Journal of Clinical & Experimental Dermatology Research

Canavese et al. J Clin Exp Dermatol Res 2011, S:2
DOI: 10.4172/2155-9554.S2-002

Research Article Open Access

# VEGF Induces IL-23 Expression in Keratinocytes through p38 Signaling

**Miriam Canavese[1,2*], Mark Peric[1], Yvonne Dombrowski[1], Sarah Koglin[1], Thomas Ruzicka[1] and Jürgen Schauber[1]**

[1]*Department of Dermatology and Allergy, Ludwig-Maximilian-University, Munich, Germany*
[2]*Harvard Medical School & MGH Cancer Center, 185 Cambridge Street, Simches Building, Boston, MA, USA*

## Abstract

**Background:** Psoriasis is a chronic inflammatory skin disorder which is associated with increased cutaneous vascular endothelial growth factor (VEGF) expression. Several studies demonstrate that VEGF plays an important role in psoriasis pathogenesis by linking angiogenesis and inflammation. We aimed to study the molecular functions of VEGF in psoriasis and dissect a link between VEGF and pro-inflammatory cytokine expression in human keratinocytes.

**Methods**: VEGF expression was evaluated in sections from lesional psoriatic skin by immunohistochemistry. Cultured keratinocytes were transfected with a VEGF expression plasmid and changes in pro-inflammatory cytokine expression were investigated by qPCR, ELISA and dot-blot. Protein phosphorylation profiles in lysates from cells over-expressing VEGF were analysed to identify the underlying signaling pathways. Specific inhibitors or siRNA knockdown were used in confirmatory experiments.

**Results:** In lesional psoriatic skin, VEGF and the pro-inflammatory cytokine IL-23 are both strongly expressed by epidermal keratinocytes. VEGF over-expression in cultured keratinocytes resulted in increased IL-23 and IL-6 mRNA transcript abundance and protein expression. At the same time VEGF over-expression strongly increased phosphorylation of p38 MAPK, CREB and HSp27. Inhibition of p38 MAPK by SB203580 blocked VEGF induced IL-23 expression while siRNA mediated knockdown of CREB or HSp27 showed no effect.

**Conclusions**: VEGF up-regulates pro-inflammatory IL-23 and IL-6 secretion through p38 MAPK in epidermal keratinocytes in psoriasis. Targeting VEGF and/or p38 MAPK could lead to novel anti-inflammatory treatments for this chronic skin disease.

**Keywords:** Psoriasis; Vascular endothelial growth factor; p38 MAPK; Interleukin 23

**Abbreviations:** VEGF: Vascular Endothelial Growth Factor; NHEK: Normal Human Epidermal Keratinocytes; IL-23: Interleukin 23; IL-6: Interleukin 6; MAPK: Mitogen-Activated Protein Kinase; PBGD: Porphobilinogen Deaminase

## Introduction

Psoriasis is a chronic inflammatory skin disease which is driven and maintained by multiple components of the immune system [1]. While most recent studies highlight the role of Th17 cells in psoriasis pathogenesis there is also evidence that in the course of the disease disturbed angiogenesis and skin inflammation are closely linked [2]. Alterations of the cutaneous vasculature and microcirculation such as increased permeability and dilatation of dermal capillaries are among the earliest detectable histological features during the development of psoriatic plaques [3].

From this perspective, vascular endothelial growth factor (VEGF) might play an important role in the pathophysiology of psoriasis as VEGF influences vascular permeability and mediates pro-inflammatory activity by inducing vascular leakage [4,5]. Already, in non-involved, non-lesional skin significant over-expression of several VEGF isoforms was observed in psoriasis patients as compared with healthy skin of volunteers [6]. Among resident cells in skin keratinocytes are a major source of VEGF and in psoriatic plaques epidermal VEGF expression is strongly increased [3].

A major role of VEGF in the pathogenesis of psoriasis was further corroborated by the phenotype of transgenic mice with epidermis-specific over-expression of VEGF. These mice showed enhanced skin vascularity and capillary permeability and the characteristic Koebner phenomenon with induction of chronic psoriasis-like lesions by unspecific skin irritation [3,7,8]. In addition, in a different psoriasis model, anti-VEGF treatment of mice, already displaying disease symptoms, resulted in an overall improvement of the psoriatic lesions [9]. Moreover, some patients with psoriasis receiving anti-VEGF treatment for cancer showed complete remission of their cutaneous symptoms [10]. Taken together, these findings indicate that VEGF may play an important role in the pathogenesis of psoriasis. However, the exact mechanisms of VEGF induced inflammation in psoriasis are unknown.

