Increased MAPK and NF-κB expression of Langerhans cells is dependent on TLR2 and TLR4, and increased IRF-3 expression is partially dependent on TLR4 following UV exposure

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Abstract. Toll-like receptors (TLRs) and epidermal Langerhans cells (LCs) play a crucial role in innate and adaptive immunity. To date, the pattern of TLR expression has not been fully analyzed. The effects of ultraviolet (UV) light on TLR expression and the downstream signaling cascades of human LC have not been examined. In this study, we purified human epidermal LCs using a density gradient centrifugation method and an immunomagnetic microbead method. We found that cultured purified LCs from human skin express mRNAs encoding TLR2, TLR4, TLR5 and TLR7-9. The expression of TLR2 and TLR4 protein was confirmed by Western blot analysis. The results showed for the first time that UV exposure up-regulated the mRNA and protein expression of TLR2 and TLR4 in human LCs. We also found that UV exposure-induced up-regulated MAPK and NF-κB/p65 expression was dependent on TLR2 and TLR4, and up-regulated IRF-3 expression was partially dependent on TLR4. In conclusion, UV light up-regulates the expression of TLR2, TLR4 and downstream signaling molecules MAPK, NF-κB/p65 and IRF-3 in human LCs. This suggests that UV light has a significant effect on skin immune responses.

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Abbreviations: TLRs, toll-like receptors; LCs, Langerhans cells; UV, ultraviolet; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor-κB; MAPK, mitogen-activated protein kinases; IRF-3, IFN regulatory factor-3

Key words: Toll-like receptors, Langerhans cells, ultraviolet

Introduction

Currently, 10 toll-like receptors (TLRs) are known to be expressed in humans. TLRs are a group of evolutionary conserved proteins belonging to the IL-1R superfamily, characterized by the extracellular leucine-rich repeat domain and an intracellular portion that shares homology with the cytoplasmic domain of the IL-1 receptor (toll/IL-1 receptor domain) (1,2).

The signaling cascades that occur after TLR activation have been the subject of intense research. There appear to be two distinctive pathways, the myeloid differentiation factor 88 (MyD88)-dependent and MyD88-independent pathways. In the MyD88-dependent signaling pathways, the toll/IL-1 receptor domain of TLR interacts with MyD88. This interaction activates IL-1 receptor-associated kinase, which in turn activates tumor necrosis factor receptor-activated factor 6 and then activates transforming growth factor β-activating kinase-1. At this point, the MyD88-dependent signaling pathways diverge, one trail leading to nuclear factor-κB (NF-κB) activation and the other to mitogen-activated protein kinase (MAPK) signaling pathways. In MyD88-independent pathways, the activation of TLR3 and TLR4 activates IFN regulatory factor-3 (IRF-3), involves the adaptor proteins TRIF and TRAM, then up-regulates the expression of IFN-inducible genes. The activation of TLR3 and TLR4 also activates NF-κB, and involves TRIF and tumor necrosis factor receptor-activated factor 6 (1,3).

Epidermal Langerhans cells (LCs) are bone marrow-derived MHC class II antigen-possessing cells that constitute 1-3% of epidermal cells (4). There is a long-standing belief that LCs play an important role in a multitude of cutaneous immune responses, including innate and adaptive immune responses (5). TLRs are expressed on immune cells, such as monocytes, dendritic cells and granulocytes, but the pattern of TLR expression on LCs has yet to be fully analyzed.

The effects of ultraviolet (UV) light on the TLR expression and downstream signaling cascades of human LCs have never been examined. In this study, we analyzed the expression pattern of the 10 identified human TLRs in cultured human LCs. Furthermore, the expression of TLR2, TLR4 and the downstream signal transduction molecules of LCs exposed to UV light were also investigated.

