DOI:10.1111/j.1600-0625.2011.01393.x
www.blackwellpublishing.com/EXD

Innate immune sensing 2.0 – from linear activation pathways to fine tuned and regulated innate immune networks

Thomas Volz, Susanne Kaesler and Tilo Biedermann

Department of Dermatology, Eberhard Karls University, Tübingen, Germany
Correspondence: Tilo Biedermann, MD, Professor of Dermatology, Department of Dermatology, Eberhard Karls University, Liebermeisterstrasse 25, 72076 Tübingen, Germany, Tel.: +49-7071-29-80836, Fax: +49-7071-29-7463-4117, e-mail: tilo.biedermann@med.uni-tuebingen.de

Abstract: The innate immune system is based on pathogen recognition receptors that bind conserved microbial molecular structures, so called pathogen-associated molecular patterns (PAMPs). The characterization of the innate immune system was long based on a linear step-wise concept of recognition, activation pathways and effector defense mechanisms. Only more recently it was recognized that the innate immune system needs regulatory elements, sideways and crosstalks that allows it to fine tune and adapt its response. Thus, it is an emerging field within innate immunity research to try to understand how the immune outcome of innate immune sensing is regulated and why immune responses can be substantially different, even though the same PAMPs may have been 'sensed' at the surface organs such as the skin. Only the expansion of the innate immune system from 'pure' linear activation pathways to fine tuned and regulated innate immune networks allows us to integrate the generation of gradually accentuated and qualitatively different effector and tolerogenic immune responses. This article provides a review of the basic concepts and players of the innate immune system and will present some of the newer data defining the innate immune networks effectively regulating the immune homoeostasis and immune effector mechanisms with special focus on the skin as one of the organs involved in regulating the immune interface between the environment and the organism.

Key words: cytokines – dendritic cells – innate immune sensing – pathogen-associated molecular patterns – pattern recognition receptors

Accepted for publication 6 October 2011

Introduction

The complex and challenging task of the mammalian immune system is to detect and defeat host-threatening 'non-self' such as pathogens while avoiding damage to the host by uncontrolled immune activation in response to 'self'. The immune system of vertebrates can be roughly divided into two major branches – innate and adaptive immunity – to fulfil this task. Adaptive immune responses occur at later stages of infections and are characterized by the activation of highly antigen-specific lymphocytes and contribute to immunological memory. In contrast, the innate immune system provides components specialized on early and rapid sensing of invading microorganism such as bacteria, fungi and viruses and act as first line of defense (1,2). This innate immune system as part of the mammalian concept to fight pathogens was discovered about 15 years ago and characterized in depth ever since. It was soon understood that classes of microbial structures, called 'pathogen-associated molecular patterns' (PAMPs), bind to so called pathogen recognition receptors (PRR). Consequently, the innate immune system triggers intracellular activation pathways mostly initiating the transcription of genes that code for pro-inflammatory cytokines and cellular constituents. Today PRR are grouped into classes such as the Toll-like receptors (TLRs), the C-type lectin receptors (CLRs), NOD-like receptors (NLRs) and the RIG-like helicases (RLRs) (3). PRRs are germline-encoded proteins each detecting unique microbial PAMPs. PAMPs are expressed only in microbes but not in vertebrates thus enabling the innate immune system to discriminate self from non-self (3,4). Numerous PAMPs have been characterized in recent years and represent highly conserved molecules being often essential for survival of microorganisms thus allowing only limited to none structural alteration by the microbe to avoid innate immune recognition (5,6). Characterizing functional consequences of PAMP recognition by the innate immune system, over years, research focused on a linear step-wise concept of (i) recognition of PAMPs, (ii) activation pathways and (iii) effector defense mechanisms that include the development and education of specific immune phenotypes of the adaptive immune system (7–9). Only more recently it was recognized that the innate immune system needs regulatory elements, sideways and crosstalks that allow it to fine tune its response. Thus, it is an emerging field within innate immunity research to try to understand how the immune outcome of innate immune sensing is regulated despite its very conserved basis and why immune responses can be substantially different, even though the same type of PAMP may have been 'sensed' at surface organs such as the skin. An increasing body of evidence indicates that the outcome of innate immune sensing depends (i) on the eliciting microbe, (ii) the organ and cell type of microbial exposure and (iii) the circumstances of innate immune sensing. The latter are determined by the composition of cellular response elements, the combinative innate immune sensing of different PAMPs by several PRRs and the micromilieu that influences the process of innate immune sensing. Only the expansion of the concept of the innate immune system from one of 'pure' linear activation pathways to a widened concept of fine tuned and regulated innate immune networks allows us to integrate the generation of gradually accentuated and qualitatively different effector and tolerogenic immune responses. This article provides a review of the basic concepts and players of the innate immune system and will present some of the newer data defining the innate immune networks effectively regulating the immune homoeostasis and immune effector mechanisms with special focus on the skin as one of the organs involved in regulating the immune interface between the environment and the organism.

The discovery of the innate immune system: pathogen recognition by toll-like receptors

A groundbreaking conceptual framework how pathogens initiate activation of the immune system was postulated in 1989 by Janeway (10). According to his concept, conserved microbial structures are detected by germline-encoded receptors in vertebrates thus initiating an innate immune response. The existence of the anticipated germline-encoded receptors in vertebrates was proven in 1997 by identification of a human homologue to the drosophila gene product *Toll* and consequently termed toll-like receptor (TLR) (11). The gene '*Toll*' was originally identified being essential for dorso-ventral polarity in the fruitfly *Drosophila melanogaster*. Later it could be shown that the protein encoded by *toll* also plays a critical role in antifungal defense of fruit flies and mutants carrying a non-functional *toll* gene succumb to fungal infections (12). The relevance of mammalian TLRs in pathogen recognition was demonstrated by the identification of lipopolysaccharide (LPS) as an essential component of the cell wall of Gram-negative bacteria being a ligand for TLR4 (13). In a rapid series of publications further members of the TLR-family were identified and their ligand specificity was deduced. Up to date 10 functional human and 12 functional murine TLRs have been identified (4,14). Differences between mice and human TLRs exist as it could be shown that TLR10 is non-functional in mice because of a retrovirus insertion (15). In contrast, the genes encoding TLR11, TLR12 and TLR13 are represented in the human genome only by a pseudogene (16).

Toll-like receptors can be grouped according to their subcellular distribution (Fig. 1) with TLR1, TLR2, TLR4, TLR5, TLR6 and TLR11 being expressed on the cellular surface. In contrast, TLR3, TLR7, TLR8 and TLR9 are detected in intracellular compartments such as in the endoplasmatic reticulum (ER) and endolysosomes (17). The common feature of TLRs localized at intracellular compartments is their sensing of nucleic acids. TLR3 recognizes double-stranded RNA derived from viruses and is also activated by the synthetic analogue polyinosinic–polycytidylic acid (poly I:C) (14,18). Activation of TLR3 plays a key role in host defence against herpes virus (19). Single-stranded RNA of viral origin is recognized by TLR7 and TLR8 leading to the induction of a strong anti-viral immune response (20,21). This activation pathway is also triggered by small molecular compounds, imiquimod and resiquimod (R-848), developed to treat a disease elicited by viruses, the genital warts (22,23). However, the consequences of TLR-induced inflammation by imiquimod are not restricted to anti-viral activity but can in addition be used in topical treatment of actinic keratoses or superficial basal cell carcinomas demonstrating the potential of TLR agonists in a therapeutic setting (24).

