Journal of Microbiology, Immunology and Infection (2016) 49, 409–416

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

ORIGINAL ARTICLE

Tacrolimus suppresses atopic dermatitis-associated cytokines and chemokines in monocytes

Kai-Ting Chang [a,b,c,d,h], Hugo You-Hsien Lin [e,f,h], Chang-Hung Kuo [a,b,c,*,h], Chih-Hsing Hung [a,b,c,d,g,*,h]

[a] *Department of Pediatrics, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan*
[b] *Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*
[c] *Department of Pediatrics, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan*
[d] *Department of Pediatrics, Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan*
[e] *Division of Nephrology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan*
[f] *Department of Internal Medicine, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan*
[g] *Department of Pediatrics, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan*

Received 15 January 2014; received in revised form 14 June 2014; accepted 24 July 2014
Available online 12 October 2014

KEYWORDS
atopic dermatitis;
chemokine;
cytokine;
tacrolimus

Background: Calcineurin inhibitors (CNIs) exhibit remarkable efficacy in atopic dermatitis (AD). Tacrolimus, one type of CNI, is prevalently used to treat AD. AD is a chronic inflammatory disease that exhibits predominant infiltration of T-helper type 2 (Th2) cell in the acute phase and a mixed Th1 and Th0 cell pattern in chronic lesions. Cytokines such as tumor necrosis factor-α (TNF-α), Th2-related chemokines [e.g., macrophage-derived chemokine (MDC)/CCL22 and I-309/CCL1], Th1-related chemokines [e.g., interferon γ-induced protein 10 (IP-10)/CXCL10], and neutrophil chemoattractant growth-related oncogene-α (GRO-α)/CXCL1 are involved in the pathogenesis of AD. However, whether tacrolimus modulates the expression

* Corresponding authors. Department of Pediatrics, Kaohsiung Medical University Hospital, Kaohsiung Medical University, 100 Tzyou First Road, Kaohsiung, Taiwan.
E-mail address: pedhung@gmail.com (C.-H. Hung).

[h] All authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jmii.2014.07.006

of AD-associated cytokines and chemokines remains unknown. The intracellular mechanisms of tacrolimus are also unclear.
Methods: Human monocytic cell line THP-1 cells were pretreated with tacrolimus and stimulated with lipopolysaccharide (LPS). The MDC, I-309, IP-10, GRO-α, and TNF-α concentrations of the cell supernatants were measured using enzyme-linked immunosorbent assay. Intracellular signaling was investigated using the Western blot analysis.
Results: Tacrolimus suppressed the expression of MDC, IP-10, I-309, GRO-α, and TNF-α in LPS-stimulated THP-1 cells in a dose- and time-dependent manner. All three mitogen-activated protein kinase (MAPK) inhibitors and the nuclear factor-κB inhibitor suppressed LPS-induced MDC, I-309, and TNF-α expressions in THP-1 cells. Only MAPK inhibitors suppressed LPS-induced expression of IP-10 and GRO-α. Tacrolimus suppressed the LPS-induced phosphorylation of MAPK-extracellular signal-related kinase (ERK).
Conclusion: Tacrolimus suppressed LPS-induced MDC, I-309, IP-10, GRO-α, and TNF-α expressions in monocytes through the MAPK-ERK pathway; thus, tacrolimus may yield therapeutic efficacy by modulating AD-associated cytokines and chemokines.

Introduction

Atopic dermatitis (AD) is a chronic inflammatory disease in children. It is highly pruritic and frequently seen in infants and children, particularly among patients with atopy. The pathophysiology of AD is complex, including skin barrier dysfunction,[1] immune responses to allergens, the impairment of antimicrobial defense, and interactions among susceptible genes and the environment.[2] The amplification cycle of atopic skin inflammation contributes to the disease.[3] Patient scratching induces mechanical trauma, resulting in the production of proinflammatory cytokines such as interleukin-1α (IL-1α), IL-1β, tumor necrosis factor-α (TNF-α), and granulocyte-macrophage colony-stimulating factor (GM-CSF). Subsequently, the chemoattractants and adhesion molecules are upregulated, directing the recruitment of leukocytes toward the skin lesions.[4] Both T-helper cell type 2 (Th2)-related and T-helper cell type 1 (Th1)-related cytokines contribute to the biphasic inflammatory phase in AD, including an initial Th2-dominant phase preceding a chronic Th0- and Th1-dominant phase.[5] Compared with the skin and blood of healthy patients, AD patients exhibit increased expression of Th2-cytokines IL-4, IL-5, IL-13, and decreased expression of Th1-cytokine interferon-γ (IFN-γ) in skin lesions and peripheral blood during the acute stage of the disease.[2] Chronic lichenified AD skin lesions have fewer IL-4 and IL-13 expressions but significantly greater IL-5, GM-CSF, IL-12, and IFN-γ expressions than acute AD skin lesions.[6] IL-12 switches initial Th2 immune responses to Th1 immune responses in the transformation from the acute to the chronic phase of AD.[2,6,7] Tissue damages among AD lesions might release neutrophil chemoattractants. The expression of neutrophil chemoattractants contributes to the recruitment, activation, and proliferation of neutrophils among AD lesions.[8]

