

Received Date : 20-Oct-2013

Accepted Date : 17-Mar-2014

Article type : Letter to the Editors

TLR2 and TLR4 expression in atopic dermatitis, contact dermatitis and psoriasis

Rüdiger Panzer¹, Conrad Blobel¹, Regina Fölster-Holst, Ehrhardt Proksch

Department of Dermatology, University of Kiel, Germany.

¹These authors contributed equally to the work

Corresponding author:

Ehrhardt Proksch MD, PhD

Department of Dermatology

University Hospitals of Schleswig-Holstein

Campus Kiel

Arnold-Heller-Str. 3

24105 Kiel

Germany

Tel. +49 431 597-1505

Fax +49 431 597-1611

E-mail: eproksch@dermatology.uni-kiel.de

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi:

10.1111/exd.12383

This article is protected by copyright. All rights reserved.

Abbreviations used:

TLR: toll-like receptor

Keywords:

atopic dermatitis, skin barrier, innate immunity, toll-like receptors

ABSTRACT

The aim of the study was to investigate the expression of Toll-like receptor (TLR) 2 and 4 on keratinocytes in atopic dermatitis, contact dermatitis, and psoriasis by PCR and by immunohistochemistry including confocal microscopy. Confocal microscopy revealed a granular intracellular expression pattern for TLR 2 and a homogenous intracellular expression pattern for TLR 4 in normal and diseased skin. TLR 2 was constitutively expressed in the suprabasal layers in normal skin, but limited to the basal epidermis in diseased skin. TLR 4 expression was concentrated to the basal layers in normal skin, whereas it was pronounced in upper layers in diseased skin. The shift in the TLR expression may be related to the disturbed skin barrier and a need for enhanced immune surveillance because of invading microbes. Also, there must be a balance between sufficient immune response and overstimulation.

Background

Toll-like receptors (TLR) are an important part of the innate immunity in humans and regulate antimicrobial defense (1). Human keratinocytes express TLR1, 2, 3, 5 and 9 constitutively (2-7). TLR4 has been described to be expressed constitutively and non-constitutively in keratinocytes (2-6,8). TLR2 and TLR4 recognize lipoteichoic acid (gram-positive bacteria) and lipopolysaccharide (gram-negative bacteria), respectively (9). Usually, TLR2 and TLR4 are localized to the cell membrane (4,6,10). In contrast, cytoplasmatic TLR2 and TLR4 expression in keratinocytes was reported (5). In inflammatory skin diseases the disturbed physical skin barrier may influence the state of the immune barrier and *vice versa* (11-13). Skin infections occur more frequently in atopic dermatitis than in psoriasis. (14) TLR might

contribute to the disordered immune activity in atopic dermatitis (15-17) and contact dermatitis (18,19). Data about TLR expression in atopic and contact dermatitis are lacking. TLR2 has been described to be increased or not detected in psoriasis (5).

Questions

We asked whether TLR2 and TLR4 are expressed constitutively by keratinocytes, asked for the epidermal and subcellular localization and whether the expression is altered in atopic dermatitis, contact dermatitis and psoriasis. We have chosen TLR2 and TLR4, as these two receptors together comprise recognition of both gram-positive and negative bacteria.

Design

Lesional skin samples were taken from atopic dermatitis (n = 9), contact dermatitis (n = 12), psoriasis (n = 12) and control subjects (n = 5). Paraffin-embedded sections were incubated with TLR2 or TLR4 antibodies (ab47840 and ab47093, Abcam, Cambridge, UK). As secondary antibodies swine anti rabbit and Cy2 (Jackson, Baltimore, USA) were used. Strength of immuno staining was determined by grading 0 to 3 for each layer. Significances were determined by paired t-test. RNA was isolated from untreated HaCaT cells, primary human keratinocytes, THP-1 cells, primary monocytes and lymphocytes (immune cells serving as positive control) and from homogenized normal skin using standard protocol.

