

Effects of the Janus Kinase Inhibitor CP-690550 (Tofacitinib) in a Rat Model of Oxazolone-Induced Chronic Dermatitis

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Key Words

Janus kinase inhibitor · Animal model · Contact dermatitis · Psoriasis · Atopic dermatitis

Abstract

The effect of CP-690550 (tofacitinib), a new Janus kinase (JAK) inhibitor, was evaluated in chronic allergic dermatitis. Allergic contact dermatitis was induced in rat ears by repeated application of oxazolone. This dermatitis was accompanied by sustained ear swelling and marked epidermal hyperplasia. In the induced ear, a lot of inflammatory cells infiltrated into the dermis site and the amounts of interferon (IFN)- γ , tumor necrosis factor (TNF)- α , and interleukin (IL)-22 were elevated. Orally administered CP-690550 significantly suppressed ear swelling as well as epidermal thickening, and the effect at 10 mg/kg was comparable to that of cyclosporin A and etanercept. These results suggest a great potential of CP-690550, a JAK inhibitor, as a treatment for chronic dermatitis featuring epidermal hyperplasia (in the pathogenesis of which IFN- γ , TNF- α and IL-22 play a role) such as psoriasis and chronic atopic dermatitis.

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Introduction

Psoriasis and atopic dermatitis are common, chronic, inflammatory skin disorders. Psoriatic skin is characterized by prominent epidermal hyperplasia, which results

from dysregulated proliferation and differentiation of epidermal keratinocytes driven by infiltrating lymphocytes and their released cytokines [1]. In particular, type 1 T cells consisting of T-helper (Th)1 and T-cytotoxic (Tc)1 cells have attracted attention as psoriasis patients have type 1 bias in lesional skin and peripheral blood [2]. Among type 1 cytokines, interferon (IFN)- γ has been indicated to be crucial to the pathogenesis of psoriasis [3, 4]. Another type 1 cytokine, tumor necrosis factor (TNF)- α , has also been recognized as an important factor in the pathogenesis of this disease, as anti-TNF- α biologics such as etanercept have been found extremely effective in treating psoriasis [5]. In addition, Th17 cells were also recently discovered to contribute to the development of psoriasis [6]. Expression of Th17-producing cytokines, interleukin (IL)-17 and IL-22, which is increased in psoriatic skin lesions, was subsequently reduced on treatment with cyclosporin A or etanercept [7, 8].

Atopic dermatitis, like psoriasis, results from disrupted skin homeostasis and dysregulated immune response [9]. Histopathologically, activated mast cells, eosinophils and IL-4-, IL-5- and IL-13-producing Th2 cells are observed in acute lesional skin [9], and are thought to play a key role in the development of dermatitis. On the other hand, chronic atopic lesions have increased numbers of IL-12 and IFN- γ mRNA-expressing cells, which suggests the contribution of Th1 cells to the characteristic symptoms like thickened skin, lichenification and fibrotic papules, where epidermis is hyperplastic [9–11]. Recent studies revealed

that IL-22 mRNA level also increases in chronic atopic lesions [12]. Therefore, T cells and cytokines implicated in the pathogenesis of psoriasis and atopic dermatitis have been appreciated as potential therapeutic targets, and indeed immunosuppressive agents targeting T cells such as tacrolimus and cyclosporin A have demonstrated efficacy in alleviating chronic inflammatory skin disorders [5, 13].

Cytokines exert biological effects by binding to their cognate receptors, which activate Janus kinases (JAKs), a small family of cytoplasmic protein tyrosine kinases comprising JAK1, JAK2, JAK3, and tyrosine kinase 2, and then signal transducers and activators of transcription (STAT) proteins to induce specific gene expression [14, 15]. Genetic studies have established that different classes of cytokine receptors preferentially use any one or combination of four JAK family members and a specific downstream STAT protein [15]. JAK1 and JAK3 are both required for signaling through common γ -chain-containing receptors for cytokines including IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21, which are integral to lymphocyte function [16]. JAK1 also plays a key role in signal transduction of proinflammatory cytokines such as IL-6 and IFN- γ [16]. JAK2 and tyrosine kinase 2 are known to transmit the signals derived from IL-12 and IL-23 receptors [14]. Therefore, the inhibition of JAK activities may be a promising therapeutic strategy for immune and inflammatory disorders.

CP-690550 (tofacitinib) is a novel oral JAK inhibitor which inhibits all four JAK family members with nanomolar potency [17]. This study describes the effect of CP-690550 in the rat model of oxazolone-induced chronic dermatitis where epidermal thickening may be induced through the contribution of IFN- γ , TNF- α and IL-22, and investigates the potential of JAK inhibitor therapy for chronic, inflammatory skin disorders such as psoriasis and atopic dermatitis.

Materials and Methods

Animals

Sprague Dawley rats (female, 6 weeks old) purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan) were housed under specific pathogen-free conditions, fed a standard laboratory diet, given water ad libitum, and then used for respective experiments after an appropriate acclimation period. All animal experimental procedures were approved by the Committee for Animal Experiments of Astellas Pharma Inc.