The migration of the inflammatory cells is regulated by the interaction of chemokines and chemokine receptors.[9] AD patients have a high percentage of $CD4^+$ T lymphocyte bearing CCR4 receptors, which can bind to Th2-related chemokines macrophage-derived chemokine (MDC) and thymus and activation-regulated chemokine (TARC).[10] Subsequently, these CCR4-containing Th2 lymphocytes are attracted to the AD lesions.[11] In patients diagnosed with AD, the serum levels of TARC and MDC are increased and positively correlated with severity.[12] In addition, I-309/CCL1 is a Th2-related chemokine. I-309 is increased in AD patients, particularly during the acute phase and can be a useful marker for distinguishing AD from psoriasis.[13] IFN-γ-induced protein 10 (IP-10)/CXCL10 is a Th1 cell-related chemokine and its production can be stimulated by the IFN-γ produced in Th1 cells. IP-10 consequently attracts and recruits additional activated lymphocytes.[14] The chemotactic action of IP-10 plays a role in both innate and adaptive immunity.[15] Growth-related oncogene-α (GRO-α)/CXCL1 is a powerful neutrophil chemoattractant, which also plays a crucial role in chronic inflammation and several autoimmune diseases.[16] TNF-α is a proinflammatory cytokine and is considered a potential biomarker of AD.[17] TNF-α levels rapidly increase after mechanical trauma and skin barrier disruption, inducing the production of chemoattractants and adhesion molecules, and subsequently facilitating the recruitment and proliferation of leukocytes in the skin.[4]

Calcineurin inhibitors (CNIs), such as tacrolimus, are widely used as topical agents to treat AD by modulating T-cell activity. Tacrolimus inhibits the phosphatase activity of calcineurin, blocks nuclear translocation of the transcription factor-nuclear factor of activated T cells (NFAT), and inhibits the production of cytokines.[18]

According to the results of pharmacokinetic studies, the systemic exposure to tacrolimus after ointment application was low and highly variable. When the size of the body surface area treated by tacrolimus increased, the systemic exposure to tacrolimus increased proportionally.[19,20] In the study on a group of young children treated with topical 0.03% tacrolimus two times daily for 2 years, Mandelin et al[21] found that the concentration of tacrolimus was less than 1.0 ng/mL in 98% of blood samples. Topically applied tacrolimus has the highest absorption in the initial stage of AD when the skin barrier is extremely impaired. When the disease improves and epidermal barrier recovers, penetration of tacrolimus into the skin decreases.[22] Significant

absorption of topical tacrolimus into circulation was found in diseases with profound skin barrier damage (e.g., Netherton syndrome, lamellar ichthyosis, pyoderma gangrenosum). In patients with severe skin barrier dysfunction, the serum levels of tacrolimus after topical application could reach 20—40 ng/mL.[23]

T-cell subpopulations, mast cells, and eosinophils participate in the immunological mechanisms of AD.[24] Monocytes also contribute to the initiation and progression of this disease. The high-affinity receptor for immunoglobulin E (FcεRI) is upregulated in atopic monocytes in patients with AD. The FcεRI-bearing monocytes may be recruited into the dermis by chemotactic signals. They differentiate into macrophages, tissue-specific dendritic cells such as Langerhans cells (LCs), and inflammatory dendritic epidermal cells (IDECs).[25,26] Macrophages, LCs, and IDECs present antigens to T cells and initiate T-cell-mediated immune responses.[27] Besides, monocytes from most AD patients show a hyperactivated status.[28] They are activated through the recognition of microbial molecules by Toll-like receptors (TLRs), and subsequently produce large amounts of proinflammatory cytokines. The well-known microbial ligands are peptidoglycan and lipoteichoic acid for TLR2, and lipopolysaccharide (LPS) for TLR4.[29]

The suppressive effect of tacrolimus on the expression of cytokines in T cells has been widely studied. However, whether tacrolimus modulates the expression of Th1- and Th2-related chemokines, neutrophil chemoattractant, and TNF-α in monocytes remains unknown. In this study, we investigated the modulatory effects of tacrolimus on TNF-α, Th1- and Th2-related chemokines, and neutrophil chemoattractant expressions in monocytes. In addition, we explored the detailed mechanisms about the effects of tacrolimus on the expressions of these LPS-induced cytokines and chemokines in monocytes.

Materials and methods

Cell preparation

The human monocytic cell line THP-1 cells (American Type Culture Collection, Rockville, MD, USA) were cultured in Roswell Park Memorial Institute-1640 medium (Sigma Chemical Co., St. Louis, MO, USA) supplemented with 10% fetal bovine serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin at 37°C, and 5% CO_2 in a humidified incubator. The cells were centrifuged at 1500 rpm for 5 minutes and resuspended in fresh medium in 24-well plates at a concentration of 10^6/mL for 24 hours prior to experimental use. The cells were pretreated with tacrolimus (Sigma Chemical Co.) at various concentrations (0.5 ng/mL, 5 ng/mL, and 50 ng/mL) for 2 hours before LPS stimulation (0.2 μg/mL; *Escherichia coli*; Sigma Chemical Co.). The cell supernatants were collected 24 hours and 48 hours after LPS stimulation.

Cell viability assay

The cytotoxic effects of tacrolimus on THP-1 cells at various concentrations (0.5 ng/mL, 5 ng/mL, and 50 ng/mL) were investigated using the WST-1 Cell Viability and Proliferation Assay Kit (Roche Applied Science, Mannheim, Germany) according to the manufacturer's instructions.