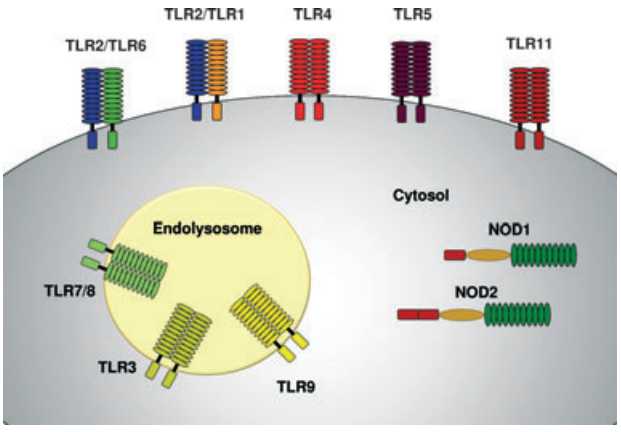

Figure 1. Subcellular distribution of toll-like receptors (TLRs) and NOD proteins. TLRs sensing bacterial cell wall components such as lipoproteins (TLR2/1, TLR2/6), lipopolysaccharide (TLR4) or distinct microbial proteins such as flaggelin (TLR5) or profilin (TLR11) are located at the cell membrane. TLR11 is only expressed in mice but not in humans. In contrast, nucleic acid sensing TLRs (TLR3, TLR7, TLR8 and TLR9) are found in intracellular compartments (endolysosomes). The NOD-like receptors NOD1 and NOD2 sensing peptidoglycan-derived muropeptides are expressed in the cytosol.

Non-mammalian DNA of bacterial or viral origin has been shown to have high amounts of unmethylated CG-rich motifs potently activating immune cells (25,26). TLR9 could be identified as the key receptor involved in recognition of bacterial CpG-rich DNA motifs (27). Further investigations could demonstrate species-specific differences between murine and human CpG motifs required for TLR9 activation (28). Among the cell surface expressed TLRs, TLR2 and its co-receptors TLR1 and TLR6 are especially important in the process of cutaneous innate immune sensing in response to Gram-positive bacteria and the relevance and consequences of TLR2 activation including also newer data will be discussed below. TLR4 as the dominant pathogen recognition receptor for Gram-negative bacteria is unique in regard to its ability to transmit signals via at least two distinct activation pathways, partly based on the recruitment of different accessory molecules. As this represents an important element of regulating innate immune sensing, this will also be covered later. Another important PRR expressed at the cell surface is TLR5. TLR5 was identified to be activated by the bacterial protein flagellin being an essential molecule in building bacterial flagella (29). TLR11 that is only expressed in mice but not in humans has been shown to be essential in sensing uropathogenic bacteria although the exact ligand has not been identified yet (30). Furthermore, a profilin-like molecule of *Toxoplasma gondii* is detected by TLR11 (31).

This system of pattern recognition from pathogenic microbes and viruses was soon expanded. First of all, it was understood that also non-pathogenic microbes are sensed by the innate immune system and the term microbe-associated molecular pattern (MAMPs) was introduced not to exclude innate sensing of non-pathogenic microbes by the abbreviation PAMPs. Moreover, during the last years, it was demonstrated that also non-microbial ligands of endogenous origin can bind to these recognition receptors. These ligands are mostly released during inflammation or destruction such as cell death. Thus, the presence of the endogenous sterile PRR ligands also stands for danger situations in the host. Different ligands have been shown to bind to innate immune receptors thus eliciting immune responses identical or at least similar to 'PAMPs' among them heat-shock proteins (Hsp), uric acid, hyaluronic acid and HMGB1 (32,33). Obviously, situations of PAMPs recognition by PRR concomitantly provoke DAMPs release and consequently possibly also combinatorial innate immune sensing already shaping the outcome.

Innate immune recognition shapes adaptive immunity

It was soon recognized that the two major branches of the mammalian immune system – the innate and the adaptive immune system – are not two mutually exclusive biological systems. In fact it has been shown that innate immune signals critically influence and shape adaptive immune responses in many ways (34). On a

cellular level, antigen-presenting cells (APC) such as monocytes, macrophages and dendritic cells (DC) play a central role in the transition of innate immune signals into adaptive immunity (35,36). DC, which express high levels of PRRs like TLRs and NLRs, are located at the surface of interface organs like the skin or the gut acting as immune sentinels. After having encountered a microbial stimulus, DC undergo a maturation process that includes upregulation of MHC and co-stimulatory molecules as well as cytokine secretion. Furthermore, DC start emigrating out of the tissue where they became activated and migrate into the draining lymph node (37,38). In the lymph node, DC prime naïve T cells which then undergo a polarization process into various specialized subtypes (e.g. Th1, Th2, Th17 or iTreg). T-cell polarization relies in large parts on the presence of cytokines being present during DC–T cell interaction, and DC are the main producers of T-cell polarizing cytokines (39,40). A key role for the induction of IFN-γ producing Th1 cells has been determined for IL-12p70 secreted by DC. In contrast, Th2 cells are polarized in the presence of IL-4 and the absence of IL-12p70 (39,41). Th17 cells are induced in the presence of IL-6, TGF-β, IL-1β and IL-23 acting in various combinations (42,43). Furthermore, production of IL-23 is necessary for maintenance of Th17 cells demonstrating a central role for this DC-derived cytokine in the polarization of adaptive immunity (42,44). Besides priming of effector T-cell phenotypes, DC are also capable of inducing various subsets of regulatory T cells. Different types of tolerogenic DC have been determined that are characterized by the degree of maturation, expression of co-stimulatory molecules and the dominant cytokines they produce (45–48). DC-derived cytokines also play a crucial role in mediating tolerogenic immune responses and in inducing regulatory T cells as previously shown for the priming of effector T cells. Interleukin-10 produced by activated DC is central in mediating tolerance to environmental antigens by inducing Tr1-like regulatory T cells (49). As underlying mechanisms, autocrine effects of DC-derived IL-10 resulting in reduced priming of effector T-cell responses have been reported, and IL-10 treatment of DC has been shown to render these cells into a tolerogenic DC phenotype (50,51). Indeed, as functional *in vivo* consequence of IL-10 acting on DCs and T cells during T-cell polarization, the induction of Tr1 cells could be shown *in vivo* (52). The induction of regulatory T cells as a consequence if innate immune sensing seems to be a double-edged sword as it not only allows surface immunity to tolerate non-pathogenic bacteria but also represents a mechanism of immune evasion. Thus, it has been shown that some pathogens like *Yersinia pestis* and *Bordetella pertussis* efficiently hamper priming of an anti-microbial effector T-cell response by the induction of regulatory T cells (53,54).

These data demonstrate that DC-derived cytokines are critical in shaping an adaptive immune response. The cytokine profile will either promote priming of different types of effector and memory T cells or favour polarization of regulatory T-cell phenotypes (55,56). Innate immune signals triggering cytokine release by DC therefore play a dominant role in determining T-helper cell polarization, immunity and tolerance (Fig. 2).

Innate immune sensing – regulating and fine tuning a highly conserved system

Detection of PAMPs and activation of innate immune receptors is the first step in the initiation of an innate immune response and the subsequent activation of adaptive immunity. This early event can be critically influenced by a variety of factors on both the microbial and the host side fundamentally shaping the resulting outcome. As PAMPs are highly conserved among species and there seems to be a fixed set of receptors to sense these PAMPs by the host, the need for regulation and fine tuning at different levels seems obvious and essential to discriminate 'dangerous' from 'harmless' and to shape also adaptive immune responses of different qualities (Th1, Th2, Th17, Treg).

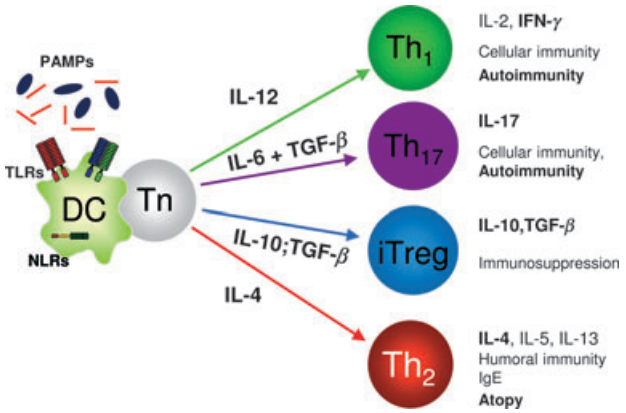

Figure 2. Dendritic cells (DC) at the interface between innate and adaptive immunity. DC located at surface organs like the skin or the gut act as immune sentinels detecting microbes by sensing the presence of pathogen-associated molecular patterns (PAMPs). After migration into the draining lymph nodes, DC activate naive T-helper cells. PAMP activated DC determine T-helper (Th) cell polarization into various specialized subtypes. Th cell polarization is driven by, e.g. DC secreted cytokines and costimulatory molecules.

