The Effect of Interleukin-21 on the Gene Expression of Psoriasis-associated Chemokines in Human Keratinocyte Cell Line, HaCaT

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Directed by Professor Min-Geol Lee

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Hyunjoong Jee

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This certifies that the Master's Thesis of
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The Graduate School
Yonsei University

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Hyunjoong Jee

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Psoriasis is a chronic inflammatory skin disease that affects approximately 1-3% of the population worldwide. In psoriasis, the stimulation of local innate immune response initiates inflammation, and in turn, initiates the adaptive immune response leading to the production of a panel of cytokines, chemokines and growth factors that eventually culminate in epidermal hyperplasia. Studies of recent have successfully linked the Interleukin (IL)-23/Th17 axis and keratinocytes, and have revealed that an increased expression of CCL20 from keratinocytes can recruit CCR6+ Th17 cells, and that the lesional cytokine milieu persistently activates keratinocytes to produce CCL20.

IL-21, recently, has been found to play an important role in differentiation and

function of T-cells. The production of IL-21 is mainly restricted to CD4+ T cells, Th17 and T-follicular helper (T_{FH}) cells. In association to psoriasis, IL-21 was reported to be overexpressed in psoriatic skin and causes epidermal hyperplasia and inflammation when injected intradermally into mice.

This study sought to clarify other effects of IL-21 in psoriasis, including its effect on the expression of psoriasis-related chemokines and antimicrobial peptides in keratinocytes, using microarray analysis and real time RT-PCR. Microarray analysis revealed statistically significant increase in the expression of CCL20 and CXCL8 upon keratinocyte stimulation with IL-21. Via real time quantitative PCR, we confirmed a significant increase in the expression of CCL20 and CXCL8 upon HaCaT stimulation with IL-21 – evidence supporting its role in the recruitment of Th17 cells and neutrophils to psoriatic skin. No change was observed in the expression of antimicrobial peptides upon IL-21 stimulation.

We hope this further elucidation of the role of IL-21 aids in the advent of yet another potential therapeutic target in psoriasis.

Key words : interleukin-21 (IL-21), chemokine (C-C motif) ligand 20 (CCL20), HaCaT, psoriasis

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I. INTRODUCTION

Psoriasis, affecting approximately 1-3% of the population, is a common chronic skin disease.[1] In psoriasis pathogenesis an over-reaction of local innate immune response initiates inflammation with subsequent involvement of adaptive immune response leading to the production of a panel of cytokines, chemokines and growth factors that induce epidermal hyperplasia.

In the last few years, the discovery of interleukin (IL)-23/Th17 axis in the pathophysiology of psoriatic diseases shifted the cytokine paradigm from Th1 to Th17 cytokines, focused mainly on IL-17 and IL-22.[2] T-helper 17 cells are distinct from Th1 and Th2 cells in their differentiation and maintenance conditions, as well as in their cytokine profiles. Th17 cells have also been shown to secrete other cytokines in addition to IL-17 and IL-22, including

IL-21 and tumor necrosis factor (TNF). A recent breakthrough in understanding the pathogenesis of psoriasis has been made with studies that successfully linked the IL-23/Th17 axis and keratinocytes.[3] Th17 and Th1 cells preferentially express the chemokine receptors CC chemokine receptor (CCR) 6 and CXC chemokine receptor (CXCR) 3, and are attracted by their ligands, CC chemokine ligand (CCL) 20 and CXC chemokine ligand (CXCL) 10, respectively. CCL20 and CXCL10 production by epidermal keratinocytes is enhanced in psoriatic lesions, which may induce the recruitment of Th17 and Th1 cells to these lesions.[4] CXCL8 (also known as IL-8), one of the common ligands for CXCR1 and CXCR2, has also been identified to be overexpressed in epidermal keratinocytes of the psoriatic skin and acts as an important chemoattractant for neutrophils, which may eventually cause an aggregation of neutrophils in the epidermis known as Munro abscesses.[5]

Recently, IL-21 has been found to play a pivotal role in differentiation and function of T-cells.[6] In particular, IL-21 drives an inflammatory T-cell response by triggering the production of IL-17, which is thought to be a crucial cytokine for inflammatory processes.[7] The novel class I cytokine IL-21 is a member of the common γ-chain receptor family, and its production is mainly restricted to CD4+ T cells, Th17 and T-follicular helper (T_{FH}) cells.[8, 9] Ligation of IL-21 to its receptor IL-21Rα promotes B cell dependent IgG production, enhances expansion of CD8+ cells and their cytotoxic capacity, and augments naive CD4+ T cell differentiation towards effector T-cells.[10]

Several recent reports of IL-21 and IL-21R in relation to the immortalized human keratinocyte cell line (HaCaT), the human keratinocytes, and the gut epithelial cells have revealed new roles for the novel cytokine and its receptor. IL-21 production and IL-21R expression was confirmed in HaCaT via flow cytometry, and IL-21R mRNA was shown to be 4.7-fold greater in the epidermis of biopsy specimens from patients of systemic sclerosis compared to healthy controls.[11, 12] Finally, in a recent study, IL-21 was shown to promote the synthesis of CCL20 by the gut epithelial cells in patients with inflammatory bowel disease.[13]

In regards to psoriasis, IL-21 was reported to be overexpressed in psoriatic skin and can cause epidermal hyperplasia and inflammation when injected intradermally into mice. Moreover blockade of IL-21 with a human antibody against IL-21 reduced the epidermal thickness and the expression of Th1 and Th17 genes in the well-characterized model of human psoriasis-xenograft mouse.[14 - 16] However, besides the induction of epidermal hyperplasia, other effects of IL-21 in regards to keratinocytes in psoriasis has not yet been fully elucidated.

The objective of this study was to investigate a new role of IL-21, expressed by T helper cells, including Th17 cells, in inducing the expression of certain psoriasis-related chemokines, such as CCL20, by the HaCaT cells. By doing so, we attempted to demonstrate its possible role in creating a vicious cycle between the human keratinocytes with Th17 cells culminating in a

pathogenesis that connects the innate and the adaptive immunity in this chronic inflammatory disease.

II. MATERIALS AND METHODS

1. Reagents

Recombinant human IL-21 was purchased from Peprotech (Peprotech, Rocky Hill, NJ, USA). Human recombinant TNF-α, IFN-γ, IL-17A, IL-1β, IL-22 was purchased from R&D Systems (R&D Systems, Minneapolis, MN, USA).

2. Cell culture

A. HaCaT cells

The HaCaT cells, human keratinocyte cell lines, were cultured in Isocove's Modified Dulbecco's Medium (IMDM) (Gibco, Carlsbad, CA, U.S.A.), supplemented with 10% fetal bovine serum (Gibco) and penicillin-streptomycin 100 IU-100 μg/mL (Gibco) grown on 100 cm^2 plate and incubated with 5% CO_2 at 37℃. Before performing cytokine stimulation experiments, the growth medium was changed for serum-free RPMI 1640 and the experiments were initiated on 70-80% confluence of HaCaT cells in 12-well plates.

