLR1* I602S mutation, a functional TLR1 knockout, were protected from *M. leprae* infection, suggesting that *M. leprae* may have utilized TLR1 signaling to enhance its pathogenesis [12]. These findings underline the complexity of the interaction between TLRs and *M. leprae* pathogenesis through evolution and provide additional proof that TLRs are involved in bridging the gap between innate and adaptive immunity.

Syphilis

Syphilis is a contagious, sexually transmitted disease caused by the obligate human pathogen *Treponema pallidum* [76]. There are three stages of syphilis. In primary syphilis, a painless genital ulcer, called a chancre, appears 18–21 days after infection. Secondary syphilis can appear as various cutaneous eruptions—macular, papular, or polymorphous—often with lesions on the palms and soles. Tertiary syphilis occurs 3–5 years after infection. Patients may develop gummas, or necrotic lesions in the skin, mucous membranes, bones, or joints. Other complications of syphilis include neurologic and cardiac involvement.

It is appreciated that the outer cell wall structures of spirochete bacteria like *T. pallidum* are vastly different from the typical outer membranes of Gram-negative bacteria [226]. It is thought that *T. pallidum* has developed multiple strategies to evade the host immune response. For instance, *T. pallidum* lacks LPS and contains a paucity of immunogenic proteins compared to other spirochete bacterium [227]. Thus, during syphilitic infection, *T. pallidum* membrane lipoproteins (LPs) serve as principal proinflammatory mediators [35]. Indeed, it was demonstrated that *T. pallidum* LPs stimulated TLR2- and TLR4-expressing immature murine dendritic cells (DCs) to release proinflammatory cytokines such as IL-12, IL-1β, TNFα, and IL-6. It was long thought that opsonization of spirochete bacteria was essential for *T. pallidum* clearance but mechanistic studies were missing until Silver et al. recently demonstrated that TLR-MyD88 signaling is crucial for phagocytosis and bacterial clearance [227]. MyD88-deficient animals exhibited increased inflammation with a stronger infiltration of neutrophils and lymphocytes but still harbored a high bacterial load due to the inability of MyD88$^{-/-}$ macrophages to opsonize *T. pallidum*. Consistent with these findings, a recent clinical study found that *TLR1*, *TLR2* and *TLR6* polymorphisms are associated with an increased risk of neurosyphilis development, suggesting that the TLR1/TLR2 and TLR2/TLR6 heterodimers are important in protecting against *T. pallidum* [16].

Yersinia pestis

Y. pestis is a gram-negative bacillus that causes plague, a disease that killed millions of people in the "Black Death" pandemic. It is transmitted by the bite of the rat flea *Xenopsylla cheopis*. Clinically, painful buboes form in the axillae or groin, although other skin lesions such as vesicles, plaques, petechiae, and purpura can be seen. *Yersinia* outer membrane protein, V antigen, targets TLR2 and CD14 on the surfaces of APCs [228]. Interestingly, *Y. pestis* has specific variations in its LPS lipid A structure to evade TLR4-mediated host immune recognition [229].

Lyme Disease

Lyme disease is a tick-borne illness caused by the spirochete *Borrelia burgdorferi* and is loosely divided into three stages. The primary stage is characterized by constitutional symptoms and erythema chronicum migrans. The second stage occurs for 5–6 months after the rash resolves. In the tertiary phase, cardiac, neurologic, and rheumatologic complications can occur. Like other spirochetes such as *T. pallidum*, *B. burgdorferi* does not have LPS in its outer membrane structure to stimulate TLR4. *B. burgdorferi* outer surface protein A (OspA) stimulates TLR2 to activate inflammatory signaling [36]. Stimulation with *B. burgdorferi* lysate was found to increase the expression of TLR1 and TLR2 in all peripheral blood monocytes and human brain cells, but not neurons [230]. Consistent with the aforementioned *in vitro* data, TLR2 deficient animals harbored much higher loads of *B. burgdorferi* and TLR2$^{-/-}$ macrophages produced lower levels of proinflammatory cytokines [231]. Peripheral blood monocytes (PBMCs) isolated from patients with TLR2 Arg^{753}Gln mutations also secreted less proinflammatory cytokines [37]. Interestingly, the lower levels of TNFα and IFNγ were protective against late stages of disease such as lyme arthritis development.