Dimerization of pathogen recognition receptors to modulate PAMP specificity

One very important TLR for the cutaneous innate immune system is TLR2, because several constituents of Gram-positive bacteria that colonize or infect the skin bind to TLR2. TLR2 has been shown to be a pattern recognition receptor sensing a wide repertoire of PAMPs derived from bacteria, parasites, fungi and viruses (3,4). The sensing and discrimination of this variety of PAMPs are in part explained by the ability of TLR2 to build homodimers or heterodimers with either TLR1 or TLR6 (57). The TLR2–TLR1 heterodimer has been shown to detect triacetylated lipoproteins (LPP) whereas TLR2–TLR6 heterodimers bind diacetylated LPP. Crystallographic studies have revealed structural insight into these differential binding modes using synthetic lipopeptides with two (Pam2Cys) or three (Pam3Cys) acetyl side chains (58,59). While two side chains are buried in pockets formed by the TLR2 molecule, the structure of TLR1 forms a kind of hydrophobic tunnel binding the third acetyl side chain of tri-acetylated LPP. The crystallographic structure of TLR6 does not show this tunnel formation thus being not available to bind Pam3Cys laying the basis for the discrimination of di- or tri-acetylated lipopeptides. Interestingly, it has been demonstrated that TLR2 can bind LPP independent of TLR1 or TLR6, presumably forming TLR2-TLR2 homodimers (60,61). Next to bacterial LPP, lipoteichoic acid (LTA) of Gram-positive bacteria such as *Staphylococci* spp. has been described as TLR2 ligand (62,63). There is, however, rising evidence that residual LPP in the LTA preparations are responsible at least for large parts of the observed immunostimulatory activity

(64,65). Peptidoglycan (PGN), the major component of the cell wall of Gram-positive bacteria, has been initially attributed to interact with TLR2 initiating potent cellular activation (62,66). However, using highly purified PGN, these TLR2 activating properties of PGN have been lost (67). Thus, it has been proposed that bacterial LPP and other yet unidentified 'contaminants' are responsible for PGN activity on TLR2 (67). It could be demonstrated that indeed LPP embedded in the polymeric PGN purified by conventional means are responsible for TLR2 activation as PGN derived from *Staphylococcus aureus* deficient in lipidation of LPP loses much of its immunostimulatory capacity (68,69). The quality and functional relevance of LTA and PGN as PAMPs and TLR2 ligands have still to be determined, as there is evidence for the interactions also in the absence of LPP (70,71).

TLR2 can furthermore interact with PRR outside the TLR-family to detect fungal-derived PAMPs. As one example, dectin-1, an important member of the CLRs, which has been shown to be essential in the detection of yeast zymosan, functions in conjunction with TLR2 leading to innate immune activation (72).

Thus, the homo- and heterodimerization as well as combination of different innate immune recognition receptors represent a decision step in regard to the 'on' and 'off' of innate activation pathways and should be looked upon as an important level of shaping the quality of immune responses driven by microbial signals.

Accessory molecules as regulatory elements in pathogen recognition

Cell-surface-located toll-like receptors (TLR1, 2, 4, 5, 6) are mainly involved in the recognition of microbial cell wall components. Bacterial endotoxin also termed LPS being an essential component of the outer membrane of Gram-negative bacteria is bound by TLR4 (13,73). LPS is sensed in a complex with the accessory molecule MD-2 binding to TLR4. LPS binding protein (LBP) and CD14 are also involved in LPS-mediated activation of TLR4. Interestingly, CD14 has been shown to be critical for the activation of TLR4 by smooth LPS but not rough LPS types allowing the discrimination of classes of ligands and consequently the modulation of downstream signalling pathways and immune phenotypes (74,75). Recently, CD14 has been identified to act also as a co-receptor for TLR7 and TLR9 (76). This concept of fine tuning of the pathogen recognition system by accessory molecules gained further attention and today includes CD36. CD36 has been shown to be critical for the detection of R-MALP and LTA by the TLR2/6 heterodimer while being dispensable for sensing of the lipopeptides Pam2Cys and Pam3Cys by TLR2/6 or TLR2/1, respectively (77). As a result murine macrophages deficient in CD36 produced significantly less pro-inflammatory cytokines in response to LTA and R-MALP and mice carrying a non-functional CD36 could not control an infection with *S. aureus* (77).

These data demonstrate that accessory molecules like CD14 and CD36 act as modulatory elements in the early process of pathogen recognition by fine tuning immune responses elicited after activation of the same PRR by different ligands.

TLR signalling as possible target for the regulation of innate activation pathways

Activation of TLRs leads to the transcription of cytokine genes finally determining the induced immune response. TLR ligation results in production of pro- or anti-inflammatory cytokines, induction of type I interferons and cellular alterations, such as enhanced expression of co-stimulatory molecules on the cell surface of APC. All TLRs exhibit a Toll/IL1-R (TIR) domain at their cytoplasmic part being crucial for downstream signalling. The TIR domain-containing adaptor proteins MyD88, TRIF, TIRAP (Mal) and TRAM bind to the TLR–TIR domain through TIR–TIR interaction thereby transforming PRR-ligation into a intracytoplasmic signalling cascade (9). Two major signalling pathways triggered by TLRs have been determined: The MyD88 pathway is activated by all TLRs with the exception of TLR3 resulting in induction of the transcription factor NFκB via several steps involving IRAK4 and TRAF6. Furthermore, the MyD88 pathway leads to activation of the MAP kinases p38, ERK and Jnk. Signalling via MyD88 finally initiates production of pro-inflammatory cytokines, such as TNFα, IL-6 and IL-12. The adaptor molecule TIRAP (Mal) is required for TLR2- and TLR4-mediated activation of MyD88 while TLR5, TLR7 and TLR9 activation of MyD88 does not require Mal (78,79). The second major pathway is the TRIF pathway activated by TLR3 and TLR4. Signalling via TRIF mainly induces type I interferons via TRAF3 and IRF 3 but also activates NFκB-induced genes (14,79). TLR4 but not TLR3-induced activation of the TRIF pathway relies on the presences of the adaptor molecule TRAM. Interestingly, in the absence of CD14, rough LPS engages only MyD88-dependent responses involving the TLR4/MD2 complex. In the presence of CD14, both smooth and rough LPS initiate MyD88-dependent and MyD88-independent responses indicating the regulation of PRR signalling through ligand-dependent engagement of accessory molecules (74,75). More complexity to the field of TLR signalling has been added by the discovery that substantial differences in TLR signalling induced by the same TLR exist in different cell types. The induction of large amounts of type I interferons in plasmacytoid DCs (pDCs) but not in conventional DC (cDC) has been mapped to signalling via IRF 7 exclusively in pDCs but not in cDCs (80). The TLR signalling pathways outlined have to be tightly regulated to avoid uncontrolled activation resulting in deleterious inflammation. Several distinct mechanisms regulating TLR signalling have been identified. An alternatively spliced short form of MyD88 termed MyD88s is expressed after LPS stimulation and inhibits IL-1R/TLR signalling (81). It could be demonstrated that MyD88s fails to interact with IRAK-4 thus preventing IRAK-1 phosphorylation finally inhibiting the activation of NFκB (82). As MyD88s is not constitutively expressed but upregulated soon after TLR4 stimulation, this demonstrates an 'intrinsic' negative feedback mechanism to prevent over-activation of this TLR signalling pathway. Another protein regulating TLR signalling at the level of the IRAKs is Toll-interacting protein (TOLLIP). TOLLIP has been shown to potently decrease TLR2- and TLR4-mediated signalling by suppressing phosphorylation and kinase activity of IRAK-1 (83).

