

## REVIEW

# Dissecting the Role of Infections in Atopic Dermatitis

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**In patients with atopic dermatitis the skin is highly susceptible to infection by bacteria, fungi and viruses. Increasing knowledge about the complex immune network that regulates anti-microbial responses has helped to dissect further the role of infections in atopic dermatitis. Conserved patterns of microbes are recognized by the innate immune system, which mediates microbicidal activity, either directly or through inflammatory responses. New evidence suggests that components of the innate immune system, such as anti-microbial peptides, humoral lectins, nucleotide-binding oligomerization domain-containing (NOD) proteins, and Toll-like receptors not only protect from microbial invasion, but contribute to skin inflammation in atopic dermatitis. In addition, atopic patients tend to develop Th2-dominated immune responses that weaken anti-microbial immunity. This impairment of an appropriate anti-microbial defence compounded by amplified microbe-driven innate and adaptive immune responses leads to the vicious circle of skin inflammation. New microbial management in atopic dermatitis will foster a well-balanced microbial flora, which establishes natural defence mechanisms to maintain immuno-surveillance of the skin. In addition to anti-microbial therapies, other innate immune stimuli may suppress pro-inflammatory signals and help to break the vicious circle of cutaneous inflammation. To elucidate further these different interactions of the skin immune system and microbes in atopic dermatitis, clinical studies and further efforts in basic research are needed. *Key words: innate immunity; pattern recognition receptors; Toll-like receptors; Th2 cells; chemokine receptors.***

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*Abbreviations used:* AD: atopic dermatitis; CAMP: cationic anti-microbial peptides; CLA: cutaneous lymphocyte antigen; DC: dendritic cells; HPV: human papilloma virus; IL: interleukin; IFN: interferon; LPS: lipopolysaccharide; MBL: mannan-binding lectin; NOD: nucleotide-binding oligomerization domain-containing; PAMP: pathogen-associated molecular pattern; PRR: pattern recognition recep-

tors; SNP: single nucleotide polymorphism; Th: T-helper; TLR: Toll-like receptors; TNF: tumour necrosis factor

## INTRODUCTION

Patients suffering from atopic dermatitis (AD) tend to develop disseminated viral infections, such as eczema molluscum, eczema vaccinatum or eczema herpeticum, whereas these diseases do not complicate other inflammatory skin diseases, such as psoriasis (1, 2). Taken alone, this susceptibility to viral cutaneous infections would already point towards an immune defect in AD skin. In addition, multiple studies have demonstrated that several types of bacteria, especially *Staphylococcus aureus* (*S. aureus*), can be detected on the skin of more than 90% of patients with AD. Common clinical manifestations of bacterial skin infections in patients with AD include impetigo contagiosa and paronychias (1, 3). Colonization by *Malassezia spp.* and skin infections by *Candida albicans* indicate that fungi, especially members of the yeast family, are also major invaders of AD skin. These observations lead to the obvious and puzzling question as to whether patients with AD have a general defect in the control of skin infections. A possible failure of bacterial defence mechanisms becomes especially evident when looking at the numerous non-lesional *S. aureus* colonizations detected in patients with AD: more than half of our patients with AD are carriers of *S. aureus* on non-lesional skin, whereas in less than 20% of control groups *S. aureus* is identified on non-lesional skin (4). Some authors even consider these skin infections to be opportunistic infections indicative of an inherent immune defect.

### *Dysfunctional microbial defence in patients with atopic dermatitis*

The skin, like other “interface” organs to the environment, controls integrity of the organism by providing the first line of defence as well as the adaptive immune responses that prevent harm and control microbial invasions. The cellular composition of the skin and molecular response mechanisms developed during evolution are adapted to a variety of microbial strategies attacking the skin. Consequently, a complex network of multiple cell types and molecular pathways has developed and contributes to epidermal and dermal defence mechanisms.

### *Dysfunctional epidermal barrier*

Barrier dysfunction, dry skin and increased trans-epidermal water loss, possibly as a consequence of reduced ceramides, are characteristic findings in patients with AD. Ceramides are sphingolipid constituents of lamellar sheets present in the intercellular spaces of the stratum corneum. These structures mainly provide the barrier properties of the skin.

A decrease in the amount of ceramides results in reduced levels of sphingosine in the stratum corneum, which normally exerts a potent anti-microbial effect on *S. aureus* (5). The high susceptibility of AD skin to cutaneous infections could therefore be a consequence of a dyscomposition and subsequent dysfunction of the outermost layer of the epidermis. Indeed, skin resistance to infection increases and even systemic infections occurring through the skin are reduced, when barrier dysfunction and dry skin are effectively treated (6). Increasing knowledge about lipid mediators and the initiation of specific regulations of signal transduction pathways by lipid mediators may help to integrate the “dry skin concept” into the current understanding of the regulation of inflammation (7).