B. Normal human epidermal keratinocytes (NHEK)

The adult normal human epidermal keratinocyte (NHEK) was purchased from Modern Cell & Tissue Technologies (MCTT, Seoul, Korea). NHEK was cultured in EpiLife medium (Gibco) supplemented with HKGS kit (Gibco) comprised of insulin growth factor-1 (10 ng/ml), transferrin (5 μg/ml), bovine

pituitary extract (2.4 μg protein/ml), hydrocortisone (0.18 μg/ml), epidermal growth factor (0.2 ng/ml), and gentamycin/amphotericin B. NHEK was grown on 100 cm^2 plate and incubated with 5% CO_2 at 37℃. Before performing IL-21 stimulation experiments, the growth medium was changed for supplement-free primary keratinocyte medium and the experiments were initiated on 70-80% confluence of NHEK in 6-well plates.

3. Microarray experiments and data analysis

Total RNA from NHEK with or without IL-21 treatment was extracted using TRIzol reagent (Life Technologies, Carlsbad, California, USA) and RNA quality was evaluated with Agilent's 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Total RNA was amplified and labeled using Agilent's Low RNA Input Linear Amplification kit PLUS, then amplified RNA was hybridized using Agilent's Gene Expression Hybridization kit on the Agilent Human GE 4 X 44K (V2). Data were collected by Agilent's DNA microarray scanner and Feature Extraction Software. Data analysis was conducted by using Agilent's GeneSpring software according to manufacturer's recommendation. Briefly, raw data were normalized using Lowes Normalization method and low signals compared to the background were removed before statistical analysis. Then t-test and corresponding false discovery rate was calculated to identify the statistical difference in genes expressed between the IL-21 treated and untreated

groups (treated 3, untreated 3). FDR less than 0.01 was defined as statistically significant.

4. Reverse transcription polymerase chain reaction and real-time PCR

Total RNA from HaCaT with or without IL-21 treatment was extracted using TRIzol reagent (Life Technologies) and cDNA was made using TaKaRa PrimeScript® RT Master Mix (Takara Bio Inc, Otsu, Shiga, Japan). Then, cDNA was amplified with CCL20 and IL-21 receptor primers, combined with real time qPCR kit. Relative expression of CCL20 and IL-21 receptor was normalized relative to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression level. Primer sequences used in this study are as follows: CCL20 forward 5'- TACTCCACCTCTGCGGCGAAT CAGAA-3', reverse 5'- GTGAAACCTCCAACCCCAGCAAGGTT-3'; CXCL8 forward 5'- ATGACTTCCAAGCTGGCCGTGGCT -3' reverse 5'- TCTCAGCCCTCTTCAAAAACTTCTC -3'; DEFB4A forward 5'- CCAGCCATCAGCCATGAGGT -3' reverse 5'- GGAGCCCTTTCTGAA TCCGCA -3'; S100A7 forward 5'- CAACTTCCTTAGTGCCTGTGACAA -3' reverse 5'- CCAGCAAGGACAGAAACTCAGAA -3'; S100A12 forward 5'- AAAGGAGCTTGCAAACACCATC -3' reverse 5'- CAGGCCTTGGAA TATTTCATCAA -3'. Real-time PCR was performed using an Applied Biosystems StepOnePlus Real-Time PCR System (Applied Biosystems, Foster

City, CA, USA). Ct values were based on thresholds determined by the StepOnePlus software.

5. Statistical analysis

Differences between groups were compared using the Student t test, one-way ANOVA test, analysis of variance, and Wilcoxon tests. SPSS version 19.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses.

III. RESULTS

1. IL-21 enhances the expression of psoriasis-associated chemokines CCL20 and CXCL8 (IL-8) genes in keratinocytes

The effect of IL-21 on human keratinocytes was demonstrated via microarray analysis (Table 1). According to the acquired data, a statistically significant increase in gene expression was observed in the psoriasis-associated chemokines CCL20 and CXCL8 compared to the untreated control (1.1 and 1.6 fold change, respectively). On the other hand, psoriasis-related antimicrobial peptide genes, such as DEFB4, S100A7, and S100A12 either showed no change or a decrease in expression with IL-21 stimulation compared to the untreated control (1.0, 0.9, and 0.7 fold change, respectively).

Table 1. Microarray fold change values of selected chemokine/chemokine receptor genes, inflammatory genes, and antimicrobial peptide genes

FCH	Gene	FCH	Gene	FCH	Gene	FCH	Gene	FCH	Gene	FCH	Gene
9.2	CCL11	1.1	CXCL2	2.3	IL2RG	1	IL2RG	1.4	S100P	0.9	DEFB110
2.4	CCL21	1.1	CXCL2	1.8	IL10	0.9	IL12B	1.4	DEFA3	0.9	DEFB115
2.1	CCL3 *	1.1	CXCR3 *	1.6	IL8 *	0.9	IL13	1.3	DEFA8P	0.9	DEFB121
2	CCL19	1	CCL17 *	1.6	IL9	0.9	IL16	1.3	DEFB105B	0.9	DEFB130
1.9	CCL1	1	CCL21	1.3	IL17A	0.9	IL17REL	1.2	S100A2	0.9	DEFB131
1.7	CCL3L3	1	CXCL5	1.3	IL1A	0.9	IL18	1.2	S100B	0.8	S100A1
1.6	CCL4	0.9	CCL13	1.3	IL1RL2	0.9	IL18RAP	1.2	DEFB124	0.8	S100A9 *
1.6	CXCL1 *	0.9	CCL16	1.3	IL24	0.9	IL1R1	1.2	DEFB128	0.8	S100G
1.5	CCL4L1	0.9	CCL18	1.3	IL6	0.9	IL2RA	1.1	DEFB104B	0.8	DEFB103B
1.5	CCR7	0.9	CCL24	1.3	IL7	0.9	IL2RA	1	S100A3	0.8	DEFB107A
1.5	CXCL6	0.9	CCL25	1.3	IL9R	0.9	IL2RB	1	S100A11	0.8	DEFB110
1.4	CXCL9 *	0.9	CCL27 *	1.2	IL10RA	0.9	IL3	1	S100A13	0.8	DEFB113
1.3	CCL22 *	0.9	CCR1	1.2	IL13RA2	0.9	IL3RA	1	S100PBP	0.8	DEFB114
1.3	CCR2 *	0.9	CCR6 *	1.2	IL18R1	0.9	IL5	1	DEFA7P	0.8	DEFB116
1.3	CCR4 *	0.9	CCR9	1.2	IL32	0.9	IL6R	1	DEFB1	0.8	DEFB122
1.3	CX3CL1 *	0.9	CXCL10 *	1.1	IL11	0.9	IL6ST	1	DEFB4	0.8	DEFB133
1.3	CXCL11 *	0.9	CXCL13	1.1	IL12RB2	0.8	IL8RB	0.9	S100A4	0.8	DEFB134
1.3	CXCL12	0.9	CXCR4	1.1	IL1B	0.7	IL2	0.9	S100A7 *	0.8	DEFB135
1.2	CCL5 *	0.8	CCL2 *	1.1	IL1R2	0.7	IL31	0.9	S100A7L2	0.7	S100A8 *
1.2	CCR5 *	0.8	CCL7	1.1	IL4R	0.7	IL4	0.9	S100A10	0.7	S100A12 *
1.2	CX3CR1 *	0.8	CCL8	1.1	IL8RA	0.6	IL12A	0.9	DEFA11P	0.7	S100B
1.2	CXCL3	0.8	CCL23	1	IL10RB	0.6	IL7R	0.9	DEFA4	0.7	DEFA9P
1.2	CXCR6 *	0.8	CCL26	1	IL13RA1	0.5	IL16	0.9	DEFA6	0.7	DEFB136
1.1	CCL16	0.8	CCR8	1	IL1RAP	0.1	IL32	0.9	DEFB106B	0.6	DEFB112
1.1	CCL19	0.7	CCR3	1	IL27RA			0.9	DEFB108B	0.5	S100A5
1.1	CCL20 *	0.6	CXCL14								
1.1	CXCL16 *										

