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The Role of Toll-like Receptors in the Pathogenesis and Treatment of Dermatological Disease

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Toll-like receptors (TLR) are crucial players in the innate immune response to microbial invaders. These receptors are expressed on immune cells, such as monocytes, macrophages, dendritic cells, and granulocytes. Importantly, TLR are not only expressed by peripheral blood cells, but their expression has been demonstrated in airway epithelium and skin, important sites of host–pathogen interaction. Host cells expressing TLR are capable of recognizing conserved pathogen-associated molecular patterns, such as lipopolysaccharide and CpG DNA, and their activation triggers signaling pathways that result in the expression of immune response genes and cytokine production. As TLR are instrumental in both launching innate immune responses and influencing adaptive immunity, regulation of TLR expression at sites of disease such as in leprosy, acne, and psoriasis may be important in the pathophysiology of these diseases. Furthermore, since TLR are vital players in infectious and inflammatory diseases, they have been identified as potential therapeutic targets. Indeed, synthetic TLR agonists such as imiquimod have already established utility in treating viral pathogens and skin cancers. In the future, it seems possible there may also be drugs capable of blocking TLR activation and thus TLR-dependent inflammatory responses, providing new treatment options for inflammatory diseases.

Key words: innate immunity/skin/TLR
J Invest Dermatol 125:1–8, 2005

In response to pathogen exposure, a host employs both the innate and adaptive arms of the immune system to protect against infection. The innate immune response utilizes both physical barriers such as skin and mucosal epithelium as a means of avoiding infection and rapid cellular responses enacted by dendritic cells (DC), monocytes, natural killer cells, granulocytes, and epithelial cells to protect a newly infected host. These cells express pattern recognition receptors that mediate responses to pathogen-associated molecular patterns (PAMP) that are conserved among microorganisms. Human Toll-like receptors (TLR) are one such family of pattern recognition receptors capable of initiating innate immune responses and influencing subsequent adaptive immune responses (Medzhitov *et al*, 1997). Currently, 10 TLR are known to be expressed in humans, and the microbial ligands for many of these receptors have been identified (Fig 1). The ligands include molecules uniquely found in microbes such as bacterial cell wall components. More specifically, TLR4 mediates host responses to bacterial lipopolysaccharide (LPS) from Gram-negative bacteria such as *Escherichia coli*, whereas TLR2 mediates responses to peptidoglycan from Gram-positive bacteria such as *Staphylococcus aureus* (Poltorak *et al*, 1998; Yoshimura *et al*, 1999). In addition, TLR2/1 heterodimers mediate responses to tri-acylated lipoproteins, and TLR2/6 heterodimers mediate responses to di-acylated lipoproteins (Brightbill *et al*, 1999; Ozinsky *et al*, 2000). Not all TLR, however, mediate innate responses to components of bacterial cell walls. For instance, TLR9 mediates the response to unmethylated CpG DNA found in bacterial genomes, whereas TLR3 mediates the response to viral double-stranded RNA (Hemmi *et al*, 2000; Alexopoulou *et al*, 2001). Furthermore, TLR5 is involved in mediating the host response to bacterial flagellin, and recently single-stranded RNA was identified as the ligand for TLR8 in humans and TLR7 in mice (Hayashi *et al*, 2001; Diebold *et al*, 2004; Heil *et al*, 2004).

TLR are transmembrane proteins with the extracellular portion composed of leucine-rich repeats, whereas the intracellular portion shares homology with the cytoplasmic domain of the IL-1 receptor. When TLR are activated by ligand exposure, the intracellular domain of the TLR may trigger a MyD88-dependent pathway that ultimately leads to the nuclear translocation of the transcription factor NFκB. NFκB then acts to modulate expression of many immune response genes (Takeda *et al*, 2003).

In MyD88-dependent signaling, MyD88 interacts with the Toll/IL-1 receptor (TIR) domain of the cytoplasmic portion of the TLR (Medzhitov *et al*, 1998). This interaction then facilitates MyD88 association with IL-1 receptor-associated kinase (IRAK), a serine–threonine kinase, which in turn activates tumor necrosis factor receptor-activated factor 6 (TRAF6) (Suzuki *et al*, 2002). TRAF6 may then activate the

Abbreviations: DC, dendritic cells; LC, Langerhans cells; LPS, lipopolysaccharide; TLR, Toll-like receptor