### ANTI-MICROBIAL PEPTIDES

Anti-microbial peptides represent an important molecular mechanism of defence. Not only fungi, such as *Penicillium notatum*, produce antibiotics, but secretion of anti-microbial compounds is a highly conserved method among different species to defeat microbes even before forming relevant colonies on the host's surface. Among others,  $\alpha$ - and  $\beta$ -defensins, cathelicidin and thrombocidin have been discovered to display anti-microbial activity. These anti-microbial peptides are cationic in nature (Cationic Anti-Microbial Peptides, CAMP). They destroy bacteria virtually by forming holes into the oppositely charged cell walls (8, 9). During inflammation, granulocytes secrete  $\alpha$ -defensins, thrombocytes produce thrombocidin and resident skin cells are stimulated to release  $\beta$ -defensins and cathelicidin. Because of the increased susceptibility to bacterial and viral infections in patients with AD, the presence of these anti-microbial peptides was analysed in normal control skin, AD, and psoriasis. Compared with controls, an up-regulation of  $\beta$ -defensins and cathelicidin could be detected in psoriatic skin. In contrast, up-regulation of  $\beta$ -defensins and cathelicidin was significantly lower or absent in AD skin (10). These data indicated that there is a defect in the first line of defence against bacteria ( $\beta$ -defensin) and viruses (cathelicidin) in inflamed AD skin. However, it is still an unresolved issue as to whether there is a primary defect in patients with AD to produce CAMPs or whether this is a result of the composition of inflammatory mediators in AD.

The hallmark Th2 cytokine, interleukin (IL)-4, dominates AD inflammation in the early phase. Apparently,

it also has the capacity to suppress the production of CAMPs in the skin (11).

Another human anti-microbial peptide, dermcidin, has been described and is exclusively secreted by sweat glands (12, 13). In contrast to CAMPs, secretion of dermcidin is constitutive and not regulated by inflammation. Despite the constitutive secretion, dermcidin and dermcidin-derived peptides were found to be significantly reduced in patients with AD compared with healthy controls (14). This indicates a possible inherent defect of the innate anti-microbial defence system in patients with AD and further research will throw light on the biology of dermcidin and its peptides.

### INNATE IMMUNITY RECOGNIZES PATHOGEN-ASSOCIATED MOLECULAR PATTERN

The response pattern of innate immunity is rapidly initiated upon recognition of a broad set of danger signals. Resident cells of the outer organs at the interface between organism and environment are the first to respond to microbial “danger signals” and these direct response mechanisms are called “innate immune responses” (15). In contrast to adaptive immune responses of T and B lymphocytes, innate immunity cannot adjust its response towards the pathogen and develop a specific memory. The innate immune system recognizes unique and widely distributed regular patterns in microbes. These molecular patterns are often derived from structures that are inevitable for the survival of microbes, such as major parts of the bacterial cell wall. So called “pathogen-associated molecular pattern” (PAMP) from these evolutionarily conserved microbial substances is expressed solely by microbes of the same class. PAMP is recognized by specific host-pattern-recognition molecules, such as the class of lectin proteins. The latter bind to native or opsonized pathogens at extracellular sites and mediate their elimination through recruitment of anti-microbial factors, such as complement. Another class of pattern-recognition molecules is involved in induction of microbicidal host responses through intracellular signalling events. This group includes nucleotide-binding oligomerization domain-containing proteins (NOD) and Toll-like receptors (TLR).

### *Humoral lectins of the innate immunity*

Humoral lectins are part of the innate immune system and are closely linked to the complement system. This is demonstrated by the mannan-binding lectin (MBL) pathway that does not involve antibodies to destroy pathogens, but functions similarly to the classical complement activation cascade. MBL is a serum protein of the collectin family, also known as C-type lectins, which binds to mannose-containing carbohydrates on microbial surfaces. These are outer surface components of Gram-positive and

Gram-negative bacteria, of fungi, and of protozoa such as *Leishmania major* (16). Following MBL activation by microbial mannose-containing carbohydrates, MBL and MBL-associated serine proteases form a complex that activates complement 4. Subsequently, the activation cascade proceeds into the classical complement activation pathway, finally resulting in the destruction of the microbe (17). Patients with inherited deficiencies in MBL or MBL-associated serine protease are highly susceptible to infections and also tend to develop immune diseases, such as lupus erythematosus or rheumatoid arthritis, more frequently (16, 18).

MBL deficiency is occasionally observed in patients with AD, indicating that a defect in innate microbial defence mechanisms may allow bacteria especially to function as a triggering factor of this disease (19, 20). However, a correlation of MBL deficiency with atopy has not been detected in analysing a larger cohort of children with recurrent respiratory infections (21).

Another class of lectins is the ficolins, consisting of L-ficolin, M-ficolin and H-ficolin. They are structurally related to collectins and also involve the complement system to allow opsonization and phagocytosis of microbes (16). In contrast to MBL, no gene polymorphisms or deficiencies have been described for ficolins, but reduced serum levels of L-ficolin were strongly associated with atopy in children with recurrent respiratory tract infections (21). Taken together, collectins and ficolins, similar to anti-microbial peptides, contribute to the immuno-surveillance by the skin, helping directly to destroy and eradicate microbes. Importantly, the functionality of humoral lectins is not dependent on inflammation.

