

Mechanistic insights into topical tacrolimus for the treatment of atopic dermatitis

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Abstract

More than 15 years have passed since the clinical launch of topical tacrolimus for the treatment of atopic dermatitis. Its efficacy and safety have been clearly demonstrated in many global and domestic short-term and long-term clinical trials. Although the prolonged external application of steroids causes many adverse reactions including cutaneous atrophy, no such reactions occur with the use of topical tacrolimus. Therefore, the therapeutic guidelines recommend a combined topical treatment with tacrolimus and steroids. Tacrolimus is a potent immunosuppressant. However, recent studies have revealed its diverse action on the cardinal pathomechanisms of atopic dermatitis. In this review, we summarize the mechanistic role of tacrolimus in various aspects of allergic inflammation including mast cell activation, innate allergic response, pruritus, sensory nerve activation, and skin barrier dysfunction.

KEYWORDS

atopic dermatitis, cytokine, inflammation, itch, sensory nerve, skin barrier, tacrolimus

1 | INTRODUCTION

Tacrolimus, a calcineurin inhibitor, was discovered in the biomolecules produced by the actinomycete strain *Streptomyces tsukubaensis* in 1984. Tacrolimus mainly acts on T cells by inhibiting the activation of both the T helper (Th) 1 and Th2 phenotypes.¹ The production of Th1 [interferon- γ (IFN- γ) and interleukin (IL)-2] and Th2 (IL-4 and IL-5) cytokines is potently inhibited by tacrolimus.¹ Tacrolimus also suppresses histamine release from mast cells.^{2,3} Since its first launch in Japan in 1999, topical tacrolimus is currently approved and available in over 75 countries for the treatment of atopic dermatitis (AD). Topical tacrolimus has expanded the treatment options for AD, as it can be used on patients who are unresponsive to topical steroids and for patients who cannot receive steroids due to side effects.

The cardinal complaints of AD include pruritus, chronic inflammation, and dry skin with abnormal skin barrier function, which progress chronically with remissions and exacerbations.⁴ A Th2 cytokine-mediated allergic response is involved in its onset and progression, and scratching due to severe pruritus of the lesion destroys skin barrier function, resulting in a vicious cycle (the itch-scratch cycle) that further exacerbates symptoms.⁵ The current disease pathogenesis is supported by recent genomewide association studies (GWAS). Results of GWAS

conducted in Europe, China, and Japan have suggested that gene clusters associated with barrier function and Th2 inflammation function as the disease-susceptibility genes of AD.⁶ Moreover, the condition of patients with AD is significantly improved by administration of anti-IL-4 receptor α antibodies.⁷ Notably, IL-31, which is preferentially produced by Th2 cells, induces the itch sensation. The pruritus of AD is significantly suppressed by anti-IL-31 receptor A antibody administration.⁸

More than 15 years have passed since the launch of tacrolimus ointment, and many new insights regarding the control of AD progression have come to light. In this review, we summarize recent mechanistic findings of tacrolimus, including suppression of itching, inhibition of allergic inflammation, and improvement in skin barrier function. The advancements in our understanding of the pathomechanisms of AD warrant an investigation of new potential uses of topical tacrolimus in its treatment.

2 | SUPPRESSION OF PRURITUS BY TACROLIMUS

Pruritus significantly reduces quality of life in patients with AD.⁹ Because the itch-scratch cycle in AD exacerbates and sustains the symptoms, it is thought that skin lesions can be improved by avoiding

pruritus and the subsequent scratching behavior.^{5,10} Topical tacrolimus has been reported to alleviate pruritus in adult and pediatric patients with AD¹¹⁻¹⁴ and to contribute to the resolution of the itch-scratch cycle.¹²

2.1 | Suppression of sensory nerve activation by tacrolimus

Skin sensory nerves are distributed in the dermo-epidermal junction and the dermal compartment in healthy controls.^{15,16} In the lesional skin of AD, the sensory nerve fibers extend widely into the epidermis and are believed to be highly susceptible to external stimulation.¹⁵

