

The Production IL-21 and VEGF in UVB-irradiated Human Keratinocyte Cell Line, HaCaT

Hyemin Kim, Jae Seung Kang and Wang Jae Lee*

Department of Anatomy and Tumor Immunity Medical Research Center, Seoul National University College of Medicine, Seoul 110-799, Korea

Background: Ultraviolet B (UVB) induces multiple inflammatory and carcinogenic reactions. In skin, UVB induces to secrete several kinds of inflammatory cytokines from keratinocytes and also increases angiogenic process via the modulation of vascular endothelial growth factor (VEGF) production. Interleukin-21 (IL-21) is an inflammatory cytokine and produced by activated T cells. The biologic functions of IL-21 have not yet extensively studied. **Methods:** In the present study, we investigate the production of IL-21 from human keratinocyte cell line, HaCaT and its biological effect after exposure to UVB. **Results:** First, we confirmed the IL-21 production and its receptor expression in HaCaT. And then, the change of IL-21 and VEGF production in HaCaT by UVB irradiation was examined. Not only IL-21 but also VEGF production was enhanced by UVB irradiation. Next, to determine relationship of enhanced production of IL-21 and VEGF, we detected VEGF production after neutralization of IL-21. VEGF production was reduced by IL-21 neutralization, which indicates that the IL-21 is involved in the VEGF production. **Conclusion:** Taken together, our results suggest that IL-21 and VEGF production is enhanced by UVB irradiation in HaCaT. In addition, it seems that IL-21 plays a role in the angiogenic process in skin via the modulation of VEGF production.

[Immune Network 2010;10(2):75-80]

INTRODUCTION

Interleukin (IL)-21 is a member of the type I cytokine family with significant sequence homology to IL-2, IL-4, and, in par-

tical, IL-15 (1-3). IL-21 is also the most recently described member of the common γ -chain cytokine family, and found to be a potent immunomodulatory cytokine (4). IL-21 receptor (IL-21R) has been reported to associate with the common γ chain, a property it shares with the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15. The intracellular signaling pathway of IL-21R involves Janus kinase 1 (Jak1), Jak3, signal transducer and activator of transcription 1 (Stat1), Stat3, Stat5a and stat 5b (5-7). IL-21 was first noted to be produced by activated CD4+ T cells, whereas expression of its receptor was somewhat broader (8,9). IL-21 serves a critical role for immunoglobulin production and terminal B cell differentiation, acts as a T cell comitogen and can drive the expansion of CD8+ T cells, can negatively regulate dendritic cell function and plays an essential role in the differentiation of Th17 cells (10). Also, IL-21 is implicated in the pathogenesis of autoimmunity (11,12) and exhibits potent actions as an antitumor agent (13).

The process of new blood vessel formation or angiogenesis is essential for tumor growth and wound healing as it is critical for supply of oxygen and nutrients. Especially, vascular endothelial growth factor (VEGF) has an essential role in angiogenesis. VEGF is thought to play an important role in the increased vascular permeability and angiogenesis associated with tumor malignancies. It augments neovascularization and tumor growth in cells transfected with the VEGF gene versus control cells (14). Overexpression of VEGF and both of its receptors has been documented in a number of animal and human tumors (15). VEGF is also overexpressed

Received on March 23, 2010. Revised on April 8, 2010. Accepted on April 13, 2010.

© This is an open access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Corresponding Author. Tel: 82-2-740-8208; Fax: 82-2-745-9528; E-mail: kinglee@snu.ac.kr

Keywords: HaCaT, UVB, IL-21, VEGF

by epidermal keratinocytes in certain non-neoplastic processes of the skin which are characterized by increased microvascular permeability and angiogenesis, e.g. cutaneous wound healing (16) and psoriasis (17). Recently, up-regulation of VEGF was also found in bullous pemphigoid, erythema multiforme, and dermatitis herpetiformis (18), which are all associated with hyperpermeable dermal microvessels. In addition, members of the type I cytokine family such as IL-2 and IL-4, which is analogy to IL-21, are suggested to affect the formation of new blood vessels (19-21).

Ultraviolet B (UVB) induces multiple inflammatory and carcinogenic reactions like angiogenesis. In skin or human keratinocyte cells, UVB induces to secrete inflammatory cytokines such as IL-1 α , IL-6 and IL-8 (22,23). In addition, cutaneous angiogenesis is enhanced by exposure to UVB irradiation (24) and UVB-induced skin angiogenesis is mediated by upregulating of VEGF (25). In present study, we examined the production and regulation of IL-21 and VEGF by UVB irradiation in a human keratinocyte cell line, HaCaT.