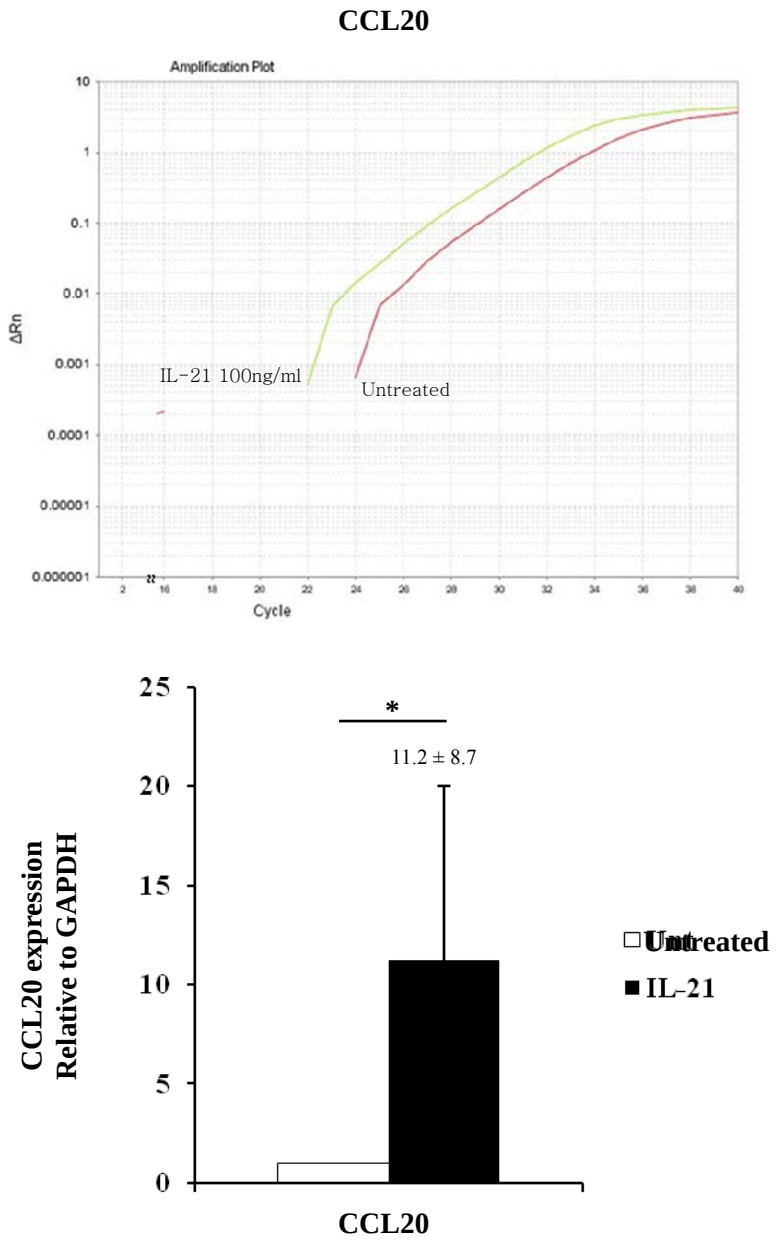
*** FDR < 0.05**

Abbreviations: FCH, fold change; FDR, false discovery rate

2. IL-21 enhances the expression of psoriasis-associated chemokines CCL20 and CXCL8 in HaCaT cells

In attempt to verify the microarray analysis results, expression levels of mRNA of CCL20 and CXCL8 were determined in the absence and presence of IL-21 stimulation via RT-PCR. In accordance to previous microarray results, an 11.2-fold statistically significant increase in CCL20 gene expression (Figure 1A) and a 2.1-fold increase in CXCL8 gene expression compared to the untreated group were observed (Figure 1B).

A)


CCL20
Amplification Plot
ΔRn
Cycle
IL-21 100ng/ml
Untreated
CCL20 expression
Relative to GAPDH
*
11.2 ± 8.7
Untreated
IL-21
CCL20

B)