A new mechanism of controlling TLR signalling has been elucidated in recent years demonstrating that microRNAs (miRNAs) act on a post-transcriptional level to target components of the TLR signalling pathways (84). MicroRNAs have been shown to exert their function by decreasing target mRNA levels thus providing an early and fast acting mode of controlling signalling pathways (85). Most miRNAs are upregulated in response to NFκB activation induced by TLR signalling and have been shown to negatively regulate TLR signalling by targeting crucial adaptor molecules such as MyD88, IRAK1 and TRAF6 by miR-146 and miR-155, respectively (86,87).

These few examples show that within the TLR signalling pathways many distinct mechanisms at various levels are involved to tightly control the resulting activation of pro-inflammatory genes and cytokine expression.

Other players, partners and modulators: innate sensing by NOD-like receptors

During the last years, intracellular pattern recognition receptors distinct from TLRs have been identified. These receptors have been grouped into two major classes named RIG-I-like receptors (RLRs) and NLRs. Both RLRs and NLRs are exclusively expressed in the cytoplasm thus allowing detection of PAMPs derived from microbes that are not detected by surface located PRR. The family of RLRs consists of RIG-I-helicase, MDA5 and LGP2 that are involved in recognition of RNA viruses by sensing viral double-stranded RNA (3). The family of NLRs consists of several subgroups of receptors that share some common structural features. All NLRs possess three well-defined domains with distinct functions. At the N-terminus, the CARD or Pyrin domain is responsible for protein interactions, while the central located NACHT domain mediates nucleotide binding and oligomerization. Detection of PAMPs is mediated by a variable number of leucine rich repeats (LRR) located at the C-terminus (6,88). NLRs can be further divided into two major subgroups – the family of NALPs or NLRPs and the NOD proteins (6). The NLRPs activate a multiprotein complex termed the inflammasome resulting in caspase-mediated processing of pro-IL-1β into its active form IL-1β (89). The NOD proteins NOD1 and NOD2 have been shown to play an essential role in bacterial recognition by detecting PGN-related molecules from Gram-positive or Gram-negative bacteria (90). PGN fragments containing the amino acid meso-diaminopimelic acid (meso-DAP) are sensed by NOD1 (91). The minimal activating PGN structure for NOD1 is γ-D-glutamyl-meso-DAP (iE-DAP), a dipeptide primarily found in PGN of Gram-negative bacteria (92). Muramyldipeptide (MDP) composed of *N*-acetylmuramic acid, L-alanine and D-glutamate (D-Glu) has been shown to fulfil the minimal requirements to activate NOD2 (93,94). The MDP structure can be found in the PGN network of Gram-positive and Gram-negative bacteria, and a variety of mainly synthetic molecules have been investigated to identify NOD2-ligand interactions (95). Our group could demonstrate that highly purified monomeric PGN activates NOD2 thus showing for the first time NOD2 activation in response to a natural PGN breakdown product of *S. aureus* highlighting the importance of this pathway in pathogen recognition (69).

The downstream signalling of NOD1 and NOD2 is significantly different from signalling initiated by the members of the TLR-family. NOD proteins interact with the seronine/threonine kinase RICK through their CARD domains resulting in CARD–CARD associations (96). RICK then leads to polyubiquitinylation of the inhibitor of IKB kinase γ (IKKγ). This finally leads to phosphorylation of IKKβ resulting in activation of NFκB by translocation into the nucleus. NOD proteins also activate the MAP kinase pathway resulting in activation of p38 and ERK, although the detailed pathway is unknown so far (97,98).

Innate immune sensing – combination on multiple levels is key

Based on the variety of ligands and receptors, one possible mechanism of regulating the outcome of innate immune sensing is combinatorial recognition as a code for differential activation pathways. The term 'combinative innate immune sensing' reflects this modulation of innate immune sensing on various levels, and examples will be presented below. Combinative innate immune sensing may have its roots on the microbial side as most microbes express multiple PAMPs. These PAMPs may be either detected by PRRs of the same family, e.g. by different TLRs, or activate PRR of distinct families critically shaping the resulting outcome. PRR expression on host cells is indispensable for pathogen sensing and therefore tightly regulated. Despite the importance of DC and macrophages as the dominant immune sentinels, epithelia lining the surface of interface organs like the skin or the gut are constantly exposed to microorganisms. Close interaction of haematopoietic and resident epithelial cells in response to a fungal pathogen has been shown to be critical for innate immune responses demonstrating a cellular level of combinative innate immune sensing. The cytokine milieu being present during innate immune sensing can also critically influence the resulting outcome and will therefore add another layer to the field of combinative innate immune sensing.

Combinative innate immune sensing – TLR combination

After the identification of distinct TLR-specific ligands, it became obvious that many microbes express multiple PAMPs activating different TLRs (4). Mycobacteria, for example, can activate TLR2 by lipoarabinomannan (LAM) and TLR9 by bacterial DNA containing CpG-rich motifs. The Gram-negative bacterium *Neisseria meningitides* triggers TLR2, TLR4 and TLR9 by expressing outer cell wall proteins (porins), LPS and CpG-DNA. Fungal pathogens like *Candida albicans* activate TLR2 and TLR4 with phospholipomannan and mannans (99). As activation of multiple TLRs may result in augmented or regulated and even different downstream signalling pathways, immune responses may significantly differ compared to those elicited after triggering the respective single TLRs solely. It could be demonstrated that the TLR4 agonist LPS preferentially induces IL-12p70 production resulting in Th1 responses while the TLR2 agonist Pam3Cys fails to induce IL-12p70 resulting in shaping a Th2 response. The underlying signalling pathways have been elucidated, and differential activation of the MAP kinase pathway in particular p38 and c-fos has been accounted for this observation (100,101). Synergistic effects in cytokine induction in response to TLR ligands have also been described for the cytokine IL-12p70 resulting in enhanced Th1 priming capacity (102). Identifying such collaborative TLR agonists may facilitate the development of effective vaccines and may be a prerequisite in cancer immunotherapy (103). Furthermore, cooperation of TLR2 and TLR9 activation has been demonstrated to yield optimal anti-infectious immune responses in two infectious models with either *Toxoplasma gondii* or *Mycobacterium tuberculosis* demonstrating *in vivo* evidence for the need of combinative TLR signalling in successfully defeating infectious diseases (104,105).

In contrast, simultaneous stimulation of DC with defined TLR2 and TLR4 ligands inhibited Th1 driving cytokine production initiated by the TLR4 agonist because of release of IL-10 produced in response to TLR2 activation acting in an autocrine manner (106). A critical role of the interplay of multiple TLRs has been shown for the fungal pathogen *C. albicans* in an *in vivo* infection model

as TLR2 activation has been demonstrated to be critical for IL-10 production and regulatory T-cell numbers (107). Our group has recently shown that a lysate of the non-pathogenic Gram-negative bacterium *Vitreoscilla filiformis* (Vf lysate) induces high levels of IL-10 and low levels of IL-12p70 in DC. This differential cytokine production could be mapped to TLR2 and TLR4 activation, respectively. Analysing the impact of *V. filiformis* activated DC revealed induction of IL-10 producing Tr1 cells efficiently suppressing T effector cell proliferation (108). This immunomodulatory effect could also be demonstrated in a clinical trial on atopic dermatitis (AD) patients resulting in significant improvement of AD lesions after topical treatment with Vf lysate (109). These data display how activation of multiple TLR pathways can exert antagonistic functions also demonstrating a kind of hierarchy of TLR agonists finally resulting in a specific immune response clearly distinct from that induced by a single TLR agonist.