Table I. Sequences of primers used for RT-PCT.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
TLR1	TTT GAA AAT TGT GGG CAC CTTACT G	AAG CAA CAT TGA GTT CTT GCA AAG C
TLR2	TGT GAA CCT CCA GGC TCT G	GTC CATATT TCC CAC TCT CAG G
TLR3	AGC CGC CAA CTT CAC AAG	AGC TCT TGG AGATTT TCC AGC
TLR4	ACA GAA GCT GGT GGC TGT G	TCT TTA AAT GCA CCT GGT TGG
TLR5	CAT GAC CAT CCT CAC AGT CAC AAA G	GGG CATAAC TGA AGG CTT CAA GG
TLR6	CAT GAC GAA GGATAT GCC TTC TTT G	TAT TGA CCT CAT CTT CTG GCA GCT C
TLR7	TTG GCT TCT GCT CAA ATG C	CTA AAG GTT GGA ATT CAC TGC C
TLR8	GTC GAC TAC AGG AAG TTC CCC	GGG TAA CTG GTT GTC TTC AAG C
TLR9	GTG CCC CAC TTC TCC ATG	GGC ACA GTC ATG ATG TTG TTG
TLR10	CTT GAC CAC AAT TCATTT GAC TAC TC	CAT CTT CCT TCT CTA GAT TGG GGA TC
GAPDH	AAC CAT GAG AAG TAT GAC AAC AGC	CAT GTG GGG CCA TGA GGT CCA CCA C

Materials and methods

Media and reagents. The culture medium used was DMEM (Hyclone) with 10% (v:v) fetal calf serum (Gibco). TLR2 siRNA (h), sc-40256; TLR4 siRNA (h), sc-40260; siRNA transfection reagent, sc-29528; siRNA transfection medium, sc-36868; and control siRNA-A, sc-37007, were all purchased from Santa Cruz Biotechnology. Mouse anti-TLR2 antibody was purchased from InvivoGen. Goat anti-TLR4 antibody, mouse anti-GAPDH antibody, and horseradish peroxidase-linked anti-rabbit, anti-goat or anti-mouse second antibody were purchased from Santa Cruz Biotechnology. Rabbit anti-IRF3 antibody, rabbit anti-MAPK antibody and rabbit anti-NF-κB/p65 antibody were purchased from Cell Signaling Technology. FITC-conjugated monoclonal mouse anti-human CD1a antibody was obtained from AbD Serotec.

LC isolation from human skin. LCs were prepared using the density gradient centrifugation and immunomagnetic microbead methods. Briefly, human foreskin was separated and treated with 0.5% dispase (Roche) for 16 h at 4˚C. The epidermis was separated and incubated in 0.25% trypsin (Sigma) and 0.02% EDTA for 5 min at room temperature. An epidermal cell suspension was obtained by vigorous pipetting of the epidermal sheets. The epidermal cell suspension was slowly layered on top of a Histopaque-1077 (Sigma) layer. Centrifugation was performed at 2,000 x g for 30 min at room temperature. The middle layer from the Histopaque-1077 interface containing LCs was slowly aspirated off. Centrifugation was performed again at 1,500 x g for 10 min at room temperature. The cells were then treated with monoclonal mouse anti-human CD1a MicroBeads (Miltenyi Biotec GmbH) for 20 min at 4˚C. The Large Cell Separation Column was placed in the MiniMACS Separation Unit (both from Miltenyi Biotec GmbH). Magnetically labeled cell suspension was pipetted onto the column. The column was removed from the separator and the cells were flushed out using the plunger supplied. LC purity was revealed by flow cytometric analysis. LC viability was determined by trypan blue staining. Human LCs were incubated in culture plates for 4 h and up to 72 h.

UV irradiation. After incubation for 24 h, LCs were washed once with phosphate-buffered saline pH 7.4 (PBS) and exposed to UV light (UVB and UVA) in a thin layer of PBS using two SS-04P lamps (Sigma Corporation), which were placed 30 cm above the flasks. The UVB dose was set as 30 mJ/cm^2 and the UVA dose was set as 10 J/cm^2. After UV irradiation, PBS was replaced by the culture medium. Control cells were maintained under identical culture conditions without UV exposure.

TLR2 and TLR4 siRNA transfection of LC. TLR2 siRNA (h) is a pool of 3 target-specific 20-25 nt siRNAs designed to knock down TLR2 gene expression. TLR4 siRNA (h) is a pool of 4 target-specific 20-25 nt siRNAs designed to knock down TLR4 gene expression. siRNA transfection reagent is a highly efficient reagent for siRNA delivery with minimal cellular damage. siRNA transfection was carried out according to the manufacturer's protocol. Briefly, 2x10^6 cells/well were seeded and incubated for 24 h. siRNA duplex was resuspended in 330 μl of the RNAse-free water (10 μM solution). Then, solution A and B were prepared as follows: for solution A, 6 μl siRNA duplex solution was diluted into 100 μl siRNA transfection medium. For solution B, 6 μl siRNA transfection reagent was diluted into 100 μl siRNA transfection medium. Solutions A and B were mixed gently and incubated for 30 min (transfection mixture). After washing once with siRNA transfection medium, the cells were treated with transfection mixture or control siRNA-A, then incubated for 6 h. The transfection mixture was replaced by the culture medium and the cells were incubated for an additional 24 h.

