



A murine model of epicutaneous protein sensitization is useful to study efficacies of topical drugs in atopic dermatitis

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ABSTRACT

We studied the suitability of our murine model for the treatment trials of atopic dermatitis (AD). In this model topical application of ovalbumin (OVA) together with bacterial superantigen, staphylococcal enterotoxin B (SEB) induces a cutaneous disease resembling AD. Injured mouse skin was treated with three different drugs: a class III corticosteroid, a calcineurin inhibitor and a type 4 phosphodiesterase inhibitor. One-week treatment with corticosteroid and phosphodiesterase inhibitor remarkably decreased both epidermal and dermal thickness, whereas the calcineurin inhibitor affected only the epidermal thickness. All investigated drugs reduced the infiltration of eosinophils and mast cells onto OVA/SEB sensitized skin areas, whereas CD4+ and CD8+ T cells as well as CD11c+ dendritic cells variously diminished after corticosteroid and calcineurin inhibitor treatments. Cutaneous expression of interleukin -4, -13, -10 and interferon- γ also decreased differently depending on drug type. Interestingly, the calcineurin inhibitor and phosphodiesterase inhibitor increased total IgE antibodies and decreased SEB-specific IgG2a antibodies in OVA/SEB sensitized mice. All these drugs can ameliorate cutaneous inflammation, although the degree of recovery depends on the type of the drug. In summary, our results show that this mouse model can be used to test new topical treatments for AD.

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1. Introduction

Atopic dermatitis (AD, also called atopic eczema) is a chronic inflammatory skin disease affecting about 10 million people in the world and its incidence is continuously increasing in westernized countries [1,2]. Extrinsic AD is characterized by intensely pruritic eczematous skin lesions, along with elevated IgE levels and is often associated with a family history of atopic diseases such as asthma and allergic rhinitis [3,4]. Cutaneous AD lesions are infiltrated with CD4+ T cells which in the acute phase predominantly secrete Th2 cytokines IL-4, IL-5 and IL-13, whereas in chronic lesions IFN- γ -producing Th1 cells dominate. In addition, tissue and serum eosinophilia as well as dermal mast cell infiltration are characteristic features of AD. Finally, latest studies suggest that in addition to defects in adaptive immunity also innate immunity defects are important factors predisposing people to AD [5,6].

In recent years, it has been suggested that epidermal skin barrier defects play a significant role in AD. Using a murine model of AD, it has

been shown that skin barrier dysfunction induced by tape-stripping enhances allergen sensitization leading to increased IgE levels, Th2-dominated skin inflammation and even airway hyper-reactivity [7,8]. Previous studies have been shown that loss-of-function mutations in skin barrier genes encoding filaggrin are associated with severe AD and development of asthma in AD patients [9–11]. In addition, mice which have disturbed skin barrier because of transgenic cutaneous expression of human apolipoprotein C1 spontaneously develop symptoms of atopic dermatitis [12].

Patients with AD are particularly susceptible to *Staphylococcus aureus* infections, in part due to lack of endogenous antimicrobial peptides, the human cathelicidin product LL-37 and human β -defensins 2 and 3 [13,14]. Defective killing of *S. aureus* in AD patients is associated with reduced mobilization of human β -defensin-3 [15]. *S. aureus* can initiate or aggravate skin inflammation by secreting superantigens which are potent T cell activators and can stimulate up to 35% of T cells without antigen processing [16] and finally augment skin inflammation and the production of Th2- and Th1-type cytokines [7,17]. One of superantigens, staphylococcal enterotoxin B (SEB), increases the production of allergen specific IgE and also specific IgE directed toward SEB in the murine model of AD [7]. In addition, antimicrobial IgE antibodies including SEB-specific IgE levels are elevated among AD subjects with high total IgE levels [18].

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Current topical treatment possibilities for AD are topical corticosteroids as a standard treatment and topical calcineurin inhibitors (macrolide immunosuppressants) such as tacrolimus or pimecrolimus as a second-line therapy [1]. Previously, only spontaneous mouse models of AD have been used to investigate topical drugs in AD [19,20]. Here we show that our mouse model of AD caused by repeated epicutaneous ovalbumin (OVA) and SEB exposures is valuable and repeatable model to test topical drugs in AD. We investigated the efficacy of a potent corticosteroid (betamethasone-17-valerate), a calcineurin inhibitor (tacrolimus) and the type 4 phosphodiesterase inhibitor (cipamfylline) in the murine model of AD.

2. Materials and methods

2.1. Sensitization and treatment protocol

Female BALB/c mice (aged 6–8 weeks) were obtained from Scanbur A/S (Karlslunde, Denmark) and maintained under pathogen-free conditions. All animal experiments were approved by State Provincial Office of Southern Finland. Mice were sensitized topically applying 100 µl of OVA/SEB solution onto a sterilized gauze (1 × 1-cm patch) which was then attached to the shaved and tape-stripped skin as earlier described [7]. OVA and SEB were purchased from Sigma-Aldrich Co (St. Louis, MO) and dosed at 100 and 2.5 µg/patch, respectively. Drugs were applied to the skin during the third sensitization week together with OVA/SEB mixture in 75% acetone/PBS solution. In the first step we investigated the action of betamethasone-17-valerate and in the second phase the impact of tacrolimus and cipamfylline. Drug concentrations were as follows: betamethasone-17-valerate (a strong corticosteroid, Sigma-Aldrich Co) 15 µg/100 µl, tacrolimus (FK-506, Sigma-Aldrich Co) 100 µg/100 µl and cipamfylline (type 4 phosphodiesterase (PDE-4) inhibitor; Leo Pharma A/S, Ballerup, Denmark) 100 µg/100 µl. Patches were changed two times during the first and second sensitization week and three times during the last week. Phosphate-buffered saline (PBS) was used as a vehicle during the first and second sensitization week and 75% acetone in PBS during the treatment week. There were two resting weeks between sensitization weeks. Mice were killed by CO₂-inhalation after sensitization and treatment protocol. Blood samples were taken for antibody analysis and skin biopsies from treated skin areas for RNA isolation and histology 24 h after the protocol. Eight mice were sensitized in all investigated groups.