MATERIALS AND METHODS

Cells and UVB irradiation

The human keratinocyte cell line, HaCaT was obtained from American Type Culture Collection (Manassas, VA, USA). Cells were cultured in RPMI 1640 supplemented with 2 mM L-glutamine, 100 units/ml penicillin, 100 μ g/ml streptomycin, and 10% heat-inactivated fetal bovine serum. This cell line in the log phase of growth was used for experiments. Cells were exposed to UVB irradiation (290~320 nm) using bank of lamps (Waldmann, Schweningen, Germany). The irradiance of the lamps was measured with a photometer (Waldmann, Schweningen, Germany).

RT-PCR

Total RNA was isolated from 5×10^6 cultured cells using easy BLUETM, and cDNA was made using Power cDNA synthesis kit. Then, cDNA was amplified with IL-21 primers (5'-GAG ATC CAG TCC TGG CAA-3', 5'-GCA AGT TAG ATC CTC AGG AA-3'; product=480 bp). Cycling conditions for IL-21 were 1 min at 95°C, 1 min at 56°C, and 1 min at 72°C for 40 cycles. PCR products were separated on 1.2% agarose gel, stained with ethidium bromide and visualized under UV light.

Flow cytometry

Surface flow cytometry was used to detect the expression of

IL-21 receptor (IL-21R). HaCaT cells were collected, washed twice with 0.15 M phosphate-buffered saline (PBS), and incubated with anti-human IL-21R monoclonal antibody (R&D system, Minneapolis, MN, USA). And then cells were incubated with FITC-conjugated anti-mouse IgG (Sigma, St. Louis, MO, USA), and analyzed with flow cytometer. Intracellular flow cytometry was performed to detect the production of IL-21. HaCaT cells were collected after UVB irradiation, washed twice with 0.15 M PBS. After fixing with fixation buffer (2% paraformaldehyde in PBS) for 20 min on ice, cells were permeabilized with permeabilization buffer (0.1% saponin in PBS) for 30 min on ice. Then, cells incubated with FITC-conjugated anti-goat IgG (Sigma, St. Louis, MO, USA) for 30 min on ice, washed twice with permeabilization buffer, and analyzed with flow cytometer. Flow cytometric data was obtained using an Epics ALTRA (Beckman coulter, Brea, CA, USA) after processed by the Expo32 program (Beckman coulter, Brea, CA, USA).

ELISA

Culture supernatants of HaCaT cells were collected after UVB irradiation. The VEGF concentration in the culture supernatants was measured by ELISA (R&D). VEGF ELISA was performed according to the manufacturer's instructions.

RESULTS

Expression of IL-21 and its receptors on the surface of HaCaT cells

It is reported that the interaction between keratinocytes and inflammatory cells are one of the important factors on the pathogenesis of Psoriasis, chronic inflammatory disease of the skin (26). In addition, IL-21 receptor is expressed on a variety of immune cells as well as non-immune cells (27). Therefore, we investigated the expression of IL-21 and its receptor on human keratinocyte cell line, HaCaT by flow cytometry analysis. As shown in Fig. 1, we found the spontaneous IL-21 production and IL-21R expression in HaCaT.

The enhancement of IL-21 production from HaCaT by UVB irradiation

There are several reports regarding the pivotal role of IL-21 in chronic inflammatory diseases, including rheumatoid arthritis, inflammatory bowel disease, and psoriasis. Therefore we investigated whether IL-21 is produced in skin under inflammatory condition by UVB, which can lead inflammatory

