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How Effective Is Tacrolimus in the Imiquimod-Induced Mouse Model of Psoriasis?

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TO THE EDITOR

The imiquimod (IMQ)-induced mouse model has become a widely used standard to model human psoriasis since its introduction in 2009 and seems to mirror psoriasis in many pathogenetic, clinical, and histological features (van der Fits et al., 2009). Because of various advantages, the number of publications based on this model has increased exponentially in the past 7 years (Hawkes et al., 2017). However, van der Fits et al. already stated that the model's response to antipsoriatic drugs still needs to be shown. A review article in this journal addressed a lack of validation of the model resembling human psoriasis and thus its applicability for therapeutic testing (Hawkes et al., 2017).

The immunomodulatory drug tacrolimus (TAC) is commonly used for the topical treatment of dermatitis because it lacks important side effects of corticosteroids (e.g., skin atrophy). However, the efficacy of topically applied TAC has not yet been achieved in the most common plaque-type psoriasis, in contrast to facial and inguinal psoriasis, and its efficacy after systemic administration (Scheinfeld, 2004).

Here, we investigated how effective topical TAC treatment is in the IMQ-induced mouse model of psoriasis.

The *model was induced* successfully according to the standard regime as reported by van der Fits et al. by daily topical administration of IMQ to the right ear and flank (Supplementary Figure S1, left column, online) in BALB/c mice. Of note, the model could not be induced in hairless SKH1 mice, which are widely used in dermatology research (Supplementary Figure S1, right column). Detailed information on materials and methods is provided in the Supplementary Material online. Experiments were approved by the State Office of Health and Social Affairs, Berlin, Germany.

To evaluate the *therapeutic efficacy of TAC* in the IMQ model, psoriasis-like dermatitis was induced in 33 mice. Mice were treated topically with TAC and various control substances, following three different treatment protocols (Figure 1a–c). Clinical parameters were quantified daily, including transepidermal water loss, erythema, skin hydration, and ear thickness. Additionally, skin lesions were clinically scored using a modified psoriasis area and severity index. As final readout parameters, skin was examined histologically, epidermal thickness was measured, and infiltrating T-lymphocytes were counted in a randomized, blinded manner.

In the *first treatment protocol*, which has been routinely used in this model (Doppalapudi et al., 2017; Jain et al., 2016), mice were treated daily starting on day 3 for 5 consecutive days (Figure 1a). TAC was applied on six mice, in two different concentrations (0.03%, 0.003%) at the dermatological standard dose of 2 mg ointment/cm^2. However, no effects on any of the parameters measured were achieved (Figure 1, left column; Supplementary Figure S2 online). By contrast, six mice topically treated with dexamethasone developed significant improvements in ear thickness and less infiltrating T cells. However, no differences in other parameters measured on the flank were observed. The phenomenon of skin region-dependent treatment success has been observed before (Schaper et al., 2013).

The possible causes of lack of TAC efficacy include too low dose, too short treatment period, and too late onset of intervention. To address these possibilities, we repeated the experiment using a *second treatment protocol* with a higher amount of TAC ointment and the highest commercially available concentration for topical treatment (0.1%, 10–40 mg/cm^2, n = 5). We also