Results

Real-time PCR with subsequent gel electrophoresis showed a constitutive expression of TLR2 and TLR4 mRNA in HaCaT cells, primary human keratinocytes and normal human skin (Fig. S1a and b). The localization of the two TLR proteins in the epidermal layers was quite different, as shown by immunohistology. TLR2 protein was constitutively and uniformly expressed in all nucleated epidermal layers in control skin. In atopic dermatitis, in contact dermatitis and in psoriasis staining was faint in the granular and upper spinous layers and increased towards lower spinous and basal layers. (Fig. 1) TLR4 was expressed in normal skin constitutively in the entire nucleated epidermis, but concentrated in the upper tip of the basal layer. In atopic dermatitis, in contact dermatitis and in psoriasis TLR4 staining was low in

the basal and the lower and mid spinous layers and increased towards the upper spinous and the granular layers (Fig. 2)

(Fig. 2) Immunohistological staining for TLR4 was weaker than for TLR2. Supporting this TLR4 mRNA showed much higher crossing points in real time PCR. Comparison of crossing-points gained with different primers is difficult. But the analogy of PCR and histologic results suggests a lower TLR4 expression. This may be due to the less frequent infection and the more efficient clearance of the skin with gram-negative bacteria by antimicrobial peptides.

In atopic dermatitis, contact dermatitis and psoriasis, TLR4 expression was continuously increasing from basal to upper spinous layers including *stratum granulosum*. (Fig. 2) Epidermal barrier disruption in diseased skin facilitates invasion of pathogens activating the innate immune barrier in the upper epidermal layer as shown for antimicrobial proteins (20,21). This is in accordance with the lower frequency of gram-negative bacteria especially in physiologic skin colonisation. The lower risk of overstimulation would explain why upregulation in the upper cell layers occurs for TLR4 but not for TLR2.

In atopic patients TLR2 expression is downregulated in macrophages (22). Our study showed no decrease in keratinocytes of atopic or contact dermatitis, as we initially expected. The missing upregulation of TLR2 may contribute to the susceptibility for gram-positive skin infection in atopic skin. This is in line with the previously described impaired innate immune response in atopic skin comprising impaired function of TLR2 signaling pathway compared to psoriasis (20,23,24).

In contrast to other authors our data showed no upregulation of TLR2 expression in psoriasis, possibly due to a differential expression during the course of the disease, e. g. caused by IFN- γ and TNF- α which have been shown to upregulate TLR2 and TLR4 in renal epithelial cells (25). In contrast, Curry et al. showed TLR2 downregulation in keratinocytes by TNF- α (4). Responsiveness of cells for TLR ligands results in part from the localization of TLRs within the cells (5,26). Usually, TLR2 and TLR4 are localized to the cell membrane, although cytoplasmatic expression in keratinocytes was reported (5). Our immunofluorescence

microscopy studies showed that the expression pattern of TLR2 in normal and diseased skin was prevalently intracellular with mainly perinuclear coarse granules (Fig. S2a). This expression pattern in keratinocytes has not been described yet and may be due to a localization in cell organelles, like endoplasmic reticulum or Golgi apparatus as described for TLR9 (27). Cytoplasmic expression may also be a method to avoid overstimulation gaining more specificity as suggested for other TLR. (28). Increase of cytoplasmic TLR 2 and TLR4 expression after stimulation with TLR ligands results in lower reactivity (29). The expression of TLR4 was also prevalently intracellular, but more homogenous with fine grains instead of coarse granula (Fig. S2a) We could not find the previously described “chicken-wire” appearance (4). The intracellular localization of TLR2 and TLR4 in keratinocytes in normal and diseased skin *in vivo* may avoid a constant state of proinflammatory activation as it has been shown in intestinal cells (28,29). The more homogenous cytoplasmic pattern could be due to an expression of TLR4 bound to very small organelles like lysosomes.

In summary, our data showed strong evidence for a constitutive intracellular expression of TLR2 and TLR4 in human keratinocytes *in vitro* and *in vivo*. TLR2 expression was shifted to lower layers and TLR4 was shifted to upper layers in diseased skin compared to control skin.

ACKNOWLEDGEMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 617/A7; DFG PR 272/6-1) given to Ehrhardt Proksch. We thank Dr. Felix Scholz for scientific advices and Claudia Neumann and Graziella Francesca Podda for excellent technical assistance.

R. Panzer designed the research, analyzed the data and wrote the paper. C. Blobel performed the research, analyzed the data and wrote the paper. E. Proksch and R. Foelster-Holst designed the research and wrote the paper.

CONFLICT OF INTERESTS

The authors state no conflict of interest.

References

- (1) Hertz CJ, Wu Q, Porter EM *et al.* Activation of Toll-like receptor 2 on human tracheobronchial epithelial cells induces the antimicrobial peptide human beta defensin-2. *J Immunol* 2003; **171**: 6820-6826.
- (2) Mempel M, Voelcker V, Kollisch G *et al.* Toll-like receptor expression in human keratinocytes: nuclear factor kappaB controlled gene activation by *Staphylococcus aureus* is toll-like receptor 2 but not toll-like receptor 4 or platelet activating factor receptor dependent. *J Invest Dermatol* 2003; **121**: 1389-1396.
- (3) Lebre MC, van der Aar AM, van Baarsen L *et al.* Human Keratinocytes Express Functional Toll-Like Receptor 3, 4, 5, and 9. *J Invest Dermatol* 2006.
- (4) Curry JL, Qin JZ, Bonish B *et al.* Innate immune-related receptors in normal and psoriatic skin. *Arch Pathol Lab Med* 2003; **127**: 178-186.
- (5) Begon E, Michel L, Flageul B *et al.* Expression, subcellular localization and cytokinic modulation of Toll-like receptors (TLRs) in normal human keratinocytes: TLR2 up-regulation in psoriatic skin. *Eur J Dermatol* 2007; **17**: 497-506.
- (6) Pivarcsi A, Bodai L, Rethi B *et al.* Expression and function of Toll-like receptors 2 and 4 in human keratinocytes. *Int Immunol* 2003; **15**: 721-730.
- (7) Baker BS, Ovigne JM, Powles AV *et al.* Normal keratinocytes express Toll-like receptors (TLRs) 1, 2 and 5: modulation of TLR expression in chronic plaque psoriasis. *Br J Dermatol* 2003; **148**: 670-679.
- (8) Song PI, Park YM, Abraham T *et al.* Human keratinocytes express functional CD14 and toll-like receptor 4. *J Invest Dermatol* 2002; **119**: 424-432.
- (9) Takeuchi O, Hoshino K, Kawai T *et al.* Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. *Immunity* 1999; **11**: 443-451.
- (10) Mogensen TH, Paludan SR, Kilian M *et al.* Live *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis* activate the inflammatory response through Toll-like receptors 2, 4, and 9 in species-specific patterns. *J Leukoc Biol* 2006.
- (11) Mudnakudu NK, Babina M, Worm M. Opposing effects on immune function and skin barrier regulation by the proteasome inhibitor bortezomib in an allergen-induced eczema model. *Exp Dermatol* 2013.
- (12) Hatano Y, Adachi Y, Elias PM *et al.* The Th2 cytokine, interleukin-4, abrogates the cohesion of normal stratum corneum in mice: implications for pathogenesis of atopic dermatitis. *Exp Dermatol* 2013; **22**: 30-35.