Candidal Infections

Candida albicans is a dimorphic fungi that causes cutaneous and mucocutaneous candidiasis and causes severe infections in immunocompromised individuals (see Chap. 19). It has been demonstrated that the immune response against

yeast phospholipomannans and glycans involves TLR2, causing upregulation of TNFα via the NF-κB pathway [38, 39]. Candidal cell polysaccharide mannan most likely activates TLR4 as anti-CD14 and anti-TLR4 antibodies (but not anti-TLR2 antibodies) blocked mannan-induced cytokine production [38, 39]. When stimulated with *C. albicans*, TLR4 defective macrophages expressed lower levels of neutrophil chemokines and impaired neutrophil recruitment [232]. Consistent with the animal model data, killing of *C. albicans* in human keratinocytes was shown to be dependent on TLR2 and TLR4 [51]. More recent work has also implicated a role for TLR7 in IL-12 production in response to fungal RNA [233]. TLR7 and TLR9 deficient animals harbored higher fungal load compared to wild type animals but whether this was dependent on IL-12 was not studied. Together, these studies suggest that TLRs work differently to foster an immune response against *C. albicans* – TLR4 activation leads to recruitment of neutrophils; TLR2 mediates the production of TNFα and TLR7 is important in the IL-12 response against candidal infections.

Herpes Simplex Virus

Viruses are obligate intracellular parasites that rely on host protein machinery to complete their replication cycles (see Chap. 17). Due to their intracellular location, viral nucleic acids are usually recognized in intracellular components such as endolysosomes by various TLRs. Viral proteins released during replication may also stimulate TLRs on cell surfaces. Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are double-stranded DNA (dsDNA) viruses that commonly infect skin and mucosa. HSV-1 generally produces vesicular outbreaks at the orolabial or ocular mucosa, whereas HSV-2 typically infects genital mucosa and renders patients more susceptible to other sexually transmitted infections. However, both strains of the virus can infect either physical location.

Herpes simplex virus glycoproteins gH/gL and gB have been shown to stimulate TLR2 and activate NF-κB signaling [40, 234]. TLR2-mediated NF-κB activation, however, may have detrimental effects as TLR2 knockout mice with decreased cytokine responses are resistant to HSV encephalitis [42]. Plasmacytoid dendritic cells recognize HSV through TLR9 to activate interferon production [235, 236]. In contrast to TLR2 deficient animals, TLR9−/− were more susceptible to HSV infection [237, 238]. Furthermore, TLR2/TLR9 double knockout animals exhibited 100 % mortality and had decreased NK cells as well as global cytokine levels. Thus, while TLR9 plays a protective role against HSV infection, the role of TLR2 is complex and further dissection of its role in different cell types is necessary. The importance of TLR signaling is further demonstrated by the fact that a HSV-1 protein, ICP0, that is expressed early during infection accelerates the degradation of MyD88 and inhibits NF-κB activation [239]. Interestingly, Iwasaki et al. [240] showed that HSV is detected in a serial recognition system by DCs – viral glycoproteins are first detected by TLR2 and then viral DNA is recognized by intracellular TLR9. The authors suggested that this serial recognition system helps to mount an optimal antiviral response. Together, this body of work indicates that while TLR2 and TLR9 may have differential effects on the antiviral response, they also work synergistically and the loss of both receptors leads to detrimental effects in the host.

Other than the TLR2 and TLR9 interaction, TLR3, which recognizes dsRNA, has also been shown to play an important role against HSV infection [44]. Vaginal inoculation of $TLR3^{-/-}$ mice led to higher viral loads in the central nervous system compared to healthy controls. Of note, global cytokine production was unaltered in $TLR3^{-/-}$ mice but $TLR3^{-/-}$ astrocytes were unable to produce type I IFN after HSV infection, thereby rendering the host susceptible to extensive CNS infection. Importantly, TLR3 is also protective against HSV in humans as children born with TLR3 deficiencies were more susceptible to HSV encephalitis [10].

Autoimmune Diseases- SLE

The autoimmune connective tissue diseases (AI- CTDs) are a group of clinical disorders that all have circulating autoantibodies (autoAbs) (see Chap. 30). Such disorders include systemic lupus erythematosus (SLE), dermatomyositis, systemic sclerosis, rheumatoid arthritis, mixed connective tissue disease, Sjögren's disease and more [76]. SLE is a disease commonly seen in dermatology, in which patients may exhibit several key diagnostic signs and symptoms, including antinuclear antibody positivity, malar and discoid rashes, photosensitivity, oral ulcers, arthritis, serositis, and renal, neurologic, hematologic, and immunologic disorders. It is generally accepted that IFNα and pDCs contribute to the pathogenesis in SLE – pDCs recognize self-nucleic acids in a TLR7 and TLR9 dependent manner, which leads to the upregulation of IFN production as well as B cell production of anti-DNA and anti-RNP antibodies [57, 61]. These autoantibodies maybe directed against self antigens such as small nuclear ribonuclear protein particles (SnRNP) called U1 and Sm and this interaction leads to the formation of immune complexes with DNA or RNA from dying cells [241]. Recent evidence suggests that TLR7, TLR8 and TLR9 play key roles in mediating an abnormal immune response mediated by pDCs and neutrophils to endogenous ligands, leading to chronic activation that triggers autoimmunity in the skin [57, 242].

