

The effect of pimecrolimus on innate immunity in subjects with atopic dermatitis: a double-blind, randomized, vehicle-controlled study

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MADAM, Patients with atopic dermatitis (AD) are at increased risk of *Staphylococcus aureus* and herpes simplex infections.¹ Subjects with AD respond to inflammation with lower amounts of the antimicrobial peptides (AMPs) cathelicidin (LL-37) and human β -defensin (HBD)-3. This defect has been attributed to elevated interleukin (IL)-4 and IL-13,² and may explain in part why AD innate immunity is ultimately too low to kill *S. aureus*.³ Pimecrolimus, an effective topical treatment for AD, has been shown to inhibit the serine/threonine phosphatase calcineurin, and prevent nuclear translocation of nuclear factor of activated T cells, and thus activation of T cells.⁴ Previously, our group showed that application of pimecrolimus to normal human keratinocytes *in vitro* induced cathelicidin and HBD-3 in response to bacterial products.⁵ Our current study sought to determine if pimecrolimus would have a similar effect after topical application to the skin of subjects with AD. In addition, we sought to determine if IL-13 levels, which are elevated in AD, were affected by pimecrolimus therapy *in vivo*.

Forty subjects with AD were enrolled in a double-blind, randomized controlled, 3-week study to apply either pimecrolimus or vehicle twice daily to lesional and nonlesional skin (Table S1; see Supporting Information). Target lesional and nonlesional sites were selected and 2-mm punch biopsies were used to measure IL-13, HBD-3 and cathelicidin mRNA expression. Quantification of IL-13, HBD-3 and cathelicidin mRNA was performed by quantitative reverse transcriptase-polymerase chain reaction (PCR) (Data S1; see Supporting Information), and their expression was calculated as relative to the house-keeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). All statistical analysis was performed using GraphPad Software (GraphPad Prism version 5.01 for Windows; San Diego, CA, U.S.A.; <http://www.graphpad.com>) (Data S2; see Supporting Information).

As expected, IL-13 levels were significantly increased in lesional vs. nonlesional skin (Fig. 1a, $P = 0.007$) of subjects with AD. HBD-3 levels were higher in lesional than nonlesional skin (Fig. 1b, $P < 0.0001$). This increase has been reported previously,⁶ but the magnitude of this increase in AD is less than in other inflammatory conditions such as psoriasis. Although lesional, inflamed skin would be expected to have elevated expression of AMPs, cathelicidin levels showed no

significant difference between lesional and nonlesional AD skin (Fig. 1c), thus reflecting the defect of innate immunity in AD.

After 3 weeks of pimecrolimus treatment there was no significant change in IL-13 in lesional skin; however, expression of IL-13 in the post-treatment pimecrolimus group was significantly reduced in comparison with the vehicle-treated group (Fig. 2a, $P = 0.01$). HBD-3 mRNA was significantly less in lesional skin following treatment with pimecrolimus compared with vehicle (Fig. 2b, $P = 0.004$). Cathelicidin mRNA expression showed no significant change but trended lower (Fig. 2c). There was no change in LL-37, HBD-3 or IL-13 expression in treated nonlesional skin (data not shown).

Our study sought to define better the influence of pimecrolimus on skin innate immune defence. Specifically, we sought to determine the effect of pimecrolimus on the expression of two important AMPs and the Th2 cytokine IL-13. Our results reveal that unlike corticosteroids, which suppress cathelicidin,⁷ pimecrolimus has a minimal effect on cathelicidin. This finding is partially contradictory to a recent publication which found both cathelicidin and HBD-3 to be suppressed following pimecrolimus treatment.⁸ The methodology of that study differed from ours in that it evaluated protein abundance by immunostaining while we applied quantitative PCR. However, both the current study and the previous report of skin samples from subjects with AD show a different response than our prior observation that pimecrolimus can induce HBD-3 and cathelicidin in cultured human keratinocytes. The difference between results from prior experiments on normal keratinocytes and our current findings may reflect intrinsic differences in the keratinocytes from patients with AD (such as filaggrin mutations), or a difference between the local environment *in vitro* and *in vivo*. Furthermore, the inability to detect an increase in AMPs in AD skin may be due to the anti-inflammatory action of pimecrolimus leading to a decrease in the stimulatory signals for cathelicidin expression. Supporting this conclusion are our observations that after 3 weeks, IL-13 levels in the pimecrolimus group were lower than in the vehicle group, an observation supported by prior work.^{9,10} Our study, the largest to date looking at the effects of pimecrolimus on IL-13, corroborates this effect, and suggests that any capacity of this drug to induce AMPs in keratinocytes is negligible when applied to inflamed skin, but at least in the case of cathelicidin the use of this immunomodulator does not further suppress the innate immune response.