In a murine model of contact hypersensitivity to picryl chloride, Kido et al¹⁷ reported that topical tacrolimus remarkably suppressed inflammatory cell infiltration and nerve fiber elongation, as well as substance P and calcitonin gene-related peptide expression. The above responses can also be suppressed using topical betamethasone valerate, but only topical tacrolimus significantly inhibited scratching behavior.¹⁷ Samukawa et al¹⁸ examined the reactions of a contact hypersensitivity model to dinitrofluorobenzene and demonstrated that topical tacrolimus significantly inhibited scratching behavior, sensory nerve elongation, nerve growth factor mRNA expression, and preprotachykinin mRNA expression, suggesting that the improvement in scratching behavior on using topical tacrolimus may be due to its inhibitory action on sensory nerve activation. Mite-induced murine dermatitis model also revealed that topical betamethasone valerate did not inhibit scratching behavior but it was inhibited by topical application of more potent clobetasol propionate. Topical tacrolimus reduced the scratching behavior as potently as topical clobetasol propionate.¹⁹

Some studies have pointed out the possibility that tacrolimus may directly act on the sensory nerves and inhibit their activation.^{20,21} Kim et al²⁰ performed immunohistologic examinations of expression levels of substance P, nerve growth factor, and neurotrophin-3 in the lesional skin of patients with AD at pre- and post-treatment with topical tacrolimus. The expression of sensory nerve-derived peptides decreased significantly after treatment.²⁰ Pereira et al²¹ cultured porcine dorsal root ganglion-derived sensory nerve cells and showed that tacrolimus administration causes sensory nerve cell desensitization and suppression of substance P release by inhibiting calcium influx into the nerve cells through transient receptor potential vanilloid 1 (TRPV1) phosphorylation. They suggested that the skin irritation caused by application of topical tacrolimus might be attributable to the direct action of tacrolimus on the sensory nerves, as a remarkable amount of calcium influx into the nerve cells was noted at a very early time point following the addition of tacrolimus into the culture.²¹ These studies prompted us to speculate that the sensory side effect of tacrolimus and its antipruritic effect are due to its direct effects on cutaneous nerve endings.²¹

2.2 | Suppression of IL-31 by tacrolimus

Many reports have recently shown that Th2 cell-derived IL-31 acts as a mediator of pruritus in AD.²²⁻²⁴ Dillon et al²⁵ were the first to

demonstrate that IL-31 transgenic mice showed extensive scratching and AD-like skin lesions, which has led to the consideration of IL-31 as a mediator of pruritus. IL-31 levels are elevated in the serum and the affected skin of patients with AD, and are significantly correlated with clinical symptoms and severity in AD.²⁶⁻²⁸ IL-31 receptors have been shown to be colocalized with TRPV1 and transient receptor potential ankyrin 1 (TRPA1) receptors in sensory nerve cells.²⁹ Murota et al³⁰ reported that topical tacrolimus inhibited the AD-like skin lesions that developed spontaneously in NC/Nga mice in concert with the down-regulation of cutaneous IL-31 levels. Notably, cutaneous IL-31 levels were strongly and significantly correlated with the number of intraepidermal nerve fibers.³⁰ These studies suggested that tacrolimus might alleviate itching not only by sensory nerve desensitization, but also by decreasing IL-31 levels.³⁰

3 | SUPPRESSION OF ALLERGIC INFLAMMATION BY TACROLIMUS

3.1 | The innate immune response and tacrolimus

Allergic inflammation has been thought to be induced by IgE antibody production and local infiltration of activated inflammatory cells such as eosinophils and Th2 cells, which generate a Th2 cytokine-rich milieu and further accelerate the acquired immune response.^{31,32} However, innate-type allergies are mainly attributable to the production of IL-18, IL-25, and IL-33, or thymic stromal lymphopoietin (TSLP), which do not require the production of IgE antibodies.^{31,32} Innate lymphoid cells (ILC) are categorized into three subsets, ILC1, ILC2, and ILC3, and they play a critical role in innate-type allergic response.³³ Among them, ILC2 cells are particularly important, as they induce severe allergic inflammation by releasing large amounts of IL-5 and IL-13 in response to IL-25, IL-33, and TSLP.³⁴ Recently, numerous reports have demonstrated that IL-25, IL-33, and TSLP are involved in the pathogenesis of AD.^{5,35-38}