By contrast, other innate response mechanisms need to induce inflammation in order to defeat microbes. Cells of the interface organs carry pattern recognition receptors (PRR) that mediate intracellular signalling after PAMP binding. A signalling cascade results in gene transcription, finally regulating protein production within host cells. Secretion of an array of pro-inflammatory mediators consecutively displays effective microbicidal activity. PRR that induce pro-inflammatory gene transcription are the NOD proteins or TLR. NOD proteins and TLR are very important molecules for anti-microbial innate immunity, but are also involved in the induction of some (auto)-inflammatory responses.

#### *Nucleotide-binding oligomerization diamin proteins*

NOD proteins are cytosolic PRR, and recognize PAMPs that cross the plasma membrane. A large number of NODs is described and genetic variations of some of them have been associated with the development of inflammatory diseases or increased susceptibility to microbial infection. Together with TLR, NOD1 and NOD2 seem to play an important role in the early detection of PAMP. NOD1

and NOD2 bind degradation products of bacterial peptidoglycans (diaminopimelic acid containing molecules and muramyl dipeptides) that are cell wall components of Gram-positive and Gram-negative bacteria (22). After ligand binding to NOD1 and NOD2, a signal transduction cascade is initiated, which causes translocation of NF- $\kappa$ B to the nucleus. NF- $\kappa$ B induces the transcription of genes of anti-microbial peptides, pro-inflammatory cytokines, and chemokines and is crucial for effective anti-microbial host responses.

Naturally occurring gene variations can be analysed by screening single base mutations in the DNA of certain genes. The functional relevance of these polymorphisms can be demonstrated by detecting a significant association of polymorphisms to a disease.

Three groups demonstrated independently, that single nucleotide polymorphisms (SNP) at the NOD-2 gene cause a loss of function and are associated with Crohn's disease (3 SNPs). This indicates that reduced innate response mechanisms can lead to severe inflammatory diseases (23–25). A subsequent study analysed these NOD2 polymorphisms in correlation with atopic diseases and one of them showed a significant association with AD (26). The precise role of NOD2 is not understood, but experiments with NOD2-deficient mice showed that NOD2 is crucial for mucosal and intestinal innate immunity (27). As peptidoglycans are major cell wall components of *S. aureus*, the pattern recognition receptors NOD1 and NOD2 are interesting molecules for AD research. Moreover, *NOD1* is located on chromosome 7q14-p15, a region that was described as an atopy susceptibility locus (28). Consequently, SNP and haplotypes of *NOD1* have been analysed in three large German cohorts asking for associations with atopic diseases and sensitizations. Among the atopic diseases, there are the typical type I allergy-related disorders, such as allergic rhinitis, correlated with IgE sensitizations and diseases, such as asthma or AD, which are co-dominated by cellular delayed-type immune reactions. Interestingly, two from 11 *NOD1* SNPs were found to be associated exclusively with AD, but not with rhinitis or specific IgE. Three SNPs of four that showed significant transmission from parents to children for AD and asthma were also not associated with rhinitis or specific IgE. Among the *NOD1* haplotypes that were analysed, one showed significant association with AD (29). These findings support the clinical observation that there are independent traits for the different atopic diseases.

Together, these data indicate that altered innate immune mechanisms can contribute to AD and allergic asthma, both diseases of interface organs that are in constant contact with microbes. It is important to note that NOD are especially important, when synergizing with TLR and TLR activation-dependent signalling events, the most potent innate response pathways regulating anti-microbial immune responses (22).

### Toll-like receptors

Toll and TLR were first identified in fruit flies and later across species. Toll was described as a gene important for the development of *Drosophila* flies by Christiane Nüsslein-Volhard's group in 1985 and for its impact on research in biology/immunology she was awarded the Nobel Prize in 1995 (30). This discovery initiated an unusual and almost inflationary array of discoveries in modern science. Plants, reptiles, insects and mammals carry these Toll or TLRs. Today, there are 11 different TLRs known and found to be expressed either at the cell surface or the endosomes (Fig. 1). Over the years it was discovered that TLRs are often crucial for the first-line anti-microbial defence an organism needs for survival. Several important well-known or newly discovered ligands for TLR were identified and published in the most respected scientific journals. It was a milestone discovery that resistance to lipopolysaccharide (LPS)-induced shock was due to mutations in a specific LPS receptor, named TLR4 (31). Following the description of TLR4, several microbial substances and their specific TLRs were identified, demonstrating the complexity and diversity of this most important pattern recognition family of the innate immune system. TLR2 was characterized by binding bacterial lipopeptides (32), viral double-stranded RNA to be the ligand for TLR3 (33), bacterial DNA was found to activate TLR9 (34), and bacterial Flagellin specifically binds to TLR5 (35). Imidazoquinolines had already entered the clinic and become a dermatological standard treatment of human papilloma virus (HPV)-induced warts (36). However, it was only recently that the mode of action of imidazoquinolines was detected. Imidazoquinolines, like naturally occurring single-stranded viral RNA, bind to TLR7 and TLR8, inducing local inflammation that allows eradication of viruses (37–40).