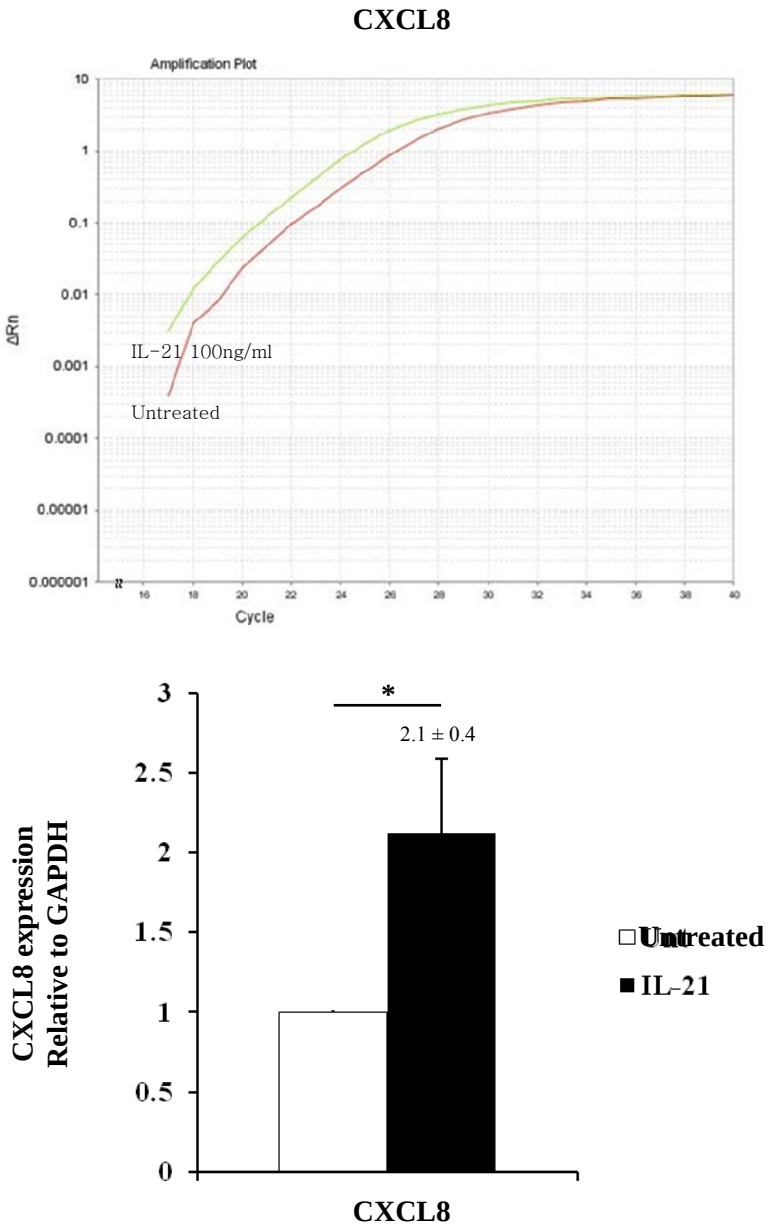
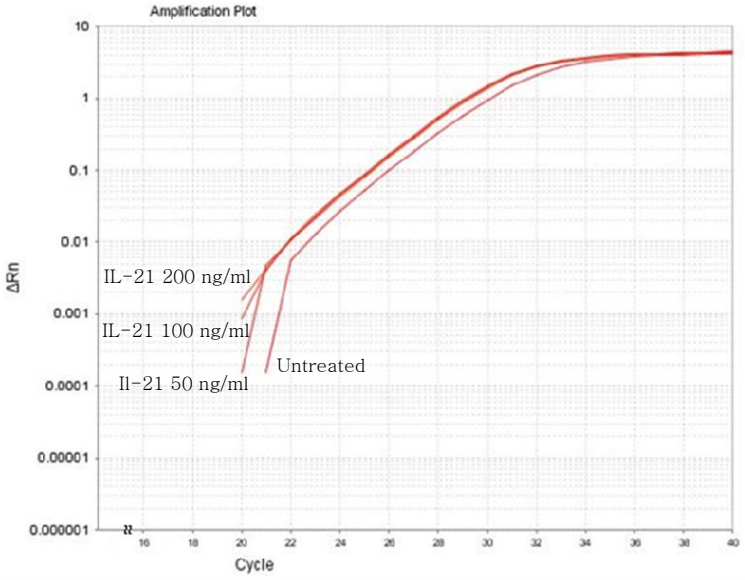

CXCL8
Amplification Plot
ΔRn
Cycle
IL-21 100ng/ml
Untreated
CXCL8 expression
Relative to GAPDH
*
2.1 ± 0.4
Untreated
IL-21
CXCL8

Figure 1. IL-21 enhances the expression of psoriasis-associated chemokines CCL20 and CXCL8 in HaCaT cells. HaCaT cells were either left untreated or treated with IL-21 (100 ng/ml) for an incubation period of 6 h. Then, CCL20 and CXCL8 expressions were evaluated via quantitative RT-PCR. (A) CCL20 Ct value for untreated sample: 33.24; IL-21 100 ng/ml 6 h treated sample: 31.13 (*upper panel*). An 11.2-fold increase was observed in the expression of CCL20 compared to the control ($P < 0.05$) (*lower panel*). (B) CXCL8 Ct value for untreated sample: 25.93; IL-21 100 ng/ml 6 h treated sample: 24.23 (*upper panel*). A significant 2.1-fold increase in the expression of CXCL8 was also observed compared to the control ($P < 0.05$) (*lower panel*).

3. IL-21 in different concentrations (50, 100, and 200 ng/ml) significantly enhance expression of CCL20 in HaCaT cells

Upon acquisition of evidence in regards to the enhancement of gene expression of CCL20 and CXCL8 with the stimulation of 100 ng/ml IL-21 via quantitative RT-PCR, it was necessary to conduct an investigation using several doses of the reagent prior to further studies, in order to establish and confirm the appropriate concentration of IL-21 when demonstrating its effect on the HaCaT cells. The IL-21 treated group, with doses of 50, 100, and 200 ng/ml, showed a significant increase in CCL20 mRNA expression compared to the control (Figure 2) ($P < 0.05$). Also taking into consideration previously established condition standards where most studies were conducted with 100 ng/ml concentration of IL-21, experiments henceforth was performed using 100 ng/ml of IL-21 for stimulation of the HaCaT cells.

CCL20


Amplification Plot
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
ΔRn
IL-21 200 ng/ml
IL-21 100 ng/ml
Il-21 50 ng/ml
Untreated
16
18
20
22
24
26
28
30
32
34
36
38
40
Cycle