Reverse transcription-polymerase chain reaction (RT-PCR). The Takara RNA PCR kit (Takara) was used in the study. After incubation for 48 h, cultured LCs were lysed and mRNA was extracted with TRIzol reagent (Gibco) according to the manufacturer's instructions. mRNA was reverse transcribed (20 μl total volume) at 42˚C for 60 min. For the analysis of TLR1-10 mRNA expression, thermal cycling was initiated with a first denaturation step of 95˚C for 10 min, and continued with 40 cycles of 95˚C for 15 sec, 58˚C for 20 sec and 72˚C for 30 sec. For the analysis of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA expression, 35 cycles of 95˚C for

1 min, 52˚C for 30 sec and 72˚C for 50 sec were performed. The primers (Takara) used for the amplification of TLR1-10 and GAPDH are described in Table I. Products were fractionated by 1.5% agarose gel electrophoresis, stained with ethidium bromide and viewed on an ultraviolet transilluminator. Results were representative of at least three independent experiments.

Western blot analysis. Western blot analysis was performed as previously described (6). Briefly, after incubation for 48 h, cultured LCs were washed once with PBS and lysed on ice. After homogenization of the lysates, 30 μg of proteins were electrophoresed on 10% sodium dodecyl sulfate-polyacrylamide gel. The proteins were transferred onto an apolyvinylidene fluoride (PVDF) membrane. The membrane was incubated with primary antibody in 2% BSA in TBST overnight at 4˚C, then incubated with secondary antibody for 45 min at room temperature. The bands were visualized chemiluminescently (Pierce). Results were representative of at least three independent experiments.

Flow cytometry. After incubation for 4 h, isolated and purified human LCs were incubated with FITC-conjugated CD1a antibody for 30 min at room temperature. The labeled cells were then analyzed with a FACScalibur (Becton-Dickinson) using CellQuest software (Becton-Dickinson). The results were expressed as the mean ± SD of three independent experiments.

Statistical analysis. Data were analyzed using SPSS 12.0 software. The statistical level of significance was set at $p<0.05$. When two means were compared, significance was determined by the Student's t-test ($p<0.05$). When multiple means were compared, significance was determined by one-way analysis of variance in conjunction with the Newman Keuls *post hoc* test ($p<0.05$).

Results

Purity and viability assays of LCs. LCs were isolated and purified using the density gradient centrifugation and immunomagnetic microbead methods. The epidermal cell suspension contained 3.24±0.37% of CD1a FITC-positive cells as revealed by flow cytometric analysis (Fig. 1A). Using the isolation and purification technique, it was possible to obtain 86.72±1.23% purity of LCs (Fig. 1B). The results of trypan blue staining showed that 96.12±1.45% of LCs cultured for 4 h were viable, and 85.47±0.93% of LCs were still viable after 48 h of culture.

TLR2, TLR4, TLR5, TLR7, TLR8 and TLR9 mRNA are expressed on cultured human LCs. The TLR1-10 mRNA expression of the LCs was investigated after 48 h of culture by RT-PCR. The results revealed the expression of TLR2, TLR4, TLR5, TLR7, TLR8 and TLR9 mRNA, but not of TLR1, TLR3 and TLR10 mRNA. The expression of TLR2, TLR4, TLR7, TLR8 and TLR9 mRNA was weak as compared to TLR5 mRNA expression. TLR6 mRNA expression was very weak in the cultured LCs (Fig. 2A). The expression of GAPDH mRNA was used as an internal control.

Next, we examined the protein expression of TLR2 and TLR4 in cultured human LCs. The expression of TLR2 and TLR4 protein was confirmed by Western blotting (Fig. 2B).