Following TLR ligation, pro-inflammatory pathways are activated downstream of TLR, the most prominent being the NF- $\kappa$ B and type 1 interferon (IFN) pathways. Today, the signalling cascades induced by TLR ligation

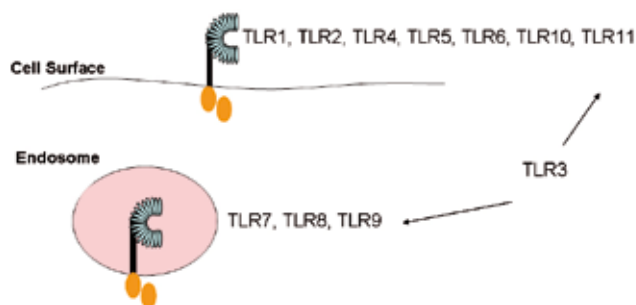


Fig. 1. Toll-like receptors (TLR) in humans are expressed either at the cell surface or in the endosomes in many different cells, which are present in particular on organs communicating constantly with the environment. There are 11 different known TLR, which bind structurally conserved molecular patterns of essential microbial structures. Some TLR also bind endogenous ligands. TLR3 is present both on the surface and in the endosomes.

are well defined and a MyD88 dependent pathway leading to NF- $\kappa$ B translocation to the nucleus next to a MyD88 independent pathway responsible for type 1 IFN production has been characterized (41, 42). TLRs and TLR-dependent signal transduction molecules are expressed in cells of "interface" organs. Therefore, PAMPs from microbes can bind to TLR on skin cells and activate, among other cell types, keratinocytes, Langerhans' and dendritic cells, mast cells and neutrophils. This receptor ligation turns on an inflammatory signalling cascade within the target cells and its organs (Fig. 2). This inflammatory response induced by the TLR system is set up to delete microbes before these can enter the organism. Mice deficient in specific TLR or downstream signalling molecules are susceptible to severe infections (43). In conclusion, PAMP recognition by TLR is probably more sensitive, more specific, and more potent in sensing and responding to microbes than any other PAMP recognition system described thus far.

### CONSEQUENCES OF TOLL-LIKE RECEPTOR ACTIVATION FOR ATOPIC DERMATITIS

As stated above, colonization with *S. aureus* can be detected in more than 90% of patients with AD and is believed to enhance skin inflammation. Major cell wall components of *S. aureus* bind to TLR2 and mice deficient in TLR2 are highly susceptible to *S. aureus* infection (44). Mouse studies also indicate that complete loss of function of one TLR, or signalling molecules downstream of TLR, results in severe disease phenotypes (45). However, altered TLR2 activity due to gene variations may underlie reduced microbial defence or enhanced PAMP-induced inflammation in patients with AD (Fig. 2). Indeed, polymorphisms of TLR2 have been described in patients with severe AD and the TLR2 gene was detected in studies that were set up to discover genes responsible for atopy and/or allergy (46, 47). In theory, loss of function polymorphisms of TLR2 may allow infections to spread on AD skin resulting in enhanced AD inflammation. In contrast, gain of function polymorphisms may be responsible for aggravated AD inflammation *per se*. Therefore, it is still under debate whether and in what respect TLR polymorphisms are of clinical relevance for patients with AD pointing towards the need for further research in this field.

Activation of TLR is an important and highly effective "danger signal" that not only initiates effective defence mechanisms but also leads to an excess of inflammation as in allergic asthma (48). Moreover, TLR-mediated pro-inflammatory activation signals are also involved in auto-immune diseases (49). Under some circumstances, endogenous ligands of TLRs take over the stimulatory potential of PAMPs and drive auto-immune diseases, such as TLR9 activation in lupus erythematosus (50).