Enzyme-linked immunosorbent assay

The MDC, I-309, IP-10, GRO-α, and TNF-α levels of the cell supernatants were determined using commercially available enzyme-linked immunosorbent assay-based assay systems (R&D Systems, Minneapolis, MN, USA) according to the protocols recommended by the manufacturer. The samples were examined with a Dynatech MRX plate reader at 450 nm and 540 nm using Revelation software (Dynatech Laboratories Ltd., Chantilly, VA, USA).

Western blotting and c-Jun mitogen-activated protein kinase activity assay

After pretreatment with or without tacrolimus (5 ng/mL and 50 ng/mL) for 2 hours, the cells were stimulated using LPS (0.2 μg/mL) for 1 hour and were lysed with equal volumes of ice-cold lysis buffer. After centrifuging at 13,000*g* for 15 minutes, cell lysates were used to determine the protein concentration using a BCA Protein Assay Reagent Kit (Thermo Fisher Scientific, Waltham, MA, USA); the concentration of each sample was equalized using a lysis buffer. Equal amounts of cell lysates were analyzed using Western blot with anti-mitogen-activated protein kinase [anti-MAPKs; p38, extracellular signal-related kinase (ERK), and c-Jun N-terminal kinase (JNK)], anti-phospho-MAPK (phospho-p38, phospho-ERK, and phospho-JNK), anti-p65k, and anti-phospho-p65 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The immunoreactive bands were visualized using a horseradish peroxidase-conjugated secondary antibody and an enhanced chemiluminescence system (Amersham Pharmacia Biotech, Piscataway, NJ, USA).

Statistical analyses

All data are presented as mean ± standard deviation. The variations between the experimental and control groups were analyzed using the Mann—Whitney *U* test. A p value <0.05 was considered indicative of significant between-group variations.

Results

Tacrolimus suppressed LPS-induced MDC and I-309 expressions in THP-1 cells

We investigated how tacrolimus affected the expression of Th2-related chemokines. Fig. 1 indicates that the LPS-induced MDC expression in THP-1 cells was inhibited by tacrolimus in a time- and dose-dependent manner (0.5—50 ng/mL after 24 hours and 48 hours of LPS stimulation; Fig. 1A and B). Tacrolimus alone did not affect MDC expression. Similarly, the LPS-induced I-309 expression in THP-1 cells was also inhibited by tacrolimus in a time- and dose-dependent manner (0.5 ng/mL after 48 hours of LPS stimulation; 5—50 ng/mL after 24 hours and 48 hours of LPS

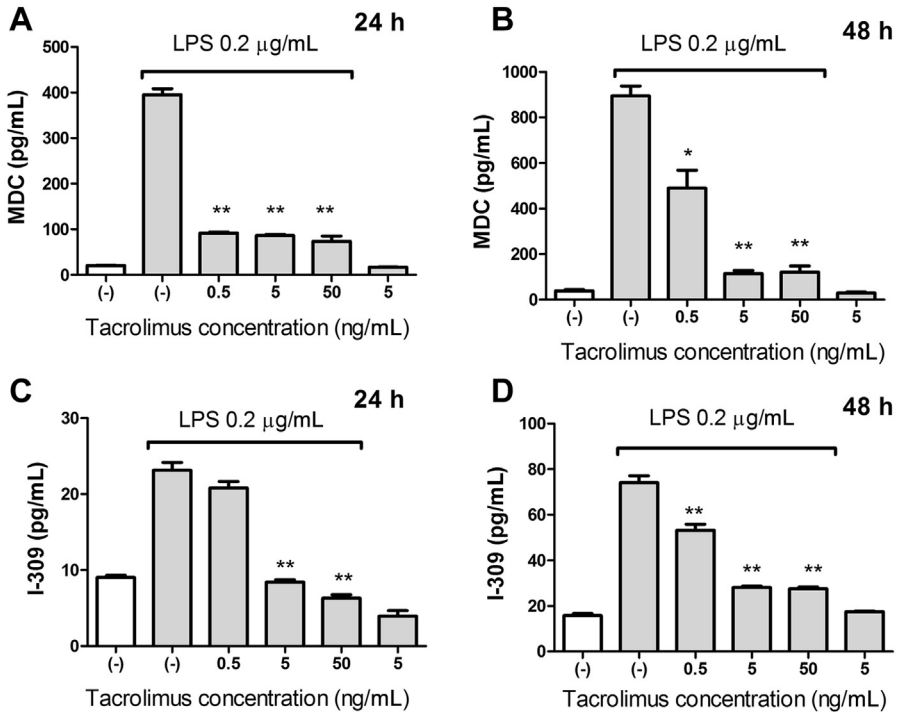

Figure 1. Tacrolimus suppressed lipopolysaccharide (LPS)-induced Th2-related chemokines expression in human monocytes. Tacrolimus suppressed macrophage-derived chemokine (MDC) expression in THP-1 cells after (A) 24 hours and (B) 48 hours of LPS stimulation. Results presented are the mean ± SD of independent experiments. *$p < 0.01$ and **$p < 0.005$ between groups of LPS treatment with and without tacrolimus pretreatment. Tacrolimus suppressed LPS-induced I-309 expression in THP-1 cells after (C) 24 hours and (D) 48 hours of LPS stimulation. Results presented are the mean ± SD of independent experiments. *$p < 0.01$ and **$p < 0.005$ between groups treated with LPS with and without tacrolimus pretreatment. SD = standard deviation.

stimulation; Fig. 1C and D). Tacrolimus alone did not affect I-309 expression.