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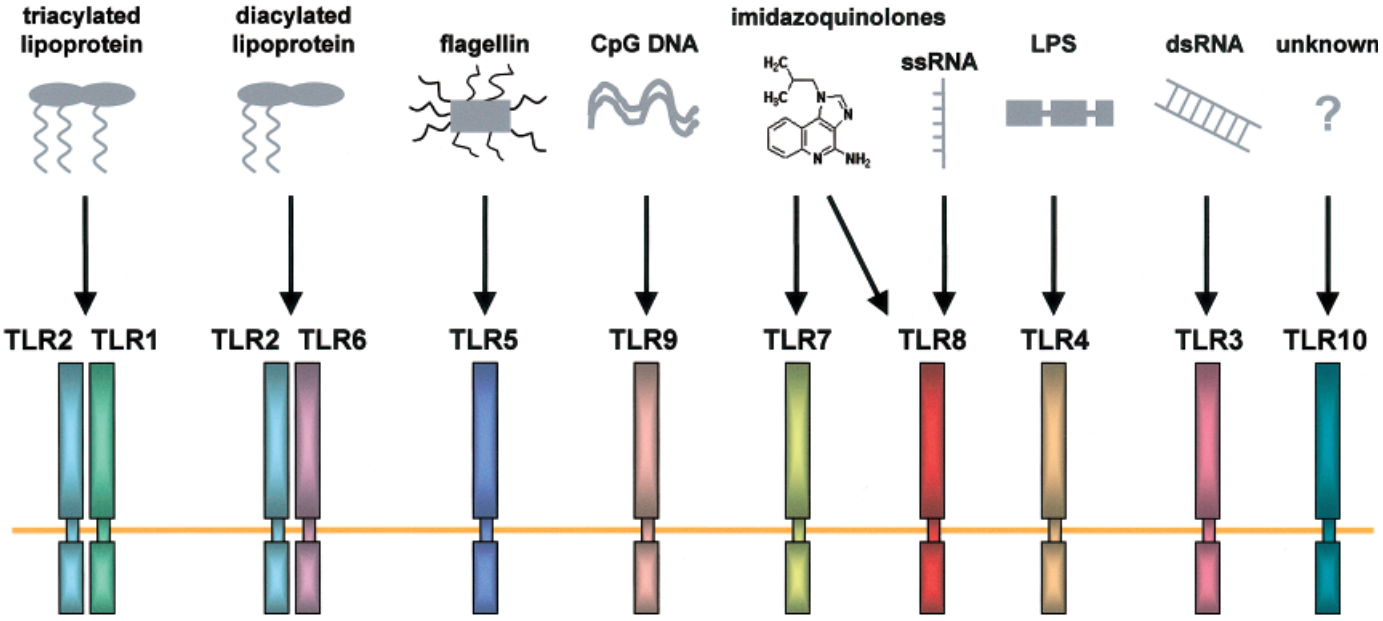

Figure 1
Human Toll-like receptors display specificity in their recognition of pathogen-associated molecular patterns and/or synthetic compounds.

IKK complex that leads to the phosphorylation and consequent degradation of IκB. Once IκB is ubiquitinated and degraded, the transcription factor NFκB is available for nuclear translocation (Woronicz *et al*, 1997).

Although an important player in innate immune responses, MyD88 is not required for recognition of some microbial ligands, and not all TLR signaling is completely MyD88-dependent (Fig 2). There is some evidence that TLR3 and TLR4 also trigger a MyD88-independent pathway that involves IRF-3 to ultimately produce interferon (IFN)-β (Doyle *et al*, 2002). Subsequent studies have revealed that the TLR4-mediated activation of IRF-3 involves the adaptor proteins TRIF and TRAM (Hoebe *et al*, 2003; Yamamoto *et al*, 2003a). Likewise, TRIF was also found to mediate TLR3-dependent activation of IRF-3, but TRIF has also been shown to mediate the TLR3-dependent MyD88-independent activation of NFκB through TRAF6 (Yamamoto *et al*, 2003b; Jiang *et al*, 2004). Furthermore, there are other receptors that act independently of MyD88 to recognize conserved microbial patterns. These include Nod1 and Nod2, which are intracellular receptors for peptidoglycan that act independently of TLR and MyD88 to activate NFκB (Girardin *et al*, 2003a, b; Inohara and Nunez, 2003).

Consequences of TLR Activation

TLR activation contributes to host inflammatory responses. The activation of NFκB allows for transcription of immunomodulatory genes, including the genes for various cytokines and chemokines. The production of cytokines and chemokines in turn triggers inflammation through the recruitment of host immune cells and activation of antimicrobial defenses. Although this may aid the host in clearing an infection, inflammation triggered through TLR may also harm the host through the damage of host tissues or the development of septic shock.