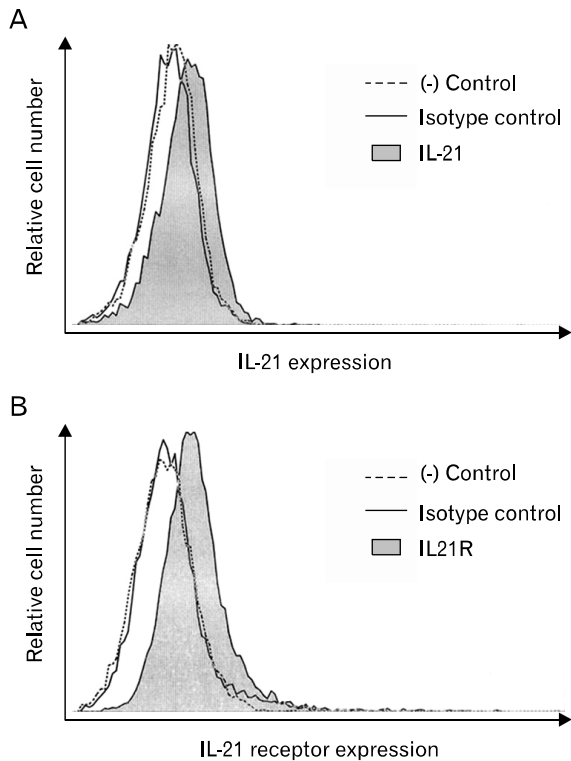


Figure 1. IL-21 production and its receptor expression in human keratinocyte cell line, HaCaT. (A) HaCaT cells (1×10^6) were harvested, intracellular IL-21 was measured by flow cytometry as described in *Materials and Methods*. (B) HaCaT cells (1×10^6) were harvested, the surface expression of IL-21 receptor was examined by flow cytometry as described in *Materials and Methods*.

responses and production of various inflammatory cytokines and chemokines (28). To determine the optimal dose of UVB, the changes of IL-21 mRNA expression were examined after cells were exposure to various ranges of UVB from 100 to 300 J/m^2 . IL-21 mRNA expression was increased in a dose-dependent manner of UVB. Since IL-21 mRNA expression was peaked under 150 J/m^2 of UVB, we investigated IL-21 production at proteins level by intracellular flow cytometry analysis. As shown in Fig. 2B, IL-21 production was increased by UVB irradiation.

The secretion of VEGF was enhance by UVB in HaCaT cells

Angiogenic process is an essential step for the regulation of inflammatory responses. We already reported IL-18 production is up-regulated from HaCaT by UVB irradiation. In addition, IL-18 is a reported as one of the angiogenic factors. Therefore, we investigated whether VEGF expression is regu-

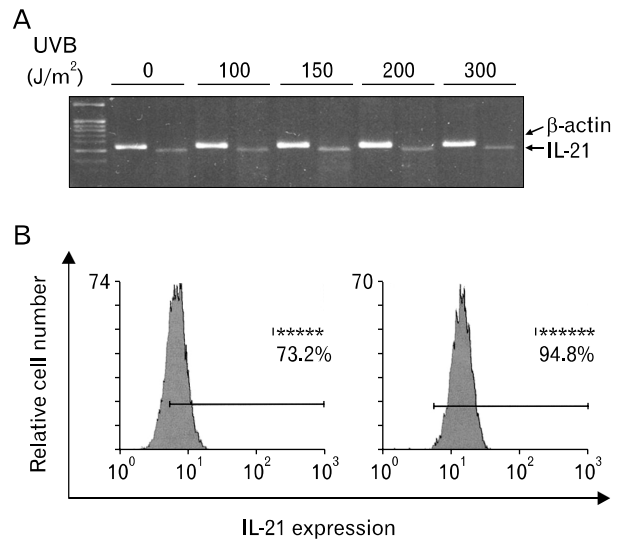


Figure 2. IL-21 production from HaCaT cells by UVB irradiation. (A) HaCaT cells (7×10^6) were exposed to 0, 100, 150, 200, 300 J/m^2 of UVB and incubated for 6 hrs. After, total RNA was isolated and cDNA was synthesized, RT-PCR for IL-21 was performed. β -actin was used as a loading control. (B) HaCaT cells (3×10^6) were exposed to 150 J/m^2 of UVB and incubated for overnight. After cells were harvested, IL-21 production was measured by intracellular flow cytometry.

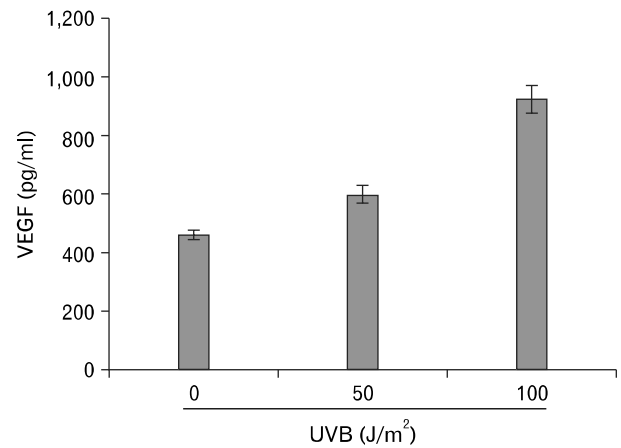


Figure 3. VEGF production from human keratinocyte cell line, HaCaT by UVB. HaCaT cells (3×10^6) were exposed to 50, 100 J/m^2 of UVB and incubated for overnight. Then, culture supernatants were collected, and VEGF concentration was measured by ELISA.

lated by UVB irradiation. As we expected, VEGF production is increased by UVB irradiation in a dose-dependent manner (Fig. 3).