## Materials and Methods

### Study design

To study the pro-inflammatory function of VEGF in psoriasis, the expression levels of VEGF and IL-23p19 in human psoriatic skin biopsies were analysed by immunohistochemistry. Subsequently, primary human keratinocytes (NHEK) were transfected with a VEGF expression vector. Pro-inflammatory cytokines in primary keratinocyte over-expressing VEGF were profiled by qPCR, ELISA and dot-blot. Analyses of protein phosphorylation profiles in lysates from cells over-expressing VEGF were performed to identify the underlying signaling pathways which were then confirmed by siRNA knockdown.

***Corresponding author:** Miriam Canavese, Harvard Medical School & MGH Cancer Center, 185 Cambridge Street, Simches Building, Boston, MA 02114, USA, Tel: 6177244955; Fax: 617 724 2662; E-mail: miriamcanavese@yahoo.com

**Received** October 07, 2011; **Accepted** November 01, 2011; **Published** November 06, 2011

**Citation:** Canavese M, Peric M, Dombrowski Y, Koglin S, Ruzicka T, et al. (2011) VEGF Induces IL-23 Expression in Keratinocytes through p38 Signaling. J Clin Exp Dermatol Res S2:002. doi:10.4172/2155-9554.S2-002

### Patient samples

Skin biopsies were taken from patients attending our psoriasis clinic. 4mm punch biopsies were taken from untreated lesional psoriatic skin. Skin biopsies from healthy (non psoriatic) volunteers served as controls. Sample acquisition was approved by the committee on investigations involving human subjects at the Faculty of Medicine, Ludwig-Maximilian-University, Munich. For all procedures informed written consent was obtained.

### Immunohistochemistry

Paraffin sections from human psoriatic lesions or healthy skin were incubated overnight with either a polyclonal VEGF antibody (Santa Cruz) or anti-human IL-23p19 (BioLegend) followed by incubation with a HRP-conjugated anti-rabbit antibody (Dako). Staining was visualized by DAB or the REAL™ Detection System (Dako). Sections were analysed with a TissueFAXS System (TissueGnostics).

### Cell culture and transfection protocol

Normal human epidermal keratinocytes (NHEK) were grown in EpiLife culture medium (Cascade Biologics) under standard tissue culture conditions. NHEK were transfected with a VEGF expression vector (Origene) or a control plasmid using FuGene HD Transfection Reagent (Roche) and Opti-MEM I (Gibco). The transfection complex was incubated at room temperature for 20 minutes. 100µL of transfection complex were then added to cells maintained in 900µL of fresh EpiLife medium free of growth supplements and antibiotics. After 24h, culture supernatants were collected and analysed for VEGF and IL-6 by ELISA (PeproTech).

### mRNA transcript quantification

Total RNA was extracted using Trizol (Invitrogen) and reverse transcribed using the DyNAmo cDNA Synthesis Kit (Finnzymes). mRNA transcript abundance was measured using a LightCycler 2.0 system and the Universal Probe Library System (Roche). Porphobilinogen deaminase (PBGD) was used as housekeeping gene in a duplex qPCR reaction. Induction relative to the vehicle treated control was calculated using the comparative Ct method (ΔΔCt).

### Dot blot

Cell lysates from NHEK transfected with VEGF expression vector or control plasmid were spotted onto an Invitrolon™ PVDF membrane (Invitrogen). Membranes were stained with an antibody detecting IL-23p19 (BioLegend) and re-probed with a HRP-conjugated secondary antibody (Dako). Stained protein was visualized using the Amersham ECL Plus (GE Healthcare).

### Proteome profiler array

Cell lysates from NHEK transfected with VEGF expression vector or control plasmid were analysed using the Proteome Profiler™ Human Phospho-Kinase Array Kit (R&D System).

### RNA interference

NHEK were transfected with siRNA oligonucleotides against CREB1 or HSPB1 or a non-targeted control using Lipofectamine™ RNAiMAX Reagent (Invitrogen) 6h after transfection with VEGF expression vector or control plasmid. Cells were maintained at 37°C under standard tissue culture conditions for 24 h prior to further analysis.

### Statistical analysis

All statistical analyses were performed using GraphPad Prism 4.0 (GraphPad Software Inc.). Analysis of variance (ANOVA) followed by Dunnett or Bonferroni´s post-tests was used to calculate statistical differences. Values of $P < 0.05$ were considered significant and all data are displayed as means ± SD of results from a single representative experiment performed in duplicate and/or triplicates. Each experiment was repeated at least twice to confirm reproducibility.