One of the stimulants of the innate allergic response is skin surface microbiota. It is well known that the atopic skin is highly colonized with *Staphylococcus aureus*.³⁹ Staphylococcal enterotoxin B (SEB), a potent superantigen, induces T-cell proliferation and IL-33 production as well as IL-31 production.^{29,40,41} Tacrolimus inhibits the SEB-induced T-cell proliferation in a dose-dependent manner.⁴⁰ Savinko et al⁴¹ administered SEB to ovalbumin (OVA)-sensitized mice and confirmed that the mRNA expression of IL-33 and its receptor ST2 was upregulated. Notably, this was significantly suppressed by topical tacrolimus, but not by topical betamethasone. Considering that the *Staphylococcus aureus* colony count in the skin of patients with AD is significantly decreased after topical tacrolimus treatment,³⁹ topical tacrolimus may be effective in inhibiting the IL-33-mediated innate allergic response by reducing *Staphylococcus aureus* colonization and SEB production.^{40,41} Coadministration of IL-33 makes the OVA-sensitized murine asthma more resistant to steroid treatment.⁴² In the presence of IL-33 and IL-31, human eosinophils and fibroblasts produce much higher amounts of chemokines, which may promote the chronicity of AD.⁴³ Topical tacrolimus

may counteract the steroid resistance and chronic progression of AD by inhibiting IL-31 and IL-33 expression.^{30,41}

Toll-like receptors (TLRs) work as sensors for microbial products.^{44,45} The polymorphism of the TLR2 gene is associated with the severe phenotype of AD.^{44,45} Antiga et al⁴⁶ reported that (i) TLR1 expression is increased while TLR2 expression is decreased in the skin lesions of adult patients with AD, and (ii) topical tacrolimus normalizes the TLR1/TLR2 imbalance by downregulating TLR1 expression and upregulating TLR2 expression, which eventually enhances the antibacterial activity of the skin tissue.

Periostin is a matricellular protein belonging to fasciclin family, and its stromal expression is strongly upregulated by IL-4 and IL-13.⁴⁷ In addition, the expression of periostin is closely linked to chronicity of allergic inflammation.⁴⁷ Levels of tear periostin are also significantly elevated in atopic keratoconjunctivitis and are associated with giant papilla formation.⁴⁸ Of note, topical tacrolimus reduces the tear periostin levels with clinical improvement of atopic keratoconjunctivitis.⁴⁸

3.2 | Suppression of chronicity by tacrolimus

In AD, repeated scratching turns the erythematous acute lesions into lichenified chronic and intractable ones.⁴ The lichenified and thickened skin lesions are a result of epidermal acanthosis and dermal fibrosis.⁴⁹ Lan et al⁴⁹ demonstrated that tacrolimus significantly downregulated the proliferation of human fibroblasts and their production of collagen I, both of which were stimulated by transforming growth factor β -1. Caproni et al⁵⁰ quantified the profile of inflammatory cell infiltration in the lesional skin before and after treatment with topical tacrolimus and topical hydrocortisone butyrate. The number of infiltrated CD3 + , CD4 + , or CD8 + lymphocytes, eosinophils, and neutrophils, but not CD1a+ cells or mast cells, was significantly decreased on application of topical tacrolimus compared with that of topical hydrocortisone butyrate.⁵⁰ Moreover, topical tacrolimus significantly reduced the number of infiltrated cells expressing IL-4, IL-5, IL-13, IFN- γ , IL-12, CCR3, and CXCR4, respectively, which was observed more clearly with tacrolimus than with hydrocortisone butyrate.⁵⁰ These results coincide with the fact that tacrolimus inhibits not only IFN- γ , IL-4, and IL-17 production from purified human memory CD4 + T cells, but also the differentiation of naïve T cells into Th1, Th2, and Th17 memory cells.⁵¹

In a 10-year follow-up study, topical tacrolimus significantly reduced the affected body surface area and comorbidity in patients with active asthma and rhinitis with improvement of bronchial hyper-reactivity.⁵² In addition, responders to tacrolimus showing a $\geq 60\%$ improvement of the affected body surface area exhibited a significant decrease in serum IgE levels,⁵² suggesting that effective, long-term control using topical tacrolimus may terminate the vicious cycle of the allergic march.