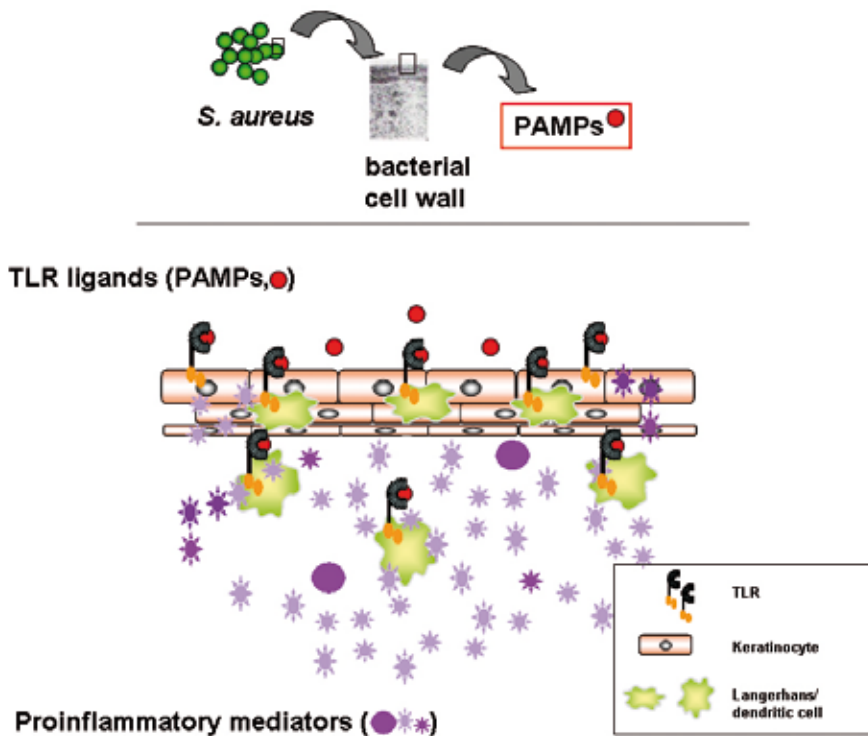


Fig. 2. Structurally conserved molecular pattern of the cell wall of Gram-positive bacteria include lipoproteins with the potency of binding to Toll-like receptor (TLR)2 (upper diagram). These TLR ligands bind to TLR that are expressed by several resident cells of the epidermis and dermis and induce the production and secretion of pro-inflammatory mediators, such as interleukin (IL)-6 or tumour necrosis factor (TNF).

These findings indicate that TLR activation may also be responsible for aggravation and chronicity of inflammatory diseases of outer organs, such as AD.

Taken together, PAMP activation in AD may be of importance in several ways. In the early phase of AD, reduced PAMP-mediated defence mechanisms lead to a higher microbial load on AD skin. In later phases, sustained inflammation may be triggered by repetitive TLR activation leading to aggravated and sustained AD inflammation. Importantly, TLR activation is also considered to be a “danger signal” that induces activation and involvement of the adaptive immune system. This “danger signal” is recognized by T lymphocytes after a process of transition from “innate inflammation”. PAMP activation leading to transition of relevant “immune” information to the adaptive immune system is another example of immune-networking relevant for inflammation in AD.

*Transition of inflammation to adaptive immunity by dendritic cells*

T cells residing in lymph nodes are most effectively activated by dendritic cells (DC) and DC have a strong impact on the nature of the developing adaptive immunity (51). Among other cell types, TLR signals can activate DC that will then undergo maturation, migrate to lymph nodes, and deliver important activation signals to T cells. Today, several subtypes of DC are characterized. These DC subtypes are either defined by haematopoietic lineage, the organ source or can be characterized by the functional consequences of DC interactions with T cells.

After an initial contact to DC, activated T cells also undergo a developmental process and turn into functionally distinct subtypes. These functional consequences of DC contact to T cells are best defined for CD4+ T cells, the T helper (Th) cells. The cytokine profile of Th cells detected after activation by DC characterizes the functional DC phenotype.

*DC phenotypes and the TH1/TH2 paradigm*

A major breakthrough for understanding regulated inflammatory immune responses was the discovery of

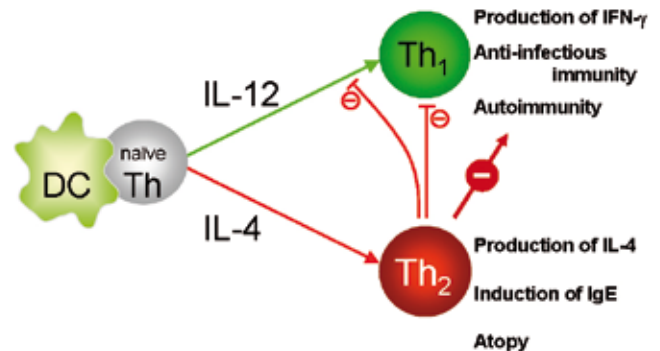


Fig. 3. Activated dendritic cells (DC) migrate from the skin to skin-draining lymph nodes where DC activate naive CD4+ T helper cells (Th cells). Among other factors, cytokines are most dominant inducers of polarized Th-cell subtypes. IL-12 is produced by DC and induces Th1 cells, whereas IL-4 is the most dominant regulator of Th2 development. Th1 cells are characterized by the production of interferon-γ, mediate auto-immune diseases and anti-infectious immunity. By contrast, Th2 cells produce IL-4, induce IgE production in B lymphocytes and suppress Th1 responses.

polarized Th-cell subsets in 1986 by Mosmann et al. (52). The authors characterized a subset of IFN  $\gamma$  producing CD4<sup>+</sup> T cells that were named Th1 cells and another population of CD4<sup>+</sup> T cells secreting interleukin (IL-4) 4, the Th2 cells. This Th1-Th2 paradigm opened a new dimension in the understanding of adaptive immunity. Th1 dominated immune responses were found to be important and effective for anti-infectious immunity, tumour immunity, and also in auto-immunity (Fig. 3). In contrast, Th2 cells are important modulators of inflammation, induce the humoral response in B lymphocytes, but are also involved in allergic and atopic diseases (51). IL-4, the major Th2 cytokine, most potently induces IgE production in B cells and suppresses anti-infectious immune responses by down-regulating anti-microbial peptides and inhibiting Th1 immunity (11, 51). Moreover, it was demonstrated that IL-4 and IL-4-producing Th2 cells can even be used to treat unwanted Th1-mediated inflammatory skin diseases such as contact dermatitis and psoriasis (53, 54). This led to the belief that Th2 dominated immunity as in atopic individuals allows cutaneous infection to spread, because effective immune responses to microbes are inhibited by IL-4 (Fig. 3). The underlying mechanism leading to this dysregulation is only partially understood, but antigen presenting cells, especially DC, seem to play a major role.