Tacrolimus suppressed LPS-induced IP-10 expression in THP-1 cells

We subsequently investigated how tacrolimus affected the Th1-related chemokine, IP-10. Tacrolimus (5—50 ng/mL) suppressed LPS-induced IP-10 expression in THP-1 cells after 24 hours and 48 hours of LPS stimulation. Tacrolimus alone did not affect IP-10 expression (Fig. 2A and B).

Tacrolimus suppressed LPS-induced GRO-α expression in THP-1 cells

We also investigated how tacrolimus affected the chemoattractant GRO-α expression. Tacrolimus (5—50 ng/mL) suppressed the LPS-induced GRO-α expression in THP-1

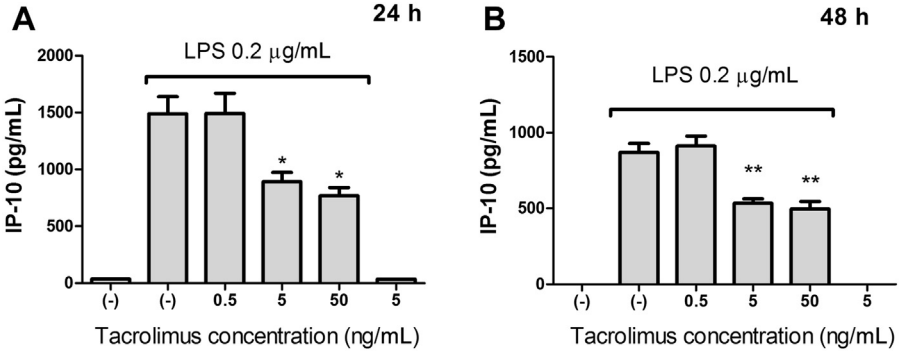

Figure 2. Tacrolimus suppressed lipopolysaccharide (LPS)-induced Th1-related chemokine expression in human monocytes. Tacrolimus suppressed interferon γ-induced protein 10 (IP-10) production in THP-1 cells after (A) 24 hours and (B) 48 hours of LPS stimulation. Results presented are the mean ± standard deviation of independent experiments. *$p < 0.01$ and **$p < 0.005$ between groups treated with LPS with and without tacrolimus pretreatment.

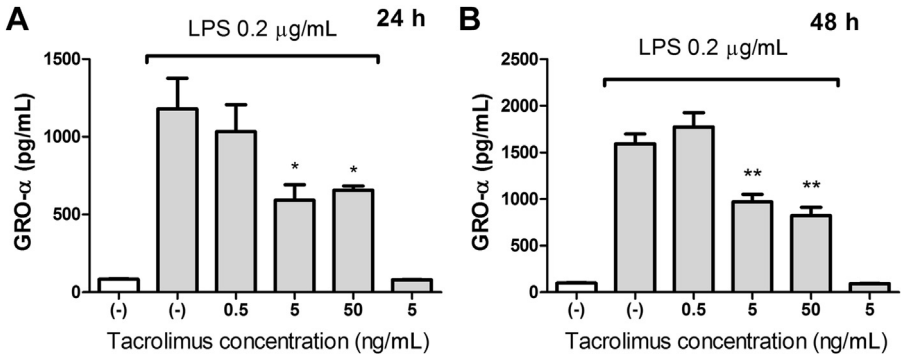

Figure 3. Tacrolimus suppressed lipopolysaccharide (LPS)-induced neutrophil chemoattractant expression in human monocytes. Tacrolimus suppressed growth-related oncogene-α (GRO-α) expression in THP-1 cells after (A) 24 hours and (B) 48 hours of LPS stimulation. Results presented are the mean ± standard deviation of independent experiments. *$p < 0.05$ and **$p < 0.005$ between groups treated with LPS with and without tacrolimus pretreatment.

cells after 24 hours and 48 hours of LPS stimulation (Fig. 3A and B). Tacrolimus alone did not affect the GRO-α expression.

Tacrolimus suppressed LPS-induced TNF-α expression in THP-1 cells

We subsequently investigated how tacrolimus affects the LPS-induced TNF-α production in THP-1 cells. Fig. 4 shows that LPS-induced TNF-α expression in THP-1 cells was inhibited by tacrolimus (0.5 ng/mL after 48 hours of stimulation; 5—50 ng/mL after 24 hours and 48 hours of stimulation). Tacrolimus alone did not affect the TNF-α expression (Fig. 4A and B).

Tacrolimus exhibited no cytotoxic effect on THP-1 cells

Because tacrolimus exerted suppressive effects on MDC, I-309, IP-10, GRO-α, and TNF-α, we investigated whether the suppressive effect of tacrolimus on the expression of chemokines and cytokines resulted from its cytotoxic effect on THP-1 cells. A cell viability test indicated that tacrolimus exerted no cytotoxic effect on THP-1 cells (Fig. 5).

Tacrolimus suppressed LPS-induced phosphorylated ERK expression in THP-1 cells

The stimulation of TLRs activates MyD88-dependent pathways, including nuclear factor-κB (NF-κB) transcription factors, and the MAPKs ERK, p38, and JNK pathways.[30] We previously reported that the LPS-induced MDC,[31] I309,[31,32] IP-10,[32,33] GRO-α,[34] and TNF-α[35] expressions in monocytes involved the MAPK and NF-κB pathways. We then investigated whether tacrolimus suppressed LPS-induced MDC, I309, IP-10, GRO-α, and TNF-α expressions through the MAPK and NF-κB pathways. We observed that tacrolimus (5 ng/mL and 50 ng/mL) suppressed the LPS-induced phosphorylation of MAPK-ERK (Fig. 6A), but not that of MAPK-p38 (Fig. 6B) or JNK (Fig. 6C), and exerted no effect on the LPS-induced phosphorylation of NF-κB-p65 (Fig. 6D) in THP-1 cells. Densitometry analysis results are shown below each Western blot data. Therefore, tacrolimus may suppress LPS-induced MDC, I309, IP-10, GRO-α, and TNF-α expressions through the MAPK-ERK pathway.