2.2. Skin histology

Skin biopsies were fixed in 10% buffered formalin and embedded in paraffin. Skin sections of 4 µm were cut and stained with haematoxylin and eosin (H&E) for inflammatory cells and skin thickness measurements or with o-toluidine blue for mast cell counts, and examined under light microscopy (Leica DM 4000B, Wetzlar, Germany). Inflammatory cell types were counted in 15 high-power fields (HPF) at 1000×, and mast cells at ×400 magnification.

2.3. Immunohistochemistry

Skin specimens were embedded in Tissue-Tek oxacalcitriol compound (Sakura Finetek Europe B.V., Zoeterwoude, the Netherlands) and quick frozen on dry ice. Staining of CD3+, CD4+, CD8+ and CD11c+ cells was made as earlier described [7,21,22]. Briefly, the skin samples were cut into 4-µm frozen sections, fixed with cold acetone and stained using the ChemMate (DakoCytomation, Glostrup, Denmark) staining kit. Primary anti-mouse monoclonal antibodies were obtained from BD PharMingen (San Diego, CA). Macrophages were stained by similar techniques with a purified rat anti-mouse F4/80 antibody (clone BM4007S, Acris Antibodies, Herford, Germany).

Biotin-conjugated secondary antibodies were purchased from Vector Laboratories Inc. (Burlingame, CA; anti-rat IgG (H+L) for T and F4/80 cells or from BD PharMingen (anti-hamster cocktail for CD11c cells). Epidermal and dermal areas were counted at 400× magnification.

2.4. Cytokine analysis by RT-PCR

Quickly-frozen skin samples were kept in –70 °C before extraction of total RNA. RNA isolation was made as earlier described with a slight modification: we used Eurozol (EuroClone, Sizzano, Italy) instead of Trizol in the homogenization step [22] and RNA content was measured by NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE). cDNA was synthesized from 0.5 µg of total RNA in 25 µl reaction mixture with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Reaction conditions were: at 25 °C for 20 min and at 37 °C for 120 min in a thermal cycler (GeneAmp 2720, Applied Biosystems). PCR primers and probes were obtained from Applied Biosystems and quantitative real-time PCR was performed with 7500 Fast Real-Time PCR System and SDS Software v.1.4.0 (Applied Biosystems). The gene expression between different samples was normalized with endogenous 18S rRNA, and the target gene expression was calculated by the comparative C_T method according to the instructions of Applied Biosystems.

2.5. IgE and IgG2a antibody measurements

Total IgE levels in sera were analyzed by sandwich ELISA using the standard BD PharMingen protocol [22]. Serum levels of antigen specific antibodies were measured by the straight ELISA method [7,22]. Briefly, plates were coated with antigens in 0.05 M NaHCO₃ (pH 9.6) overnight at +4 °C, washed with PBS-Tween 20 (0.05%) and blocked (3% BSA/PBS) for 2 h at 20 °C. Serial dilutions of sera in dilution buffer (1% BSA/PBS) were added after washing and incubated overnight at +4 °C. After washing, biotin-conjugated rat anti-mouse IgE or IgG2a monoclonal antibody (2 µg/ml) was incubated for 2 h at 20 °C. Subsequently, streptavidin-horseradish peroxidase (1/4000 in 1% BSA/PBS) was added after washing for 30 min at 20 °C. Finally, ABTS substrate solution (Kirkegaard & Perry Laboratories, Gaithersburg, MD) was added to washed plates and optical densities were read with automated ELISA reader at 405 nm (Labsystems Multiskan, Thermo Electron, Turku, Finland). Coating concentrations were 100 µg/ml OVA for OVA-specific IgE, 2 µg/ml OVA for OVA-specific IgG2a and 2 µg/ml SEB for both SEB-specific IgE and IgG2a. Serial dilutions were 1/40, 1/120 and 1/360 for OVA-specific IgE; 1/200, 1/600, 1/1800 and 1/5400 for OVA-specific IgG2a; 1/20, 1/60, 1/180 and 1/540 for SEB-specific IgE; 1/50, 1/150, 1/450 and 1/1350 for SEB-specific IgG2a. Monoclonal antibodies and streptavidin-horseradish peroxidase were obtained from BD PharMingen.

2.6. Statistics

The data were expressed as means (±SEM), and differences between means were analysed with Graph Pad Prism- software using a Student's *t*-test with a two-tailed test of significance. A Mann–Whitney *U*-test was used when variances were different between groups for unpaired comparisons. Differences at *P*<0.05 were considered to be statistically significant.