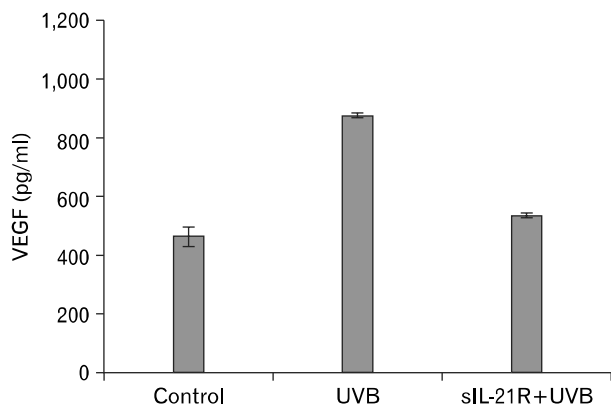


Figure 4. Inhibition of UVB-induced VEGF production by the treatment of soluble IL-21 receptor. HaCaT cells (3×10^6) were pre-treated with sIL-21R for 2 hrs before exposure to 100 J/m^2 of UVB. After UVB irradiation, cells were incubated for overnight. And then, culture supernatants were collected, and the concentration of VEGF was measured by ELISA.

IL-21 production was involved in the VEGF production

As we have already shown that IL-21 production and its receptor expression and VEGF production is increased by UVB irradiation, we further investigated the relationship between IL-21 and VEGF production by UVB irradiation. When the autocrine effect of IL-21 on UVB-irradiated HaCaT was blocked by soluble IL-21 receptor, we found complete inhibition of UVB-induced VEGF production (Fig. 4).

DISCUSSION

In the present study, we investigated the production of IL-21 and VEGF in a human keratinocyte cell line, HaCaT by UVB irradiation. And IL-21 is involved in the secretion of VEGF by UVB exposure. Interleukin (IL)-21 is a pleiotropic cytokine with immune-regulatory properties and immune-therapeutic capacity. A recent study demonstrated that IL-21 receptor is present on normal and tumor endothelial cells and can mediate angiostatic properties (21). Although several inflammatory cytokines such as $\text{TNF-}\alpha$, IL-6 and IL-8 are secreted by UVB (28), there was no report of the production of IL-21 by UVB irradiation. However, we showed the exposure of UVB to human keratinocyte cell line, HaCaT induced significant production of IL-21 and increased the surface expression of IL-21 receptors. Nurieva *et al.* represented that IL-21 acts as an autocrine cytokine and was involved in the generation of inflammatory T cells (29). Thus, increased expression of both

intracellular IL-21 and its surface receptors by exposure to UVB on HaCaT cells (Fig. 1 and 2) is suspected to expand the activity of IL-21.

Vascular endothelial growth factor (VEGF) stimulates the growth of new blood vessels, so it is involved in not only tumor growth and metastasis but also wound healing. VEGF elicits a strong angiogenic response in a variety of in vivo models. VEGF participates in the angiogenic responses by increasing microvascular permeability. Also, VEGF stimulate several endothelial cell responses in cell culture including proliferation, migration, survival, and secretion of matrix-degrading enzymes (14). Acute ultraviolet B (UVB) irradiation of the skin results in erythema, vasodilation, edema, and angiogenesis, which is associated with the expression of VEGF by epidermal keratinocytes (30). Sublethal and physiologically relevant doses of UVB increased VEGF mRNA and protein levels upon irradiation of quiescent keratinocytes. The UVB-induced overexpression of VEGF is dependent on de novo protein synthesis and occurs via release of soluble mediators, which subsequently turn on VEGF expression (31). It is unclear whether VEGF is required for the damage or repair process that occurs in the skin on UVB exposure. However, a study with VEGF-overexpressing transgenic mice reported that VEGF promotes the cutaneous damage that occurs after UVB exposure and the author mentioned VEGF signaling pathway might serve as a novel target for the prevention of UVB-induced photodamage (30). Our result also indicated the elevated secretion of VEGF by UVB irradiation, but the effect of produced VEGF is considered to be further studied.