Combinative innate immune sensing – TLR–NLR combination

Peptidoglycan is an essential cell wall component of virtually all Gram-positive and Gram-negative bacteria and is sensed by the innate immune receptors NOD1 and NOD2 (91–93). Thus, PGN and PGN fragments are present on epithelial surfaces in high concentrations, but the innate immune sentinels remain mute in response to these PAMPs (69). However, in case of infection or barrier disturbance, innate immune recognition of bacteria *in vivo* activates both the TLR pathway and the NOD pathway. Early reports demonstrated synergistic effects in cytokine production of different monocytic cell lines after stimulation with the synthetic NOD2 agonist MDP in combination with LPS resulting in enhanced TNFα or IL-8 levels (110,111). Synergistic effects of NOD1 and NOD2 ligands combined with LPS on cytokine production of DC have been reported later (112,113). It could be shown that dual activation of DC with TLR and NOD agonists lead to amplified IL-12p70 production being crucial for driving enhanced IFN-γ production in T cells resulting in enforced Th1 responses (113). Most of the investigations on deciphering the outcome of stimulating NOD proteins have been performed using synthetic muropeptides such as MDP or triDAP representing prototypic ligands for NOD2 and NOD1, respectively. Our group recently demonstrated that a highly purified muropeptide derived from PGN of *S. aureus* is sensed by NOD2 (69). This muropeptide represented the monomeric PGN structure and was thus termed PGN monomer. Interestingly, DC stimulated with PGN monomer alone remained completely mute in respect of cytokine production. Only when DC were activated with PGN monomer in the presence of TLR agonists like *S. aureus* LTA or *Salmonella minnesota* LPS, those dual activated DC displayed significantly enhanced IL-12p70 and IL-23 production compared to TLR agonist-stimulated cells. Coculture experiments with naïve Th cells identified the priming potential of these DC: DC activated by TLR ligands and PGN monomer predominantly primed Th1 and Th17 cells while suppressing Th2 responses (69). These data show that a single pathogen such as *S. aureus* is detected by two different families of innate immune receptors. As a result, anti-microbial immune responses are amplified by the host to effectively defeat the invading microbes.

Controversial results on synergetic effects on cytokine production induced by TLR and NOD agonist have been obtained using NOD2 knock-out mice showing elevated IL-12p70 levels in splenocytes and CD11b+ macrophages solely in responses to TLR2 ligands (114). Interestingly, it could be later shown that chronic stimulation of NOD2 with MDP results in induction of tolerance to subsequent TLR stimulation (115,116). These results demonstrate that spatio-temporal activation of TLR and NOD proteins is crucial in determination of the resulting immune response.

Combinative innate immune sensing – cellular combination

Regulation of innate immune sensing by the presence or absence of PRR on different cell types most likely represents a general mechanism. There is an essential requirement for cells at the interface to sense and to defend microorganism only when appropriate, demanding for a fine tuned system also capable to avoid unwanted activation against harmless or even beneficial microbes (117,118). One level to achieve this fine tuning is the concerted interaction of different cells within the epithelial architecture or even of cells from different compartments. One proof-of-concept study demonstrating this type of regulated cellular communication was recently published (119). Weindl et al. infected a three-dimensional model of reconstituted human epithelium with *C. albicans* and could show that protection from *Candida* invasion and tissue injury was mediated by the addition of polymorphonuclear leukocytes (PMNs). Interestingly, *in vivo*, PMNs are among the first leucocytes recruited to sites of infection and inflammation. In this model, we could show that the presence and innate activation of PMN potently upregulated TLR4 on epithelial cells. Moreover, TLR4 upregulation was directly responsible for defending *Candida* by the epithelium, because the addition of blocking TLR4-antibody or a knock-down of epithelial TLR4 by RNA interference abrogated PMN-induced *C. albicans* defense. Most importantly, soluble PMN-derived factors were sufficient to increase epithelial TLR4 expression and effective *Candida* defense. These data demonstrate an indirect mechanism of innate immune regulation by a crosstalk between cells of different compartments: Cells at the interface with direct contact to both non-pathogenic and pathogenic microbes remain mute unless instructed to become highly responsive to innate immune signals by cells from another compartment (Fig. 3). Other examples for cellular combination resulting in effective defense mechanisms have been described for the gut mucosa. Intestinal macrophages derived from recruited blood monocytes express a wide repertoire of PRRs but do not elicit an inflammatory response upon binding of TLR ligands. This ensures mucosal homoeostasis and avoids unwanted inflammation. However, in case of infection mucosal vessel, endothelia express several adhesion molecules leading to increased migration of blood monocytes into the mucosa resulting in release of pro-inflammatory mediators and the conditioning for defense of the surrounding resident cells (120).

Recently, horizontal intercellular communication in a model of *Listeria monocytogenes* infection has been reported by Dolowschiak et al. They could show that the main source of proinflammatory mediators in response to bacteria is not, as it may be expected, the infected cells themselves but it is the adjacent non-infected epithelial cells. This process of combinative innate immune sensing is based on intercellular communication via release of reactive oxygen intermediates (121).

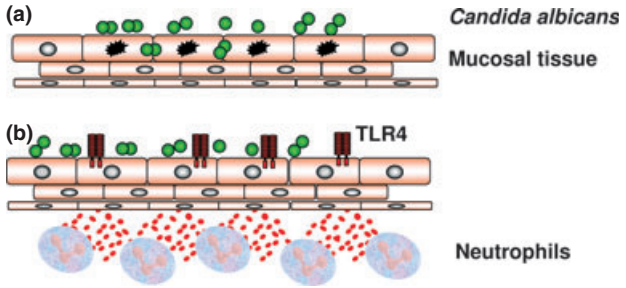

Figure 3. Cellular crosstalk enables effective pathogen defense at surface organs. *Candida albicans* infecting mucosal epithelia leads to apoptosis in epithelial cells and fungal invasion (a). Neutrophils secreting cytokines in response to *C. albicans* infection induce upregulation of TLR4 on epithelia and prevention of tissues damage and pathogen invasion (b).

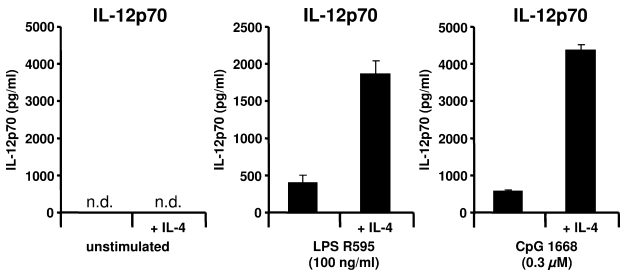

Figure 4. IL-4 enhances IL-12p70 in dendritic cells (DC) activated with different toll-like receptors (TLR) agonists. Murine BMDC were activated with *Salmonella minnesota* R595 lipopolysaccharide (100 ng/ml) or CpG 1668 (0.3 μM) in the presence or absence of IL-4 (10 ng/ml). Cytokine levels were determined after 24 h by ELISA. IL-4 significantly amplified IL-12p70 levels induced by both TLR agonists investigated. Untreated or IL-4 solely incubated DC did not produce detectable amounts of bioactive IL-12p70.

These are examples demonstrating how intercellular communication regulates innate immune responses to restrict inflammation to situations of pathogenic invasion while simultaneously providing tissues integrity and homoeostasis.