The most important underlying mechanism regulating Th1 and Th2 polarization was identified more than a decade after the initial discovery of Th1 and Th2 cells. DC were already known to be most effective in the transition from innate to acquired immunity and to be responsible for Th cell activation. However, it became clear that DC not only activate but also "educate" Th cells (Fig. 3). According to the Th1–Th2 polarization, DC that produce IL-12 and consequently induce a Th1 phenotype in Th cells were named DC1. In contrast, DC initiating Th2 cells were called DC2 (55, 56).

This immune network between innate immunity, DC, T cells, and anti-infectious immune responses of the adaptive immune system intriguingly follows a logic track. TLR ligands activate DC and initiate a maturation process that enables DC to migrate into e.g. skin draining lymph nodes. Most TLR ligands induce IL-12 production and consequently promote DC1 development. DC1 support Th cell polarization to IFN- $\gamma$  producing CD4<sup>+</sup> T cells that in turn effectively orchestrate anti-infectious immunity. In contrast, pollen-derived lipid mediators induce DC2 and consecutively Th2 cells initiating IgE production in B lymphocytes and suppression of Th1 cells (57). Moreover, there is increasing evidence that some PAMPs induce pathways that allow tolerogenic DC to develop. These DC promote the development of regulatory T cells that may be able to balance "inflammation" (58).

However, more research is needed to define tolerogenic PAMPs, the microbes delivering tolerogenic PAMPs, and the pathways that follow.

## "PROVOCATIVE" ROLE OF TH2 CELLS IN ATOPIC DERMATITIS

The concept of Th2 cell-mediated susceptibility to infection in atopic individuals is intriguing, but there is conflicting data on the role of Th2 cells in AD skin. Evidence that Th2 cells initiate the inflammatory amplification loop in AD that is finally characterized by significant co-expression of IFN- $\gamma$  questioned the suppressive role of Th2 cells on Th1 responses (59, 60). The presence of Th2 cells in skin and blood of patients with AD has been well documented (61, 62), but only recently migration of human Th2 cells to human skin could be proven experimentally (60).

### *Leukocyte recruitment – the vicious circle of cutaneous inflammation*

The understanding of the fine regulation of leukocyte migration into different tissues has increased tremendously over the last decades. A concept evolved that is known as the "multistep process of migration" and it describes in detail the subsequent steps that are necessary to allow controlled leukocyte extravasation (Fig. 4). Several receptor ligand pairs on endothelia and the corresponding leukocytes are involved in this process. First, it is necessary to reduce leukocyte velocity within vessels. Attachment of leukocytes to endothelial cells is mediated by selectins and slows down leukocytes leading to "rolling" (Fig. 4). Following the "rolling", chemokines specifically activate leukocytes through their corresponding receptors, leading to firm arrest of the leukocytes on endothelium ("activation"). Leukocytes that stick to endothelia ("adhesion") may extravasate and migrate along a chemokine gradient that places inflammatory cells in extravascular tissues such as the skin (Fig. 4) (63, 64).

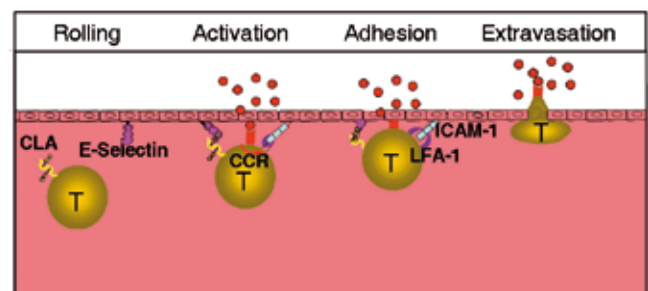


Fig. 4. Multistep process of migration. "Rolling" of T lymphocytes on human endothelium of venules is achieved by binding of the cutaneous lymphocyte antigen (CLA) to E-selectin. Subsequently, chemokines presented by endothelial cells bind to their corresponding chemokine receptors and activate T lymphocytes. This activation ("inside-out signalling") leads to conformational changes of integrins, such as LFA-1 and allows firm adhesion ("arrest") of T lymphocytes on endothelial cells. Cells that adhered stably to endothelial cells are susceptible to chemokine-mediated extravasation and migration into the tissue following a chemokine concentration gradient.

Today we understand that PAMP-mediated activation signals can directly up-regulate the expression of selectins, adhesion molecules and chemokines as part of the anti-microbial inflammatory defence system. Following recruitment, leukocytes are activated within the skin and many leukocyte subsets are again responsive to PAMP stimulation. Allergens, self-antigens and *S. aureus*-derived super-antigens are important stimuli for T cells that have been recruited into AD skin. Activation of T cells and other leukocytes within the skin leads to a further up-regulation of selectins, adhesion molecules and chemokines. Therefore, T-cell activation together with increasing PAMP recognition mediates the recruitment of more leukocytes, initiating a vicious circle of inflammation. Among the different signals that regulate the “multistep process of migration” and that mediate extravasation, especially of T cells, chemokine-mediated migration is the most specific and best regulated step and has been a target for new therapeutic approaches.