Chemicals

Oxazolone (Sigma Chemical Co. Ltd., St. Louis, Mo., USA) was dissolved in ethanol to a concentration of 2% (w/v) for initial sensitization or in vehicle [acetone:olive oil (4:1)] to 1.6% (w/v) for subsequent application to the ear. CP-690550 was prepared at Astellas

Pharma Inc. (Tsukuba, Japan) and dissolved in 50% (v/v) polyethylene glycol in water solution to appropriate concentrations. Cyclosporin A was prepared at Astellas Pharma Inc. or commercially obtained from Novartis International AG (Basel, Switzerland). The former was dissolved in a mixture of olive oil/Labrafil/ethanol, while the latter was diluted with physiological saline to prepare a 2 mg/ml solution. Etanercept, Enbrel® (Amgen, Thousand Oaks, Calif., USA) was diluted with physiological saline to 10 mg/ml.

Experimental Protocol

Chronic allergic dermatitis was induced as described in the previous report [18]. Namely, Sprague Dawley rats were sensitized by the application of 300 μ l of 2% oxazolone to the abdomen, after which 100 μ l of 1.6% oxazolone was applied to both sides of the right ear every 3 days starting from 7 days after sensitization. As a negative control, the above-described olive oil-acetone mixture was applied in the same way after sensitization. Ear thickness was measured using a dial thickness gauge (Ozaki Seisakusho Co. Ltd., Tokyo, Japan) 7 days after sensitization and then 72 h after each application of oxazolone. CP-690550 and cyclosporin A were orally administered at 5 ml/kg once a day starting from 7 days after sensitization. Etanercept was subcutaneously injected at 1 ml/kg on the day of sensitization, on days 3 and 7, and every 3 days thence. Each drug was given 1 h before each application of oxazolone.

Measurement of IFN- γ , IL-17, IL-22 and TNF- α Contents in the Ear

Samples of ear tissue extract for enzyme-linked immunosorbent assay (ELISA) were prepared according to the method described by Fujii et al. [18]. Briefly, ears were excised 6 h after the final application of oxazolone and homogenized with 0.1% (v/v) Tween 20 in phosphate-buffered saline. Homogenates were frozen at -30°C for 30 min, and then thawed in a 37°C water bath for 15 min, a procedure which was subsequently repeated twice. The samples were then sonicated for 1 min and centrifuged for 20 min at 2,000 g. Collected supernatants were centrifuged again for 15 min at 20,000 g, and the lower layer of the supernatants was collected by avoiding contamination by the upper lipid layer. Collected samples were stored at -30°C until ELISA measurements were taken for IFN- γ , TNF- α (BD Biosciences Pharmingen, San Diego, Calif., USA), IL-17 (eBioscience, San Diego, Calif., USA) and IL-22 (R&D Systems Inc., Minneapolis, Minn., USA); these measurements were carried out according to the manufacturers' instructions.

Histopathological Study and Measurement of Epidermal Thickness in the Ear

Rat ears were excised 72 h after the final application of oxazolone and fixed in 10% buffered formalin solution, embedded in paraffin by standard methods, and cut into 3- μ m-thick sections. Sliced samples were stained with hematoxylin-eosin (HE), and then assessed via light microscopy. After the microscopic fields were photographed, epidermal thickness measured as the distance from the bottom of the stratum corneum to the basement membrane in the interfollicular epidermis was determined from the mean of four random fields for which five measurements were averaged [18].

Statistical Analysis

Data are expressed as the mean $\pm$ standard error of means (SEM). Significant differences between two groups were deter-

mined using Student's t test, while those between more than two groups were assessed using Dunnett's multiple comparison test. $p < 0.05$ was considered significant.

Results

Changes in Cytokine Expression Levels in Rat Ears Receiving Repeated Application of Oxazolone

As shown in the previous report [18], the rat ear receiving topical application of oxazolone every 3 days, starting from 7 days after sensitization, exhibited significant thickening from day 10 and thereafter compared to the negative control ($p < 0.01$), with thickening increasing as application was repeated (fig. 1). Likewise, oxazolone application resulted in a significant increase in IFN- γ level in homogenized ear tissues compared to the negative control ($p < 0.01$; table 1). To further investigate the involvement of cytokines in the development of dermatitis, IL-17, IL-22 and TNF- α levels in homogenized ear tissues were measured via ELISA. Production of IL-22 and TNF- α was also significantly elevated in the ear receiving repeated application of oxazolone ($p < 0.01$), whereas the elevation of IL-17 production was slight and not significant (table 1).

Effects of CP-690550 on Oxazolone-Induced Chronic Dermatitis

We evaluated the effect of CP-690550 on oxazolone-induced chronic dermatitis. CP-690550 suppressed ear thickening and the effect at 10 mg/kg was greater than that at 1 and 3 mg/kg, in which no significant suppression was observed on days 10 and 13 (fig. 1). CP-690550 at 10 mg/kg showed a significant effect throughout the dosing period. On day 22, all doses significantly suppressed ear thickening ($p < 0.01$).