3.3 | Suppression of mast cell activation by tacrolimus

It has already been reported that tacrolimus suppresses histamine release from mast cells stimulated with anti-IgE antibodies.^{2,3} The

induction of C-C motif chemokine ligand (CCL) 1, CCL3, CCL4, and CCL18 from anti-IgE-activated mast cells is also significantly inhibited by tacrolimus but is rather enhanced by dexamethasone.⁵³ In contrast, the induction of CCL2, CCL7, CXCL3, and CXCL8 is significantly inhibited by dexamethasone but is enhanced by tacrolimus. Importantly, the combination of dexamethasone and tacrolimus suppresses the production of all chemokines, indicating that a combination of steroid and tacrolimus may be more effective than each single agent for the treatment of allergic diseases in which mast cell-derived chemokines play a major role.⁵³ Mast cells also play a key role in promoting allergic inflammation in response to δ -toxin produced by *Staphylococcus aureus* which are colonized in the lesional skin of patients with AD.⁵⁴ However, it is not known whether tacrolimus inhibits the mast cell activation induced by *Staphylococcus aureus*.

4 | EFFECTS OF TACROLIMUS ON SKIN BARRIER FUNCTION

In AD, skin barrier dysfunction is caused by a complex etiology including filaggrin gene mutations, inhibition of filaggrin expression by Th2 cytokines, and mechanical injury by scratching.^{6,10,55} Therefore, the topical application of emollients is fundamentally important in the treatment of AD.⁴

Dähnhardt-Pfeiffer et al⁵⁶ compared the treatment outcomes of topical tacrolimus and topical mometasone furoate and found

TABLE 1 Mechanism of tacrolimus

Topics	Reference
Innate and acquired immunity	
Suppression of T-cell activation	1,51
Suppression of mast cell activation	2,3,50
Suppression of T-cell proliferation induced by <i>Staphylococcus aureus</i> toxins	40
Suppression of IL-33 mRNA expression triggered by <i>Staphylococcus aureus</i> toxins	41
Correction of impairment of TLR balance	46
Epidermal and dermal function	
Effects on skin barrier function	56,57
Reduction in <i>Staphylococcus aureus</i> colonies	39
Suppression of periostin production	48
Suppression of skin tissue fibrosis	49
Pruritus	
Suppression of itching	11-14
Suppression of scratching behavior	17-19
Suppression of sensory nerve activation	20,21
Suppression of IL-31 production	30
Disease control	
Suppression of atopic dermatitis chronicity	50
Improvement in quality of life	5
Suppression of other allergy development	52