TLR activation is also involved in the phagocytosis of pathogens by host cells. TLR have been shown to sample phagosomal contents and trigger production of inflammatory cytokines after activation by TLR ligands within the phagosome (Underhill *et al*, 1999). More recently, TLR activation has also been shown to trigger internalization of pathogens as well as induce the maturation of host phagosomes (Blander and Medzhitov, 2004). The mechanism behind TLR-induced phagocytosis has been shown to involve MyD88, IRAK4, and p38, resulting in the up-regulation of scavenger receptors (Doyle *et al*, 2004). Thus, TLR activation promotes phagocytosis of pathogens and inflammatory responses to phagosome contents as well as the maturation of phagosomes, allowing for the killing of phagocytosed bacteria.

Another consequence of TLR activation is the triggering of direct antimicrobial pathways that promote the release of non-specific antibacterial molecules such as antimicrobial peptides. For example, primary human airway epithelial cells demonstrate increased β-defensin-2 production in response to TLR2 agonists, and LPS also stimulates increases in β-defensin-2 expression by tracheobronchial epithelium (Becker *et al*, 2000; Hertz *et al*, 2003; Wang *et al*, 2003). Furthermore, stimulation of an *in vitro* reconstructed epidermis with LPS has been shown to increase keratinocyte production of human β-defensin-2 mRNA by 4-fold (Chadebech *et al*, 2003). Interestingly, antimicrobial peptides are not only induced through TLR, but they may also activate cells through TLR. For instance, murine β-defensin-2 has been shown to activate DC through TLR4 (Biragyn *et al*, 2002). Thus, TLR activation is capable of promoting antimicrobial peptide production, and the release of these peptides may trigger more TLR activation. Importantly, TLR activation can also lead to the production and release of reactive oxygen and nitrogen species that aid in killing intracellular pathogens such as *Mycobacterium tuberculosis* in mice (Thoma-Uszynski *et al*, 2001).