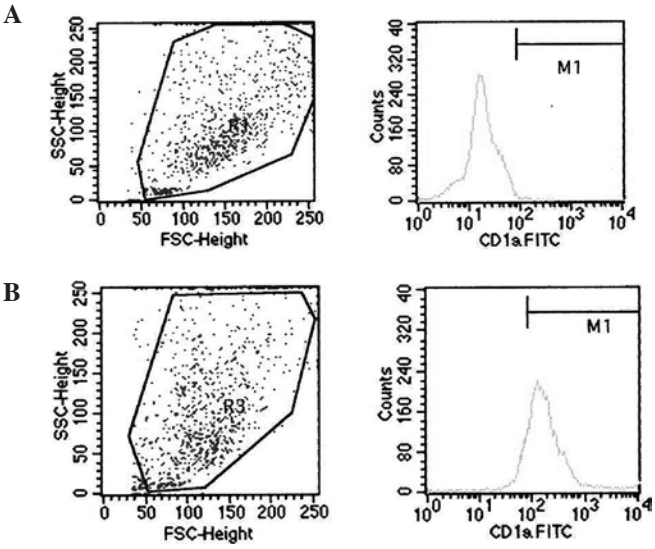

Figure 1. LC purity was revealed by flow cytometry. Mean fluorescence intensity (M1) is shown in the upper right corner of the histograms. Results are representative of three independent experiments. (A) The epidermal cell suspension contained only 3.24±0.37% of CD1a FITC-positive cells. (B) With the isolation and purification technique used, it was possible to obtain 86.72±1.23% of CD1a FITC-positive cells.

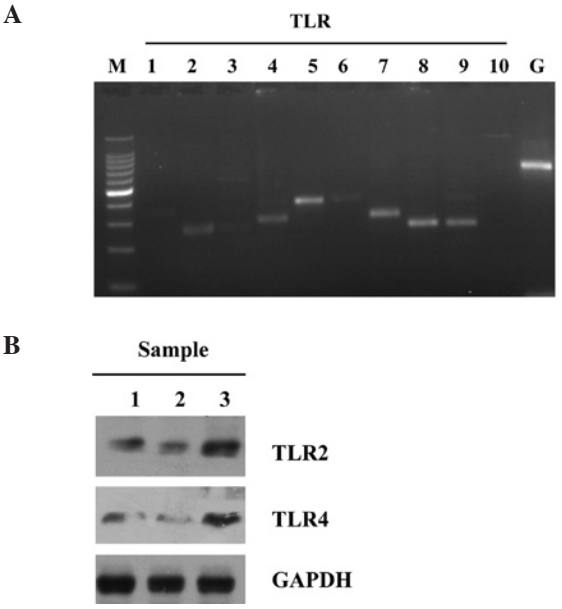

Figure 2. (A) Transcription of TLR1-TLR10 in cultured human LCs. M, DNA marker; G, internal control GAPDH. The results are representative of at least three independent experiments. (B) Protein samples from LCs were incubated with anti-TLR2, anti-TLR4 and anti-GAPDH antibody. Samples 1, 2 and 3 were representative of three independent experiments.

Increased mRNA and protein levels of TLR2 and TLR4 after UV exposure. At 24 h after UV exposure (UVA at 10 J/cm^2 and UVB at 30 mJ/cm^2), a 3.2-fold increase in TLR2 mRNA and a 3.3-fold increase in TLR4 mRNA was found compared to the controls, with GAPDH mRNA as the reference (Fig. 3A and B). At 24 h after UV exposure (UVA at 10 J/cm^2 and UVB at 30 mJ/cm^2), a 4.1-fold increase in TLR2 protein and a 2.7-fold increase in TLR4 protein was found compared to the controls, with GAPDH protein as the reference (Fig. 3C and D).