We also showed that the treatment of soluble IL-21R blocked the production of VEGF by UVB irradiation. It means that VEGF production is modulate by IL-21 and that IL-21 can function in similar to VEGF. The report that co-localization of IL-21R and VEGF was consistently seen in the skin of patients with skin sclerosis (27) is supporting our results. Recently, it is also reported that IL-21 receptor regulates wound healing and fibrosis by controlling Th2-mediated inflammation (32).

In conclusion, a human keratinocyte cell line, HaCaT expressed both IL-21 and IL-21 receptor, and the irradiation of UVB induced the increase of the IL-21 production. Also, the secretion of VEGF is elevated by exposure to UVB, and IL-21 was involved in the VEGF production. It suggests that IL-21 exhibits the angiogenetic activity by modulating VEGF when UVB is exposed to skin keratinocytes.

ACKNOWLEDGEMENTS

This study was supported by a grant of the Seoul National University Hospital (Grant #04-2007-0890).

CONFLICTS OF INTEREST

The author have no financial conflict of interest.

REFERENCES

1. Leonard WJ, Spolski R: Interleukin-21: a modulator of lymphoid proliferation, apoptosis and differentiation, *Nat Rev Immunol* 5:688-698, 2006
2. Mehta DS, Wurster AL, Grusby MJ: Biology of IL-21 and the IL-21 receptor, *Immunol Rev* 202:84-95, 2004
3. Vosshenrich CA, Di Santo JP: Cytokines: IL-21 joins the gamma (c)-dependent network? *Curr Biol* 11;R175-177, 2001
4. Brandt K, Singh PB, Bulfone-Paus S, Ruckert R: Interleukin-21: a new modulator of immunity, infection, and cancer, *Cytokine Growth Factor Rev* 18:223-232, 2007
5. Habib T, senadheera S, Winberg K, Kaushansky K: The common gamma chain (gamma c) is a required signaling component of the IL-21 receptor and supports IL-21-induced cell proliferation via JAK3, *Biochemistry* 41:8725-8731, 2002
6. Suto A, Wurster AL, Riner SL, Grusby MJ: IL-21 inhibits IFN-gamma production in developing Th1 cells through the repression of Eomesodermin expression, *J Immunol* 177:3721-3727, 2006
7. Zeng R, Spolski R, Casas E, Zhu W, Levy DE, Leonard WJ: The molecular basis of IL-21-mediated proliferation, *Blood* 109:4135-4142, 2007
8. Ozaki K, Kikly K, Michalovich D, Young PR, Leonard WJ: Cloning of a type I cytokine receptor most related to the IL-2 receptor beta chain, *Proc Natl Acad Sci U S A* 97:11439-11444, 2000
9. Parrish-Novak J, Dillon SR, Nelson A, Hammond A, Sprecher C, Gross JA, Johnston J, Madden K, Xu W, West J, Schrader S, Burkhead S, Heipel M, Brandt C, Kuijper JL, Kramer J, Conklin D, Presnell SR, Berry J, Shiota F, Bort S, Hambly K, Mudri S, Clegg C, Moore M, Grant FJ, Lofton-Day C, Gilbert T, Rayond F, Ching A, Yao L, Smith D, Webster P, Whitmore T, Maurer M, Kaushansky K, Holly RD, Foster D: Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function, *Nature* 408:57-63, 2000
10. Leonard WJ, Zeng R, Spolski R: Interleukin 21: a cytokine/cytokine receptor system that has come of age, *J Leukoc Biol* 84:348-356, 2008
11. Sawalha AH, Kaufman KM, Kelly JA, Adler AJ, Aberle T, Kilpatrick J, Wakeland EK, Li QZ, Wandstrat AE, Karp DR, James JA, Merrill JT, Lipsky P, Harley JB: Genetic association of interleukin-21 polymorphisms with systemic lupus erythematosus, *Ann Rheum Dis* 67:458-461, 2008
12. Young DA, Hegen M, Ma HL, Whitters MJ, Albert LM, Lowe L, Senices M, Wu PW, Sibley B, Leathurby Y, Brown TP, Nickerson-Nutter C, Keith JC Jr, Collins M: Blockade of the interleukin-21/interleukin-21 receptor pathway ameliorates disease in animal models of rheumatoid arthritis, *Arthritis Rheum* 56:1152-1163, 2007
13. Skak K, Kragh M, Hausman D, Smyth MJ, Sivakumar PV: Interleukin 21: combination strategies for cancer therapy, *Nat Rev Drug Discov* 7:231-240, 2008
14. Terman BI, Stoletov KV: VEGF and tumor angiogenesis, *Einstein Quart J Biol* 18:59-66, 2001
15. Folkman J: Angiogenesis in cancer, vascular, rheumatoid and other disease, *Nat Med* 1:27-31, 1995
16. Brown LF, Yeo KT, Berse B, Yeo TK, Senger DR, Dvorak HF, van de Water L: Expression of vascular permeability factor (vascular endothelial growth factor) by epidermal keratinocytes during wound healing, *J Exp Med* 176:1375-1379, 1992
17. Detmar M, Brown LF, Claffey KP, Yeo KT, Kocher O, Jackman RW, Berse B, Dvorak HF: Overexpression of vascular permeability factor/vascular endothelial growth factor and its receptors in psoriasis, *J Exp Med* 180:1141-1146, 1994
18. Brown LF, Harrist TJ, Yeo KT, Stähle-Bäckdahl M, Jackman RW, Berse B, Tognazzi K, Dvorak HF, Detmar M: Increased expression of vascular permeability factor (vascular endothelial growth factor) in bullous pemphigoid, dermatitis herpetiformis, and erythema multiforme, *J Invest Dermatol* 104:744-749, 1995
19. Sakkoula E, Pipili-Synetos E, Maragoudakis ME: Involvement of nitric oxide in the inhibition of angiogenesis by interleukin-2, *Br J Pharmacol* 122:793-795, 1997
20. Volpert OV, Fong T, Koch AE, Peterson JD, Waltenbaugh C, Tepper RI, Bouck NP: Inhibition of angiogenesis by interleukin 4, *J Exp Med* 188:1039-1046, 1998
21. Castermans K, Tabruyn SP, Zeng R, van Beijnum JR, Eppolito C, Leonard WJ, Shrikant PA, Griffioen AW: Angiostatic activity of the antitumor cytokine interleukin-21, *Blood* 112:4940-4947, 2008
22. Griswold DE, Tzimas MN: Ultraviolet B-induced inflammatory cytokine production, in vivo: initial pharmacological characterization, *Inflamm Res* 44(Suppl 2):S209-210, 1995
23. Kondo S, Kono T, Sauder DN, McKenzie RC: IL-8 gene expression and production in human keratinocytes and their modulation by UVB, *J Invest Dermatol* 101:690-694, 1993
24. Bielenberg DR, Bucana CD, Sanchez R, Donawho CK, Kripke ML, Fidler IJ: Molecular regulation of UVB-induced cutaneous angiogenesis, *J Invest Dermatol* 111:864-872, 1998
25. Yano K, Kadoya K, Kajiya K, Hong YK, Detmar M: Ultraviolet B irradiation of human skin induces an angiogenic switch that is mediated by upregulation of vascular endothelial growth factor and by downregulation of thrombospondin-1, *Br J Dermatol* 152:115-121, 2005
26. Nickoloff BJ, Qin JZ, Nestle FO: Immunopathogenesis of psoriasis, *Clin Rev Allergy Immunol* 33:45-56, 2007