3. Results

3.1. Betamethasone-17-valerate and cipamfylline significantly reduce thickness of sensitized skin areas

Skin thickening is one of general features of AD [1,2]. OVA/SEB sensitization causes a clear thickening of treated skin areas in mice (Fig. 1A). All topical drug treatments significantly decreased epidermal

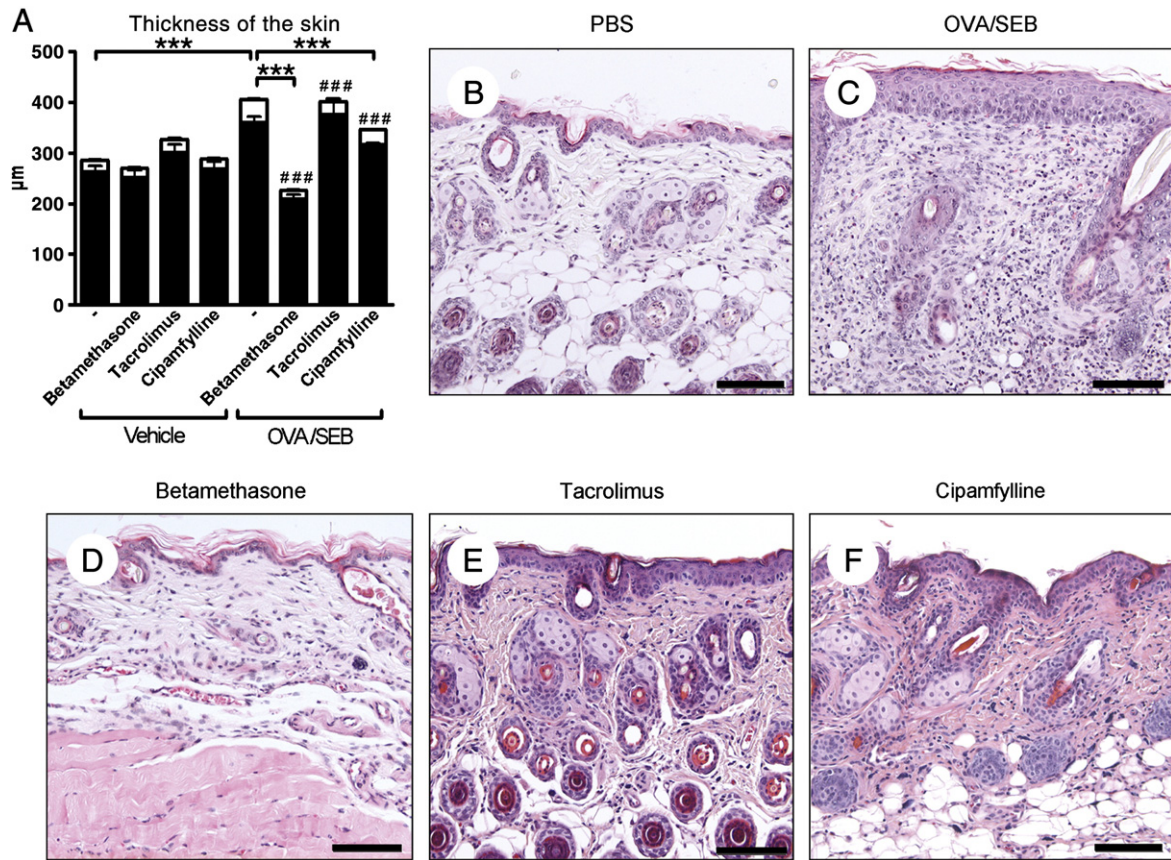


Fig. 1. Thickness of epidermis and dermis. A) Measurements were made from H&E-stained skin samples at $\times 100$ (epidermis) and at $\times 400$ (dermis) magnifications. A white section of the columns represents epidermal thickness and black part dermal thickness. Statistical significances are indicated with different symbols: # for epidermal thickness between OVA/SEB and drug-treated skin and * for whole skin thickness (as well as dermal thickness). *** $p \leq 0.001$ and ### $p \leq 0.001$. H&E stained skin of B) PBS-control, C) OVA/SEB-sensitized D) OVA/SEB-sensitized and betamethasone-17-valerate treated, E) OVA/SEB-sensitized and tacrolimus treated and F) OVA/SEB-sensitized and cipamfylline-treated skin. The columns and the error bars represent means $\pm$ SEMs ($n = 8$ mice/group). Scale bar = 100 μ m.

thickness whereas effects on dermal thickness varied (Fig. 1A–F). Both betamethasone-17-valerate and cipamfylline significantly diminished thickness of the dermis. In addition, after betamethasone-17-valerate treatment the dermis was even thinner than in their control mice (Fig. 1A). On the contrary, the dermis remained unchanged after tacrolimus treatment (Fig. 1A, E).

3.2. Topical drug administration decreases infiltration of inflammatory cells to OVA/SEB sensitized skin areas

Skin-infiltrating inflammatory cells, e.g. eosinophils and mast cells, are a prevalent feature of AD [23–25]. OVA/SEB sensitized mice showed an increased infiltration of inflammatory cells to the sensitized skin patches (Fig. 2A). All different drugs decreased the total number of infiltrating cells. However, there were differences between drugs. Betamethasone-17-valerate was the most effective and decreased infiltration of total inflammatory cells to the OVA/SEB sensitized skin significantly more than tacrolimus or cipamfylline (Fig. 2A). Cutaneous eosinophil numbers also increased after OVA/SEB treatments in this model (Fig. 2B). The efficiency of these drugs to lower eosinophils in the skin also varied: betamethasone-17-valerate induced a 5.5-fold decrease, tacrolimus a 2.8-fold decline and cipamfylline 2.3-fold drop. Increased numbers of mast cells decreased almost equally by these drugs in dermal layers of sensitized skin, although tacrolimus was less efficient than other drugs (Fig. 2C). Amounts of CD11c positive dendritic cells (Fig. 2D) and F4/80 positive macrophages (Fig. 2E) also enhanced after OVA/SEB sensitization in affected skin areas. Betamethasone-17-valerate decreased numbers of both cell types, where-

as tacrolimus affected significantly only on CD11c+ cells and cipamfylline had no significant effect on these cell types.