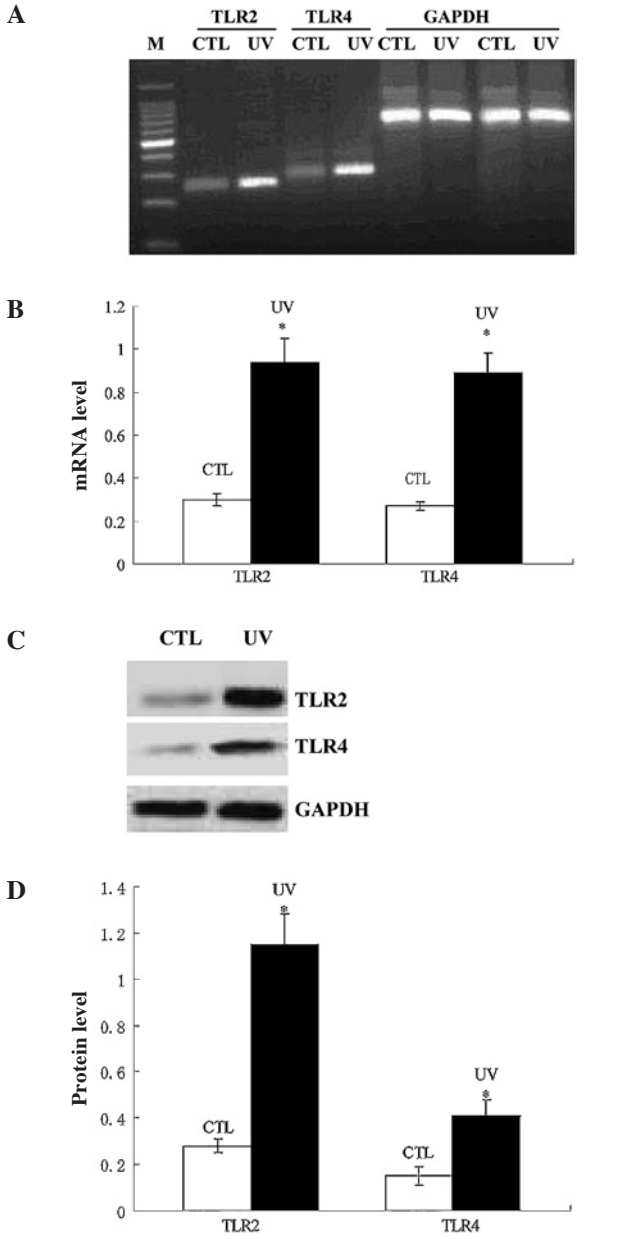

Figure 3. Effect of exposure of cultured human LCs to UV light on the mRNA and protein levels of TLR2 and TLR4. (A) The expression of TLR2 and TLR4 mRNA was investigated by RT-PCR. M, DNA marker. The results are representative of at least three independent experiments. (B) Increased mRNA levels of TLR2 and TLR4 after UV exposure ($^{*}p<0.05$ vs. CTL group). (C) The expression of TLR2 and TLR4 protein was investigated by Western blot analysis. The results are representative of at least three independent experiments. (D) Increased protein levels of TLR2 and TLR4 after UV exposure ($^{*}p<0.05$ vs. CTL group).