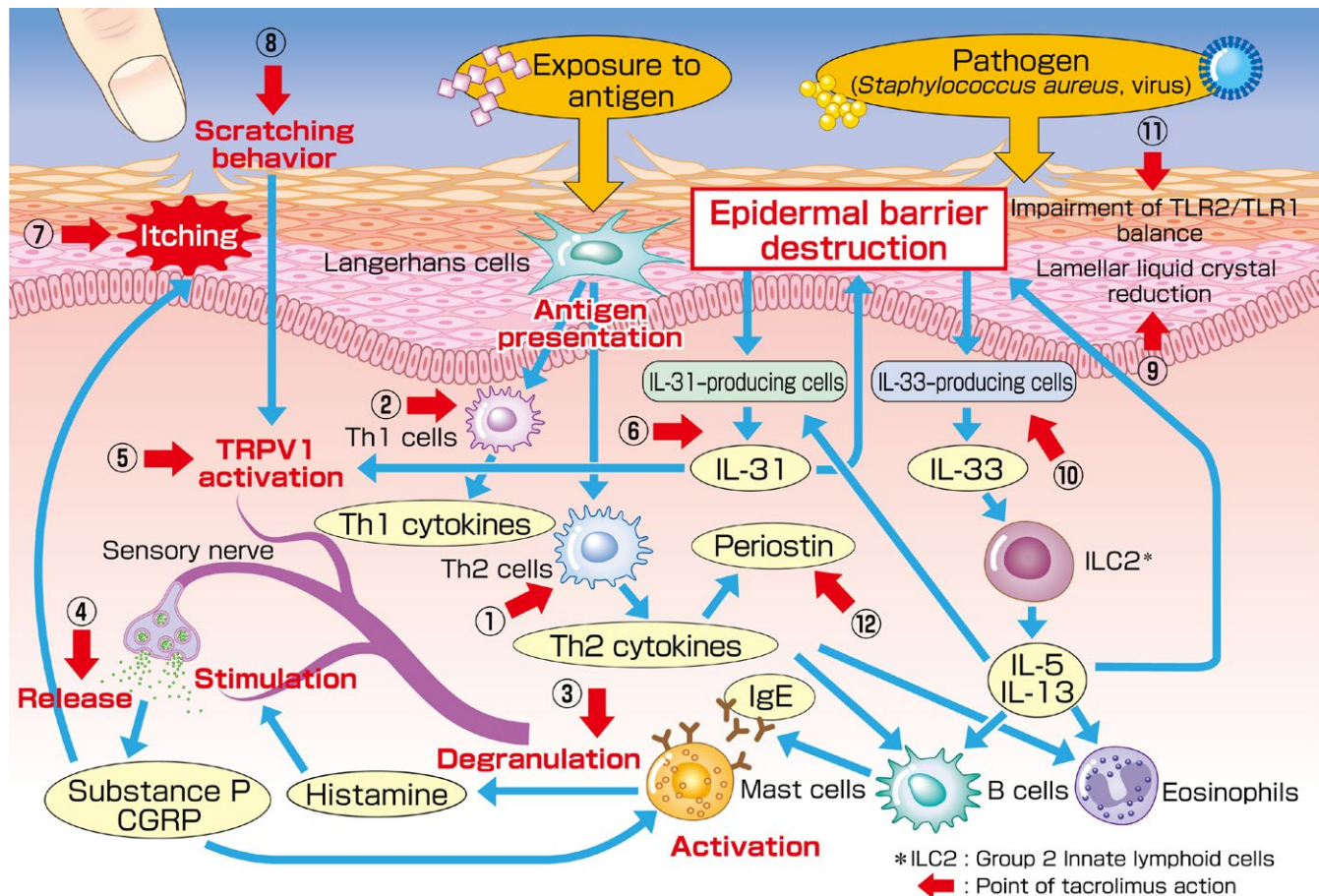


FIGURE 1 The points of action and action mechanisms of tacrolimus in the pathogenesis of atopic dermatitis. (1) Suppression of Th2 cell activation, (2) suppression of Th1 cell activation, (3) suppression of mast cell granulation, (4) suppression of primary sensory neurotransmitter release, (5) desensitization of TRPV1 receptor, (6) suppression of IL-31 production, (7) suppression of itching, (8) suppression of scratching behavior, (9) preservation of lamellar liquid crystal reduction, (10) suppression of IL-33 and ST2 receptor mRNA expression, (11) suppression of impairment of TLR2/TLR1 balance, and (12) suppression of periostin production

that both tacrolimus and mometasone improved the skin symptoms of AD to the same extent. However, topical tacrolimus, but not mometasone, significantly improved skin barrier dysfunction as assessed by transepidermal water loss and skin hydration values.⁵⁶ Moreover, semi-quantitative analysis by transmission electron microscopy revealed a significant increase in intercellular lipid lamellae after application of topical tacrolimus compared with that of topical mometasone.⁵⁶ Similar results were reported in a half-side comparative study between topical tacrolimus and topical betamethasone valerate.⁵⁷ Notably, topical tacrolimus significantly improved the skin barrier function of patients with AD, whereas it was shown to be adversely disrupted by topical betamethasone, likely because betamethasone downregulated the levels of natural moisturizing factors.⁵⁷

5 | CONCLUSION

Tacrolimus is a potent immunosuppressant that is widely used in the treatment of AD.^{1,4} In addition to its immunosuppressive activity,

recent studies have uncovered the diverse mechanistic potential of tacrolimus, including its inhibitory action on mast cells, pruritus, and innate allergic response. The new mechanistic insights into tacrolimus function mentioned in this review are summarized in Table 1 and depicted as a schematic diagram in Figure 1. Various new therapeutic approaches have been emerging in the treatment of AD.^{8,58-61} The capability of topical tacrolimus to synergistically improve AD in combination with these new therapies remains an intriguing prospect.

CONFLICT OF INTEREST

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