OVA/SEB sensitization also increased numbers of total T cells (CD3 positive cells) (Fig. 3A) as well as T helper cells (CD4 positive cells) (Fig. 3B, D) and cytotoxic/suppressor type T cells (CD8 positive cells) (Fig. 3C, H). These increases were found both in epidermal and dermal layers of sensitized skin areas. Interestingly, cipamfylline treatment did not affect any of these T cell populations. On the other hand, betamethasone-17-valerate treatment decreased more significantly CD3 and CD4 positive cells than tacrolimus, whereas tacrolimus was as effective as betamethasone-17-valerate against CD8 cells (Fig. 3A–K).

3.3. Drug administration downregulates mRNA expression of cytokines

Th2 type cytokines are important in an acute phase of AD whereas mixed Th2/Th1 type inflammation is characteristic to a chronic phase of AD [1,26]. Repeated OVA/SEB sensitization increased significantly cutaneous mRNA expression of Th2 cytokines IL-4 and IL-13 (Fig. 4A, B) and Th1 type cytokine IFN- γ (Fig. 4C). In addition, expression of regulatory cytokine IL-10 was also significantly enhanced (Fig. 4D). IL-4 and IL-13 levels significantly decreased after betamethasone-17-valerate and tacrolimus treatments, but not after cipamfylline administration (Fig. 4A, B). All drugs significantly lowered IFN- γ levels, and the effect of tacrolimus was stronger than the effects of betamethasone-17-valerate or cipamfylline (Fig. 4C). Only betamethasone-17-valerate significantly decreased expression of IL-10 in sensitized skin patches, although tacrolimus induced a non-significant lowering of IL-10 expression (Fig. 4D).