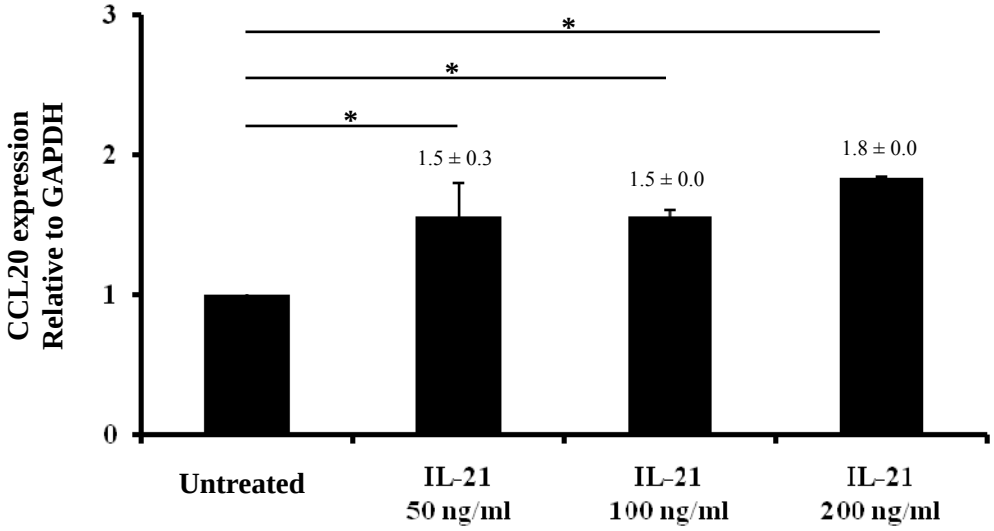
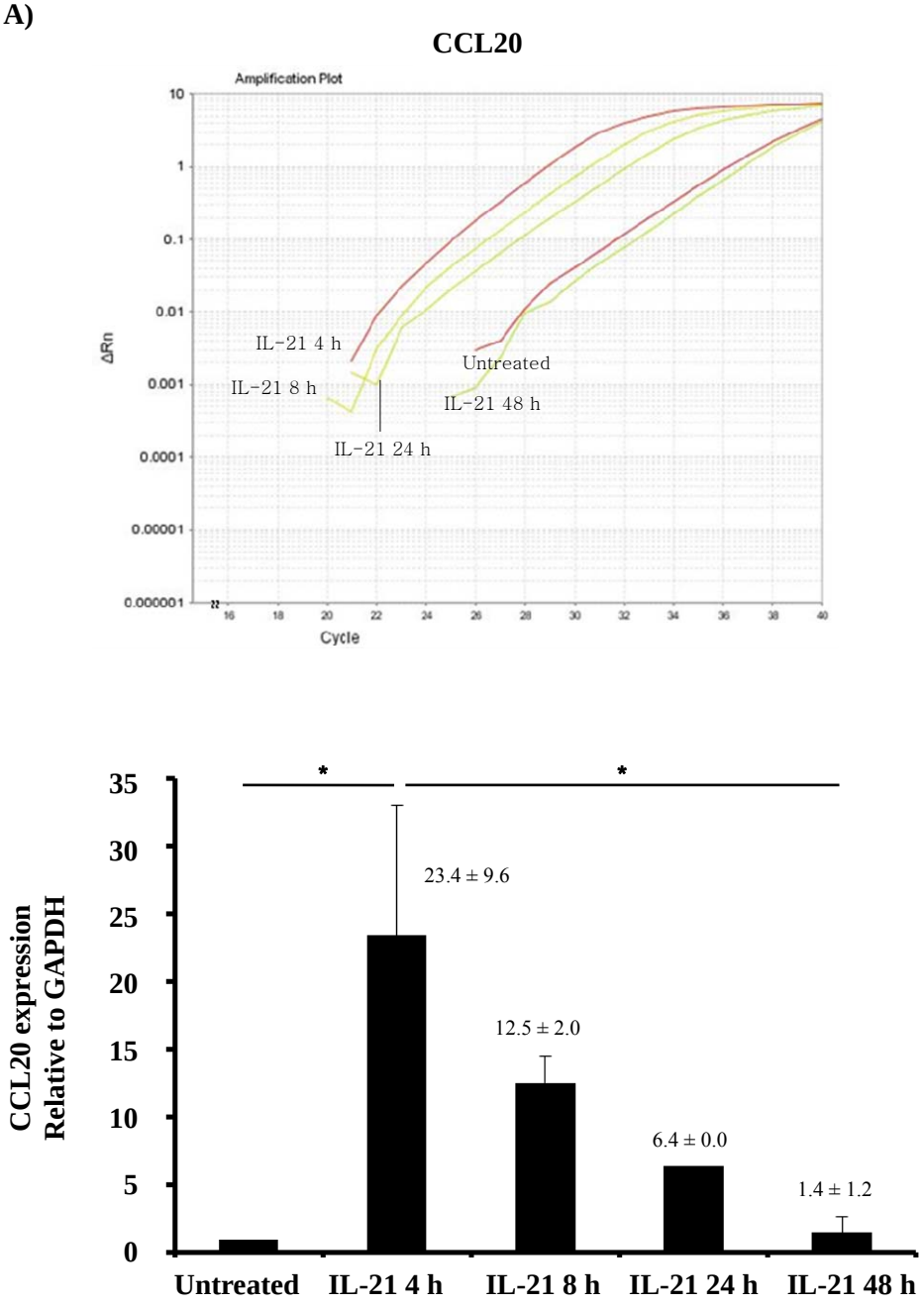

CCL20 expression
Relative to GAPDH
3
2
1
0
*
*
*
1.5 ± 0.3
1.5 ± 0.0
1.8 ± 0.0
Untreated
IL-21
50 ng/ml
IL-21
100 ng/ml
IL-21
200 ng/ml

Figure 2. IL-21 in different concentrations (50, 100, and 200 ng/ml) significantly enhance expression of CCL20 in HaCaT cells. HaCaT cells were either untreated or treated with 50, 100 and 200 ng/ml of IL-21 for an incubation period of 6 h. Then, CCL20 expression was evaluated via quantitative RT-PCR. CCL20 Ct value for untreated sample: 29.40; IL-21 50 ng/ml 6 h treated sample: 28.44; IL-21 100 ng/ml 6 h treated sample: 28.56; IL-21 200 ng/ml 6 h treated sample: 28.64 (*upper panel*). A significant increase in expression of CCL20 was observed in all three concentration groups compared to the untreated control ($P < 0.05$), with no significant difference between the dose-different IL-21 groups (*lower panel*).

4. IL-21-induced enhancement of the expression of CCL20 and CXCL8 show different time-dependent expression peaks in HaCaT cells

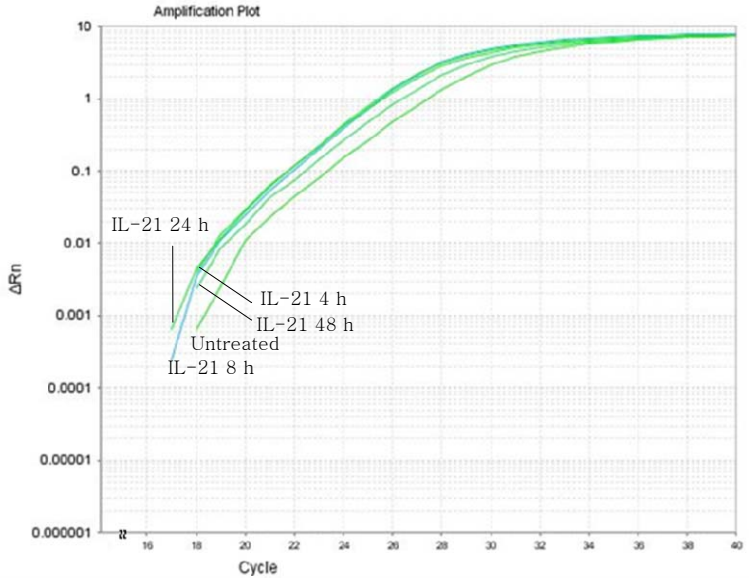
Further exploration of the expressions of CCL20 and CXCL8 in HaCaT cells were performed using different incubation intervals with IL-21 stimulation via quantitative RT-PCR. Increase in the expression of CCL20 was observed in all incubation time intervals (4 h, 8 h, 24 h, and 48 h) compared to the untreated group (Figure 3A). Expression peaked at 4 h incubation with IL-21 (an estimated 23-fold) and decreased gradually to the level of the control as incubation duration was extended to 48 h. The expression of CXCL8 was also amplified with the stimulation of IL-21 regardless of the incubation time (Figure 3B). Expression of CXCL8 peaked, however, when incubated with IL-21 for 24 h (an estimated 4-fold). A gradual increase of expression was observed prior to the peak, and a similar drop of expression level to the level of the untreated group was noticed at 48 h.