Histopathological analysis revealed that the ear receiving repeated oxazolone swelled dramatically compared to the negative control ear (fig. 2). Also the oxazolone-challenged ear showed prominent epidermal thickening and marked infiltration of inflammatory cells into the dermis, whereas only a thin epidermal layer and sparse cells were observed in the negative control ear. CP-690550 (10 mg/kg) suppressed these symptoms, and the histological picture of the ear of CP-690550-treated rat was similar to that of the negative control ear.

When epidermal thickness was measured, the ear receiving repeated oxazolone showed a significant increase compared to the negative control ($p < 0.05$; fig. 3). CP-690550 suppressed the increase in epidermal thickness at all doses, and a significant effect was observed at 10 mg/kg ($p < 0.01$).

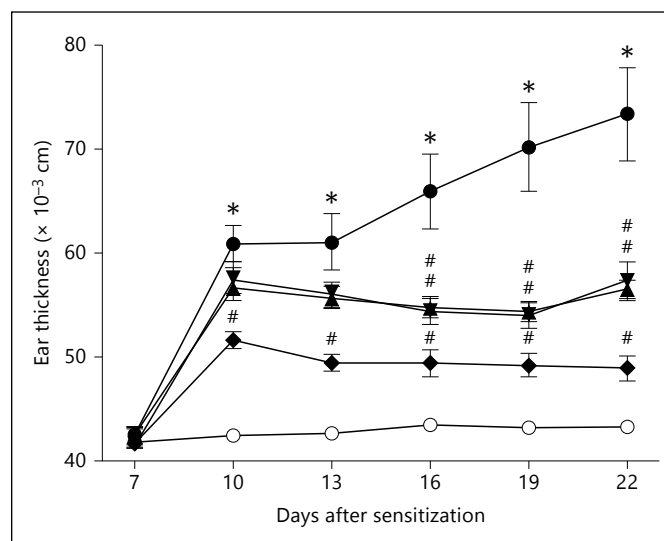


Fig. 1. Effects of CP-690550 on the change in ear thickness in an oxazolone-induced chronic dermatitis model. CP-690550 was orally administered once a day starting from 7 days after sensitization, 1 h before each application of oxazolone. Ear thickness was measured on day 7 and then 72 h after each application of oxazolone. Values represent means $\pm$ SEM for 5 or 10 rats. ○: negative control, ●: oxazolone-challenged positive control, ▼: oxazolone + CP-690550 1 mg/kg, ▲: oxazolone + CP-690550 3 mg/kg, ◆: oxazolone + CP-690550 10 mg/kg. * $p < 0.01$: significantly different from the negative control group (Student's t test). # $p < 0.01$: significantly different from the oxazolone-challenged positive control group (Dunnett's multiple comparison test).

Table 1. Changes in cytokine expression levels in rat ears receiving repeated application of oxazolone

	Cytokine production (pg/ear)			
	IFN- γ	IL-17	IL-22	TNF- α
(-) Control	287.0 $\pm$ 16.5	11.0 $\pm$ 1.2	0.0 $\pm$ 0.0	125.4 $\pm$ 15.6
Oxazolone	1,399.4 $\pm$ 221.5*	14.2 $\pm$ 1.0	15.7 $\pm$ 4.7*	410.6 $\pm$ 41.4*

Ears were excised 6 h after the final application of oxazolone or an olive oil-acetone mixture, and cytokine levels in homogenized ear tissue were measured via ELISA. Values represent means $\pm$ SEM for 6 rats. * $p < 0.01$: significantly different from the negative control group (Student's t test).

Effects of Cyclosporin A and Etanercept on Oxazolone-Induced Chronic Dermatitis

To investigate the efficacy of clinically used drugs in oxazolone-induced chronic dermatitis, we evaluated the effects of cyclosporin A and the biological anti-TNF- α