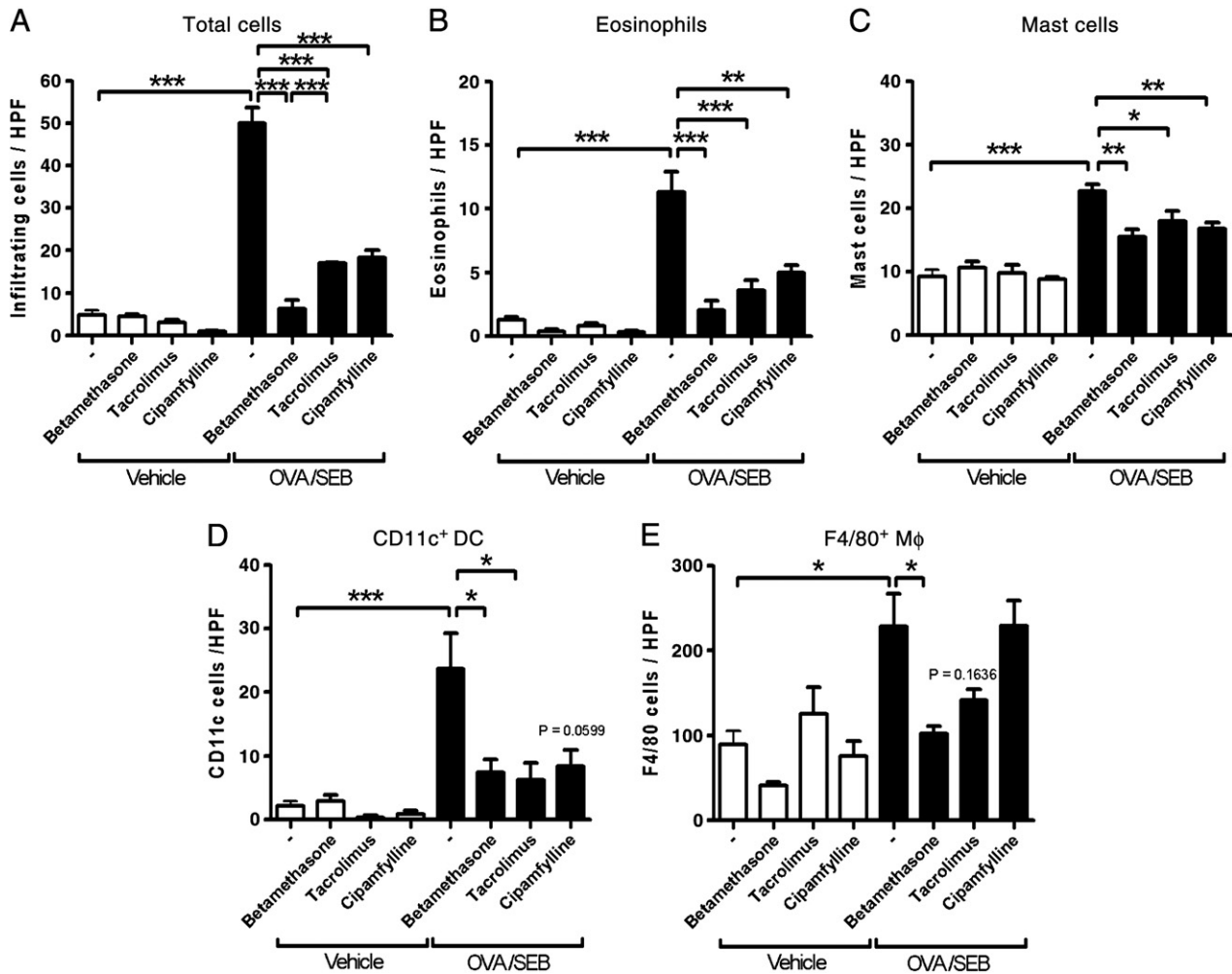


Fig. 2. The number of infiltrating cells on sensitized skin sites. A) Total infiltrating inflammatory cells and B) eosinophils were counted after H&E staining at $\times 1000$ and expressed as cells per HPF (high power field). C) Mast cells were counted after 0-toluidine staining at $\times 400$. D) CD11c⁺ dendritic cells and E) F4/80⁺ macrophages were counted after immunostaining at $\times 400$. The columns and the error bars represent means $\pm$ SEMs ($n = 4-8$ mice/group). * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$.

3.4. Tacrolimus and cipamfylline increase OVA-specific IgE whereas SEB-specific IgG2a is decreased

To evaluate systemic effects of drugs, we measured antigen- and superantigen-specific Th1 type (IgG2a) and Th2 type (IgE) antibodies in mice sera. Total IgE and both OVA- and SEB-specific IgE and IgG2a antibodies increased after repeated epicutaneous OVA/SEB sensitizations in sera (Fig. 5A–E). Unexpectedly, tacrolimus and cipamfylline significantly enhanced OVA-specific IgE levels compared to OVA/SEB sensitized mice (Fig. 5B). On the other hand, betamethasone-17-valerate treatment had no effect on these antibodies. None of drugs affected OVA-specific IgG2a antibodies after OVA/SEB sensitization (Fig. 5C). In addition, there were no significant changes in SEB-specific IgE antibody levels after different drug treatments (Fig. 5D). Interestingly, tacrolimus and cipamfylline significantly decreased SEB-specific IgG2a antibodies in OVA/SEB sensitized mice, whereas betamethasone-17-valerate had no influence (Fig. 5E).

4. Discussion

Atopic dermatitis is a life-long skin disease with unpredictable disease activity and prognosis. As a chronic and common disease, new and effective treatment methods are needed in addition to those already in use. A mouse model of AD which is reliable, reproducible and can be used to test topical drug candidates is needed. In our

murine model the protein and superantigen induced skin inflammation is similar with many aspects to human AD. The skin of AD mouse is infiltrated with CD4⁺ T cells, eosinophils and mast cells. Additionally, allergen and superantigen-specific IgE characterize the systemic response. AD patients have a defective skin barrier which allows increased allergen and pathogen penetration. It has been shown that filaggrin as well as loricrin and involucrin are important proteins for skin barrier formation and integrity [27]. In our model the dysfunction of the skin barrier is caused by tape-stripping which mimics the reduced skin barrier function and intense scratching characteristic to AD.

Three different drug preparations were used to test their efficacy in our murine model of AD. We found that those topical drugs which are already the first and second-line of therapy in AD, corticosteroid and calcineurin inhibitor, were the most beneficial in the healing of the skin inflammation. Betamethasone-17-valerate is a potent corticosteroid which has many beneficial effects on atopic eczema. However, there is a risk for local adverse effects such as skin atrophy [28]. Our results revealed that drugs studied affected differently. A characteristic feature of AD is thickening of the skin [1,2] which was also found within our OVA/SEB sensitized mice. All tested drugs decreased thickness of epidermis, whereas tacrolimus did not affect dermal thickness. A reason for this could be a relatively short treatment period (one week). On the other hand, after betamethasone-17-valerate treatment dermis was even thinner than in their control