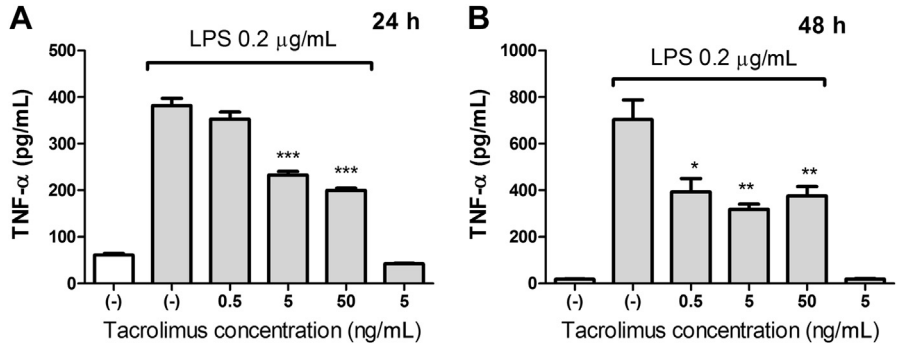

Figure 4. Tacrolimus suppressed lipopolysaccharide (LPS)-induced proinflammatory cytokine expression in human monocytes. Tacrolimus suppressed LPS-induced tumor necrosis factor-α (TNF-α) production in monocytes. After (A) 24 hours and (B) 48 hours of LPS stimulation, tacrolimus suppressed TNF-α expression in a dose-dependent manner. Results presented are the mean ± standard deviation of independent experiments. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.005$ between groups treated with LPS with and without tacrolimus pretreatment.

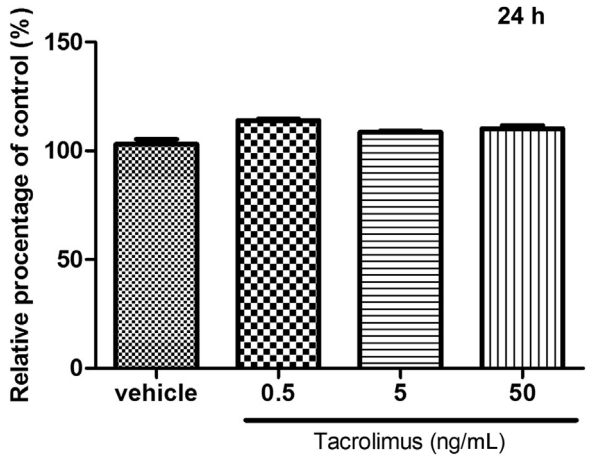

Figure 5. Tacrolimus had no cytotoxic effect on human monocytes. The treatment with tacrolimus at the concentration of 0.5 ng/mL, 5 ng/mL, and 50 ng/mL did not affect the cell viability in THP-1 cells.

Discussion

Recent clinical studies have demonstrated that topical CNIs, such as tacrolimus and pimecrolimus, were alternative drugs for treating moderate to severe AD that is unresponsive to first-line medicines. The mechanism of tacrolimus in treating AD involves inhibiting the activation of T lymphocytes and their derived Th2-related cytokines (IL-3, IL-4, IL-5, and IL-13) and Th1-related cytokines (IFN-γ, IL-12, IL-11, IL-18, GM-CSF, and TGF-β).[36] Tacrolimus binds to the FK506-binding protein and the complex inhibits calcineurin phosphatase and T-lymphocytes activation. Tacrolimus also down-regulates the synthesis of inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, by T cells.[37] Recent evidence suggests that chemokines are involved in AD. Higher amounts of Th2-related chemokines, TARC and MDC, are present in the plasma of AD patients, and their levels are positively correlated with disease severity.[38,39] The expression of I-309 is significantly high in the acute lesional skin of AD patients.[40] Th1-related chemokine IP-10 executes a powerful