## Results

In lesional psoriatic skin, VEGF and the pro-inflammatory cytokine IL-23 are both strongly expressed [11]. IL-23 is a key cytokine in psoriasis pathogenesis and mediator of Th17 cell-mediated skin inflammation [12]. When we analysed VEGF and IL-23p19 expression by immunohistochemistry strong induction of both proteins was observed in keratinocytes within psoriatic plaques (Figure 1a).

In order to investigate a possible link between VEGF and IL-23 signaling in keratinocytes, NHEK were then transfected with a VEGF expression plasmid in vitro. VEGF over-expression in keratinocytes resulted in increased secretion of VEGF (Supplementary Figure 1). At the same time, strongly increased IL-23 and IL-6 mRNA transcript abundance could be observed in these cells (Figure 1b). Dot blot and ELISA analyses further confirmed increased IL-23 p19 and IL-6 protein expression in VEGF overexpressing keratinocytes (Figure 1c).

To dissect a possible link between VEGF and IL-23 pathways, protein phosphorylation profiles in NHEK over-expressing VEGF were subsequently investigated. Among all pathways tested VEGF overexpression in human keratinocytes strongly increased phosphorylation of p38 MAPK, CREB and Hsp27 (Supplementary Figure 2).

Based on these data, keratinocytes over-expressing VEGF were treated with the p38 MAPK inhibitor SB203580 and pro-inflammatory cytokine production was analysed. As shown in (Figure 2a), p38 MAPK inhibition significantly down-regulated IL-23 and IL-6 mRNA abundance and peptide secretion in VEGF over-expressing NHEK. Noteworthy, VEGF secretion was not reduced by p38 MAPK inhibition (data not shown).

In order to investigate the role of CREB1 and Hsp27 signaling in IL-6 and IL-23 production, specific siRNAs were designed and used to transfect NHEK over-expressing VEGF. Both siRNAs down-regulated CREB1 and Hsp27 expression while VEGF secretion was not affected (data not shown). However, in contrast to p38 MAPK inhibition IL-23 or IL-6 expression was not affected by CREB1 or Hsp27 knockdown (Figure 2b).

## Discussion

Our observations suggest that VEGF up-regulates IL-23 and IL-6 expression in epidermal keratinocytes in psoriasis thereby linking cutaneous angiogenesis and T-cell mediated immune responses. As a mechanism, VEGF over-expression triggers pro-inflammatory cytokine release in keratinocytes through the p38 MAPK signaling pathway.

It is well established that in lesional psoriatic skin the activity of the p38 MAPKs is increased suggesting that p38 MAPK signalling may play a role in the pathogenesis of psoriasis [13,14]. Moreover, Johansen and colleagues demonstrated that anti-inflammatory therapy in psoriasis is associated with a reduction in p38 MAPK phosphorylation and a