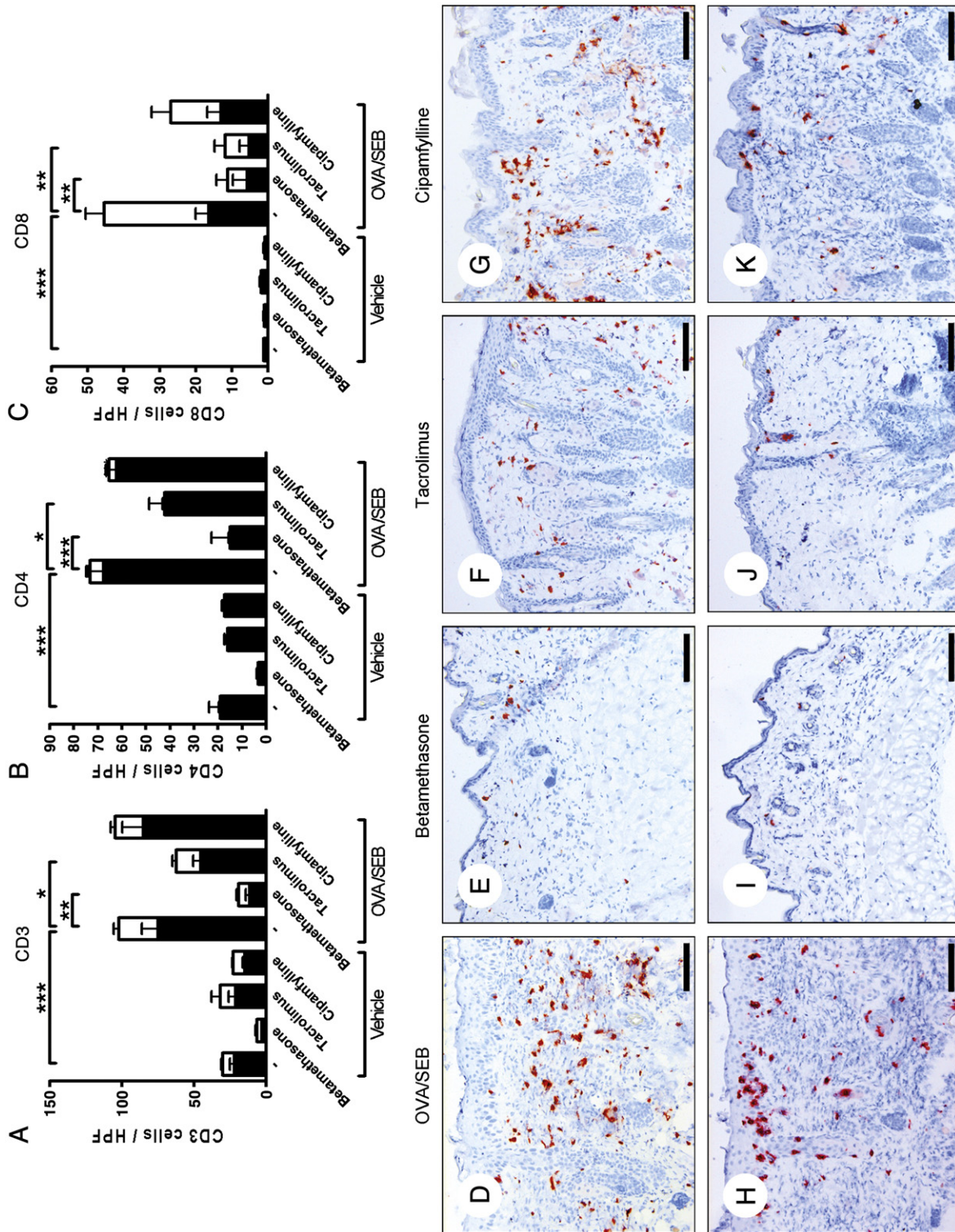


Fig. 3. Distribution of T cells in treated skin samples. Immunostained A) CD3+, B) CD4+ and C) CD8+ cells are counted at magnifications of $\times 400$. Epidermal T cells are shown with white columns and black columns are showing dermal T cells. Asterisks represent statistics between whole (epidermis and dermis) columns. The columns and the error bars represent means $\pm$ SEMs ($n = 4-8$ mice/group). * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$. D–G are showing CD4-stained skin of D) OVA/SEB-sensitized, E) OVA/SEB-sensitized and betamethasone-17-valerate treated, F) OVA/SEB-sensitized and tacrolimus treated and G) OVA/SEB-sensitized and cipamfylline treated skin. H–K are showing CD8-stained skin of H) OVA/SEB-sensitized, I) OVA/SEB-sensitized and betamethasone-17-valerate treated, J) OVA/SEB-sensitized and tacrolimus treated and K) OVA/SEB-sensitized and cipamfylline treated skin. Scale bar = 100 μ m.

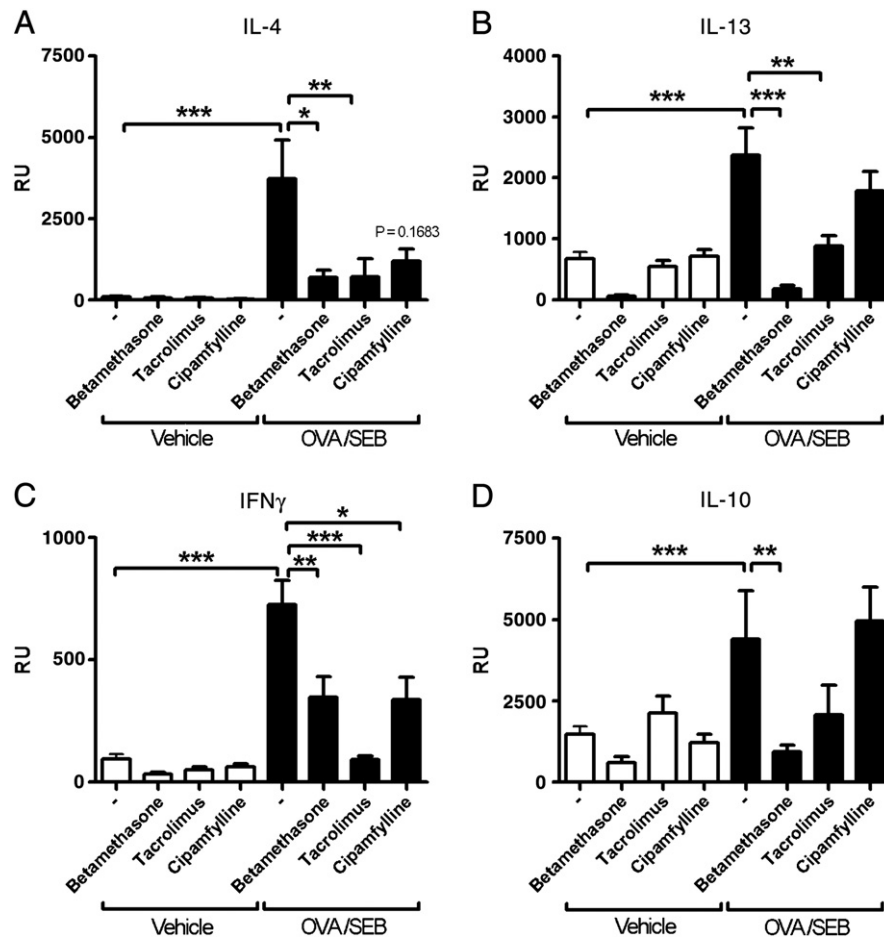


Fig. 4. The mRNA expression of different cytokines A) IL-4, B) IL-13, C) IFN- γ and D) IL-10 in affected skin patches after repeated topical application of vehicle or OVA/SEB with or without drug treatments. Results are shown as relative units (RU). The columns and the error bars represent means $\pm$ SEMs ($n = 8$ mice/group). * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$.