A)


CCL20
Amplification Plot
ΔRn
Cycle
IL-21 4 h
IL-21 8 h
IL-21 24 h
Untreated
IL-21 48 h
CCL20 expression
Relative to GAPDH
23.4 ± 9.6
12.5 ± 2.0
6.4 ± 0.0
1.4 ± 1.2
Untreated
IL-21 4 h
IL-21 8 h
IL-21 24 h
IL-21 48 h

B)

CXCL8


Amplification Plot
ΔRn
Cycle
IL-21 24 h
IL-21 4 h
IL-21 48 h
Untreated
IL-21 8 h

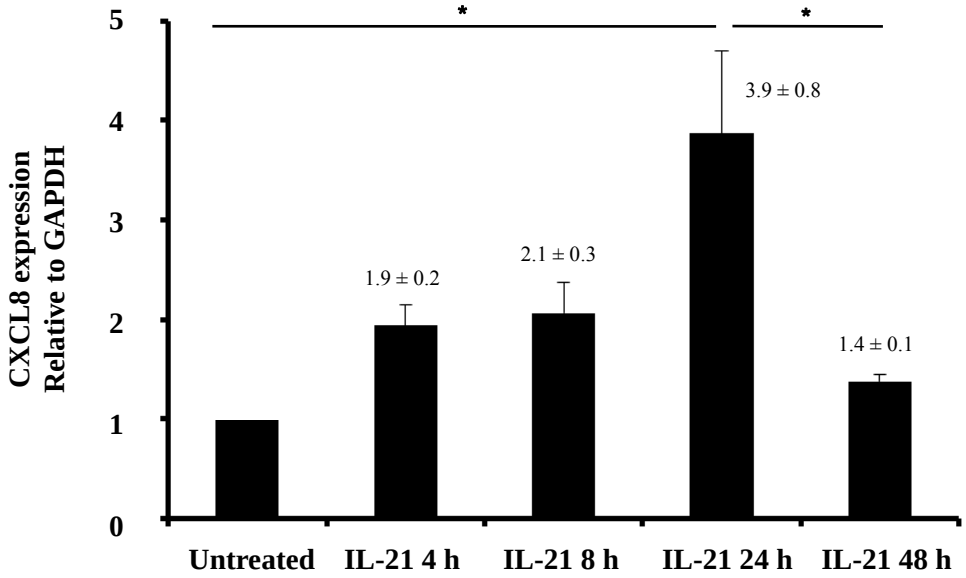
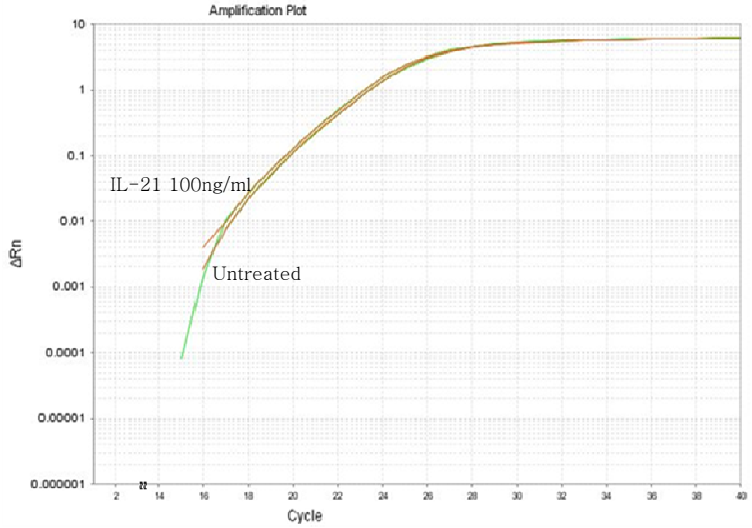

CXCL8 expression
Relative to GAPDH
*
*
3.9 ± 0.8
2.1 ± 0.3
1.9 ± 0.2
1.4 ± 0.1
Untreated
IL-21 4 h
IL-21 8 h
IL-21 24 h
IL-21 48 h

Figure 3. IL-21-induced enhancement of the expression of CCL20 and CXCL8 show different time-dependent expression peaks in HaCaT cells. HaCaT cells were either left untreated or treated with IL-21 (100 ng/ml) for an incubation period of 4, 8, 24, and 48 h. Then, CCL20 and CXCL8 expressions were evaluated via quantitative RT-PCR. (A) CCL20 Ct value for untreated sample: 36.42; IL-21 100 ng/ml 4 h treated sample: 28.98; IL-21 100 ng/ml 8 h treated sample: 30.77; IL-21 100 ng/ml 24 h treated sample: 32.33; IL-21 100 ng/ml 48 h treated sample: 36.96 (*upper panel*). Increase in the expression of CCL20 was observed in all incubation time intervals (4 h, 8 h, 24 h, and 48 h) compared to the untreated group ($P < 0.05$), with peak expression at 4 h incubation. The difference between the untreated group and the IL-21 4 h group, and the difference between the IL-21 4 h and Il-21 48 h group was statistically significant ($P < 0.05$) (*lower panel*). (B) CXCL8 Ct value for untreated sample: 27.33; IL-21 100 ng/ml 4 h treated sample: 25.49; IL-21 100 ng/ml 8 h treated sample: 25.31; IL-21 100 ng/ml 24 h treated sample: 24.92; IL-21 100 ng/ml 48 h treated sample: 26.28 (*upper panel*). Increase in the expression of CXCL8 was also observed in all incubation time intervals (4 h, 8 h, 24 h, and 48 h) compared to the untreated group ($P < 0.05$), with peak expression at 24 h incubation. The difference between the untreated group and the IL-21 24 h group, and the difference between the IL-21 24 h and Il-21 48 h group was statistically significant ($P < 0.05$) (*lower panel*).

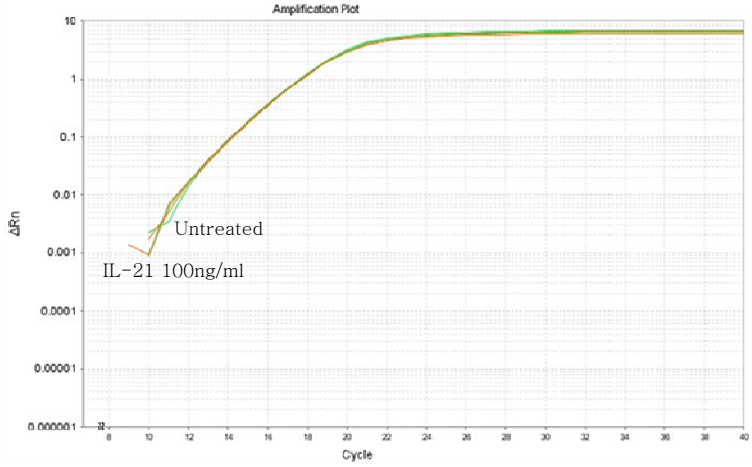
5. IL-21 does not influence the expression of psoriasis-associated antimicrobial peptides β-defensin 2, S100A7, S100A12 in HaCaT cells

In attempt to reveal whether IL-21 also affects the gene expression of psoriasis-related antimicrobial peptides β-defensin 2, S100A7, and S100A12, their mRNA expression levels were determined in the absence and presence of IL-21 stimulation via RT-PCR. In accordance to previous microarray results, no significant changes in expression were detected relative to the untreated controls (Figure 4).

