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ORIGINAL ARTICLE

Tacrolimus suppresses atopic dermatitis-associated cytokines and chemokines in monocytes

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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

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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