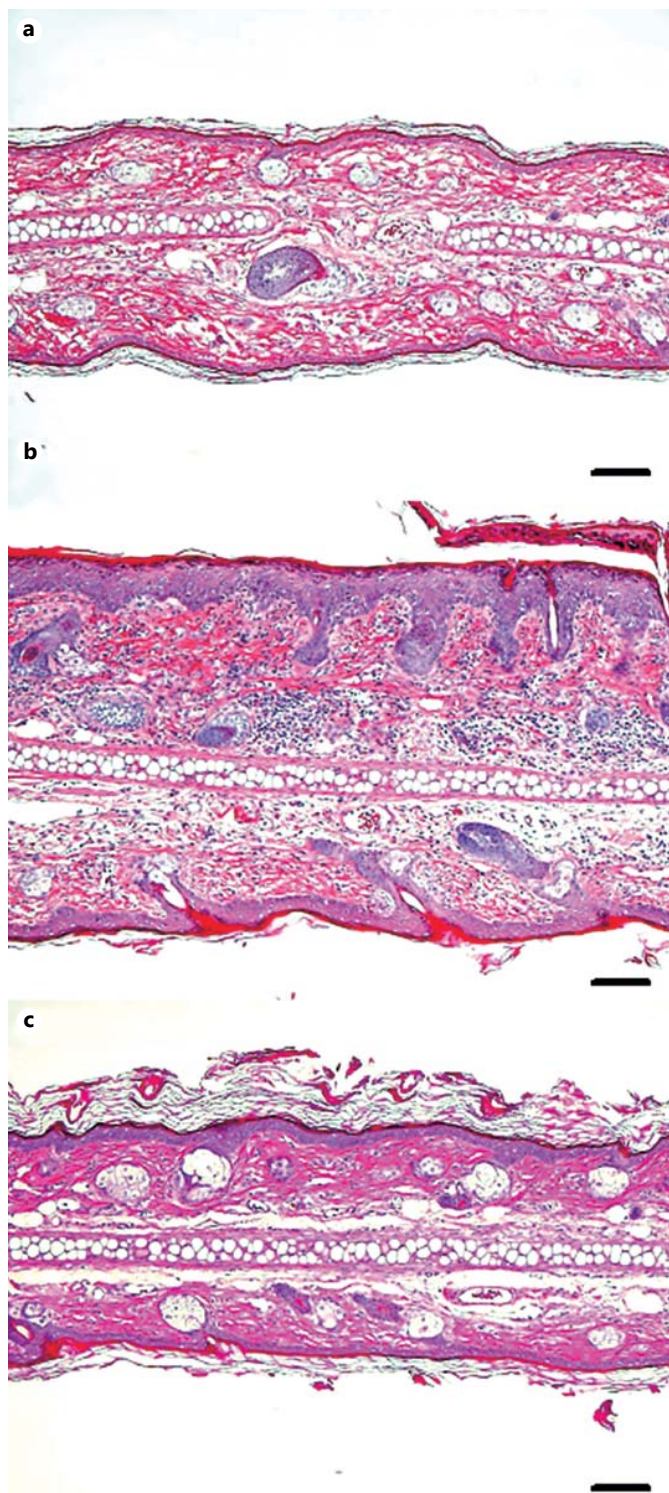


Fig. 2. Histopathological pictures of oxazolone-induced chronic dermatitis in rats with or without oral administration of CP-690550. CP-690550 (10 mg/kg) was orally administered once a day starting from 7 days after sensitization, 1 h before each application of oxazolone. Ears of rats with (c) or without (b) oral administration of CP-690550 were excised 72 h after the final application of

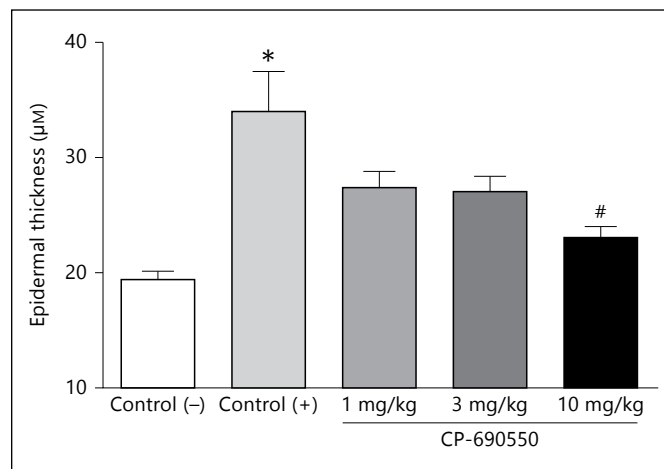


Fig. 3. Effects of CP-690550 on epidermal thickening in an oxazolone-induced chronic dermatitis model. CP-690550 (1, 3, 10 mg/kg) was orally administered once a day starting from 7 days after sensitization, 1 h before each application of oxazolone. Ears were excised 72 h after the final application of oxazolone and epidermal thickness measured following staining with HE as described in Materials and Methods. Values represent means $\pm$ SEM for 5 or 10 rats. * $p < 0.05$: significantly different from the negative control group (Student's *t* test). # $p < 0.01$: significantly different from the oxazolone-challenged positive control group (Dunnett's multiple comparison test).

agent, etanercept, in the model. Cyclosporin A (10 mg/kg) significantly suppressed ear thickening at each time point except for day 13 ($p < 0.05$ on days 10 and 19, $p < 0.01$ on days 16 and 22; fig. 4a). Etanercept at 10 mg/kg also significantly suppressed ear thickening on day 22 ($p < 0.01$), but no significant suppression was observed on the other time points (fig. 4b). Cyclosporin A and etanercept also significantly suppressed epidermal thickening on day 22 by 81 and 67%, respectively ($p < 0.05$; fig. 5b, c), and the effects were comparable with that of CP-690550 at 10 mg/kg, which produced a significant suppression of 75% ($p < 0.01$; fig. 5a).

oxazolone and stained with HE. As a negative control, an olive oil-acetone mixture was applied in the same way after sensitization and ears were excised 72 h after the final application (a). Results are representative of 5 (negative control) or 10 (oxazolone-challenged positive control and oxazolone + CP-690550 10 mg/kg) rats per group. Scale bars, 100 μ m.