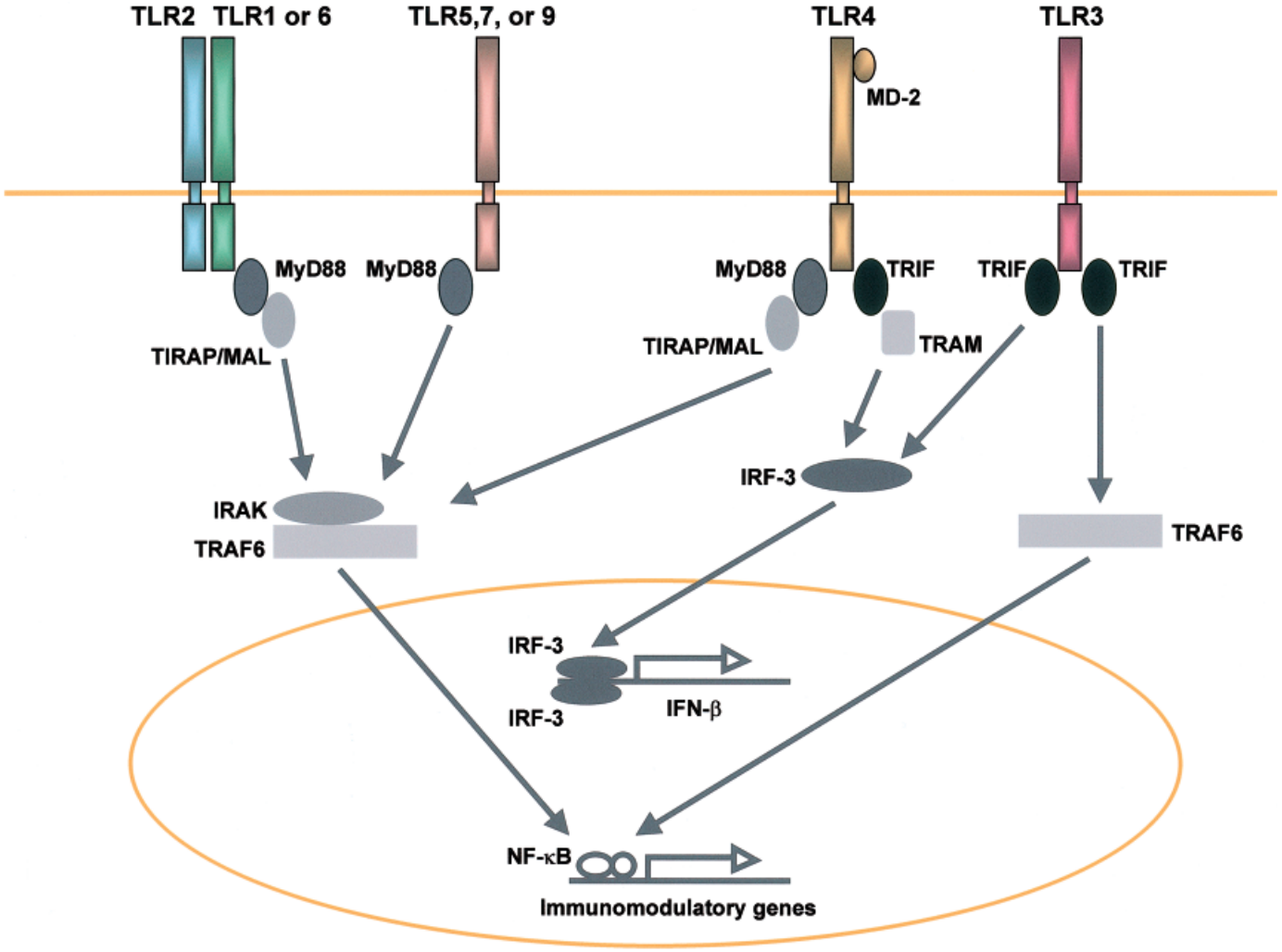

Figure 2
Human Toll-like receptor activation triggers MyD88-dependent and MyD88-independent signaling pathways resulting in gene transcription.

Furthermore, TLR activation facilitates and instructs the development of adaptive immune responses. One way TLR facilitate adaptive immunity is by increasing the levels of expression of co-stimulatory molecules such as CD80 and CD86 on DC, allowing the DC to more effectively activate T cells (Tsuji *et al*, 2000; Michelsen *et al*, 2001). Another avenue through which TLR activation influences adaptive immunity is through the production and release of cytokines. An important aspect of cytokine production is that the specific cytokines produced may instruct differentiation of T cells into Th_1 or Th_2 subsets, which guide the pattern of adaptive response the host will launch against the pathogen. For instance, human monocyte-derived DC stimulated with lipopeptide from *M. tuberculosis* secrete IL-12 over IL-10, skewing the host's adaptive immune response toward a Th_1 pattern, characterized by a cellular, cytotoxic T cell response (Thoma-Uszynski *et al*, 2000). In contrast, activation of an adaptive Th_2 immune response by cytokines such as IL-10 and IL-4 is characterized by the involvement of B cells and antibody production. Thus, the activation of TLR on DC serves not only to activate an innate immune response to a pathogen but also to instruct the pattern of the host's ensuing adaptive immune response.

TLR activation may also lead to apoptosis. LPS has been shown to induce apoptosis through TLR4 (Choi *et al*, 1998). Furthermore, the 19 kDa lipoprotein from *M. tuberculosis* has been shown to trigger apoptosis in macrophages, and activation of TLR2/6 with Mycoplasmal lipoproteins has also been shown to induce apoptotic cell death (Lopez *et al*, 2003; Into *et al*, 2004). This activation of TLR2 with bacterial lipoproteins has been shown to signal apoptosis through MyD88, Fas-associated death domain protein, and caspase 8 (Aliprantis *et al*, 2000). Importantly, the induction of apoptosis in infected cells through TLR ligands may help the host in eliminating the infection.

TLR Expression in Skin

As mentioned earlier, TLR are expressed by various cells of the innate immune system such as monocytes, macrophages, DC, and granulocytes. Moreover, as TLR are key players in the innate response to pathogens, the expression and function of TLR at sites of host-pathogen interaction is critical for host defense. This allows recognition of pathogens before they invade the bloodstream or the tissues of

internal organs. This is indeed the case as TLR expression has been demonstrated in the epithelial cells of the airway and gut. Given that the skin is another crucial interface for host encounters with microbial invaders, it seems appropriate that the skin express TLR to accomplish its job as a barrier to infection.

One study has demonstrated that normal keratinocytes express TLR1, 2, and 5 (Baker *et al*, 2003). This study utilized punch biopsies from regions of normal skin in psoriasis patients and normal breast skin obtained from donors with no known skin diseases. Antibody staining of the biopsies demonstrated cytoplasmic TLR1 and TLR2 expression throughout the epidermis with TLR2 staining most strongly on basal keratinocytes. The basal layer also demonstrated TLR5 staining. Thus, it appears that keratinocytes in different layers of the epidermis may express different TLR; as keratinocytes mature as they progress from the basal layer to the surface of the skin, their patterns of TLR expression may also change.