Abbreviations: IMQ; imiquimod; TAC; tacrolimus

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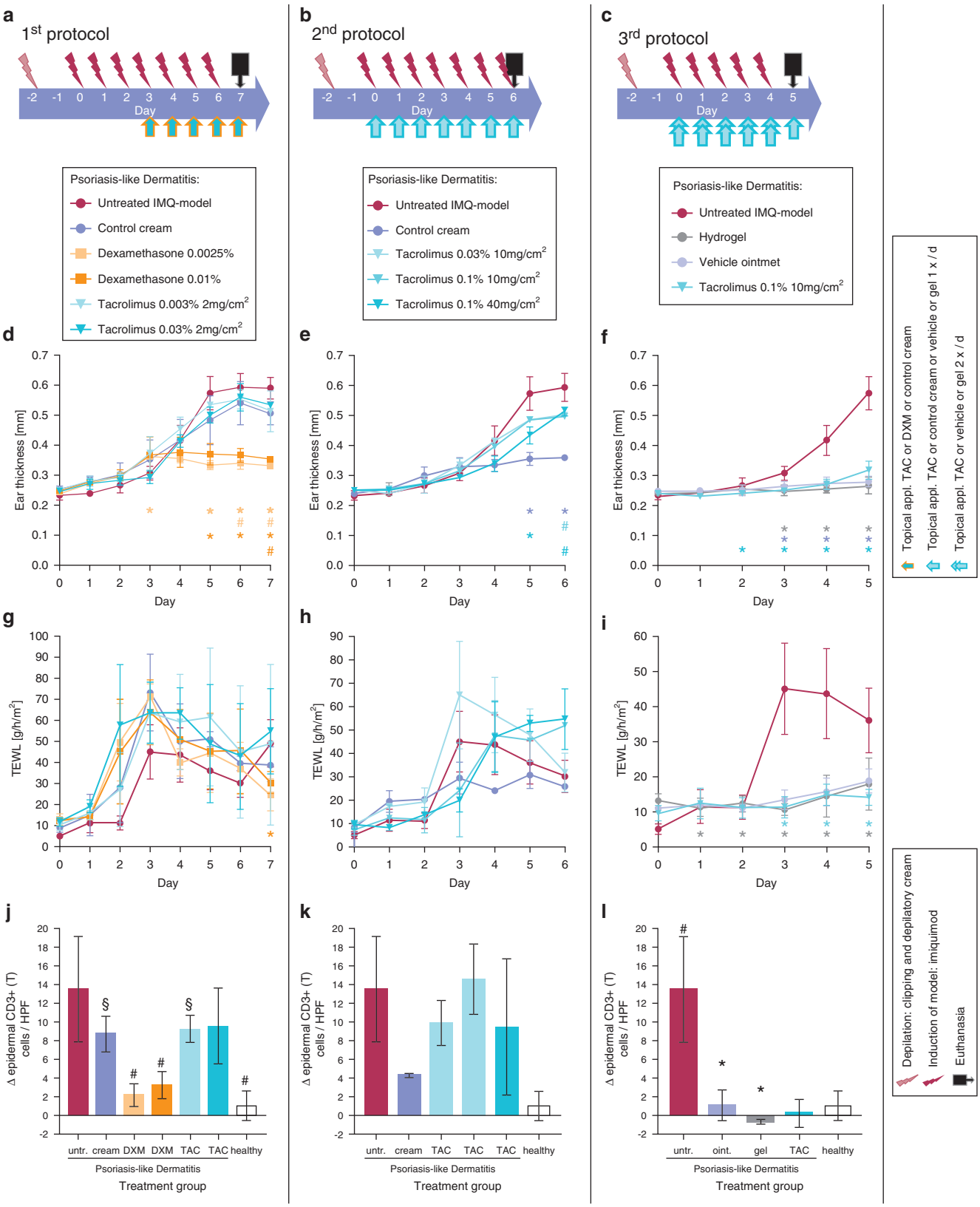

Figure 1. Clinical and histological data of treated psoriasis-like dermatitis using different protocols. (**a**–**c**) Treatment protocol schemes: data obtained using (**a**) the first protocol: treated daily starting on day 3 after induction of inflammation; (**b**) the second protocol: daily treatment started on same day 0 as induction of inflammation, or (**c**) the third protocol: treatment twice daily from day 0; (**d**–**f**) ear thickness, (**g**–**i**) transepidermal water loss (TEWL), (**j**–**l**) increase in epidermal CD3-positive cells; mean ± standard deviation; *t*-test Holm-Sidak-corrected; $P < 0.05$: * compared with the untreated IMQ model control group, # compared with the control cream- or vehicle ointment-treated control group, for histological parameters additionally (**j**–**l**): § compared with the healthy control group; additional parameters (erythema, skin hydration, PASI score, epidermal thickness), and macroscopic and histologic skin phenotype images are shown in Supplementary Figure S2. DXM, dexamethasone; HPF, high power field (400x); IMQ, imiquimod; PASI, modified psoriasis area and severity index; TAC, tacrolimus.

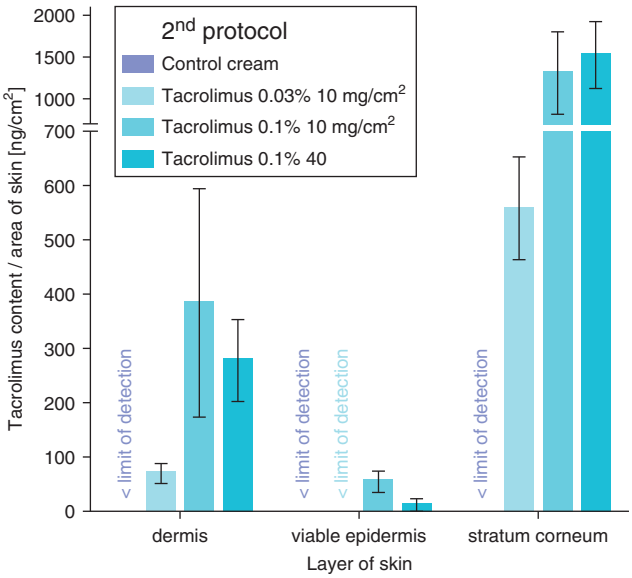

Figure 2. Tacrolimus content in different skin layers determined by LC-MS/MS. Tacrolimus content per area of skin from separated skin layers by tape stripping (stratum corneum) and subsequent heat separation of remaining viable epidermis and dermis; data were obtained from mice treated according the second treatment protocol; mean ± standard deviation. LC-MS/MS, liquid chromatography tandem-mass spectrometry.

included a prolonged treatment period of 7 days and an earlier onset of treatment, starting on the same day as model induction. This approach had been reported to be successful when using TAC on IMQ-treated C57BL/6 mice (Gabriel et al., 2016). Nevertheless, it should be kept in mind that this protocol technically constitutes a preventive rather than a therapeutic approach. Only the control cream showed a tendency to improve disease outcome (Figure 1e+k; Supplementary Figure S2, center column). In contrast to the published literature on the C57BL/6 strain (Gabriel et al., 2016), we failed to observe TAC-specific anti-inflammatory efficacy in any of the tested parameters (Figure 1, center column; Supplementary Figure S2).