- (13) Darlenski R, Kazandjieva J, Tsankov N *et al.* Acute irritant threshold correlates with barrier function, skin hydration and contact hypersensitivity in atopic dermatitis and rosacea. *Exp Dermatol* 2013.
- (14) Christophers E, Henseler T. Contrasting disease patterns in psoriasis and atopic dermatitis. *Arch Dermatol Res* 1987; **279 Suppl**: S48-S51.
- (15) Leung DY. Atopic dermatitis: new insights and opportunities for therapeutic intervention. *J Allergy Clin Immunol* 2000; **105**: 860-876.
- (16) Hamid Q, Boguniewicz M, Leung DY. Differential in situ cytokine gene expression in acute versus chronic atopic dermatitis. *J Clin Invest* 1994; **94**: 870-876.
- (17) Kupper TS, Fuhlbrigge RC. Immune surveillance in the skin: mechanisms and clinical consequences. *Nat Rev Immunol* 2004; **4**: 211-222.
- (18) Martin SF, Dudda JC, Bachtanian E *et al.* Toll-like receptor and IL-12 signaling control susceptibility to contact hypersensitivity. *J Exp Med* 2008; **205**: 2151-2162.
- (19) Schmidt M, Goebeler M. Nickel allergies: paying the Toll for innate immunity. *J Mol Med (Berl)* 2011; **89**: 961-970.
- (20) Harder J, Dressel S, Wittersheim M *et al.* Enhanced expression and secretion of antimicrobial peptides in atopic dermatitis and after superficial skin injury. *J Invest Dermatol* 2010; **130**: 1355-1364.
- (21) Jensen JM, Ahrens K, Meingassner J *et al.* Differential suppression of epidermal antimicrobial protein expression in atopic dermatitis and in EFAD mice by pimecrolimus compared to corticosteroids. *Exp Dermatol* 2011; **20**: 783-788.
- (22) Niebuhr M, Lutat C, Sigel S *et al.* Impaired TLR-2 expression and TLR-2-mediated cytokine secretion in macrophages from patients with atopic dermatitis. *Allergy* 2009; **64**: 1580-1587.
- (23) Nomura I, Goleva E, Howell MD *et al.* Cytokine milieu of atopic dermatitis, as compared to psoriasis, skin prevents induction of innate immune response genes. *J Immunol* 2003; **171**: 3262-3269.
- (24) Hasannejad H, Takahashi R, Kimishima M *et al.* Selective impairment of Toll-like receptor 2-mediated proinflammatory cytokine production by monocytes from patients with atopic dermatitis. *J Allergy Clin Immunol* 2007; **120**: 69-75.
- (25) Wolfs TG, Buurman WA, van Schadewijk A *et al.* In vivo expression of Toll-like receptor 2 and 4 by renal epithelial cells: IFN-gamma and TNF-alpha mediated up-regulation during inflammation. *J Immunol* 2002; **168**: 1286-1293.

(26) Otte JM, Cario E, Podolsky DK. Mechanisms of cross hyporesponsiveness to Toll-like receptor bacterial ligands in intestinal epithelial cells. *Gastroenterology* 2004; **126**: 1054-1070.

(27) Leifer CA, Kennedy MN, Mazzone A *et al.* TLR9 is localized in the endoplasmic reticulum prior to stimulation. *J Immunol* 2004; **173**: 1179-1183.

(28) Haas T, Metzger J, Schmitz F *et al.* The DNA sugar backbone 2' deoxyribose determines toll-like receptor 9 activation. *Immunity* 2008; **28**: 315-323.

(29) Cario E, Brown D, McKee M *et al.* Commensal-associated molecular patterns induce selective toll-like receptor-trafficking from apical membrane to cytoplasmic compartments in polarized intestinal epithelium. *Am J Pathol* 2002; **160**: 165-173.