Although not detected in the study previously discussed, other studies report expression of TLR4 on keratinocytes. Pivarcsi *et al* (2003) demonstrated TLR2 and TLR4 mRNA and protein expression in cultured human epidermal keratinocytes obtained from the skin of healthy individuals. Also, antibody staining of skin sections demonstrated the presence of TLR2 and TLR4 throughout the epidermis. Notably, the ability of Pivarcsi *et al* to detect TLR4 corroborates an earlier study that utilized cultured human keratinocytes derived from foreskin. Song *et al* (2002) detected TLR4 mRNA through both northern blot and quantitiative PCR analyses, and this TLR4 expression was shown to increase in response to treatment with LPS. In addition, flow cytometry revealed surface TLR expression on these keratinocytes, and immunostaining with TLR4 monoclonal antibody was positive. Moreover, this group demonstrated that the LPS-induced IL-8 expression by these keratinocytes was TLR4 dependent. Most recently, Mempel *et al* (2003) reported that cultured primary human keratinocytes expressed TLR1,2,3,5, and 9, but TLR4,6,7, and 8 were undetectable. These conflicting reports make it unclear if keratinocytes constituitively express TLR4. Therefore, it seems additional studies are needed to clarify TLR expression in keratinocytes.

Effector Functions of Keratinocyte TLR Activation

Studies with keratinocytes have not only demonstrated the expression of TLR, but they have also shown the ability of keratinocytes to activate innate immune responses through their TLR. Stimulation with *Candida albicans* or heat-killed *M. tuberculosis*, which possess TLR-stimulatory molecules, strongly induced NFκB in cultured keratinocytes suggesting that keratinocytes are capable of launching TLR-mediated responses (Pivarcsi *et al*, 2003). Furthermore, Mempel *et al* found stimulation of keratinocytes with *Staphylococcus aureus* caused translocation of NFκB and subsequent increased production of IL-8 and iNOS. This inflammatory response was found to be TLR2 dependent (Mempel *et al*, 2003). Thus, TLR are not merely present on keratinocytes but may be active participants in cutaneous defense through triggering NFκB activation and thus production of cytokines and chemokines. Induction of chemokines and cytokines through TLR activation promotes the recruitment of immune cells out of the circulation to sites of infection, such as the skin, and the modulation of immune cell behavior.

TLR-activated keratinocytes are also capable of modulating the host's adaptive immune response. Lebre *et al* (2003) demonstrated that supernatants of TLR-stimulated keratinocytes induced the maturation of human monocyte-derived immature DC. These now-mature DC were found to promote Th_1 immune responses from naïve T cells. Thus, keratinocytes may be able to influence the development of Th_1 or Th_2 adaptive immune responses to cutaneous pathogens. Furthermore, this suggests that keratinocytes, through their activation of DC, may play an important role in inflammation mediated by T cells in the skin.

In summary, keratinocytes play dynamic roles in host defense that extend beyond their role as a physical barrier. Keratinocytes have been shown to produce cytokine and antimicrobial peptides, recruit neutrophils, and kill microbes such as *C. albicans*. It appears now that many of these abilities are due to the activation of their TLR.

TLR Expression: When Less is Better

Although TLR expression at sites of host–pathogen interaction likely serves to protect the host from pathogens, unnecessary immune responses to commensal bacterial may harm the host. This concept is illustrated by studies of TLR expression in Langerhans cells (LC), unique DC found in the epidermis. In contrast to DC, LC have been shown to respond differently to microbial TLR ligands. For example, LPS is capable of inducing the maturation of DC but not LC. Moreover, stimulation with LPS leads to the upregulation of CD80, CD86, and HLA-DR on DC but not LC. One group linked this relative unresponsiveness of LC to lower levels of TLR expression on the LC (Takeuchi *et al*, 2003). More specifically, this group could not detect mRNA for TLR4 in LC, and the amount of mRNA encoding TLR2 found in LC was demonstrably less than that found in DC. The authors propose the diminution of TLR expression on LC found in skin, as compared with DC, may explain why commensal bacteria do not continuously trigger inflammatory responses in the skin. More recently, another group has also demonstrated differences in TLR expression and activation between LC and DC (Mitsui *et al*, 2004). LC were found to have much lower levels of TLR4 on their surfaces as compared with DC, and unlike DC their level of TLR4 expression was not upregulated after stimulation with LPS. In addition, stimulation with ligands for TLR2,4, and 9 matured splenic DC but not LC. Taken together these results suggest that the differences in expression and activation of TLR on LC may potentially explain why commensal bacteria do not continuously trigger inflammation in skin; however, greater knowledge of the role of TLR on LC is warranted before this theory may be considered a fact.