Combinative innate immune sensing – modulation by the cytokine environment

It has long been known that the cytokine milieu that is induced by the innate immune system and the adaptive immune system plays a crucial role for the phenotype and development of immune responses (40,41). Shaping the phenotype of Th cells is among the levels of regulation directly depending on activation cascades induced by the innate immune system as discussed above. While the requirements and feedback mechanisms during the process of Th cell polarization were studied in depth, much less is known about the combinatorial effects of innate and T-cell cytokines for the regulation of innate immune responses. However, the Th1 cell cytokine IFN-γ and the Th2 cell cytokine IL-4 were both shown to potently amplify the capacity of DC to produce bioactive IL-12p70 in response to microbes and microbial PAMPs (122–124) and previously unpublished, Fig. 4). These data may have clinical relevance in dermatology. Th2 cells that are abundantly present in early AD lesions secrete large amounts of IL-4. As AD skin is often infected with *S. aureus*, *S. aureus*-derived PAMPs will activate skin-residing DC and in conjunction with IL-4 lead to secretion of high amounts of IL-12p70 (125). As a consequence, these dual activated DC will predominantly induce Th1 polarization (124). These mechanisms may explain the observed cytokine switch in AD where early lesions are dominated by an Th2 secreting lymphocytic infiltrate whereas in chronic AD lesion, IFN-γ producing T-helper cells can be found (126). Future work needs to especially focus on the balance of pro- and anti-inflammatory innate cytokines that are induced by one or more PAMPs as this balance may be the regulatory basis for downstream activation and immune modulation. Because in contrast to IL-4 and IFN-γ, IL-10 and TGF-β are capable to reduce the responsiveness and pro-inflammatory potential of innate immune sentinels such as the DC. These cytokines known for their immunosuppressive function are produced by various cells either of haematopoietic origin such as different types of regulatory T cells or by resident stromal cells. TGF-β secreted by skin tumors has been shown to reduce DC mobility and emigration for the tumor environment thus suppressing effective anti-tumor immunity (127). DC treated with TGF-β are severely hampered in achieving a mature phenotype in response to danger signals such as PAMPs and display an immature phenotype even after LPS activation (Figure S1a, previously unpublished). Moreover, such treated DC fail to secrete IL-12p70 after having encountered LPS (Figure S1b). In contrast to IL-12p70, the levels of the anti-inflammatory cytokine IL-10 are not modulated by TGF-β treatment resulting in induction of a predominantly IL-10 producing DC phenotype. These data demonstrate that TGF-β not only limits innate immune activation in regard to activating inflammatory pathways but counteracts pro-inflammatory immune responses by inducing tolerogenic DC. Such silencing of pro-inflammatory innate immune pathways has profound impact on anti-tumoral immune responses and may partly explain immune escape mechanisms found in various tumors and in chronic infection (128). Moreover, these mechanisms may also contribute to the homoeostasis and integrity of surface organs, in which a constitutive secretion of TGF-β by stromal cells acts as a signal to 'hold still' until stronger signals call for defense.

Implications of innate immune signalling networks on skin immune homoeostasis, inflammation and infections

The skin as one of the major interface organs of the human body is constantly exposed to a multitude of microbial and environmental factors of which at least some may be deleterious to the host. During evolution, mechanisms have evolved to defend the host, maintain tissue integrity and keep up or reconstitute skin immune homoeostasis (129). Therefore, the principles of innate immune networking hold true also for the skin, but only some of them have been specifically addressed in skin research and specific cutaneous innate pathways and networking still need to be characterized in more detail. Invading pathogens are sensed by PRRs expressed not only on immune cells like skin-resident DC but also by keratinocytes which express functional TLRs responding to PAMPs (130,131). A major pathogen responsible for a variety of skin infections is *S. aureus*, and the analysis of consequences of innate immune sensing of this bacterium for the skin is an important focus of research (132). It has been shown that *S. aureus* activates various innate immune pathways such as the TLR2-pathway and the IL-1-MyD88 axis required for defense of cutaneous infections (133). However, the interplay of different innate immune

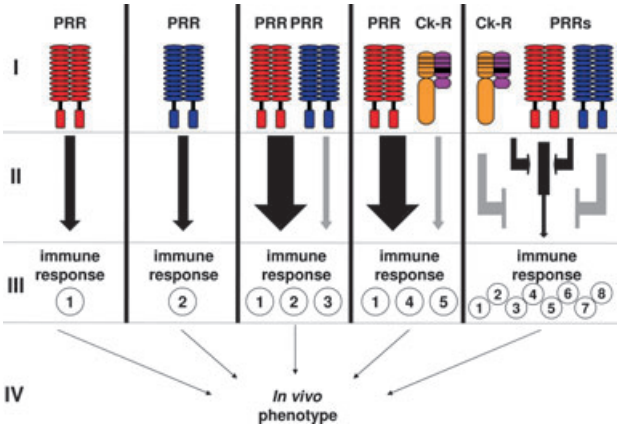

Figure 5. Innate immune networks. Activation of different single innate immune receptors such as toll-like receptors can by itself lead to different qualities of immune responses (schematically depicted as immune response 1 or 2; linear model of innate immune sensing). Dual activation may either amplify single PRR activation pathways inducing stronger immune responses (middle 1 or 2, black arrow), display an inhibition of one or two pathways (grey arrow) or modify the response shaping a different immune response outcome (middle 3). This represents one example of a model for dual combination of innate immune sensing. PRR signalling can also be modulated by cytokine receptor (Ck-R) signalling resulting in either promotion and amplification or inhibition of PRR-induced immune responses (modified response 1). Alternatively, the combination of PRR and Ck-R signals may lead to new qualities of immune responses mediated by PRR (4) and/or Ck-R (5). The combination of PRR signals and Ck-R signals represents another model for dual combination of signal recognition forming the outcome of innate immune sensing. Possible outcomes of triple signal combinations are already much broader allowing innate sensing to 'just' amplify or inhibit outcomes of single signal transduction (right, 1, 2), modify the result of dual combinations (right 3, 4, 5) or shape new qualities of immune responses unique to triple signal sensing (right 6, 7, 8). The levels of signalling and shaping an immune response are also regulated: Level I outlines ligand receptor binding under the influence of, e.g. ligand competition, monomer, homo- or heterodimer binding, single, dual or triple receptor binding and downstream signalling. Level II indicates the downstream signalling cascade that is susceptible to regulation by transcription factors and competing pathways. Level III describes the immune response that combines the results of innate sensing in several cell types together with the downstream consequences, and level IV integrates all these pathways and levels in different compartments that lead to the *in vivo* immune phenotype as a result of innate immune sensing. Functional consequences of the different immune responses (1–8) *in vivo* may be synergistic, complementary, antagonistic or unrelated. This outlines the combinatorial potential that allows a system of fixed ligand receptor pairs such as the innate immune system to exert plasticity and flexibility to fine tune its response and the outcome for the host. This outcome may be inflammation and defense or tolerance and termination of inflammation with the goal to preserve or reconstitute the host's integrity. An imbalance within this complex system will result in disease.

pathways that detect, sense and answer the confrontation of the skin with *S. aureus* still need to be defined in detail. TLRs have also been described to be essential in maintaining immune homoeostasis in the skin after injuries or wounding. As an example, activation of TLR7 and TLR9 on plasmacytoid DC has been shown to sense skin injury by release of nucleic acids and to promote wound healing (134). A network describing the interplay of two TLRs in maintaining immune homoeostasis in the skin has been described for TLR2 and TLR3. TLR3 activation after injury because of the detection of apoptotic cells induces inflammation, which is suppressed by a soluble factor derived from *S. epidermidis* or Staphylococcal LTA acting via TLR2 (135). These analyses already demonstrate that combinatorial innate immune sensing is pivotal to skin defense and integrity and more work in the very near future will elucidate crucial pathways and networks that may also allow to develop new therapeutic strategies.

Conclusion

The innate immune system has been shown to be critical for early and rapid identification of pathogens and elicitation of an appropriate immune response. Historically, pathogen-derived ligands and their respective, receptors, signalling pathways and responsive genes were identified first (Fig. 5, left). However, it is obvious that this linear activation model needed to be expanded to create a more complex model of innate immune networking that contains the combinatorial potential that allows a system of fixed ligand receptor pairs such as the innate immune system to exert plasticity and flexibility to fine tune its response and the outcome for the host. Only the latter allows fine tuning and regulation of this powerful part of the immune system. Some players involved in this innate immune network are determined; others are yet to be identified. However, the examples described in this review already allow us to draw a much clearer picture of a multivalent and well-balanced system of innate immune sensing, a new web that we would not have imagined a decade ago. We now understand that an imbalance within this complex innate network can result in disease and that further characterization of the functional interplay of the different components of this system may also allow us to develop new therapeutic strategies for defense or chronic inflammatory diseases especially of surface organs such as the skin, the lungs or the gut.

Acknowledgements

This work was supported by grants from the Deutsche Forschungsgemeinschaft (DFG Bi 696/3-3; DFG Bi 696/5-1, SFB 685 A6), Baden-Württemberg Stiftung (P-LS-AL2/4, P-LS-AL/17), IZKF-Verbundprojekt 1596-0-0 and IZKF-FACS core facility.