FIGURES

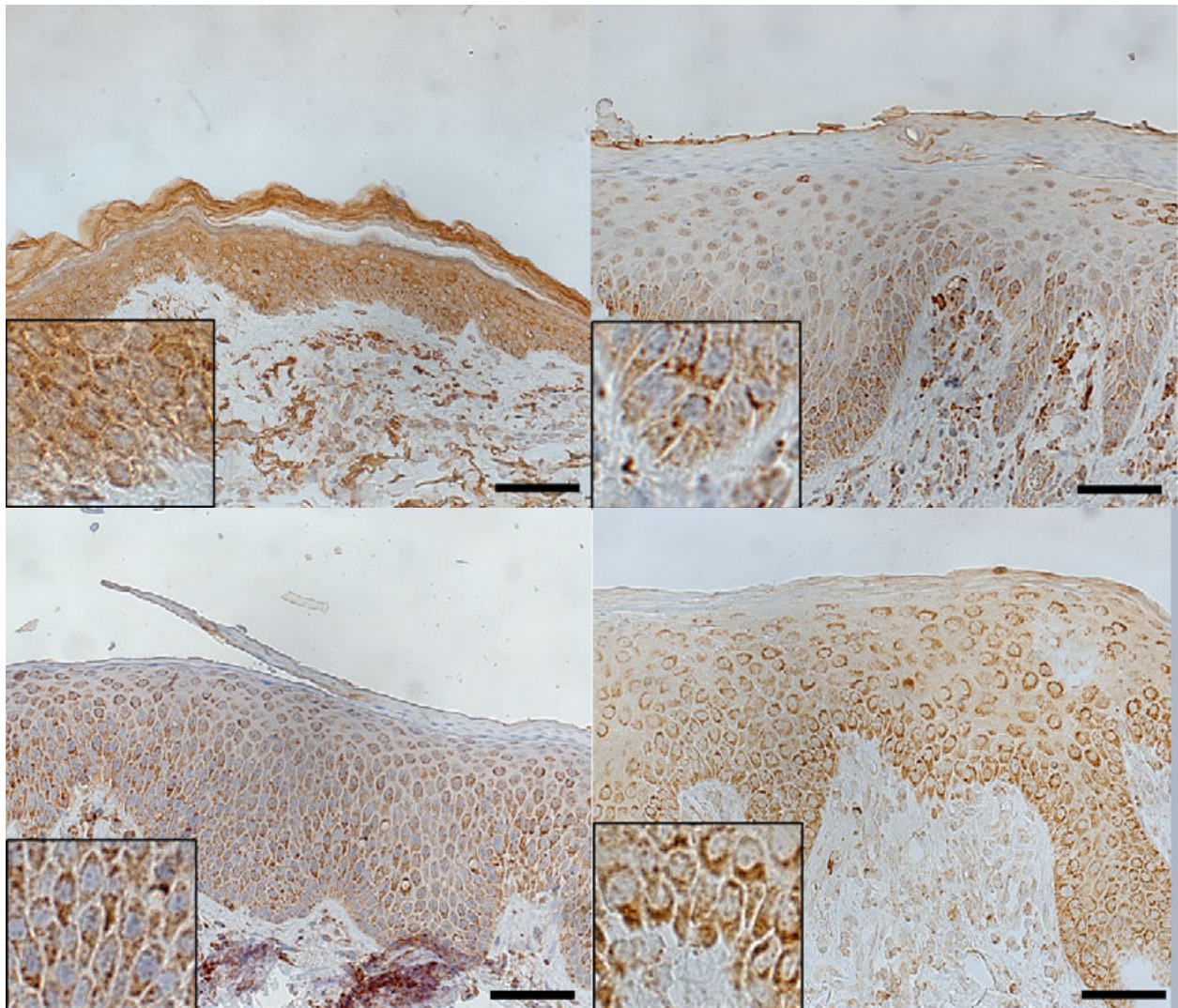


Figure 1. DAB staining for human TLR2 was performed in normal human skin of healthy volunteers (top left), atopic dermatitis (top right) contact dermatitis (bottom left) and psoriasis (bottom right). TLR2 protein was constitutively and uniformly expressed in all nucleated epidermal layers in control skin. In atopic dermatitis, in contact dermatitis and in psoriasis staining was faint in the granular and upper spinous layers and increased towards lower spinous and basal layers. (Scale bar equals 20 μ m)