TLR and Dermatological Disease

Leprosy Leprosy, a disease caused by infection with the organism *mycobaterium* varies widely in its clinical presentation, which can be correlated with the type of immune response the host has launched against *M. leprae*. The tuberculoid form of the disease is characterized by localized infection, granulomatous lesions, and the expression of type 1 cytokines that promote cell-mediated immunity. On the other end of the spectrum is the lepromatous form of the disease that is characterized by disseminated infection, disfiguring nodular lesions, and the expression of type 2 cytokines that promote a humoral immune response. A recent study demonstrated that heterodimers of TLR2/1 were activated by killed *M. leprae* (Krutzik *et al*, 2003). Since earlier studies indicate TLR2/1 heterodimers recognize triacylated lipoproteins, the genome of *M. leprae* was scanned to identify putative lipoproteins. Further experiments revealed that two lipoproteins, identified as 19 and 33 kDa, were capable of both monocyte and DC activation. Furthermore, the staining of skin lesions revealed that patients with the tuberculoid form of the disease more strongly expressed TLR2 and TLR1 within the lesion as compared with patients with lepromatous leprosy, suggesting that lepromatous patients may not be as able to activate cellular immune responses. Thus, in leprosy, the activation and regulation of TLR2 and TLR1 at the site of disease may contribute to the host's defense against *M. leprae*.

Responses launched through activated TLR, however, may not always be beneficial to the host. TLR activation may not only activate immune response genes but also apoptosis genes (Aliprantis *et al*, 2000). Therefore, the apoptosis pathway may also be important in the pathogenesis of infectious diseases. For instance, TLR2 expression was demonstrated on the surface of primary human Schwann cells. Furthermore, activation of primary Schwann cells with a synthetic peptide of the 19 kDa lipoprotein of *M. leprae* increased the number of apoptotic Schwann cells (Oliveira *et al*, 2003). Schwann cells found in leprosy skin lesions were shown to express TLR2, and apoptotic Schwann cells were demonstrated within the lesions. Thus, lipopeptides from *M. leprae* may promote TLR-induced apoptosis in Schwann cells. In light of these studies, the nerve injury observed in leprosy patients appears to be a consequence of the innate immune response to *M. leprae*.

Acne vulgaris Acne vulgaris is characterized clinically by non-inflammatory comedones and inflammatory papules, pustules, and nodules. The histological picture of an inflammatory lesion demonstrates follicular rupture, neutrophils and lymphohistiocytic infiltrates surrounding the pilosebaceous unit, and potential scar formation. In inflammatory acne, the Gram-positive microbe *Propionibacterium acnes* contributes to inflammation by inducing cytokine secretion and also releases substances such as proteases and hyaluronidases that contribute to tissue injury. Studies from our lab have shown that *P. acnes* induces IL-12 and IL-8 release from primary human monocytes that may be important in inflammatory acne, and studies of gene-disrupted mice have also demonstrated that this inflammatory response is TLR2 mediated (Kim *et al*, 2002). Importantly, TLR2 expression was also demonstrated in biopsied acne lesions, particularly in perifollicular regions, and the quantity of TLR2-positive cells detected increased with the increasing age of the lesion. This demonstration of TLR2 expression at the site of disease indicates that the inflammation triggered through TLR2 is important in the pathogenesis of acne.

Psoriasis Psoriasis is a chronic inflammatory skin disease mediated by T cells and characterized clinically by hyperproliferation of the epidermis (Baker *et al*, 1984; Hammar *et al*, 1984). It is a disease of unknown etiology, although several microorganisms have been implicated as triggers capable of initiating or exacerbating the disease (Rosenberg *et al*, 1994; Kanda *et al*, 2002; Perez-Lorenzo *et al*, 2003). Based on these implications, research into the modulation of TLR in psoriatic lesions has been pursued. One study compared TLR expression in lesional and non-lesional extensor forearm skin biopsies of untreated chronic plaque and guttate psoriasis patients (Baker *et al*, 2003). No dramatic differences in TLR expression were found. The researchers, however, noted that TLR2 appeared to be more strongly expressed in the upper epidermis of psoriasis patients, whereas TLR2 was more strongly expressed in the basal layers of normal and non-lesional skin. Also, TLR5 expression was reduced in basal keratinocytes of lesions as compared with normal skin. Another group of investigators found that the basal keratinocytes of psoriatic skin demonstrated a strong and diffuse expression of TLR1 (Curry *et al*, 2003). Since psoriasis lesions display marked epidermal thickening and mitotic activity above the basal layer of cells, these results may suggest that the differences in TLR expression in psoriatic lesions versus normal skin can be explained by the differences in age/maturity of the keratinocytes within the respective layer; as the proliferation of keratinocytes in psoriasis occurs above the basal layer, cells in the upper layers of the epidermis may be younger and exhibit less mature phenotypes with respect to TLR expression. Furthermore, the differential expression of TLR across the layers of the epidermis may have a functional role in contributing to host immune defense in skin. Importantly, these subtle variances in TLR expression within psoriatic lesions have not yet been linked to the etiology or pathogenesis of the disease.