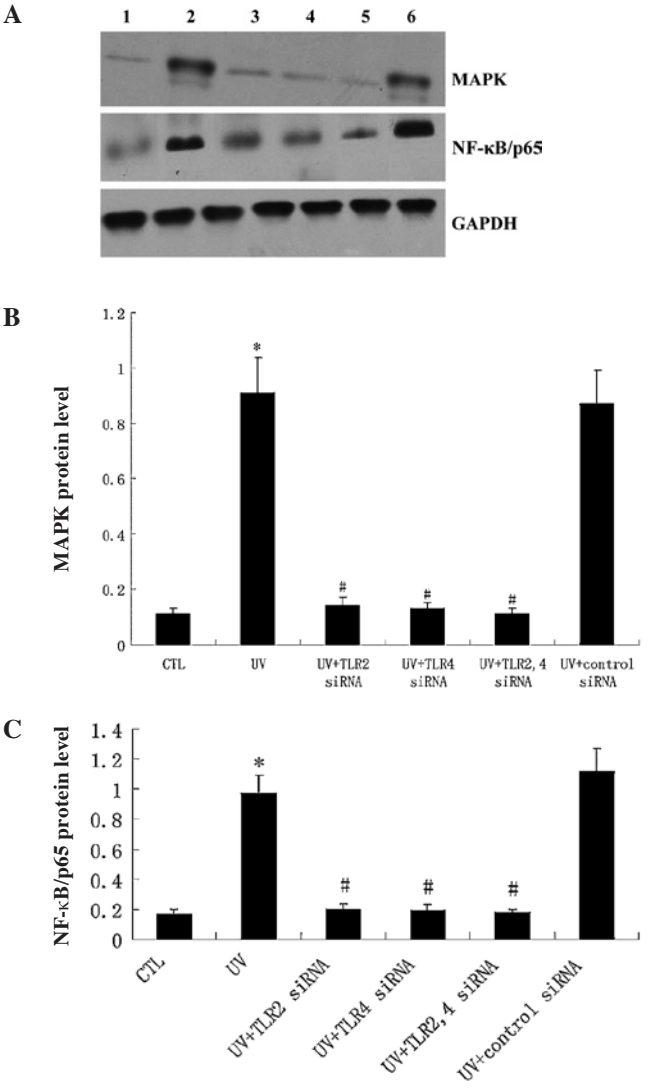

Figure 4. Role of TLR2 and TLR4 in the expression of MAPK and NF-κB protein induced by UV exposure. (A) Human LCs were exposed to UV light (UV) or not (CTL). 1, CTL group; 2, UV group; 3, UV + TLR2 siRNA group; 4, UV + TLR4 siRNA group; 5, UV + TLR2,4 siRNA group; 6, UV + control siRNA group. (B) Increased MAPK protein after UV exposure requires TLR2 and TLR4. TLR2 and TLR4 siRNA significantly decreased the expression of MAPK induced by UV exposure ($^{*}p<0.05$ vs. CTL group; $^{\#}p<0.05$ vs. UV group). (C) Increased NF-κB/p65 protein after UV exposure requires TLR2 and TLR4. TLR2 and TLR4 siRNA significantly decreased the expression of NF-κB/p65 induced by UV exposure ($^{*}p<0.05$ vs. CTL group; $^{\#}p<0.05$ vs. UV group).

Increased MAPK and NF-κB/p65 protein levels after UV exposure is dependent on TLR2 and TLR4. Human LCs were treated with TLR2 siRNA, TLR4 siRNA, TLR2 siRNA and TLR4 siRNA or control siRNA. At 24 h after UV exposure (UVA at 10 J/cm^2 and UVB at 30 mJ/cm^2), an 8.3-fold increase in MAPK protein and a 5.7-fold increase in NF-κB/p65 protein was found compared to the controls, with GAPDH protein as the reference (Fig. 4). To confirm the role of TLR2 and TLR4 in the expression of MAPK and NF-κB induced by UV exposure, TLR2 and TLR4 expression was knocked down in human LCs using siRNA. TLR2 and TLR4 siRNA significantly decreased the expression of MAPK and NF-κB/p65 induced by UV exposure (Fig. 4). These results suggest that the expression of MAPK and NF-κB induced by UV exposure is dependent on TLR2 and TLR4.

Increased IRF-3 protein level after UV exposure is partially dependent on TLR4, but not TLR2. Human LCs were treated with TLR2 siRNA, TLR4 siRNA, TLR2 and TLR4 siRNA, or control siRNA. At 24 h after UV exposure (UVA at 10 J/cm^2 and UVB at 30 mJ/cm^2), a 4.2-fold increase in IRF-3 protein was found compared to the controls, with GAPDH protein as the reference. TLR2 siRNA had no significant effect on the expression of IRF-3 induced by UV exposure, while TLR4 siRNA partially decreased the expression of IRF-3 induced