27. Monteleone G, Pallone F, Macdonald TT: Interleukin-21 (IL-21)-mediated pathways in T cell-mediated disease. *Cytokine Growth Factor Rev* 2;185-191, 2009
28. Yoshizumi M, Nakamura T, Kato M, Ishioka T, Kozawa K, Wakamatsu K, Kimura H: Release of cytokines/chemokines and cell death in UVB-irradiated human keratinocytes, HaCaT. *Cell Biol Int* 32;1405-1411, 2008
29. Nurieva R, Yang XO, Martinez G, Zhang Y, Panopoulos AD, Ma L, Schluns K, Tian Q, Watowich SS, Jetten AM, Dong C: Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. *Nature* 448;480-483, 2007
30. Hirakawa S, Fujii S, Kajiya K, Yano K, Detmar M: Vascular endothelial growth factor promotes sensitivity to ultraviolet B-induced cutaneous photodamage. *Blood* 105;2392-2399, 2004
31. Brauchle M, Funk JO, Kind P, Werner S: Ultraviolet B and H₂O₂ are potent inducers of vascular endothelial growth factor expression in cultured keratinocytes. *J Biol Chem* 271;21793-21797, 1996
32. Pesce J, Kaviratne M, Ramalingam TR, Thompson RW, Urban JF Jr, Cheever AW, Young DA, Collins M, Grusby MJ, Wynn TA: The IL-21 receptor augments Th2 effector function and alternative macrophage activation. *J Clin Invest* 116;2044-2055, 2006