Lyme disease Lyme disease is caused by infection with the spirochete *Borrelia* that is transmitted to humans via *Ixodes* ticks. Early stage Lyme disease is recognized by the skin lesion erythema migrans, a dermal inflammatory response to the spirochete. Without treatment, subsequent dissemination of the infection may occur and can lead to arthritis and carditis. Prior vaccination against Lyme disease or timely antibiotic treatment of erythema migrans can, however, prevent the development of such sequelae.

One of the antigens of *Borrelia burgdorferi* capable of stimulating an immune response is the outer surface protein A lipoprotein (OspA). One study demonstrated that TLR2 and TLR6 are necessary for OspA-induced NFκB activation (Bulut *et al*, 2001). More recent studies, however, have demonstrated that TLR2/1 heterodimers are necessary to recognize OspA and launch an effective immune response

to *B. burgdorferi*. Indeed, one study examining the effectiveness of the Lyme disease vaccine (recombinant OspA) identified individuals with low antibodies to OspA after vaccination (Alexopoulou *et al*, 2002). The macrophages of these subjects, although expressing normal levels of TLR2, had low expression of TLR1 on their surfaces. Furthermore, mice deficient in TLR2 or TLR1 also demonstrated low antibodies to OspA after vaccination. Moreover, the recognition of OspA by TLR2/1 heterodimers follows the current paradigm for TLR ligands; as OspA is a triacylated lipopeptide, one may suspect it would be recognized by TLR2/1 heterodimers that recognize other known triacylated lipopeptides.

Studies have also examined the role of TLR expression in Lyme disease. Salazar *et al* (2003) examined peripheral blood and lesional aspirates of patients with erythema migrans. Patients with erythema migrans were found to have peripheral monocytes with higher surface expression of TLR2 and TLR1. Furthermore, monocytoid DC demonstrated increased levels of TLR2 and TLR4 expression. Additionally, within erythema migrans lesions, macrophages and monocytoid and plasmacytoid DCs all exhibited increased expression of TLR1, 2, and 4. Since TLR expression levels appear to be upregulated in this early stage of Lyme disease, this suggests TLR may act early in the disease, attempting to aid the host in containing the infection within the skin.

TLR as Therapeutic Targets

Treatment of viral skin diseases The imidazoquinolones, imiquimod and R-848, were originally developed for use as nucleoside analogs. These compounds do not have, however, nucleoside-like activity, and instead have established utility as immune response modifiers. Imiquimod has been identified as a TLR7 ligand, capable of inducing NFκB through a MyD88-dependent pathway in macrophages (Hemmi *et al*, 2002). As a result, imidazoquinolones induce the production of several cytokines including IFN-α, tumor necrosis factor (TNF)-α, and IL-12p40 from peripheral blood monocytes, macrophages, and DC (Gibson *et al*, 1995). These cytokine responses induced by the imidoquinolones may then influence the host's innate and adaptive immune responses.

Early research on imiquimod focused on its ability to induce IFN-α. As IFN-α is believed to protect cells from viral infections, much research has focused on anti-viral properties of the drug. Anti-viral activity was first demonstrated in guinea-pigs infected with HSV-2. Guinea-pigs inoculated with HSV-2 were given imiquimod intravaginally for 5 d, and these guinea-pigs demonstrated reduced vaginal viral replication and fewer recurrences of the infection (Harrison *et al*, 1988). Further studies demonstrated that these imiquimod-treated guinea-pigs had enhanced T cell memory to the virus suggesting one mechanism for the drug's observed anti-viral properties (Harrison *et al*, 1994). In retrospect, this finding may be attributable to the fact that TLR activation increases expression of co-stimulatory molecules on DC, allowing DC to more effectively activate T cells.

Interestingly, in spite of all the research done with imiquimod and HSV-2, imiquimod has rarely been used to treat HSV-2 infections in humans. Instead, the drug was Food and Drug Administration (FDA) approved to treat anogenital HPV infections. One randomized, controlled trial examined skin biopsies of HPV patients treated with 5% imiquimod cream (Tyring *et al*, 1998). Examination of biopsies demonstrating wart clearance revealed elevated IFN-α, IFN-β, IFN-γ, and TNF-α mRNA as well as decreased viral DNA and mRNA. These results imply that an elevated cell-mediated Th_1 response may account for the clearing of HPV lesions, and this Th_1 response may be a consequence of imiquimod-induced cytokine production, and thus TLR7 activation.