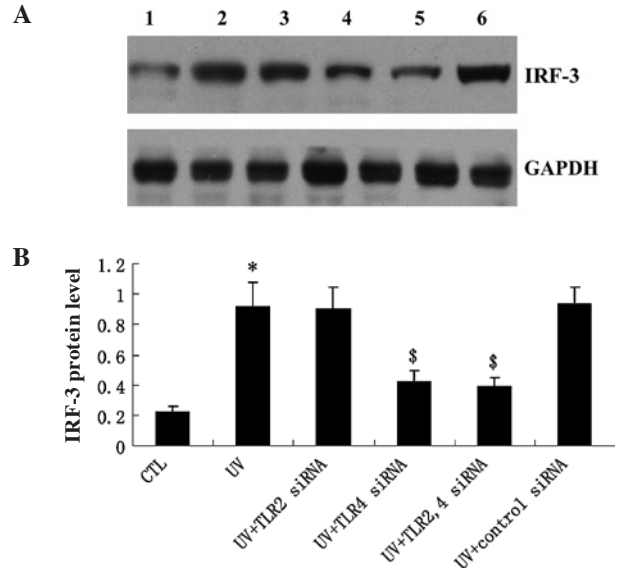

Figure 5. Role of TLR2 and TLR4 in the expression of IRF-3 protein induced by UV exposure. (A) Human LCs were exposed to UV light (UV) or not (CTL). 1, CTL group; 2, UV group; 3, UV + TLR2 siRNA group; 4, UV + TLR4 siRNA group; 5, UV + TLR2,4 siRNA group; 6, UV + control siRNA group. (B) Increased IRF-3 protein after UV exposure partially requires TLR4, but not TLR2. TLR2 siRNA had no significant effect on the expression of IRF-3 induced by UV exposure, while TLR4 siRNA partially decreased the expression of IRF-3 induced by UV exposure ($^{*}p<0.05$ vs. CTL group; $^{\$}p<0.05$ vs. UV group).

by UV exposure (Fig. 5). These results suggest that the expression of IRF-3 induced by UV exposure is partially dependent on TLR4, but not TLR2.

Discussion

As antigen-presenting cells, LCs are directly or indirectly activated with functional maturation when they encounter pathogens that invade the skin. They secrete inflammatory cytokines and chemokines to accumulate and activate other immunocompetent cells, such as NK cells and macrophages, for innate immune responses. At the same time, LCs migrate to the draining lymph nodes to induce the differentiation and activation of both $CD4^+$ and $CD8^+$ naive T cells into cytokine-producing effector T cells, then induce the following adaptive immune responses (5,7).

Previous studies on LCs were mostly established using Langerhans cell-like cells generated from bone marrow- or monocyte-derived dendritic cells, not by using genuine skin-resident LCs. To overcome these limitations, we successfully isolated and purified human epidermal LCs from the foreskin using the density gradient centrifugation and immunomagnetic microbead methods. Using this isolation and purification technique, it was possible to obtain 86.72±1.23% purity and 96.12±1.45% viability of LCs cultured for 4 h, and 85.47±0.93% viability of LCs cultured for 48 h. Mitsui *et al* used the panning method and achieved more than 95% purity of LCs from murine skin (8). Flacher *et al* used an isolation technique based on two consecutive density gradients and obtained 75.0±8.4% purity of human epidermal LCs. They then further purified the LCs by positive selection using anti-CD1a MACS and obtained more than 95% purity of LCs. However, viability assays showed that only 69.1±10.1% of LCs cultured for 24 h were viable and less than 50% of LCs were still viable after 48 h of culture (9). The establishment of an isolation and purification method would greatly contribute to our ability to successfully conduct the next series of experiments dealing with human LCs.

The pattern of TLR expression on cultured LCs has yet to be fully analyzed. Mitsui *et al* demonstrated TLR2, TLR4 and TLR9 mRNA in fresh isolated LCs obtained from murine skin. Mitsui *et al* also demonstrated TLR2 protein expression by Western blotting and TLR4 protein expression by flow cytometry (8). TLR3 mRNA was expressed in both fresh and 48-h cultured murine LCs (10). Flacher *et al* showed for the first time that freshly isolated and purified LCs from human skin express mRNA encoding TLR1, TLR2, TLR3, TLR5, TLR6 and TLR10 (9). In this study, we analyzed the expression pattern of the 10 identified human TLRs in cultured LCs using RT-PCR. The results showed that 48-h cultured human LCs expressed TLR2, TLR4, TLR5, TLR7, TLR8 and TLR9, while TLR1, TLR3 and TLR10 were undetectable. We next demonstrated TLR2 and TLR4 protein expression in cultured human LCs by Western blot analysis. The pattern of TLR expression varies markedly depending on the species. These conflicting reports make the expression of 10 TLRs in human LCs unclear. Some discrepancies remain between our and other studies, which likely result from differences in culture time or isolation and purification methods.