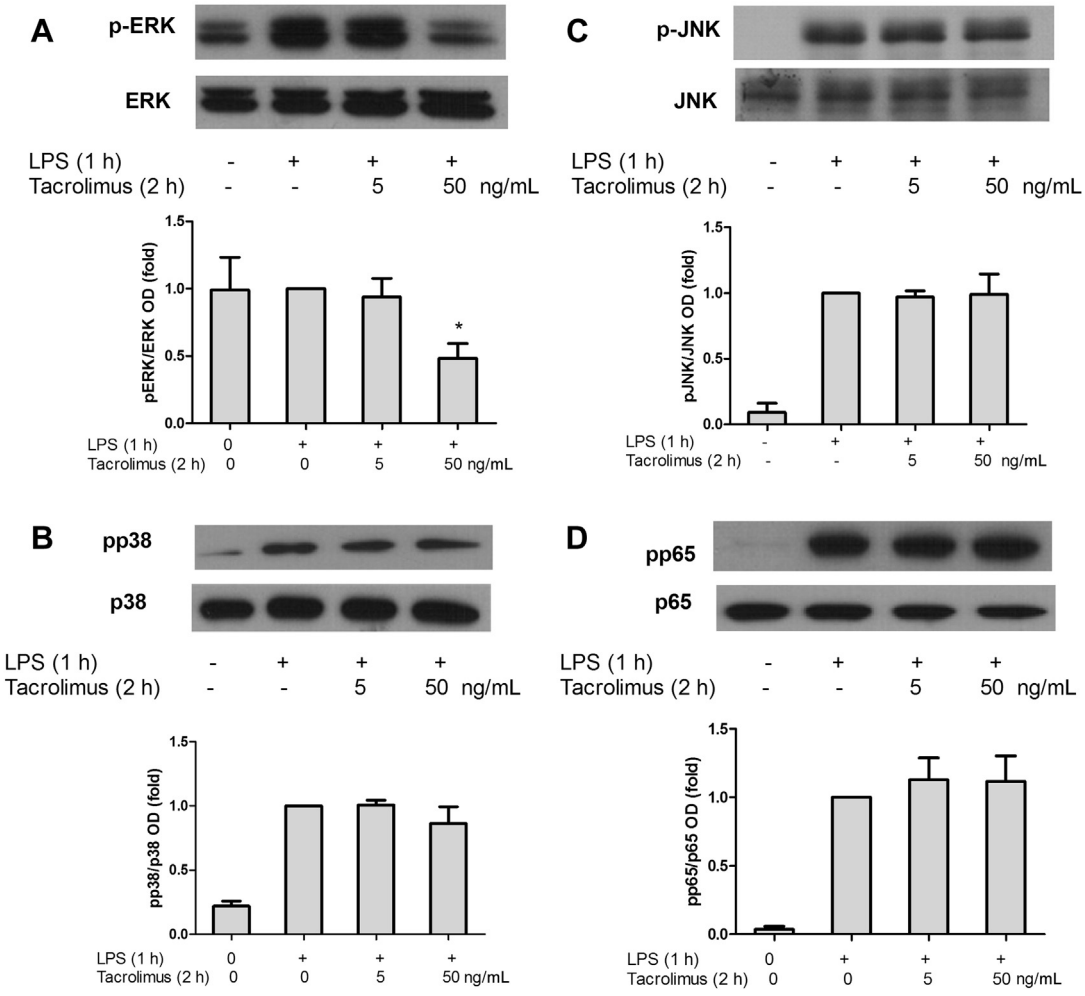

Figure 6. Tacrolimus modulates the MAPK pathway in human monocytes. Tacrolimus suppressed lipopolysaccharide (LPS)-induced phosphorylation of (A) MAPK-ERK but not (B) MAPK-p38, or (C) MAPK-JNK. Tacrolimus had no effect on LPS-induced phosphorylation of (D) NF-κB-p65 expression in THP-1 cells. Densitometry analysis results are shown below each Western blot. Results presented are the mean $\pm$ standard deviation of three independent experiments. For Western blotting analyses, one experiment representative of three is shown. *$p \leq 0.05$ compared with LPS-treated cells. ERK = extracellular signal-related kinase; JNK = c-Jun N-terminal kinase; MAPK = mitogen-activated protein kinase; NF-κB = nuclear factor κB.

chemotactic action on activated lymphocytes toward inflammatory sites, particularly during infection.[15] The chemoattractant GRO-α can trigger neutrophil and T-lymphocyte chemotaxis, particularly after tissue damage.[16] TNF-α, as a proinflammatory cytokine, plays a key role in the inflammation of AD.[17] In this study, we showed for the first time in the literature that tacrolimus can affect the function of monocytes by suppressing the expression of cytokines and chemokines. These findings suggest that in addition to inhibiting the activation of T cells, tacrolimus may exert therapeutic effects on AD by modulating the expression of AD-related cytokines and chemokines in monocytes.

Chemokines are crucial for the attraction and activation of various types of leukocytes and are also involved in the inflammatory processes of various acute and chronic inflammatory diseases. For example, the expression of Th2-related chemokine, MDC, is increased in the exhaled breath condensate and serum of asthmatic patients.[41] The expression of Th1-related chemokine, IP-10, is also increased in the airways of asthmatic patients.[42] TNF-α mediates the acute phase reaction of inflammation by activating T lymphocytes and recruiting additional inflammatory cells into target tissues during inflammatory diseases and the post-transplantation rejection effect. Medicines, such as tacrolimus, that modulate the expression of chemokines and cytokines can potentially treat inflammatory diseases.

Several studies have shown that tacrolimus not only inhibits NFAT activation by blocking calcineurin, but also affects the activation of MAPK-signaling pathways by suppressing the phosphorylation of MAPK-p38 and JNK in primary human T lymphocytes.[43] Similarly, in this study, we determined that tacrolimus inhibited the activation of the MAPK pathway by suppressing the LPS-induced phosphorylation of MAPK-ERK, but not that of MAPK-p38/JNK, and also did not have any effect on the phosphorylation of NF-κB-p65. Based on these findings and our previous reports,[31–35] tacrolimus may interfere with the MAPK-ERK pathway in monocytes to modulate the expression of chemokines and cytokines. The suppressive effect of tacrolimus in MAPK-ERK may indicate its potential to inhibit other MAPK-related inflammatory processes.[44]

In conclusion, in this study, we determined that tacrolimus suppressed the expression of AD-related chemokines and TNF-α in monocytes. According to our review of relevant research, this study is the first to suggest that tacrolimus exerts therapeutic effects by modulating the expression of AD-related chemokines and TNF-α. We also determined that this effect was achieved by suppressing the MAPK-ERK pathway. By inhibiting the expression of chemokines and TNF-α in monocytes, tacrolimus may exhibit therapeutic potential in treating other inflammatory disorders.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

This study was supported by a grant from Kaohsiung Municipal Ta-Tung Hospital, Kaoshiung, Taiwan (grant no. kmtth-101-006) and two grants from Kaohsiung Municipal Hsiao-Kang Hospital, Kaoshiung, Taiwan (grant nos. Kmhk-102-007 and Kmhk-102-010).