In summary, several potential mechanisms for imiquimod's effectiveness in treating viral infections have been proposed. Application of 1% imiquimod cream on the skin of hairless mice demonstrated increased transcription of IFN-α in the treated skin, indicating IFN-α may act locally as an anti-viral agent (Imbertson *et al*, 1998). Imiquimod has also been shown to promote maturation and migration of LC to regional lymph nodes and to inhibit production of IL-4 and IL-5 by peripheral blood mononuclear cells, thus suppressing the development of a Th_2 adaptive immune response (Wagner *et al*, 1999; Burns Jr *et al*, 2000; Suzuki *et al*, 2000). The proposed imiquimod-induced suppression of a Th_2 adaptive immune response corroborates the finding that HSV-2 antibody levels were reduced in imiquimod-treated guinea-pigs (Harrison *et al*, 1994). Additionally, imiquimod induces IL-12 production, and this promotes a Th_1 adaptive immune response that is necessary to eliminate intracellular pathogens such as viruses (Gibson *et al*, 1995). As cytokine production and Th_1/Th_2 skewing of adaptive immunity are each results of TLR activation, it appears that many of the immunomodulatory effects demonstrated by imiquimod occur as a result of TLR7 activation. Also, as other TLR ligands such as lipoproteins and LPS have demonstrated the ability to induce apoptosis, apoptosis may be one mechanism behind imiquimod's effectiveness (Meyer *et al*, 2003). The precise mechanism, however, for imiquimod's anti-viral activity in humans is not known.

Treatment of skin malignancies In addition to treating viral skin infections, there is recent research that investigates imiquimod's utility for treatment of skin cancers and precancerous lesions. There are studies and case reports that treatment with 5% imiquimod cream has been effective in treating basal cell carcinoma, actinic keratosis, Bowen's disease, and lentigo maligna (Bianchi *et al*, 2003a, b; Chen and Shumack, 2003; Giannotti *et al*, 2003; Naylor *et al*, 2003; Stockfleth *et al*, 2003). Also, other TLR ligands such as CpG (TLR9) and Taxol (TLR4) have been shown to kill tumor cells and are being used for treatment of other cancers such as melanoma (Byrd-Leifer *et al*, 2001; Wang *et al*, 2002; Lonsdorf *et al*, 2003; Krieg, 2004). Since a cellular immune response plays a role in suppressing the development and growth of cancers, it is not too outrageous that an immune response modifier such as imiquimod could be used to treat cancers. Although it is too early to state if this is a safe and effective alternative to other proven treatments, however, these studies do highlight that the potential applications for immune response modulators that act through TLR are both broad and promising.

Conclusions

TLR are crucial players in the innate immune response to microbial invaders. These receptors are expressed on immune cells, such as monocytes, macrophages, DC, and granulocytes, and at sites of host–pathogen interaction such as airway epithelium and skin. Host cells expressing TLR are capable of recognizing conserved pathogen-associated molecular patterns, and their activation triggers signaling pathways that result in the expression of immune response genes and cytokine production. The patterns of cytokines produced may then instruct the type of adaptive immune response the host will employ to fight the pathogen.

As TLR are instrumental in both launching innate immune responses and influencing adaptive immunity, regulation of TLR expression at sites of disease such as in leprosy, acne, and psoriasis may be important in the pathophysiology of these diseases. In some cases, as in leprosy, modulation of TLR expression and activation may be protective and lessen the severity of disease. TLR activation, however, may also promote excessive inflammation and apoptosis contributing to the pathology such as the nerve damage seen in leprosy patients. Additionally, the recognition of *P. acnes*, a commensal bacterium, through TLR2 induces inflammatory cytokine production that may play a role in the pathogenesis of the disease. Thus, TLR are vital players in infectious and inflammatory diseases, making them potential therapeutic targets. Indeed, the ability of TLR to combat disease has already been harnessed through the development of drugs that act as TLR agonists. To date, synthetic TLR agonists such as imiquimod have found utility in treating viral pathogens and skin cancers. Therefore, it seems possible that in the future there may be drugs capable of blocking TLR-dependent inflammatory responses, and thus new treatment options for inflammatory diseases such as acne and psoriasis.

The discovery of TLR and the development of drugs that act through them are beginning to have an impact upon our understanding and treatment of several cutaneous diseases. In spite of their importance, however, relatively few studies have addressed the role of TLR in skin. The expression of TLR on keratinocytes and their potential differential expression across the layers of the epidermis is still unclear. Also, in addition to their role in infectious skin diseases, TLR may play important roles in inflammatory skin diseases such as acne, psoriasis, atopic dermatitis, lichen planus, and lupus erythematosis, leaving this promising area of study open for future investigations.

DOI: 10.1111/j.0022-202X.2004.23459.x

Manuscript received February 25, 2004; revised June 30, 2004; accepted for publication July 2, 2004

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