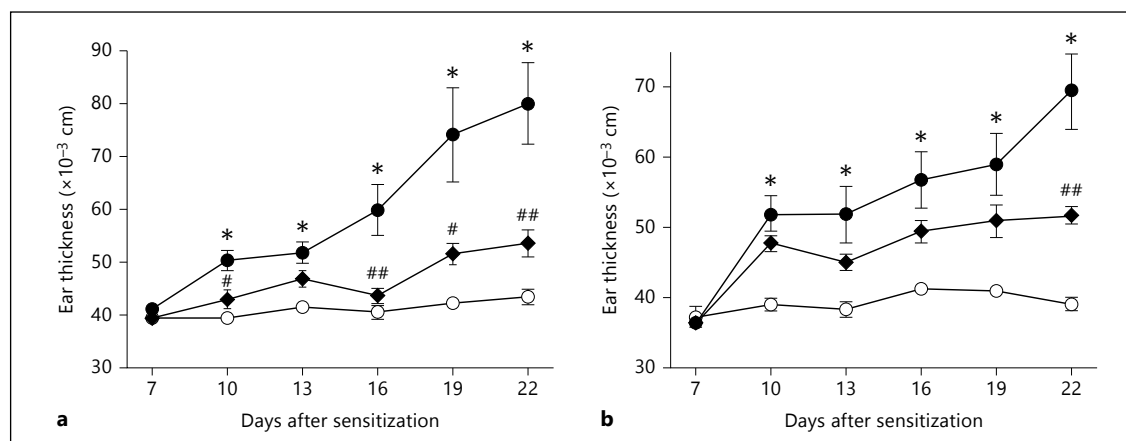


Fig. 4. Effects of cyclosporin A (**a**) and etanercept (**b**) on ear thickening in oxazolone-induced chronic dermatitis model. Cyclosporin A (10 mg/kg) was orally administered once a day starting from 7 days after sensitization, 1 h before each application of oxazolone. Etanercept (10 mg/kg) was subcutaneously injected on the day of sensitization, on days 3 and 7, and every 3 days thence, 1 h before each application of oxazolone. Ear thickness was measured on day

7 and then 72 h after each application of oxazolone. Values represent means $\pm$ SEM for 8 rats. O: negative control, ●: oxazolone-challenged positive control, ◆: oxazolone + cyclosporin A 10 mg/kg (**a**) or etanercept 10 mg/kg (**b**). * $p < 0.01$: significantly different from the negative control group (Student's *t* test). # $p < 0.05$, ## $p < 0.01$: significantly different from the oxazolone-challenged positive control group (Student's *t* test).

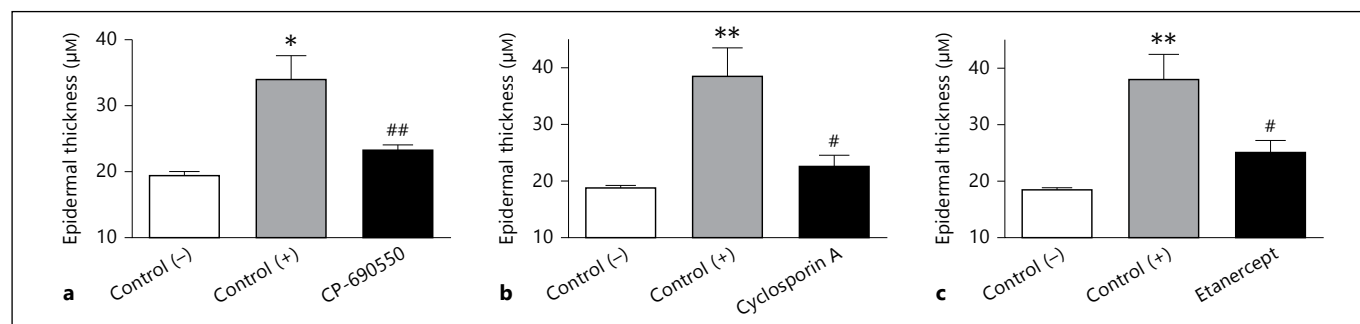


Fig. 5. Comparison of the effect of CP-690550 (**a**), cyclosporin A (**b**), and etanercept (**c**) on epidermal thickening in an oxazolone-induced chronic dermatitis model. CP-690550 (10 mg/kg) or cyclosporin A (10 mg/kg) was orally administered once a day starting from 7 days after sensitization, 1 h before each application of oxazolone. Etanercept (10 mg/kg) was subcutaneously injected on the day of sensitization, on days 3 and 7, and every 3 days thence, 1 h

before each application of oxazolone. Ears were excised 72 h after the final application of oxazolone and epidermal thickness measured following staining with HE as described in Materials and Methods. Values represent means $\pm$ SEM for 5–10 rats. * $p < 0.05$, ** $p < 0.01$: significantly different from the negative control group (Student's *t* test). # $p < 0.05$, ## $p < 0.01$: significantly different from the oxazolone-challenged positive control group (Student's *t* test).