In conclusion, we show that application of pimecrolimus for 3 weeks to AD lesional skin retained the subject's ability

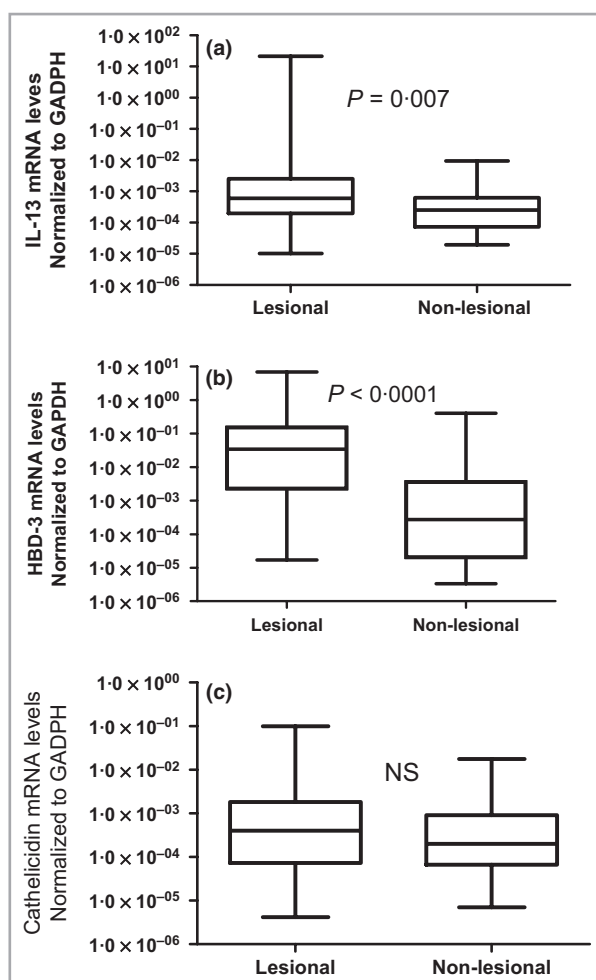


Fig 1. (a) Interleukin (IL)-13 mRNA expression in atopic dermatitis (AD) lesional vs. nonlesional skin as analysed by quantitative reverse transcriptase–polymerase chain reaction (RT-PCR). Lesional and nonlesional skin biopsy samples were collected and analysed for expression of IL-13. Values of IL-13 were normalized to the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and the data are presented as relative IL-13 expression. The boxplot display of the data shows the median (horizontal line in the middle of the box), quartiles (top and bottom of the box representing the 25th and 75th percentiles, respectively) and range (whiskers representing the minimum and maximum values). Analysis revealed significantly increased IL-13 in AD lesional in comparison with nonlesional skin ($P = 0.007$). (b) Human β -defensin (HBD)-3 mRNA lesional vs. nonlesional expression as analysed by quantitative RT-PCR and normalized to the housekeeping gene GAPDH revealed significantly increased HBD-3 levels in lesional in comparison with nonlesional AD skin ($P < 0.0001$). (c) Cathelicidin mRNA lesional vs. nonlesional expression as analysed by quantitative RT-PCR and normalized to the housekeeping gene GAPDH revealed no significant difference (NS) between cathelicidin lesional and nonlesional expression.

to produce cathelicidin and inhibited HBD-3. Additional studies are required to define further the effects of pimecrolimus on IL-13, and to investigate the timing of effects on the inflammatory milieu in patients with AD and risk of secondary infection.