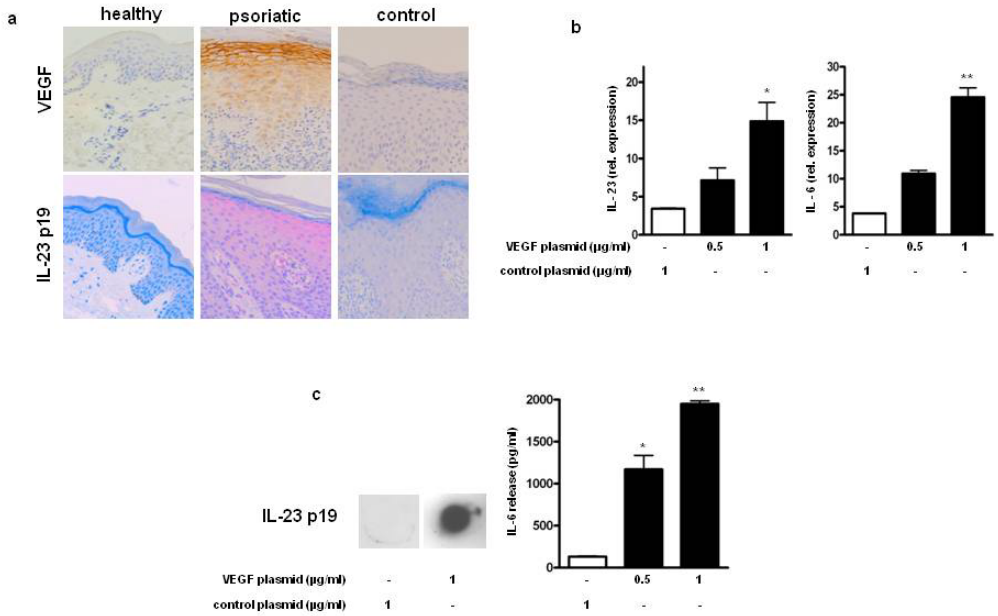


**Figure 1: Human keratinocytes over-expressing VEGF increase IL-23 and IL-6.** Immunohistochemical analyses demonstrate increased VEGF and IL23 (IL-23p19) protein expression in epidermal keratinocytes in lesional psoriatic skin in vivo **(a)**. In vitro, primary human keratinocytes (NHEK) transfected with a VEGF expression plasmid show increased IL-23 and IL-6 mRNA abundance compared to NHEK transfected with a control plasmid **(b)**. Data are means ± SD of a single experiment performed in triplicate and are representative of two to three independent experiments. In **(c)** induction of IL-23p19 protein in VEGF over-expressing NHEK was confirmed by dot blot. Increased release of IL-6 into cell culture supernatants could be demonstrated by ELISA.

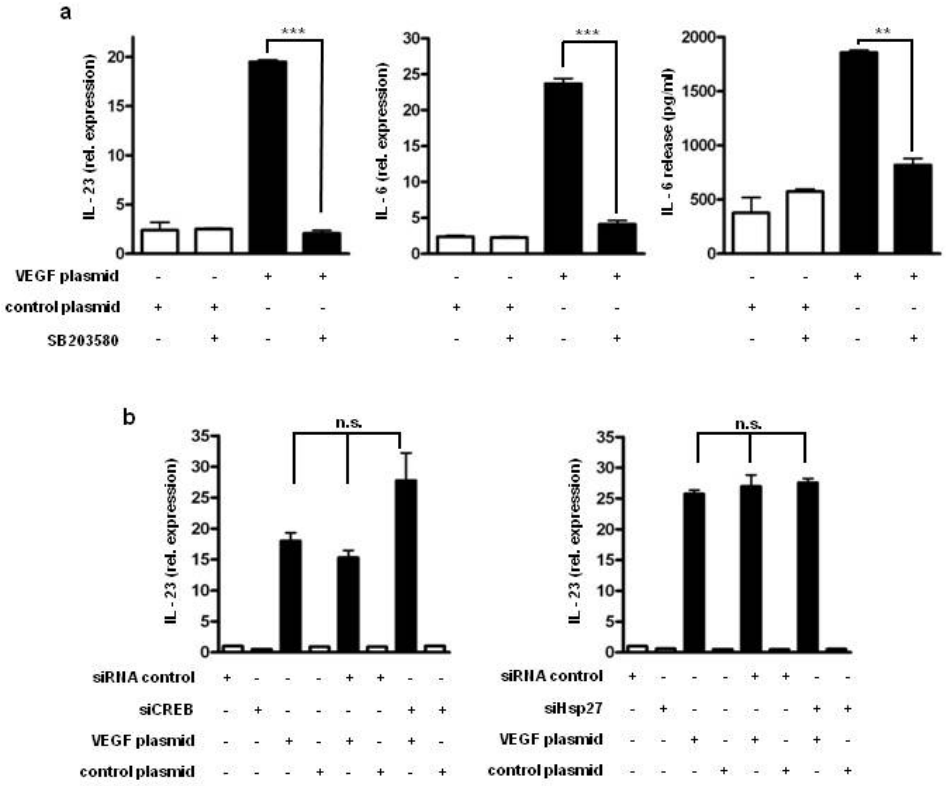


**Figure 2: VEGF increases IL-23 and IL-6 through p38 MAPK signaling in primary human keratinocytes.** Primary human keratinocytes (NHEK) were transfected with a VEGF plasmid or a control plasmid at 1 µg/ml and treated with the specific p38 MAPK inhibitor, SB203580 (1µM), for 24h. p38 MAPK inhibition blocked IL-23 and IL-6 mRNA expression in VEGF over-expressing NHEK (as measured by qPCR) **(a)**. Inhibition of CREB1 or Hsp27 by specific siRNA had no effect on VEGF induced IL-23 and IL-6 transcript abundance (as measured by qPCR) **(b)**. Data are means ± SD of a single experiment performed in triplicate and are representative of three independent experiments.

subsequent decrease in the expression of p38 MAPK regulated genes [15]. Consequently, p38 MAPK was suggested as possible target for novel therapies for psoriasis. Indeed, specific inhibitors of p38 MAPK block the secretion of cytokines such as IL-6 from human keratinocytes [16].