Previous work revealed that specific RNA sequences within snRNPs stimulate TLR7 and TLR8 to activate immune cells, such as pDCs and monocytes, to secrete high levels of IFNα and TNFα respectively [58]. Intriguingly, *TLR7* and *TLR8* are both encoded on the X chromosome, which may partially account for why 90 % of SLE cases occur in women [243]. A deletion of a single copy of *TLR7* in mice led to increased survival and reduced autoantibody production and splenocyte proliferation [244]. A direct correlation existed between TLR7 expression and autoAb production, further implicating that TLR7 plays a pathogenic role in SLE. Gene duplication of TLR7 in a specific strain of mice also led to increased autoantibody production [59]. Compared to TLR7, the role of TLR9 in SLE pathogenesis is more complex. TLR9 has been shown to bind single-stranded unmethylated CpG-DNA containing a phosphodiester backbone, a process that is inhibited by chloroquine and quinacrine, suggesting a possible mechanism for the therapeutic effect of these drugs seen in some autoimmune diseases, such as lupus [245]. Moreover, TLR9/MyD88 signaling was crucial for generation of pathogenic autoantibodies in SLE [246]. Based on these studies, it was expected that TLR9 deficient animals would exhibit less severe SLE. Paradoxically, TLR9 deficiency promoted SLE in multiple lupus models, suggesting that the role of TLR9 was more complex [69, 70]. Most recently, it was shown that although TLR9 was indeed required for autoAb formation, TLR9 also plays a role in B cell-mediated tolerance by controlling the life-span of autoreactive B cells [247]. TLR9 also suppressed TLR7-mediated autoAb production and thus has dual roles in SLE pathogenesis [248].

In support of the aforementioned animal data, SLE patients also expressed high levels of TLR7 and 9 [249]. Interestingly, chronic TLR7 and TLR9 stimulation of pDCs led to resistance to glucocorticoid treatment [60]. Inhibition of TLR7/TLR9 with a small immunoregulatory sequence in animal models improved autoantibody production as well as kidney damage and a similar inhibitor has been tested in patients with promise [62]. Other drugs targeting TLR signaling are also under development for SLE and will hopefully lead to drug regimens with more favorable side effect profiles for SLE patients in the future [250].

Melanoma and Mycosis Fungoides

Melanoma is a skin cancer caused by neoplastic transformation of melanocytes and has been increasing in incidence and mortality over the years [251]. It is thought that genetic factors and intermittent high-dose UV irradiation during childhood are both important etiologic factors in melanoma. Although melanoma only accounts for 4 % of all skin cancers, it causes more than 70 % of skin cancer related deaths as metastatic disease often carries a poor prognosis [252]. Since melanocytes express functional TLR2, 3, 4, 5, 7, 9 and 10, it has not surprising that TLR ligands have the ability to modulate melanoma pathogenesis [136, 137]. Indeed, LPS has been shown to stimulate melanocyte IL-8 production in a TLR4 dependent manner [253]. Agonists of TLR 3, 4, 7, 8 and 9 have showed promise as cancer immunotherapy agents and are regarded as having high potential by the National Cancer Institute [254].

Manipulation of TLRs is currently being investigated as a therapeutic option for melanoma as TLR agonists can activate dendritic cells in sentinel lymph nodes (SLNs) of melanoma patients [255]. In animal studies, addition of CpG DNA and poly-I:C (TLR9 and TLR3 ligands respectively) to peritumoral injections have been shown to increase cutaneous tumor rejection and animals remained tumor free after 50 days [256]. TLR7 agonists such as 852A and imiquimod have also been shown to have antitumor effects [63, 64, 66, 252]. Topical application of imiquimod in melanoma patients enhanced influx of CD4+ and CD8+ T cells to the skin as well as SLNs [252]. While commonly used as a topical agent, imiquimod has chemical properties that are not favorable for systemic administration [63], which led to the testing of other TLR7 agonists such as 852A. 852A was well tolerated in metastatic melanoma patients and induced systemic inflammatory responses [64]. In animal models, 852A had significant antitumor activity and stimulated higher levels of type I IFN release [63].