Solar UV light comprises UVB (wavelength 290-320 nm) and UVA (wavelength 320-400 nm) light. UVB hits the epidermis and, to a lesser extent, the upper part of the dermis, while UVA penetrates more deeply into human skin (11). The effects of UV light on the TLR expression and function of human LCs have never been examined. One study showed the increased transcription of the TLR2 and TLR4 genes in UVB-irradiated macrophages (12). Exposure to UV light induced clustering and internalization of cell surface receptors for IL-1 and epidermal growth factor. Exposure to UV light also activates the c-Jun amino-terminal protein kinase cascade, causing the induction of many target genes (13). Since the intracellular portion of TLRs shares homology with the cytoplasmic domain of the IL-1 receptor and the IL-1 receptor and TLR signal function through similar mechanisms, employing many of the same signal transduction molecules, we conclude that UV light also activates TLRs and downstream NF-κB and mitogen-activated protein kinase signaling molecules. Our results revealed increased mRNA and protein expression of the TLR2 and TLR4 genes in UV-irradiated human LCs. Upon engagement of a TLR by its ligand, TLR activation induced the activation of NF-κB and the c-Jun-NH2-terminal kinase, extracellular signal-regulated kinase 1 and 2, and p38 mitogen-activated protein kinases (MAPK) (3). The activation of TLR3 and TLR4 also activated IRF-3 in MyD88-independent pathways, then up-regulated the expression of IFN-inducible genes (14). In this study, we observed increased MAPK, NF-κB/p65 and IRF-3 protein levels after UV exposure. Next, we examined the mechanisms underlying the UV exposure-induced increased expression of MAPK, NF-κB/p65 and IRF-3. TLR2 and TLR4 gene expression in human LCs were knocked down using TLR2 and TLR4 siRNA. TLR2 and TLR4 siRNA prevented the

expression of MAPK and NF-κB/p65 induced by UV exposure, suggesting that the expression of MAPK and NF-κB induced by UV exposure is dependent on TLR2 and TLR4.

TLR activation may also induce the activation of IRF-3 in the MyD88-independent pathways. Our results showed that TLR2 siRNA had no significant effect on the expression of IRF-3, and that TLR4 siRNA partially decreased the expression of IRF-3 induced by UV exposure, suggesting that the expression of IRF-3 induced by UV exposure is partially dependent on TLR4, but not TLR2. We conclude that UV light induced the increased expression of IRF-3 by activating TLR4 and other TLRs. Additional studies are required to elucidate this finding.

TLRs are well documented as major initiators of innate immunity (15,16). Increasing evidence indicates that TLRs are cpable of directly or indirectly promoting adaptive immune responses, in particular T cell functions (17). TLR activation induces maturation of dendritic cells by increasing the expression levels of co-stimulatory molecules of CD40, CD80, CD86 and MHC proteins on dendritic cells, allowing the dendritic cells to more effectively activate T cells (18,19). Recent reports have indicated that TLRs on dendritic cells affect T cell diversity. Activation of TLR2 on human dendritic cells induces the release of IL-12 and IFN-γ, but not IL-10 and IL-4, generating a Th1 cytokine response (20,21). However, in other studies, the activation of TLR2 induced strong expression of Th2-associated effector molecules such as IL-13, IL-1β and GM-CSF, but low levels of Th1-associated cytokines such as IL-12, IFNα, IL-18 and IL-27 (22). The activation of TLR5 drives MyD88-dependent Th2-type immunity by promoting the secretion of IL-4 and IL-13, while the Th1-promoting cytokine IL-12 is not induced (23). Activation of TLRs also directly or indirectly influences regulatory T cell functions (24). Our study indicated that UV light activated TLR2 and TLR4 and the downstream signaling molecules MAPK, NF-κB/p65 and IRF-3. Thus, we propose that UV light promotes innate and adaptive immunity, including the maturation of LCs, T cell diversity and regulatory T cell functions through the activation of TLRs on LCs. However, this hypothesis remains to be further investigated in future studies.

In conclusion, we demonstrated increased TLR2 and TLR4 expression after UV exposure in human cultured LCs. We also found that increased MAPK and NF-κB/p65 expression was dependent on TLR2 and TLR4, while increased IRF-3 expression was partially dependent on TLR4, after UV exposure. LCs and TLRs play a significant role in innate and adaptive immunity; we hypothesize that UV light promotes innate and adaptive immunity, including the maturation of LCs, T cell diversity and regulatory T cell functions, through the activation of TLRs on LCs. The present findings contribute to the understanding of the role of UV light in skin immune responses.

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