References

1. Cork MJ, Robinson DA, Vasilopoulos Y, Ferguson A, Moustafa M, MacGowan A, et al. New perspectives on epidermal barrier dysfunction in atopic dermatitis: gene-environment interactions. *J Allergy Clin Immunol* 2006;**118**:3–21. quiz 2–3.
2. Leung DY, Boguniewicz M, Howell MD, Nomura I, Hamid QA. New insights into atopic dermatitis. *J Clin Invest* 2004;**113**:651–7.
3. Mihara K, Kuratani K, Matsui T, Nakamura M, Yokota K. Vital role of the itch-scratch response in development of spontaneous dermatitis in NC/Nga mice. *Br J Dermatol* 2004;**151**:335–45.
4. Homey B, Steinhoff M, Ruzicka T, Leung DY. Cytokines and chemokines orchestrate atopic skin inflammation. *J Allergy Clin Immunol* 2006;**118**:178–89.
5. Terui T. Analysis of the mechanism for the development of allergic skin inflammation and the application for its treatment: overview of the pathophysiology of atopic dermatitis. *J Pharmacol Sci* 2009;**110**:232–6.
6. Hamid Q, Boguniewicz M, Leung DY. Differential *in situ* cytokine gene expression in acute versus chronic atopic dermatitis. *J Clin Invest* 1994;**94**:870–6.
7. Fonacier LS, Dreskin SC, Leung DY. Allergic skin diseases. *J Allergy Clin Immunol* 2010;**125**:S138–49.
8. Choy DF, Hsu DK, Seshasayee D, Fung MA, Modrusan Z, Martin F, et al. Comparative transcriptomic analyses of atopic dermatitis and psoriasis reveal shared neutrophilic inflammation. *J Allergy Clin Immunol* 2012;**130**:1335.e5–1343.e5.
9. Moser B, Wolf M, Walz A, Loetscher P. Chemokines: multiple levels of leukocyte migration control. *Trends Immunol* 2004;**25**:75–84.
10. Pastore S, Mascia F, Mariotti F, Dattilo C, Girolomoni G. Chemokine networks in inflammatory skin diseases. *Eur J Dermatol* 2004;**14**:203–8.
11. Panina-Bordignon P, Papi A, Mariani M, Di Lucia P, Casoni G, Bellettato C, et al. The C-C chemokine receptors CCR4 and CCR8 identify airway T cells of allergen-challenged atopic asthmatics. *J Clin Invest* 2001;**107**:1357–64.
12. Leung TF, Ma KC, Hon KL, Lam CW, Wan H, Li CY, et al. Serum concentration of macrophage-derived chemokine may be a useful inflammatory marker for assessing severity of atopic dermatitis in infants and young children. *Pediatr Allergy Immunol* 2003;**14**:296–301.
13. Gros E, Bussmann C, Bieber T, Förster I, Novak N. Expression of chemokines and chemokine receptors in lesional and nonlesional upper skin of patients with atopic dermatitis. *J Allergy Clin Immunol* 2009;**124**:753.e1–760.e1.
14. Campbell JD, Gangur V, Simons FE, HayGlass KT. Allergic humans are hyporesponsive to a CXCR3 ligand-mediated Th1 immunity-promoting loop. *FASEB J* 2004;**18**:329–31.
15. Liu M, Guo S, Hibbert JM, Jain V, Singh N, Wilson NO, et al. CXCL10/IP-10 in infectious diseases pathogenesis and potential therapeutic implications. *Cytokine Growth Factor Rev* 2011;**22**:121–30.
16. Bechara C, Chai H, Lin PH, Yao Q, Chen C. Growth related oncogene-alpha (GRO-alpha): roles in atherosclerosis, angiogenesis and other inflammatory conditions. *Med Sci Monit* 2007;**13**:RA87–90.
17. Behniafard N, Gharagozlou M, Farhadi E, Khaledi M, Sotoudeh S, Darabi B, et al. TNF-alpha single nucleotide polymorphisms in atopic dermatitis. *Eur Cytokine Netw* 2012;**23**:163–5.