PF-3512676 is an immunomodulating synthetic oligonucleotide that acts as a TLR9 agonist [257]. It is currently under development for the treatment of cancer both as monotherapy and in combination therapy, as well as an adjuvant for vaccines. It acts through TLR9 receptors present on B cells and plasmacytoid dendritic cells to stimulate B-cell proliferation, IFNα and natural killer (NK) cell activity. Used alone as a therapeutic agent, PF-3512676 had a favorable safety profile but only elicited moderate response rates in patients with advanced melanoma [71]. As an adjuvant to other therapeutic modalities, PF-3512676 was shown to be safe in melanoma patients using other modes of therapy such as CTLA-4 blockade [72, 73].

TLR modulators are also being tested in other skin malignancies. Mycosis fungoides (MF) is the most common form of cutaneous T-cell lymphoma (CTCL) and is characterized by malignant clinical proliferation of skin trafficking T-cells [258]. Skin lesions in MF include patches, plaques, tumors, hypopigmented lesions, and erythroderma. Treatment options range from light therapy,

retinoids, nitrogen mustard, topical steroids to systemic interferon [65]. TLR agonists have shown promise as a therapeutic approach – a preliminary pilot study of six patients with patch and plaque stage MF treated with topical imiquimod, a TLR7 agonist, 5 % cream three times a week for 12 weeks reported a histologic and clinical response rate of 50 % [65]. A phase I clinical study administered TLR9 agonist CpG oligodeoxynucleotide (ODN) to MF patients and demonstrated antitumor activity [74]. MF patients who failed standard treatment in a subsequent study using ODN had increased pDC infiltration as well as a decrease in regulatory T cells [75]. Skin lesion regression was noted in one-third of patients but the overall clinical response assessment was limited in this study due to the small patient size. Future studies may yield promising therapies for MF patients who do not respond to standard treatment approaches.

Ultraviolet Radiation

Ultraviolet radiation (UVR) is an established carcinogen that causes genetic lesions in keratinocytes and contributes to skin cancer development (see Chap. 10) [259]. UVR causes the formation of cyclobutane pyrimidine dimers (CPDs) and DNA single-strand breaks [260], which activates DNA repair enzymes that are vital for maintaining genome integrity. Irreversibly damaged keratinocytes that cannot be repaired undergo cell death and are sloughed off to maintain an intact skin barrier. Additionally, it has long been known that UVR causes widespread immune suppression by depleting Langerhans cells (LCs), inhibiting APC antigen presentation and upregulating immunoregulatory cytokines such as IL-10 [259]. UVR stimulates the upregulation of HSPs from keratinocytes that are known to stimulate TLRs (see section "Psoriasis") and lead to the release of IL-10 and TNFα [76]. Moreover, C3H/HeJ mice that are TLR4-hyporesponsive exhibit impaired TNF-α production after UVB exposure and are resistant to UVB suppression of CHS [52]. More recent studies have demonstrated that UVR can damage self noncoding RNA that contain stem-loop structures and activate TLR3 as DAMPs [261]. Additionally, TLR signaling may determine the form of cell death that takes place after UVR damage as deficiencies in TLR4-MyD88 axis led to increased cell survival along with upregulation of markers of necroptosis [53]. Therefore, multiple TLRs are activated after UVR exposure and have multiple downstream effects that may affect the development of malignant lesions.

The power of UV light and the importance of DNA repair machinery is demonstrated in xeroderma pigmentosum (XP), a rare, autosomal recessive disorder characterized by photosensitivity, premature skin aging, and malignant tumor development due to an inability to repair DNA damage induced by UV light [76]. Gaspari et al. [262] discovered that NK cells from XP patients had a defect in IFN production in response to poly-I:C (a TLR3 ligand) stimulation. Subsequent studies have further expanded on the role of TLRs in XP and the DNA repair machinery. TLR4 deficient animals expressed higher degrees of nucleotide excision repair after UV damage due to activation of XP complementation group A (XPA) expression [54]. The ligand involved in TLR4 stimulation was not studied but it will be interesting to determine whether PAMPs or DAMPs are involved in TLR4 activation after UVL damage. In contrast to the inhibitory role of TLR4, TLR7 agonist imiquimod was shown to enhance DNA repair gene expression and decreased DNA damage detected in local lymph nodes when applied topically [66]. Other repair functions in response to UV damage has been shown to be dependent on TLRs as well as TLR3 was shown to be required for effective skin barrier repair after UVR exposure [263]. Collectively, evidence suggests that TLRs play an important role in sensing and modulating the downstream response to UVR damage. Whether these TLR modulating properties by UVR can be harnessed to protect against DNA damage and prevent tumor development in XP patients remain to be investigated.