Discussion

A previous study has shown an increased production of IFN- γ , but not IL-4, in the ear receiving repeated application of oxazolone, and suggested the involvement of Th1 in the development of dermatitis [18]. In the present study, an increased production of TNF- α in addition to IFN- γ was observed in the infected ear. TNF- α is a proinflammatory cytokine secreted by Th1 cells, as well as a variety of other cell types such as epidermal keratinocytes,

intraepidermal Langerhans cells and dermal macrophages [19, 20]. The cytokine contributes to the pathogenesis of inflammatory skin diseases by activating keratinocytes to produce chemokines and express the surface adhesion molecules, thereby promoting inflammatory cell recruitment and keratinocyte proliferation [19]. It has also been known that TNF- α and IFN- γ synergistically function to stimulate the expression of adhesion molecules and chemokine production [21, 22]. In this model, etanercept significantly suppressed ear thickening on day 22; however,

it did not show clear effects on ear thickening at the early time points (fig. 4b). This suggests that TNF- α was not involved in the acute phase of allergic dermatitis in the model, whereas it contributed to the development of chronic dermatitis. Further, we found that the level of IL-22 was also elevated in the infected ear, whereas the elevation of IL-17 production was marginal. IL-22 is preferentially produced by terminally differentiated Th17 cells, and its production has been considered to correlate with IL-17 expression [23]; therefore, our results are not in line with the general concept. This can be explained by the identification of a new T cell subset, Th22 cells, which produce IL-22 and TNF- α , but not IL-17 or IFN- γ [24]. Th22 cells detected in the skin of patients with psoriasis, chronic atopic dermatitis and allergic contact dermatitis [24] are presumed to play a central role in inflammatory skin diseases, especially those with marked epidermal acanthosis, via IL-22 production [23]. IL-22 mediates keratinocyte proliferation by downregulating terminal keratinocyte differentiation genes, which leads to epidermal hyperplasia [23]. The effect of IL-22 on keratinocyte is enhanced by TNF- α [24]. Zheng et al. [25] showed in a mouse study that IL-12 and IL-23 induced ear epidermal thickening through the production of IFN- γ and IL-22, respectively. Accordingly, IFN- γ and IL-22 may synergistically act with TNF- α on the induction of epidermal thickening in the current model of oxazolone-induced chronic dermatitis.

CP-690550 significantly suppressed ear swelling as well as epidermal thickening in the model. The compound inhibits all four JAK family kinase members, but in cellular settings it demonstrates functional specificity for JAK1- and JAK3-dependent STAT activation over JAK2-mediated pathways [17]. An in vitro study using CD4⁺ T cells showed that CP-690550 suppressed anti-CD3-induced cytokine production including IFN- γ and IL-22 [26], and the suppression of IFN- γ production was thought to result from impairment of Th1 cell differentiation by the inhibition of the IFN- γ -mediated JAK1-STAT1 and/or IL-2-mediated JAK3-STAT5 signaling pathway [27]. Since differentiation of Th22 cells is largely dependent on IL-6 and TNF- α [23], the inhibition of IL-6-mediated signaling by blockade of JAK1 may contribute to the suppression of IL-22 production. Furthermore, an in vivo mouse study showed that CP-690550 suppressed TNF- α production after LPS administration, probably through the inhibition of IFN- γ signaling by blockade of JAK1 [27]. Taken together, the inhibition by CP-690550 observed in the current model may have been achieved through the compound's inhibition of cytokines which were elevated in the infected ear. To support the hypothesis, further study on

the effect of CP-690550 on cytokine expression levels in the model needs to be conducted.