mice in line with atrophic effects of corticosteroids on skin thickness. Steroid related atrophy has not been observed with topical calcineurin inhibitors, such as tacrolimus [29]. Since calcineurin inhibitors also have side-effects, such as sensation of burning at the side of application and rare cases of malignancy have been reported [30,31], a pressure to develop and test new topical therapeutics exists.

It is well-known that eosinophils and mast cells play an important role in a chronic skin inflammation of patients with AD [25,26]. Histological examination showed that all tested drugs reduced the total number of skin infiltrating cells as well as amounts of eosinophils and mast cells. It has been earlier shown that short-term tacrolimus treatment reduced the number of eosinophils in the dermal skin lesions of patients with AD whereas the number of mast cells did not change [32]. In line with their result we found that tacrolimus strongly reduced the infiltration of eosinophils in OVA/SEB-sensitized skin and only weakly decreased the number of mast cells. The number of cutaneous CD11c positive myeloid dendritic cells also significantly decreased after administration of betamethasone-17-valerate and tacrolimus. Only betamethasone-17-valerate decreased F4/80 macrophages in OVA/SEB-sensitized skin patches. Since macrophages and dendritic cells function as antigen presenting cells reduction of these immune cells illustrates healing of cutaneous inflammation.

It has earlier been shown that *S. aureus* colonization can be erased with topical tacrolimus treatment. According to our results, superantigen-induced skin inflammation together with an allergen can be reduced by topical steroids or calcineurin inhibitors, such as betamethasone-17-valerate or tacrolimus. Since superantigens are known to be more potent in activating T cells than conventional antigens it is very promising that mice sensitized with OVA/SEB and treated with

betamethasone-17-valerate or tacrolimus showed a significant reduction of CD3+ and CD4+ T cells. Immunohistochemical staining of T cells revealed that betamethasone-17-valerate was most efficient drug type against skin infiltrating T cells. In addition, tacrolimus decreased the number of T helper and cytotoxic/suppressor T cells, whereas cipamfylline had no significant effect on these T cell types. Altogether the numbers of CD3+ and CD4+ cells in the skin are in line with cutaneous Th2 type cytokine expression. Both betamethasone-17-valerate and tacrolimus downregulated IL-4 and IL-13 as well as numbers of CD3+, CD4+ and CD8+ cells.

Earlier reports have shown the importance of different cytokines and chemokines in the development and maintenance of AD [33]. One important feature of chronic inflammation in AD patients is enhanced levels of Th2/Th1 type cytokines [1,26]. In line with this we found an increased cutaneous mRNA expression of IL-4, IL-10, IL-13 and IFN- γ after topical OVA/SEB sensitization. Betamethasone-17-valerate and tacrolimus, but not cipamfylline significantly decreased mRNA expression of Th2 cytokines IL-4 and IL-13 in treated skin patches. On the other hand, all these drugs decreased cutaneous mRNA expression of Th1 cytokine IFN- γ . Tacrolimus diminished IFN- γ levels more than betamethasone-17-valerate and cipamfylline whereas the effect of betamethasone-17-valerate was more pronounced on IL-13. In addition, only betamethasone-17-valerate down-regulated cutaneous mRNA expression of regulatory cytokine IL-10, emphasizing the superiority of betamethasone-17-valerate as a topical drug.

Systemic effects of topical drugs were studied by measuring total IgE as well as OVA- and SEB-specific IgE and IgG2a antibodies in sera. Interestingly, tacrolimus and cipamfylline, but not betamethasone-17-valerate, elicited additional increases in total IgE compared to