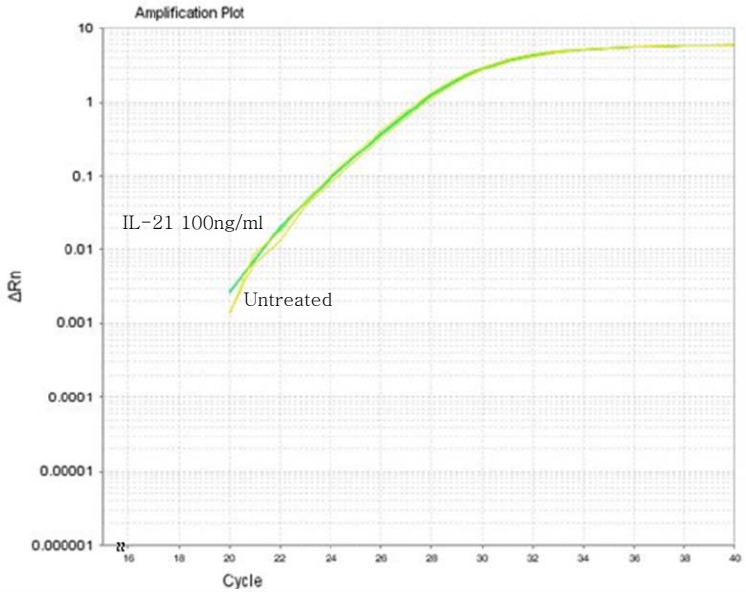
DEFB4



S100A7



S100A12


Amplification Plot
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
ΔRn
IL-21 100ng/ml
Untreated
16
18
20
22
24
26
28
30
32
34
36
38
40
Cycle

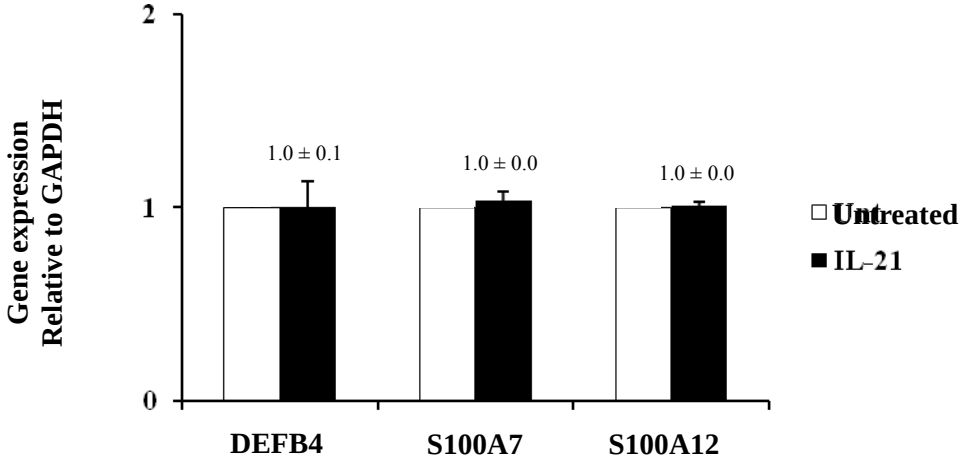

Gene expression
Relative to GAPDH
2
1
0
1.0 ± 0.1
1.0 ± 0.0
1.0 ± 0.0
DEFB4
S100A7
S100A12
Untreated
IL-21

Figure 4. IL-21 does not influence the expression of psoriasis-associated antimicrobial peptides β-defensin 2, S100A7, S100A12 in HaCaT cells. HaCaT cells were either left untreated or treated with IL-21 (100 ng/ml) for an incubation period of 6 h. Then, β-defensin 2, S100A7, and S100A12 expressions were evaluated via quantitative RT-PCR. DEFB4 Ct value for untreated sample: 23.56; IL-21 100 ng/ml 6 h treated sample: 23.83. S100A7 Ct value for untreated sample: 17.89; IL-21 100 ng/ml 6 h treated sample: 17.83. S100A12 Ct value for untreated sample: 27.46; IL-21 100 ng/ml 6 h treated sample: 27.63 (*upper panel*). No statistically significant changes in expression were observed relative to the untreated controls for all three antimicrobial peptides tested (*lower panel*).

IV. DISCUSSION

The extensive involvement, and therefore, the importance of IL-23/Th17 axis has never been more stressed in relation to the pathogenesis of chronic inflammatory skin disease of psoriasis.[3] Its crucial role in linking the innate and adaptive immunity lies in the various cytokines that are expressed by the Th17 cells that aggregate in the dermis of psoriatic skin. Previous reports have proven that when keratinocytes are stimulated by IL-17 and IL-22 – the two main cytokines expressed by the Th17 cells – not only an increase in antimicrobial peptide secretion such as LL-37 and β-defensin 2 is observed, but also an overexpression of inflammatory cell-alluring chemokines such as CCL20 and CXCL8 is demonstrated. [4, 17-20] CXCL8 binds to CXCR1 and CXCR2, attracting neutrophils to the site of inflammation, and CCL20 binds to CCR6, a chemokine receptor present on Th17 cells, and therefore pulls them towards the psoriatic skin.[21] These aggregated Th17 cells produce IL-17 and IL-22, which further stimulate the keratinocytes to promote the production of additional chemokines, consequentially creating a vicious loop of inflammatory aggravation.

IL-21, a novel cytokine originally reported to be involved in the differentiation of T cells, is produced mainly by CD4+ T-cells, Th17- and T_{FH}-cells.[6, 8-9] Until now, only a limited number of studies have been performed in regards to the role of IL-21 in the Th17-dominant inflammatory disease of psoriasis. Previous

reports have, however, illuminated the abundance of IL-21 mRNA and protein in lesional psoriatic skin, the increase in IL-21 transcript levels in peripheral blood mononuclear cells of individuals with psoriasis, and its independent role (from IL-23) in the proliferation of keratinocytes, via extracellular signal-related kinase-1 and -2 activation. IL-21, when injected intradermally in mice, induced eratinocyte hyperplasia and dermal inflammatory cell infiltration.[15]

We have therefore dedicated this study in order to clarify whether IL-21, like other cytokines of the Th17 cell, would influence the keratinocytes into overexpressing psoriasis-related chemokines and antimicrobial peptides crucial to the pathogenesis of the disease.