Cyclosporin A and etanercept also suppressed ear swelling as well as epidermal thickening in the model. Cyclosporin A is an immunosuppressant which reduces T cell function by inhibiting calcineurin, a molecule that induces transcription factors associated with the production of inflammatory cytokines [5]. The compound is approved for use in treating severe psoriasis and atopic dermatitis [5, 28]. In clinical studies, cyclosporin A successfully improved psoriatic symptoms and 70% of patients achieved psoriasis area and severity index improvement of 75% from baseline (PASI75) after approximately 12 weeks of treatment [5]. Etanercept is a soluble dimeric fusion protein consisting of two TNF receptors fused to the Fc portion of human IgG acting to competitively inhibit TNF-mediated activity [5]. Rodents are known to develop neutralizing antibodies against etanercept after repeated injection, but the limited high-dose regimen of etanercept used in the current model was active as reported in a rat model of arthritis [29], although the presence of neutralizing antibodies has to be determined. This TNF- α inhibitor showed efficacy in moderate-to-severe psoriasis with PASI75 success rates ranging from 49 to 57% after a 12-week treatment [5]. Although the efficacy of TNF- α antagonists for atopic dermatitis has been controversial, several groups reported the beneficial effect of TNF- α antagonist therapy in patients with severe, chronic atopic diseases [30, 31]. In this study, CP-690550 demonstrated comparable efficacy to that of cyclosporin A and etanercept, which suggests a potential of the compound as a treatment for chronic inflammatory skin disorders. In a recently reported phase II clinical study, CP-690550 proved effective in treating moderate-to-severe chronic plaque psoriasis with comparable efficacy to that of cyclosporin A and etanercept, and 67% of patients receiving the maximum dose of CP-690550 for 12 weeks achieved PASI75 [16]. To further support the potential efficacy of CP-690550 in atopic dermatitis, it would be valuable to evaluate the compound in an allergic scratching and IgE-mediated skin inflammation model which recapitulates the features of atopic dermatitis [10].

In conclusion, oxazolone-induced chronic dermatitis can function as a useful pharmacological model to predict the clinical efficacy of drug candidates against chronic dermatitis featuring epidermal hyperplasia (in the pathogenesis of which IFN- γ , TNF- α , and IL-22 play a role) such as psoriasis and chronic atopic dermatitis. Based on results with this model, CP-690550, a JAK inhibitor, has great potential to become a valuable treatment for such skin disorders.