In our study, p38 MAPK inhibition in VEGF over-expressing keratinocytes blocked VEGF induced IL-6 but also IL-23 expression. IL-23 is a key cytokine in psoriasis pathogenesis and responsible for subsequent Th17 cell responses. This study is the first to suggest that VEGF is involved in this pro-inflammatory cascade in psoriasis through p38 MAPK. Thus, blocking protein kinases could be a possible novel approach to psoriasis treatment that warrants further research. Furthermore, it will be interesting to investigate the factors responsible for VEGF induction in psoriatic skin. As another novel treatment option, inhibition of VEGF expression or activity could lead to amelioration of cutaneous inflammation. As VEGF is already over-expressed in developing psoriatic lesions an early anti-VEGF intervention might represent the most promising strategy.

## Acknowledgments

MC is supported by a postdoctoral fellowship from the Fritz Thyssen Stiftung.

JS has received grants from the Deutsche Forschungsgemeinschaft (Emmy Noether Programm; Scha 979/3-1; www.dfg.de) and the Fritz Thyssen Stiftung (www.fritz-thyssen-stiftung.de).

## References

1. Nestle FO, Kaplan DH, Barker J (2009) Psoriasis. N Engl J Med 361: 496-509.
2. Heidenreich R, Rocken M, Ghoreschi K (2009) Angiogenesis drives psoriasis pathogenesis. Int J Exp Pathol 90: 232-248.
3. Detmar M, Brown LF, Claffey KP, Yeo KT, Kocher O (1994) Overexpression of vascular permeability factor/vascular endothelial growth factor and its receptors in psoriasis. J Exp Med 180: 1141-1146.
4. Dvorak HF, Brown LF, Detmar M, Dvorak AM (1995) Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 146: 1029-1039.
5. Ferrara N, Gerber HP, LeCouter J (2003) The biology of VEGF and its receptors. Nat Med 9: 669-676.
6. Henno A, Blacher S, Lambert C, Colige A, Seidel L (2009) Altered expression of angiogenesis and lymphangiogenesis markers in the uninvolved skin of plaque-type psoriasis. Br J Dermatol 160: 581-590.
7. Canavese M, Altruda F, Silengo L, Castiglioni V, Scanziani E (2011) Clinical, pathological and immunological features of psoriatic-like lesions affecting keratin 14-vascular endothelial growth factor transgenic mice. Histol Histopathol 26: 285-296.
8. Xia YP, Li B, Hylton D, Detmar M, Yancopoulos GD (2003) Transgenic delivery of VEGF to mouse skin leads to an inflammatory condition resembling human psoriasis. Blood 102: 161-168.
9. Schonthaler HB, Huggenberger R, Wculek SK, Detmar M, Wagner EF (2009) Systemic anti-VEGF treatment strongly reduces skin inflammation in a mouse model of psoriasis. Proc Natl Acad Sci U S A 106: 21264-21269.
10. Akman A, Yilmaz E, Mutlu H, Ozdogan M (2009) Complete remission of psoriasis following bevacizumab therapy for colon cancer. Clin Exp Dermatol 34: e202-204.
11. Piskin G, Sylva-Steenland RM, Bos JD, Teunissen MB (2006) In vitro and in situ expression of IL-23 by keratinocytes in healthy skin and psoriasis lesions: enhanced expression in psoriatic skin. J Immunol 176: 1908-1915.
12. Lee E, Trepicchio WL, Oestreicher JL, Pittman D, Wang F (2004) Increased expression of interleukin 23 p19 and p40 in lesional skin of patients with psoriasis vulgaris. J Exp Med 199: 125-130.
13. Funding AT, Johansen C, Kragballe K, Iversen L (2007) Mitogen- and stress-activated protein kinase 2 and cyclic AMP response element binding protein are activated in lesional psoriatic epidermis. J Invest Dermatol 127: 2012-2019.
14. Arthur JS, Darragh J (2006) Signaling downstream of p38 in psoriasis. J Invest Dermatol 126: 1689-1691.
15. Johansen C, Vinter H, Soegaard-Madsen L, Olsen LR, Steiniche T (2010) Preferential inhibition of the mRNA expression of p38 mitogen-activated protein kinase regulated cytokines in psoriatic skin by anti-TNFalpha therapy. Br J Dermatol 163: 1194-1204.
16. Takeichi T, Sugiura K, Muro Y, Matsumoto K, Ogawa Y (2010) Overexpression of LEDGF/DFS70 induces IL-6 via p*38* activation in HaCaT cells, similar to that seen in the psoriatic condition. J Invest Dermatol 130: 2760-2767.

This article was originally published in a special issue, **Skin & Immune System** handled by Editor(s). Dr. Adriana T Larregina, University of Pittsburgh, USA