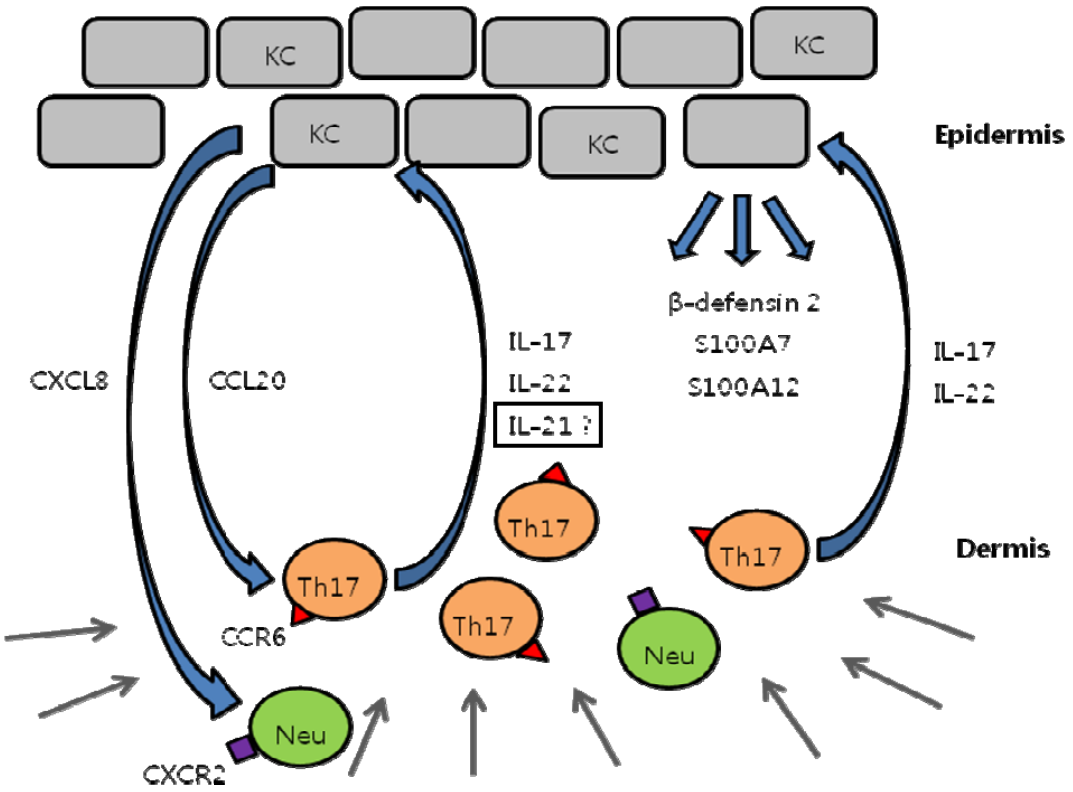
The initial microarray analysis revealed statistically significant increases in expression of certain psoriasis-associated chemokine genes such as CCL20 and CXCL8. However, previously reported antimicrobial peptides genes associated with the disease (i.e. BDEF4, S100A7, S100A12) showed no change or a decrease in expression compared to the untreated control. Interestingly, this general trend appeared to exist where more chemokine and chemokine receptor genes showed an increase in expression, while more antimicrobial peptide-related genes showed a decrease in fold change, regardless of their relevance to psoriasis.

The mRNA level study on HaCaT cells via RT-PCR further elucidated the influence of IL-21 on the keratinocyte expression of chemokines while showing no control over the production of certain antimicrobial peptides. The HaCaT

expressions of both CCL20 and CXCL8 were significantly increased when stimulated with IL-21 compared to the untreated control while β-defensin 2, S100A7, and S100A12 were unaffected. Interestingly, when expression levels were determined for different incubation time intervals, the peak expression of CCL20 preceded the peak expression of CXCL8 (4h vs. 24h incubation time, respectively). This may indicate a sequential manner of inflammatory cell infiltration, and hence the difference in chronological intensity of action in attracting either the T cells that occupy the dermis or the neutrophils that eventually are thought to immigrate to the upper epidermal level forming Munro abscesses specific to psoriasis.

V. CONCLUSION

In this study, we have further elucidated the role of IL-21 in psoriasis – IL-21 may induce a significant increase in psoriasis-related chemokines (CCL20, CXCL8), similar to the effect of other cytokines expressed by Th17 cells (i.e. IL-17, IL-22), but may not play a role in inducing psoriasis-related antimicrobial peptides (β-defensin 2, S100A7, S100A12). We hope this further elucidation of the role of IL-21 aids in the advent of yet another potential therapeutic target in psoriasis.



Abbreviations: KC, keratinocyte; Th17, T helper 17; Neu, neutrophil

Figure 5. A model indicating the potential role of IL-21 in linking innate and adaptive immunity in the pathogenesis of psoriasis.

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ABSTRACT (IN KOREAN)

HaCaT에서 건선관련 chemokine 유전자 발현에 Interleukin-21이 미치는 영향

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지현중

건선은 전세계 약 1-3%에 해당하는 인구에서 발병하는 만성 염증성 피부 질환이다. 건선의 병인은 아직까지 정확히 알려지지 않은 자극에 의해 선천면역이 염증을 일으키고 이는 결국 적응면역을 유발시켜 다양한 cytokine, chemokine, 그리고 growth factor들이 생성되어 표피의 증식을 일으키는 것으로 알려져 있다. 최근 보고들은 Interleukin (IL)-23/Th17 축과 각질세포간의 연결고리를 병인의 중요한 기전으로 설명하고 있는데, 특히 피부의 각질세포에서 과분비되는 CCL20에 의해 CCR6+ Th17 세포들이 피부병변으로 이동하게 되며, 추후 야기되는 cytokine 환경에 의해 각질세포가 자극이 되어 지속적인 CCL20의 발현을 유발한다고 보고하고 있다.

IL-21은 최근에 발견된 새로운 cytokine으로, 특별히 T세포의 분화와 기능에 중요한 역할을 함이 밝혀진 바 있으며, CD4+ T-cell, Th17-, T-follicular helper (T_{FH})-cell에 국한하여 발현되는 것으로 알려져 있다. 건선에 관련해서는 건선 피부병변에서 IL-21이 과발현되고 있음을 확인하였으며, mouse 실험을 통해 표피의 증식과 염증의 악화에 기여한다는 것이 보고 된 바 있다.

이번 실험을 통해 저자는 건선에 관하여 IL-21의 기타 역할에 대해 microarray analysis와 real time RT-PCR을 통해 알아보고자 하였고, 특히 각질세포를 자극하였을 때 건선 관련 chemokine과 antimicrobial peptide의 발현에 미치는 역할을 고찰하고자 하였다. Microarray analysis에서 확인한 CCL20과 CXCL8의 유의한 발현 증가를 RT-PCR을 통해 재확인 할 수 있었으며, 이를 통해 IL-21은 각질세포를 자극하여 발현되는 chemokine으로 적응면역의 주역인 T세포, 그리고 건선의 병인에 중요한 호중구를 병변으로 불러들이는데 중요한 요소가 될 수 있음이 확인 되었다. 반면 IL-21의 자극으로 antimicrobial peptide의 변화를 관찰 할 수는 없었다. 따라서 저자는 이 실험을 통하여 기존에 보고되지 않은 IL-21이 건선에 미치는 영향, 즉, 선천면역과 적응면역의 고리역할 할 수

있음을 밝히며, 추후 건선의 치료에 있어 잠재적인 표적의 고려 대상이 될 수 있음을 제안하는 바이다.

핵심되는 말 : interleukin-21 (IL-21), chemokine (C-C motif) ligand 20 (CCL20), HaCaT, 건선