References

- Bowcock AM, Krueger JG: Getting under the skin: the immunogenetics of psoriasis. *Nat Rev Immunol* 2005;5:699–711.
- Austin LM, Ozawa M, Kikuchi T, Walters IB, Krueger JG: The majority of epidermal T cells in psoriasis vulgaris lesions can produce type 1 cytokines, interferon- γ , interleukin-2, and tumor necrosis factor- α , defining TC1 (cytotoxic T lymphocyte) and TH1 effector populations: a type 1 differentiation bias is also measured in circulating blood T cells in psoriatic patients. *J Invest Dermatol* 1999;113:752–759.
- Bonish B, Jullien D, Dutronc Y, Huang BB, Modlin R, Spada FM, Porcelli SA, Nickoloff BJ: Overexpression of CD1d by keratinocytes in psoriasis and CD1d-dependent IFN- γ production by NK-T cells. *J Immunol* 2000;165:4076–4085.
- Ovigne JM, Baker BS, Brown DW, Powles AV, Fry L: Epidermal CD8+ T cells in chronic plaque psoriasis are Tc1 cells producing heterogeneous levels of interferon-gamma. *Exp Dermatol* 2001;10:168–174.
- Staidle JP, Dabade TS, Feldman SR: A pharmacoeconomic analysis of severe psoriasis therapy: a review of treatment choices and cost efficiency. *Expert Opin Pharmacother* 2011;12:2041–2054.
- Fitch E, Harper E, Skorcheva I, Kurtz SE, Blauvelt A: Pathophysiology of psoriasis: recent advances on IL-23 and Th17 cytokines. *Curr Rheumatol Rep* 2007;9:461–467.
- Lowes MA, Kikuchi T, Fuentes-Duculan J, Cardinale I, Zaba LC, Haider AS, Bowman EP, Krueger JG: Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. *J Invest Dermatol* 2008;128:1207–1211.
- Zaba LC, Cardinale I, Gilleaudeau P, Sullivan-Whalen M, Suárez-Fariñas M, Fuentes-Duculan J, Novitskaya I, Khatcherian A, Bluth MJ, Lowes MA, Krueger JG: Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses. *J Exp Med* 2007;204:3183–3194.
- Bieber T: Atopic dermatitis. *N Engl J Med* 2008;358:1483–1494.
- Leung DY: Atopic dermatitis: the skin as a window into the pathogenesis of chronic allergic diseases. *J Allergy Clin Immunol* 1995;96:302–318.
- Boguniewicz M, Leung DY: Pathophysiologic mechanisms in atopic dermatitis. *Sem Cutan Med Surg* 2001;20:217–225.
- Nogales KE, Zaba LC, Shemer A, Fuentes-Duculan J, Cardinale I, Kikuchi T, Ramon M, Bergman R, Krueger JG, Guttman-Yassky E: IL-22-producing ‘T22’ T cells account for up-regulated IL-22 in atopic dermatitis despite reduced IL-17-producing TH17 T cells. *J Allergy Clin Immunol* 2009;123:1244–1252.
- Simpson D, Noble S: Tacrolimus ointment: a review of its use in atopic dermatitis and its clinical potential in other inflammatory skin conditions. *Drugs* 2005;65:827–858.
- O’Sullivan LA, Liongue C, Lewis RS, Stephenson SE, Ward AC: Cytokine receptor signaling through the Jak-Stat-Socs pathway in disease. *Mol Immunol* 2007;44:2497–2506.
- Murray PJ: The JAK-STAT signaling pathway: input and output integration. *J Immunol* 2007;178:2623–2629.
- Papp KA, Menter A, Strober B, Langley RG, Buonanno M, Wolk R, Gupta P, Krishnaswami S, Tan H, Harness JA: Efficacy and safety of tofacitinib, an oral Janus kinase inhibitor, in the treatment of psoriasis: a phase 2b randomized placebo-controlled dose-ranging study. *Br J Dermatol* 2012;167:668–677.
- Meyer DM, Jesson MI, Li X, Elrick MM, Funckes-Shippy CL, Warner JD, Gross CJ, Dowty ME, Ramaiah SK, Hirsch JL, Saabye MJ, Barks JL, Kishore N, Morris DL: Anti-inflammatory activity and neutrophil reductions mediated by the JAK1/JAK3 inhibitor, CP-690,550, in rat adjuvant-induced arthritis. *J Inflamm* 2010;7:41.
- Fujii Y, Takeuchi H, Tanaka K, Sakuma S, Ohkubo Y, Mutoh S: Effects of FK506 (tacrolimus hydrate) on chronic oxazolone-induced dermatitis in rats. *Eur J Pharmacol* 2002;456:115–121.
- Krueger G, Callis K: Potential of tumor necrosis factor inhibitors in psoriasis and psoriatic arthritis. *Arch Dermatol* 2004;140:218–225.
- Tracey D, Klareskog L, Sasso EH, Salfeld JG, Tak PP: Tumor necrosis factor antagonist mechanisms of action: a comprehensive review. *Pharmacol Ther* 2008;117:244–279.
- Trefzer U, Brockhaus M, Loetscher H, Parlow F, Kapp A, Schöpf E, Krutmann J: 55-kd tumor necrosis factor receptor is expressed by human keratinocytes and plays a pivotal role in regulation of human keratinocyte ICAM-1 expression. *J Invest Dermatol* 1991;97:911–916.
- Kakinuma T, Nakamura K, Wakugawa M, Yano S, Saeki H, Torii H, Komine M, Asahina A, Tamaki K: IL-4, but not IL-13, modulates TARC (thymus and activation-regulated chemokine)/CCL17 and IP-10 (interferon-induced protein of 10kDa)/CXCL10 release by TNF-alpha and IFN-gamma in HaCaT cell line. *Cytokine* 2002;20:1–6.
- Fujita H, Nogales KE, Kikuchi T, Gonzalez J, Carucci JA, Krueger JG: Human Langerhans cells induce distinct IL-22-producing CD4+ T cells lacking IL-17 production. *Proc Natl Acad Sci USA* 2009;106:21795–21800.
- Eyerich S, Eyerich K, Pennino D, Carbone T, Nasorri F, Pallotta S, Cianfrani F, Odorisio T, Traidl-Hoffmann C, Behrendt H, Durham SR, Schmidt-Weber CB, Cavani A: Th22 cells represent a distinct human T cell subset involved in epidermal immunity and remodeling. *J Clin Invest* 2009;119:3573–3585.
- Zheng Y, Danilenko DM, Valdez P, Kasman I, Eastham-Anderson J, Wu J, Ouyang W: Interleukin-22, a Th17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature* 2007;445:648–651.
- Migita K, Miyashita T, Izumi Y, Koga T, Komori A, Maeda Y, Jiuchi Y, Aiba Y, Yamasaki S, Kawakami A, Nakamura M, Ishibashi H: Inhibitory effects of the JAK inhibitor CP690550 on human CD4(+) T lymphocyte cytokine production. *BMC Immunol* 2011;12:51.
- Ghoreschi K, Jesson MI, Li X, Lee JL, Ghosh S, Alsup JW, Warner JD, Tanaka M, Steward-Tharp SM, Gadina M, Thomas CJ, Minnerly JC, Storer CE, LaBranche TP, Radi ZA, Dowty ME, Head RD, Meyer DM, Kishore N, O’Shea JJ: Modulation of innate and adaptive immune responses by tofacitinib (CP-690550). *J Immunol* 2011;186:4234–4243.
- Bussmann C, Bieber T, Novak N: Systemic therapeutic options for severe atopic dermatitis. *J Dtsch Dermatol Ges* 2009;7:205–219.
- Lu Y, Stinnette TW, Westrick E, Klein PJ, Gehrke MA, Cross VA, Vlahov IR, Low PS, Leamon CP: Treatment of experimental adjuvant arthritis with a novel folate receptor-targeted folic acid-aminopterin conjugate. *Arthritis Res Ther* 2011;13:R56.
- Rullan P, Murase J: Two cases of chronic atopic dermatitis treated with soluble tumor necrosis factor receptor therapy. *J Drugs Dermatol* 2009;8:873–876.
- Cassano N, Loconsole F, Coviello C, Vena GA: Infliximab in recalcitrant severe atopic eczema associated with contact allergy. *Int J Immunopathol Pharmacol* 2006;19:237–240.

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