### *Chemokines*

The chemokines (from contraction of the words chemotactic and cytokines) are a family of pro-inflammatory cytokines that attract and activate specific leukocyte subtypes. The superfamily of chemokines consists of more than 40 members that are small, structurally related molecules and can be divided into four subgroups, based on the number and spacing (X) of the first two conserved cysteine residues in the amino terminus: CXC ( $\alpha$ -family), CC ( $\beta$ -family), C ( $\gamma$ -family), and CX3C ( $\delta$ -family). The corresponding receptors have been named accordingly and a new alphanumeric nomenclature of the chemokines has been introduced, according to the gene loci and the receptor to which the chemokines bind (65). Today, there are 6 CXCR receptors (CXCR1–6), 11 CCR receptors (CCR1–11), and 1 XCR and 1 CX3CR receptor. Chemokines were recognized mainly for their effects on cell trafficking, but activation, differentiation of immune cells, as well as exertion of direct anti-microbial effects, in addition to many other functions were subsequently identified as mediated by chemokines (63, 66). The regulation of cell trafficking by chemokines is considered to be the dominant system organizing lymphocyte homeostasis and lymphocyte distribution between lymphoid organs and peripheral tissues (66, 67).

### *Extravasation of Th cells into the skin in patients with atopic dermatitis*

In general, the extravasation of Th cells into the skin depends on the interaction of several receptors on Th cells and endothelial cells, as outlined in Fig. 4. Adhesion molecules, such as the cutaneous lymphocyte antigen (CLA) or LFA-1, are key players in the process of Th cell

extravasation and biologicals, or small molecular weight compounds, have been developed to interfere with these molecules and to inhibit skin homing of Th cells (60, 64, 68–71). The value of these therapeutic approaches for AD is still unclear, but is currently under investigation. In order to define potentially promising therapeutic strategies for AD, a closer look to the steps of Th-cell migration and its specificity in AD is needed.

Chemokine interactions with chemokine receptors demonstrated leukocyte subset specificity that was interpreted with some enthusiasm with regard to new therapies. Indeed, some chemokine receptors have been identified as potential therapeutic targets in AD. CCR4 and CCR10 are chemokine receptors that were described as “skin-specific” and CCR3, CCR4, and CCR8 are chemokine receptors associated with a Th2 phenotype (60, 72–76). Pre-clinical studies in humanized mouse models and analyses of human skin lesions showed that the chemokine receptor CCR4 is most powerful in mediating extravasation and skin homing of Th2 cells into human skin (60, 77). More recently, it was also shown that in addition to CCR4 ligands, CCL18, also known as PARC, recruits memory Th cells into human skin and, like CCR4 ligands, may serve as a disease marker in AD (78). Moreover, innate and adaptive immunity synergize to up-regulate the pro-inflammatory chemokine system in AD: chemokine expression is effectively triggered by PAMP activation and the expression of CCR4 ligands and CCL18 is further up-regulated by IL-4.

### *Cytokine switch to interferon- $\gamma$ in atopic dermatitis*

Many examples have demonstrated how IL-4 and IL-4-producing Th2 cells interfere with efficient anti-microbial immune responses and promote cellular inflammation not only in AD. However, microbial colonization of the skin and activation of PRR by PAMPs deliver “danger signals” that induce Th1 rather than Th2 cells. It is not known how these opposing signals co-operate. The discovery that IL-4 can act synergistically with TLR signals to induce IL-12 and Th1 cells was an important finding also for the understanding of chronic AD and the cytokine switch in AD that was described much earlier (79–81). Apparently, early AD inflammation is dominated by IL-4 producing Th2 cells, whereas increasing levels of lesional IFN- $\gamma$  are present in later phases of AD (59). These data indicate that there is counter-regulation from a Th2 to a Th1 phenotype in AD that may be regulated by TLR activation in the presence of IL-4.

Interestingly, experiments simulating this cytokine switch in AD showed that inducing IFN- $\gamma$  production in Th2 cells from AD skin does not suppress the expression and function of Th2-associated homing receptors, such as CCR4. By contrast, these former Th2 cells express even more CCR4 and in addition other Th1-associated chemokine receptors, such as CXCR3, which also

mediate skin homing (68, 82). Thus, in AD, the Th2-cell dominated immunity initiates inflammation and allows infections to spread in the early phase, whereas later, TLR stimulation adds to aggravation and further amplification of cutaneous inflammation (Fig. 5).