Conflict of interest

The authors declare no conflict of interest.

References

1 Medzhitov R, Janeway C A Jr. Cell 1997: **91**: 295–298.
2 Janeway C A Jr, Medzhitov R. Annu Rev Immunol 2002: **20**: 197–216.
3 Takeuchi O, Akira S. Cell 2010: **140**: 805–820.
4 Ishii K J, Coban C, Akira S. J Clin Immunol 2005: **25**: 511–521.
5 Gay N J, Gangloff M. Annu Rev Biochem 2007: **76**: 141–165.
6 Meylan E, Tschopp J, Karin M. Nature 2006: **442**: 39–44.
7 Palm N W, Medzhitov R. Immunol Rev 2009: **227**: 221–233.
8 Jin M S, Lee J O. Immunity 2008: **29**: 182–191.
9 Akira S, Takeda K. Nat Rev Immunol 2004: **4**: 499–511.
10 Janeway C A Jr. Cold Spring Harb Symp Quant Biol 1989: **54** (Pt 1): 1–13.
11 Medzhitov R, Preston-Hurlburt P, Janeway C A Jr. Nature 1997: **388**: 394–397.
12 Lemaitre B. Nat Rev Immunol 2004: **4**: 521–527.
13 Poltorak A, He X, Smirnova I *et al.* Science 1998: **282**: 2085–2088.
14 Kawai T, Akira S. Nat Immunol 2010: **11**: 373–384.
15 Hasan U, Chaffois C, Gaillard C *et al.* J Immunol 2005: **174**: 2942–2950.
16 Roach J C, Glusman G, Rowen L *et al.* Proc Natl Acad Sci U S A 2005: **102**: 9577–9582.
17 Blasius A L, Beutler B. Immunity 2010: **32**: 305–315.
18 Alexopoulou L, Holt A C, Medzhitov R *et al.* Nature 2001: **413**: 732–738.
19 Zhang S Y, Jouanguy E, Ugolini S *et al.* Science 2007: **317**: 1522–1527.
20 Diebold S S, Kaisho T, Hemmi H *et al.* Science 2004: **303**: 1529–1531.
21 Heil F, Hemmi H, Hochrein H *et al.* Science 2004: **303**: 1526–1529.
22 Hemmi H, Kaisho T, Takeuchi O *et al.* Nat Immunol 2002: **3**: 196–200.
23 Jurk M, Heil F, Vollmer J *et al.* Nat Immunol 2002: **3**: 499.
24 Tran H, Chen K, Shumack S. Br J Dermatol 2003: **149** (Suppl. 66): 37–39.
25 Krieg A M, Yi A K, Matson S *et al.* Nature 1995: **374**: 546–549.
26 Wagner H. Trends Immunol 2004: **25**: 381–386.
27 Hemmi H, Takeuchi O, Kawai T *et al.* Nature 2000: **408**: 740–745.