To exclude the possibility that the penetration of TAC was insufficient to induce anti-inflammatory effects, we used liquid chromatography tandem-mass spectrometry to quantify the *content of TAC in viable epidermis and dermis* as well as in the stratum corneum. Approximately 300 ng/cm^2 TAC could be detected in the dermis when using 0.1% TAC ointment (Figure 2). This is in the same order of magnitude as data from a successful treatment of atopic dermatitis skin in humans (Undre et al., 2009). Thus, we assume that the penetration of TAC was sufficient.

To further exclude the possibility that the effective TAC concentration was still too low, we conducted a *third treatment protocol*. As no topical formulation with a higher concentration of TAC is commercially available, we increased the dose by applying TAC twice daily (Figure 1c). As the control cream showed beneficial effects in the second protocol, we included hydrogel and vehicle ointment as control groups.

Surprisingly, almost no psoriasis-like phenotype developed, neither in the TAC-treated group nor in any of the control groups compared with the untreated group (Figure 1, right column; Supplementary Figure S2). This protocol thus seems inappropriate to test for anti-inflammatory treatment efficacy in the IMQ model.

To conclude, we could not show a relevant drug-specific anti-inflammatory efficacy of topically applied TAC in the IMQ-induced psoriasis model in BALB/c mice, despite sufficient penetration of the drug. Further research is needed to elucidate the apparent conflict to first reports of TAC efficacy in this model (Boakye et al., 2017; Gabriel et al., 2016; Jain et al., 2016; Thapa and Yoo, 2014; Wan et al., 2017). However, either ointment/cream vehicle controls were not reported (Boakye et al., 2017; Gabriel et al., 2016; Jain et al., 2016; Thapa and Yoo, 2014) or only a small improvement compared with vehicle control was seen in clinical scoring but not in objective parameters (Wan et al., 2017). This might have led to an overinterpretation of drug-specific effects. Furthermore, using different mouse strains like C57BL/6 might have had an impact on the response to anti-inflammatory treatments (Boakye et al., 2017; Gabriel et al., 2016; Swindell et al., 2017). Our results emphasize that a vehicle control is imperative in the IMQ model, that several objective readout parameters should be investigated, and effects in single parameters alone should not be overinterpreted (Hawkes et al., 2017).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2017.09.019.

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See related commentary on pg 246

The Anti-C1s Antibody TNT003 Prevents Complement Activation in the Skin Induced by Bullous Pemphigoid Autoantibodies

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TO THE EDITOR

Chronic skin inflammation, subepidermal blistering, and severe itching are the clinical hallmarks of bullous pemphigoid (BP). The disease is caused by autoantibodies against type XVII collagen (COL17, BP180), more specifically, the extracellular fraction of the 16th noncollagenous domain of the protein (NC16A) (Schmidt and Zillikens, 2013). Two pathways are thought to drive BP pathogenesis. *First*, autoantibody binding to COL17 leads to activation of the complement cascade, evidenced by the detection of complement deposits along the dermal-epidermal junction in patients with BP (Jordon et al., 1967, 1975) and in mouse models of the disease (Iwata et al., 2015). For example, blockade of C1q or use of noncomplement activating mutant IgG as well as C4- and C5-deficient mice (Nelson et al., 2006) protected from anti-COL17 IgG transfer-induced blistering, thus underscoring the key relevance of the classical pathway of complement in BP pathogenesis (Li et al., 2010; Nelson et al., 2006). *Second*, noncomplement-dependent pathways lead to a depletion of COL17 (Ujiie et al., 2014), facilitated by protein kinase C-regulated micropinocytosis (Iwata et al., 2016). It is currently unclear which of these two mechanisms drives inflammation and blistering in patients with BP. Yet, the clinical description of an inflammatory and a noninflammatory BP phenotype (Izumi et al., 2016) provokes the assumption that complement-mediated blistering may be one of the driving disease pathways in patients with inflammatory BP.

Despite these detailed insights into BP pathogenesis (Ludwig et al., 2013), corticosteroids are still the mainstay of treatment. Although inducing a rapid and complete clinical remission in almost all patients (Joly et al., 2002), frequently occurring relapses require (Bernard et al., 2009) prolonged corticosteroid treatment (Joly et al., 2002). Therefore, treatments maintaining the initial therapeutic response, or at least reducing the steroid dose, are urgently needed. Yet, with the exception of the anti-C5 antibody eculizumab, no complement-targeting biologicals have been approved for clinical use. In addition, eculizumab inhibits the activation of the terminal cascade driven by all three complement pathways. As BP pathology has been linked specifically to classical complement pathway (CP) activity, its selective blockade would maintain full functionality of the alternative and lectin complement

Abbreviations: BP, bullous pemphigoid; COL17, type XVII collagen; CP, classical complement pathway

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