18. Vicari-Christensen M, Repper S, Basile S, Young D. Tacrolimus: review of pharmacokinetics, pharmacodynamics, and pharmacogenetics to facilitate practitioners' understanding and offer strategies for educating patients and promoting adherence. *Prog Transplant* 2009;**19**:277—84.
19. Harper J, Smith C, Rubins A, Green A, Jackson K, Zigure S, et al. A multicenter study of the pharmacokinetics of tacrolimus ointment after first and repeated application to children with atopic dermatitis. *J Invest Dermatol* 2005;**124**:695—9.
20. Rubins A, Gutmane R, Valdmane N, Stevenson P, Foster C, Undre N. Pharmacokinetics of 0.1% tacrolimus ointment after first and repeated application to adults with moderate to severe atopic dermatitis. *J Invest Dermatol* 2005;**125**:68—71.
21. Mandelin JM, Rubins A, Remitz A, Cirule K, Dickinson J, Ho V, et al. Long-term efficacy and tolerability of tacrolimus 0.03% ointment in infants:* a two-year open-label study. *Int J Dermatol* 2012;**51**:104—10.
22. Rustin MH. The safety of tacrolimus ointment for the treatment of atopic dermatitis: a review. *Br J Dermatol* 2007;**157**:861—73.
23. Allen A, Siegfried E, Silverman R, Williams ML, Elias PM, Szabo SK, et al. Significant absorption of topical tacrolimus in 3 patients with Netherton syndrome. *Arch Dermatol* 2001;**137**:747—50.
24. Mu Z, Zhao Y, Liu X, Chang C, Zhang J. Molecular biology of atopic dermatitis. *Clin Rev Allergy Immunol* 2014 Apr 9. Epub, http://dx.doi.org/10.1007/s12016-014-8415-1.
25. Novak N. New insights into the mechanism and management of allergic diseases: atopic dermatitis. *Allergy* 2009;**64**:265—75.
26. Novak N, Allam P, Geiger E, Bieber T. Characterization of monocyte subtypes in the allergic form of atopic eczema/-dermatitis syndrome. *Allergy* 2002;**57**:931—5.
27. Werfel T. The role of leukocytes, keratinocytes, and allergen-specific IgE in the development of atopic dermatitis. *J Invest Dermatol* 2009;**129**:1878—91.
28. Mandron M, Ariès MF, Boralevi F, Martin H, Charveron M, Taieb A, et al. Age-related differences in sensitivity of peripheral blood monocytes to lipopolysaccharide and *Staphylococcus aureus* toxin B in atopic dermatitis. *J Invest Dermatol* 2008;**128**:882—9.
29. Kadowaki N, Ho S, Antonenko S, Malefyt RW, Kastelein RA, Bazan F, et al. Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J Exp Med* 2001;**194**:863—9.
30. Barton GM, Medzhitov R. Toll-like receptor signaling pathways. *Science* 2003;**300**:1524—5.
31. Huang CH, Chu YT, Kuo CH, Wang WL, Hua YM, Lee MS, et al. Effect of procaterol on Th2-related chemokines production in human monocyte and bronchial epithelial cells. *Pediatr Pulmonol* 2010;**45**:977—84.
32. Tsai MK, Jan RL, Lin CH, Kuo CH, Yang SN, Chen HN, et al. Suppressive effects of imidapril on Th1- and Th2-related chemokines in monocytes. *J Investig Med* 2011;**59**:1141—6.
33. Hung CH, Suen JL, Hua YM, Chiang W, Chang HC, Chen CN, et al. Suppressive effects of ketotifen on Th1- and Th2-related chemokines of monocytes. *Pediatr Allergy Immunol* 2007;**18**:378—84.
34. Huang CH, Jan RL, Kuo CH, Chu YT, Wang WL, Lee MS, et al. Natural flavone kaempferol suppresses chemokines expression in human monocyte THP-1 cells through MAPK pathways. *J Food Sci* 2010;**75**:H254—9.
35. Wang WL, Kuo CH, Chu YT, Huang CH, Lam KP, Huang SK, et al. Prostaglandin I(2) analogues suppress TNF-alpha expression in human monocytes via mitogen-activated protein kinase pathway. *Inflamm Res* 2011;**60**:655—63.
36. Carr WW. Topical calcineurin inhibitors for atopic dermatitis: review and treatment recommendations. *Paediatr Drugs* 2013;**15**:303—10.
37. Dutta S, Ahmad Y. The efficacy and safety of tacrolimus in rheumatoid arthritis. *Ther Adv Musculoskelet Dis* 2011;**3**:283—91.
38. Shimada Y, Takehara K, Sato S. Both Th2 and Th1 chemokines (TARC/CCL17, MDC/CCL22, and Mig/CXCL9) are elevated in sera from patients with atopic dermatitis. *J Dermatol Sci* 2004;**34**:201—8.
39. Hashimoto S, Nakamura K, Oyama N, Kaneko F, Tsunemi Y, Saeki H, et al. Macrophage-derived chemokine (MDC)/CCL22 produced by monocyte derived dendritic cells reflects the disease activity in patients with atopic dermatitis. *J Dermatol Sci* 2006;**44**:93—9.
40. Kim HO, Cho SI, Chung BY, Ahn HK, Park CW, Lee CH. Expression of CCL1 and CCL18 in atopic dermatitis and psoriasis. *Clin Exp Dermatol* 2012;**37**:521—6.
41. Leung TF, Wong GW, Ko FW, Lam CW, Fok TF. Increased macrophage-derived chemokine in exhaled breath condensate and plasma from children with asthma. *Clin Exp Allergy* 2004;**34**:786—91.
42. Medoff BD, Sauty A, Tager AM, Maclean JA, Smith RN, Mathew A, et al. IFN-gamma-inducible protein 10 (CXCL10) contributes to airway hyperreactivity and airway inflammation in a mouse model of asthma. *J Immunol* 2002;**168**:5278—86.
43. Matsuda S, Shibasaki F, Takehana K, Mori H, Nishida E, Koyasu S. Two distinct action mechanisms of immunophilin-ligand complexes for the blockade of T-cell activation. *EMBO Rep* 2000;**1**:428—34.
44. Dong C, Davis RJ, Flavell RA. MAP kinases in the immune response. *Annu Rev Immunol* 2002;**20**:55—72.