28 Bauer S, Kirschning C J, Häcker H *et al.* Proc Natl Acad Sci U S A 2001: **98**: 9237–9242.
29 Hayashi F, Smith K D, Ozinsky A *et al.* Nature 2001: **410**: 1099–1103.
30 Zhang D, Zhang G, Hayden M S *et al.* Science 2004: **303**: 1522–1526.
31 Yarovinsky F, Zhang D, Andersen J F *et al.* Science 2005: **308**: 1626–1629.
32 Kono H, Rock K L. Nat Rev Immunol 2008: **8**: 279–289.
33 Osterloh A, Breloer M. Med Microbiol Immunol 2008: **197**: 1–8.
34 Iwasaki A, Medzhitov R. Science 2010: **327**: 291–295.
35 Reis e Sousa C. Curr Opin Immunol 2004: **16**: 21–25.
36 Lee H K, Iwasaki A. Semin Immunol 2007: **19**: 48–55.
37 Banchereau J, Steinman R M. Nature 1998: **392**: 245–252.
38 Reis e Sousa C. Nat Rev Immunol 2006: **6**: 476–483.
39 Murphy K M, Reiner S L. Nat Rev Immunol 2002: **2**: 933–944.
40 Kapsenberg M L. Nat Rev Immunol 2003: **3**: 984–993.
41 Biedermann T, Röcken M, Carballido J M. J Investig Dermatol Symp Proc 2004: **9**: 5–14.
42 Bettelli E, Korn T, Oukka M *et al.* Nature 2008: **453**: 1051–1057.
43 Ghoreschi K, Laurence A, Yang X P *et al.* Nature 2010: **467**: 967–971.
44 McGeachy M J, Chen Y, Tato C M *et al.* Nat Immunol 2009: **10**: 314–324.
45 Lutz M B, Schuler G. Trends Immunol 2002: **23**: 445–449.
46 Menges M, Rössner S, Voigtlander C *et al.* J Exp Med 2002: **195**: 15–21.
47 Mahnke K, Knop J, Enk A H. Trends Immunol 2003: **24**: 646–651.
48 Tuettenberg A, Fondel S, Steinbrink K *et al.* Exp Dermatol 2010: **19**: 44–53.
49 Akbari O, DeKruyff R H, Umetsu D T. Nat Immunol 2001: **2**: 725–731.
50 Steinbrink K, Wolfl M, Jonuleit H *et al.* J Immunol 1997: **159**: 4772–4780.
51 Corinti S, Albanesi C, la Sala A *et al.* J Immunol 2001: **166**: 4312–4318.
52 Wakkach A, Fournier N, Brun V *et al.* Immunity 2003: **18**: 605–617.
53 McGuirk P, McCann C, Mills K H. J Exp Med 2002: **195**: 221–231.
54 Depaolo R W, Tang F, Kim I *et al.* Cell Host Microbe 2008: **4**: 350–361.
55 Kalinski P, Hilkens C M, Wierenga E A *et al.* Immunol Today 1999: **20**: 561–567.
56 Qi H, Denning T L, Soong L. Infect Immun 2003: **71**: 3337–3342.
57 Ozinsky A, Underhill D M, Fontenot J D *et al.* Proc Natl Acad Sci U S A 2000: **97**: 13766–13771.
58 Jin M S, Kim S E, Heo J Y *et al.* Cell 2007: **130**: 1071–1082.
59 Kang J Y, Nan X, Jin M S *et al.* Immunity 2009: **31**: 873–884.
60 Buwitt-Beckmann U, Heine H, Wiesmüller K H *et al.* Eur J Immunol 2005: **35**: 282–289.
61 Buwitt-Beckmann U, Heine H, Wiesmüller K H *et al.* J Biol Chem 2006: **281**: 9049–9057.
62 Schwandner R, Dziarski R, Wesche H *et al.* J Biol Chem 1999: **274**: 17406–17409.
63 Schröder N W, Morath S, Alexander C *et al.* J Biol Chem 2003: **278**: 15587–15594.
64 Hashimoto M, Tawaratsumida K, Kariya H *et al.* J Immunol 2006: **177**: 3162–3169.
65 Kurokawa K, Lee H, Roh K B *et al.* J Biol Chem 2009: **284**: 8406–8411.
66 Iwaki D, Mitsuzawa H, Murakami S *et al.* J Biol Chem 2002: **277**: 24315–24320.
67 Travassos L H, Girardin S E, Philpott D J *et al.* EMBO Rep 2004: **5**: 1000–1006.
68 Stoll H, Dengjel J, Nerz C *et al.* Infect Immun 2005: **73**: 2411–2423.
69 Volz T, Nega M, Buschmann J *et al.* FASEB J 2010: **24**: 4089–4102.
70 Müller-Anstett M A, Müller P, Albrecht T *et al.* PLoS One 2010: **5**: e13153.
71 Bunk S, Sigel S, Metzdorf D *et al.* J Immunol 2010: **185**: 3708–3717.
72 Brown G D. Nat Rev Immunol 2006: **6**: 33–43.
73 Alexander C, Rietschel E T. J Endotoxin Res 2001: **7**: 167–202.
74 Huber M, Kalis C, Keck S *et al.* Eur J Immunol 2006: **36**: 701–711.
75 Jiang Z, Georgel P, Du X *et al.* Nat Immunol 2005: **6**: 565–570.
76 Baumann C L, Aspalter I M, Sharif O *et al.* J Exp Med 2010: **207**: 2689–2701.
77 Hoebe K, Georgel P, Rutschmann S *et al.* Nature 2005: **433**: 523–527.
78 Yamamoto M, Sato S, Hemmi H *et al.* Nature 2002: **420**: 324–329.
79 O'Neill L A, Bowie A G. Nat Rev Immunol 2007: **7**: 353–364.
80 Honda K, Taniguchi T. Nat Rev Immunol 2006: **6**: 644–658.
81 Janssens S, Burns K, Tschopp J *et al.* Curr Biol 2002: **12**: 467–471.
82 Burns K, Janssens S, Brissoni B *et al.* J Exp Med 2003: **197**: 263–268.
83 Zhang G, Ghosh S. J Biol Chem 2002: **277**: 7059–7065.
84 O'Neill L A, Sheedy F J, McCoy C E. Nat Rev Immunol 2011: **11**: 163–175.
85 Guo H, Ingolia N T, Weissman J S *et al.* Nature 2010: **466**: 835–840.
86 Taganov K D, Boldin M P, Chang K J *et al.* Proc Natl Acad Sci U S A 2006: **103**: 12481–12486.
87 Tang B, Xiao B, Liu Z *et al.* FEBS Lett 2010: **584**: 1481–1486.
88 Inohara N, Chamaillard M, McDonald C *et al.* Annu Rev Biochem 2005: **74**: 355–383.
89 Schroder K, Tschopp J. Cell 2010: **140**: 821–832.
90 Kanneganti T D, Lamkanfi M, Nunez G. Immunity 2007: **27**: 549–559.
91 Chamaillard M, Hashimoto M, Horie Y *et al.* Nat Immunol 2003: **4**: 702–707.
92 Girardin S E, Boneca I G, Carneiro L A *et al.* Science 2003: **300**: 1584–1587.
93 Girardin S E, Boneca I G, Viala J *et al.* J Biol Chem 2003: **278**: 8869–8872.
94 Inohara N, Ogura Y, Fontalba A *et al.* J Biol Chem 2003: **278**: 5509–5512.
95 Girardin S E, Travassos L H, Herve M *et al.* J Biol Chem 2003: **278**: 41702–41708.
96 Park J H, Kim Y G, McDonald C *et al.* J Immunol 2007: **178**: 2380–2386.
97 Strober W, Murray P J, Kitani A *et al.* Nat Rev Immunol 2006: **6**: 9–20.
98 Ting J P, Duncan J A, Lei Y. Science 2010: **327**: 286–290.
99 Trinchieri G, Sher A. Nat Rev Immunol 2007: **7**: 179–190.
100 Agrawal S, Agrawal A, Doughty B *et al.* J Immunol 2003: **171**: 4984–4989.
101 Dillon S, Agrawal A, Van Dyke T *et al.* J Immunol 2004: **172**: 4733–4743.
102 Napolitani G, Rinaldi A, Bertoni F *et al.* Nat Immunol 2005: **6**: 769–776.
103 Kanzler H, Barrat F J, Hessel E M *et al.* Nat Med 2007: **13**: 552–559.
104 Bafica A, Scanga C A, Feng C G *et al.* J Exp Med 2005: **202**: 1715–1724.
105 Bafica A, Santiago H C, Goldszmid R *et al.* J Immunol 2006: **177**: 3515–3519.
106 Re F, Strominger J L. J Immunol 2004: **173**: 7548–7555.
107 Netea M G, Sutmuller R, Hermann C *et al.* J Immunol 2004: **172**: 3712–3718.
108 Volz T, Gueniche A, Guenova E *et al.* J Invest Dermatol 2007: **127**: 709.
109 Gueniche A, Knaudt B, Schuck E *et al.* Br J Dermatol 2008: **159**: 1357–1363.
110 Yang S, Tamai R, Akashi S *et al.* Infect Immun 2001: **69**: 2045–2053.
111 Wolfert M A, Murray T F, Boons G J *et al.* J Biol Chem 2002: **277**: 39179–39186.
112 Fritz J H, Girardin S E, Fitting C *et al.* Eur J Immunol 2005: **35**: 2459–2470.
113 Tada H, Aiba S, Shibata K *et al.* Infect Immun 2005: **73**: 7967–7976.
114 Watanabe T, Kitani A, Murray P J *et al.* Nat Immunol 2004: **5**: 800–808.
115 Hedl M, Li J, Cho J H *et al.* Proc Natl Acad Sci U S A 2007: **104**: 19440–19445.
116 Watanabe T, Asano N, Murray P J *et al.* J Clin Invest 2008: **118**: 545–559.
117 Artis D. Nat Rev Immunol 2008: **8**: 411–420.
118 Sansonetti P J, Medzhitov R. Cell 2009: **138**: 416–420.
119 Weindl G, Naglik J R, Kaesler S *et al.* J Clin Invest 2007: **117**: 3664–3672.
120 Smith P D, Smythies L E, Shen R *et al.* Mucosal Immunol 2011: **4**: 31–42.
121 Dolowschiak T, Chassin C, Ben Mkaddem S *et al.* PLoS Pathog 2010: **6**: e1001194.
122 Hochrein H, O'Keeffe M, Luft T *et al.* J Exp Med 2000: **192**: 823–833.
123 Biedermann T, Zimmermann S, Himmelrich H *et al.* Nat Immunol 2001: **2**: 1054–1060.
124 Guenova E, Volz T, Sauer K *et al.* Eur J Immunol 2008: **38**: 3138–3149.
125 Biedermann T. Acta Derm Venereol 2006: **86**: 99–109.
126 Grewe M, Walther S, Gyufko K *et al.* J Invest Dermatol 1995: **105**: 407–410.
127 Weber F, Byrne S N, Le S *et al.* Cancer Immunol Immunother 2005: **54**: 898–906.
128 Flavell R A, Sanjabi S, Wrzesinski S H *et al.* Nat Rev Immunol 2010: **10**: 554–567.
129 Kupper T S, Fuhlbrigge R C. Nat Rev Immunol 2004: **4**: 211–222.
130 Kollisch G, Kalali B N, Voelcker V *et al.* Immunology 2005: **114**: 531–541.
131 Niebuhr M, Baumert K, Werfel T. Exp Dermatol 2010: **19**: 873–877.
132 Fournier B, Philpott D J. Clin Microbiol Rev 2005: **18**: 521–540.
133 Miller L S, Cho J S. Nat Rev Immunol 2011: **11**: 505–518.
134 Gregorio J, Meller S, Conrad C *et al.* J Exp Med 2010: **207**: 2921–2930.
135 Lai Y, Di Nardo A, Nakatsuji T *et al.* Nat Med 2009: **15**: 1377–1382.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. TGF-β inhibits dendritic cells (DC) maturation in response to toll-like receptors agonists. Murine CD11c$^+$ BMDC were treated with TGF-β for 72 h and stimulated with lipopolysaccharide (LPS) R595 (1 μg/ml) for the last 24 h. Unstimulated DC with or without TGF-β treatment displayed intermediate levels of MHC class II expression and low CD86 expression as determined by FACS analysis indicative for an immature phenotype (a, upper panel, left columns). After stimulation with LPS, DC not pretreated with TGF-β readily matured as shown by MHC class II and CD86 upregulation, while TGF-β-treated DC were hampered in achieving a mature phenotype (a, upper panel, right columns). Intracytoplasmic FACS displayed IL-12p40 secretion only by LPS stimulated DC not receiving pretreatment with TGF-β while TGF-β nearly completely inhibited IL-12p40 secretion in response to LPS activation (a, lower panel). (b) Production of IL-12p70 but not IL-10 was almost completely inhibited by pretreatment of DC with TGF-β as determined by ELISA.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.