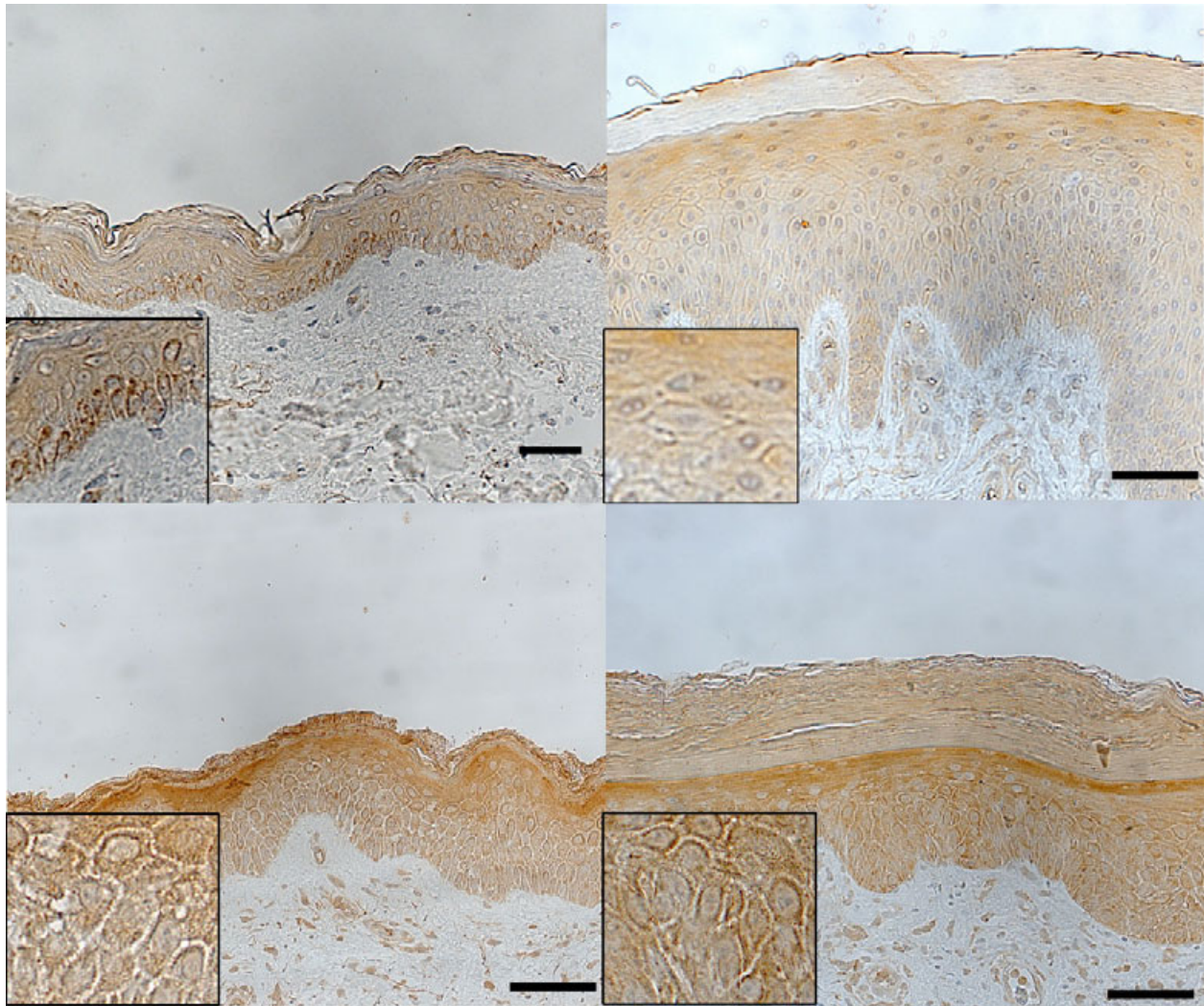


Figure 2. DAB staining for human TLR4 was performed in normal human skin (top left), atopic dermatitis (top right) contact dermatitis (bottom left) and psoriasis (bottom right). TLR4 was expressed in normal skin constitutively in the entire nucleated epidermis, but concentrated in the upper tip of the basal layer. In atopic dermatitis, in contact dermatitis and in psoriasis TLR4 staining was low in the basal and the lower and mid spinous layers and increased towards the upper spinous and the granular layers (Scale bar equals 20 μ m).