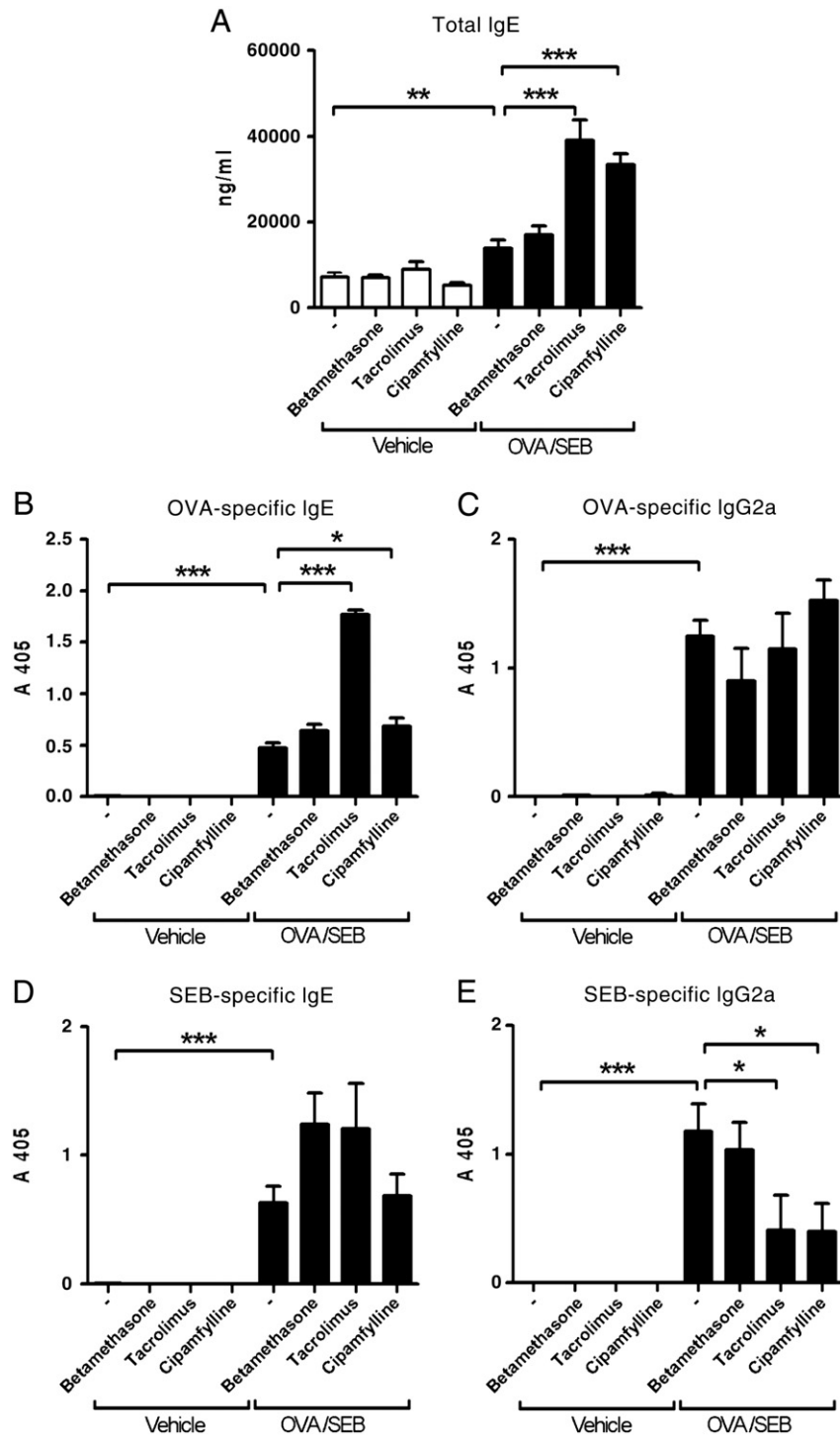


Fig. 5. Antibody levels in mice sera after repeated topical application of vehicle or OVA/SEB with or without drug treatments. A) Total IgE-levels are shown as ng/ml. B) Ova-specific IgE, C) Ova-specific IgG2a, D) SEB-specific IgE and E) SEB-specific IgG2a levels are shown as optical density values obtained at 405 nm. The columns and the error bars represent means $\pm$ SEMs ($n=8$ mice/group). * $p<0.05$; ** $p<0.01$ and *** $p<0.001$.

untreated OVA/SEB mice during the third sensitization week. Moreover, tacrolimus induced significantly higher OVA-specific IgE levels compared to cipamfylline and betamethasone-17-valerate. On the other hand, tacrolimus and cipamfylline decreased SEB-specific IgG2a antibodies in OVA/SEB sensitized mice whereas betamethasone-17-valerate had no effect. In a recent study an almost significant decrease in serum IgE was seen in a subgroup of tacrolimus treated patients with elevated serum IgE at baseline and a sustained good treatment result in AD, whereas in patients with less favourable AD response

to tacrolimus, serum IgE tended to increase [34]. It should be noted, however, that in the present study mice were treated topically only for one week compared to several months in the long-term treatments of AD in human. Thus, it is possible that early increase in IgE levels exists also in human studies before the skin barrier function is improved and penetration of allergens through the skin is prevented. Further studies are needed to evaluate whether the early increase in IgE levels also exists in clinical trials with tacrolimus and other topical drugs.

In addition to the model described in the present study also other experimental models have been developed and used to examine specific features of AD. In the spontaneous AD models certain strains of mice NC/Nga mice [35], DS-Nh mice [36], NC/F mice [37] and NOA mice [38], develop AD-like skin lesions spontaneously under conventional conditions. Drawbacks of these spontaneous models are the low morbidity of the dermatitis and difficulties to standardize these models in different laboratories. This may be due to difficulties to define and standardize microbial constituents which are required for the development of the AD-like skin lesions in the conventional animal-housing conditions. Also several genetically modified knockout or transgenic mice have been used to investigate mechanisms of AD [39]. There are, however, limitations to use genetically modified mice in which only one relevant cytokine or specific immune gene is disrupted or over-expressed when evaluating efficacies of different treatment options.

Our earlier studies showed that epicutaneous sensitization with protein allergen induces the most features of AD, including cutaneous Th2/Th1 inflammation, T cell predominance and elevated serum IgE levels [7]. Present study showed clear-cut responses to betamethasone-17-valerate and tacrolimus, both of which are efficient and established treatments in human AD. The finding that cipamfylline is topically efficacious in murine model of AD is a promising one since current treatment modalities for AD are restricted when compared for example to psoriasis. We propose that the mouse model of AD caused by repeated epicutaneous OVA and SEB exposures is valuable and reproducible model to test topical drugs in AD.

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