Conclusion

Since the discovery of TLRs more than 20 years ago, the family of PRRs continues to grow and be implicated in human disease. Evidence continues to accumulate to suggest that TLRs, the most well characterized group of PRRs, play an essential role in bridging innate and adaptive immune responses. Up to 13 mammalian TLRs have been identified and it is believed that TLRs 1–10 are functional in humans and that TLRs not only respond to PAMPs but also endogenous ligands produced after tissue damage coined DAMPs. Both PAMPs and DAMPs can contribute to the activation of TLRs, which has downstream effects on both innate and adaptive immunity (Fig. 2.2). Dysregulation in TLR activation can lead to the development of dermatological diseases such as psoriasis and allergic contact dermatitis. Thus, TLRs play an integral role in countless dermatologic diseases but many questions remain and future studies are necessary to address precise molecular mechanisms that are involved. It is certain that many more discoveries will be made to further characterize and understand this group of receptors, their role in skin diseases, as well as the potential to manipulate signaling through these TLRs to use them for diagnostic and treatment purposes.

Questions

1. Which of the following represent a negative regulator (inhibitor) of TLR function?
   A. IRAK-M
   B. TOLLIP
   C. SOCS-1
   D. All of the above
   E. None of the above

Correct answer: D-All of the above. IRAK-M, TOLLIP and SOCS-1 are all TLR negative regulators

2. Which skin disease have TLR negative regulators been associated?
   A. Non-melanoma skin cancer
   B. Psoriasis
   C. Atopic Dermatitis
   D. Cutaneous T-cell lymphoma

Correct answer: (C)-TOLLIP mutations have been associated with Atopic dermatitis. However, the exact role of these mutations in the pathophysiology of this common skin disease remains unclear

3. How do TLRs mediate pro-inflammatory cytokine production in acne vulgaris?
   A. PAMPs from P. acnes activate TLR2 and TLR4, inciting the production of pro-inflammatory cytokines
   B. PAMPs from S. aureus induce TLR2 activation
   C. TLRs are not involved in the pathophysiology of acne
   D. PAMPs from the pilosebacious unit activate TLR7,8,9

Correct answer: (A)-*P. acnes* microbial products such as LPS and peptidoglycan activate TLR2 and TLR4 to active the production of proinflammatory cytokines in the skin. It is thought that P. acnes strains in healthy controls may regulate TLR expression differently when compared to P. acnes strains in acne vulgaris patients

4. In allergic contact dermatitis (ACD), what is the predominant TLR involved in the pathophysiology of nickel allergy?
   A. TLR4
   B. TLR7
   C. TLR2
   D. TLR9
   E. None of the above

Correct answer: (A) Nickel, cobalt and palladium can bind and activate human TLR4s and activation of CHS. dependent on histidine residues that are specifically found in human TLR4, thus explaining why mice are naturally resistant to nickel-induced CHS

5. Why are mice genetically resistant to ACD to Nickel?
   A. Nickel does not penetrate mouse skin
   B. Their TLR are not activated by nickel
   C. Their Tregulatory cells suppress the response
   D. Mice have a high level of nickel in their diet

Correct answer: (B)-TLR4 in mice lacks the amino acid histidine in the extracellular domain. In humans, TLR4 normally expresses the amino acid histidine. TLR4 activation by nickel is dependent on histidine residues that are specifically found in human TLR4, thus explaining why mice are naturally resistant to nickel-induced CHS

6. How are TLRs involved in DNA repair?
   A. TLR sense DNA damage
   B. TLR activation directly induces a DNA repair response
   C. TLR activation triggers inflammation, which may stimulate DNA repair
   D. TLR7 agonists applied can increase DNA repair in the skin
   E. All of the above
   F. None of the above

Correct answer: (D)-TLR engagement may stimulate DNA repair by multiple mechanisms. This phenomenon is relevant to UV light exposure, and recovery of skin derived antigen presenting cells

7. Which of the following diseases is associated with impaired TLR signaling via TLR3?
   A. Discoid lupus
   B. Alopecia areata
   C. Psoriasis
   D. Xeroderma pigmentosa

Correct answer: (D)-XP patients NK cells are defective in IFN production in response to TLR3 stimulation

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