#### ANTI-MICROBIAL THERAPY IN ATOPIC DERMATITIS: CLINICAL EVIDENCE

Intriguing clinical evidence and our understanding of innate immune response mechanisms that are partially dysfunctional in patients with AD suggest that anti-microbial therapy should be very effective in AD. Analysing the effect of anti-microbial treatments in controlled studies, however, revealed conflicting data on the value of systemic or topical anti-microbial treatment modalities for inflammation in AD (3). A controlled study applying systemic cloxacillin or erythromycin resulted in *S. aureus* clearance and significant and sustained clinical improvement even 6 months following cessation of antibiotic therapy (83). In contrast, another study in selected patients who had no obvious signs of skin infection demonstrated no improvement after flucloxacillin treatment in symptoms or clinical appearance of AD (84). There are five double-blind placebo-controlled studies on combinations of topical corticosteroids with topical antibiotics in the literature, two studies demonstrating significant benefit, two showing no significant advantage, and one demonstrating a significant benefit in a combination of bacteriological and clinical scores (85–89). Topical application of the antibiotic mupirocin alone was analysed in unselected patients with AD using a double-blind placebo-controlled cross-over study and revealed significantly reduced clinical severity of AD, which even lasted for 4 weeks after treatment was

stopped (4). These latter data helped to promote further studies on topical antibacterial treatment strategies, such as antiseptic formulations like gentian or crystal violet (90), chlorhexidine or triclosan in topical formulations or in antibacterial soap (91, 92), or the use of silver-coated textiles and silk fabric with anti-microbial activity (93, 94), which proved to be clinically effective.

#### *New microbial management in atopic dermatitis*

The data from clinical studies on anti-microbial therapy in AD suggest (i) that anti-microbial treatments should be applied in patients with AD with apparent or recurrent skin infections; and (ii) that new strategies and studies on this subject are needed. Indeed, increasing knowledge about the innate immune system provides new evidence to define prophylactic and therapeutic strategies to regulate rather than eradicate microbes in patients with AD. From studies of the gastrointestinal tract, we know today that tolerance, resistance and inflammation are possible response patterns that are initiated by different bacteria and molecular pathways derived thereof (95). For a long time, it was believed that non-pathogenic bacterial gut flora is simply ignored by the (innate) immune system. More and more studies indicate that there is an active dialogue between members of the commensal microflora and the host mucosal immune system. This cross-talk affects immunological tolerance and homeostasis within the gut and may explain the development of inflammatory disorders, such as Crohn's disease. Some data suggest that a, yet to be defined, well-balanced commensal gut microflora may be important for disease prophylaxis not only of the gut.

Daily intake of probiotic lactobacillus bacteria reduced the prevalence of AD at the age of 2 years by 50% and this effect was stable until the age of 4 years (96, 97). Even though these studies need to be confirmed, several investigations indicate that food supplementation with probiotic bacteria beneficially influences the course of AD in children with and without food allergy (98–100). The mechanism of this kind of treatment is not understood, but inflammatory responses can be detected after treatment with probiotic bacteria, indicating that an innate immune response pattern may underlie the therapeutic effect (101). Taken together, microbes in contact with interface organs, such as the gut and the skin, seem to have a key role in balancing inflammation and disease development. Better understanding of this well-balanced system of surface microflora and its communication with our immune system will help to define different forms of bacterial stimuli. Further research may identify “the good”, “the bad” and “the ugly” bacteria or molecular patterns in respect to inflammation in AD. Treatment strategies eradicating all bacteria in patients with AD, such as the use of systemic antibiotics, may abolish “good” stimuli as well the “the

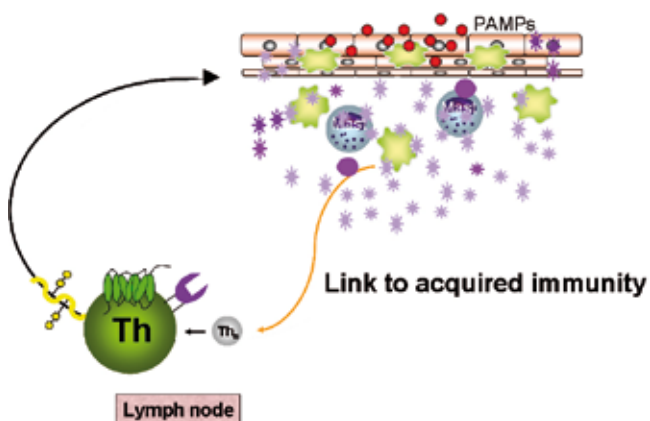


Fig. 5. Skin inflammation triggered by pathogen-associated molecular pattern (PAMPs) link innate to adaptive immunity by regulating the Th cell phenotype (see Fig. 3) and by up-regulating homing receptors on Th cells. Up-regulation of skin-specific homing receptors, such as cutaneous lymphocyte antigen and CCR4, or additional functional homing receptors, such as CXCR3, enables more Th cells to home to the skin and to aggravate skin inflammation.



bad” and “the ugly” and therefore fail. These therapeutic measures may need to be replaced by gentler approaches that support a potentially beneficial microflora on the skin. We may even be able to design “prophylactic” treatment strategies to create a balanced skin flora or apply specific compounds that support the natural defence mechanisms that prevent skin infection and that trigger modulating innate immune-response pathways. Suppressing pro-inflammatory signals that are part of an anti-microbial response in parallel with deleting the triggering microbes will lead us to the exit point from the vicious circle of cutaneous inflammation.

The different phases of microbial influence in AD inflammation need to be addressed in detail in future clinical studies with patients having AD and in basic research aiming towards the development of new treatment modalities.

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