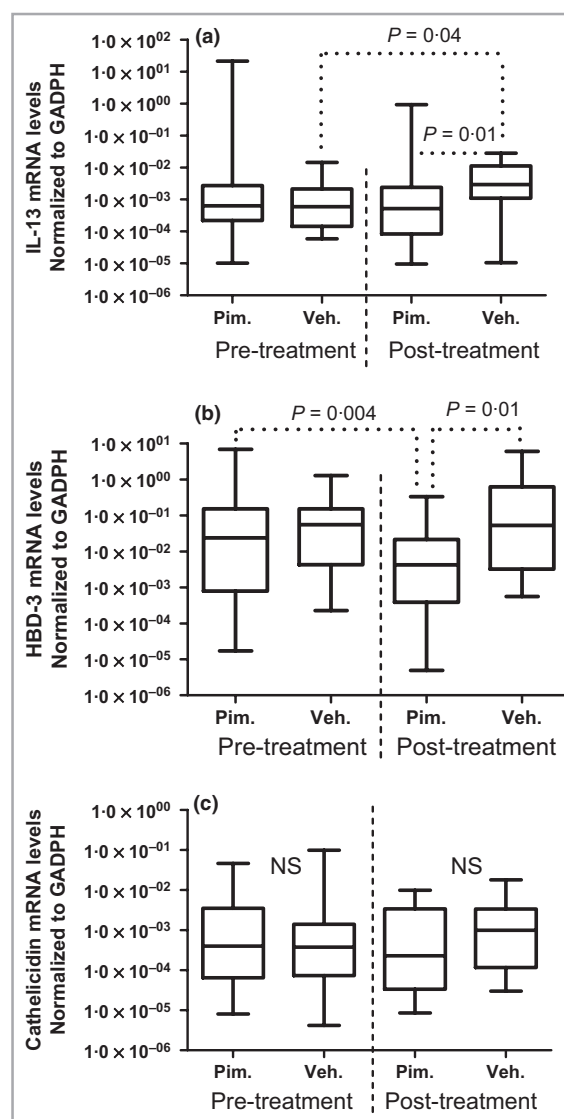


Fig 2. (a) Interleukin (IL)-13 mRNA pre- and post-treatment expression as analysed by quantitative reverse transcriptase–polymerase chain reaction (RT-PCR). Lesional skin biopsy samples were collected and analysed for expression of IL-13. Values of IL-13 were normalized to the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and the data are presented as relative IL-13 expression. The boxplot display of the data shows the median (horizontal line in the middle of the box), quartiles (top and bottom of the box representing the 25th and 75th percentiles, respectively) and range (whiskers representing the minimum and maximum values). Analysis revealed no significant change in IL-13 in lesional skin with pimecrolimus treatment. Of note, IL-13 in the post-treatment pimecrolimus group was significantly reduced in comparison with vehicle ($P = 0.01$). (b) Human β -defensin (HBD)-3 pre- and post-treatment expression as analysed by quantitative RT-PCR and normalized to the housekeeping gene GAPDH revealed a significant reduction in HBD-3 in lesional skin after pimecrolimus treatment ($P = 0.004$). (c) Cathelicidin mRNA lesional pre- and post-treatment expression as analysed by quantitative RT-PCR and normalized to the housekeeping gene GAPDH revealed no significant difference (NS) in cathelicidin mRNA levels in lesional skin after treatment. Pim., pimecrolimus; Veh., vehicle.

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Supporting Information

Additional Supporting Information may be found in the online version of the article:

Table S1. Descriptive summary of subject characteristics by diagnostic group.

Data S1. Reverse transcriptase–polymerase chain reaction methods supplement.

Data S2. Statistical supplement.

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BRAF mutation analysis of only one metastatic lesion can restrict the treatment of melanoma: a case report

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MADAM, The RAS-RAF-MEK-ERK-mitogen activated protein kinase (MAPK) pathway is a crucial signalling pathway for the growth of malignant cells. BRAF (v-raf murine sarcoma viral oncogene homolog B1) mutations, especially V600E¹ and to a lesser extent V600K, are frequently present in melanoma.^{2,3} Consequently, therapeutic inhibition of this pathway has become an attractive approach for the treatment of patients with melanoma. Indeed, several investigational drugs have been shown to block this pathway to a more or less significant extent.⁴ For example, vemurafenib (Zelboraf™; Roche Pharma AG, Grenzach-Wyhlen, Germany), an orally available selective inhibitor of the oncogenic BRAF V600E kinase, has demonstrated impressive clinical efficacy even in advanced disease.⁵ As a result, Zelboraf™ received approval from the U.S. Food and Drug Administration for the treatment of the corresponding patients in August 2011.⁶ In contrast to vemurafenib, MEK 1/2 inhibitors targeting the downstream molecules of BRAF signalling should allow the treatment of patients harbouring activating mutations in any of the upstream molecules of MEK. Notably, the combination of the MEK 1/2 inhibitor GSK 1120212 with the mutation-specific BRAF inhibitor GSK 2118436 has demonstrated encouraging antitumour activity in patients with mutated V600.⁷ Currently, clinical trials that are testing inhibitors of the respective MAPK pathways are based on the identification of a mutation in one selected melanoma lesion, either primary or metastasized. Once the respective drugs are approved, clinical practice will likely follow this standard as well. However, the following case report raises concerns associated with this current practice.

A 75-year-old woman presented herself in October 2009 with an ulcerated melanoma on the back. Her medical history included hypertonus, diabetes with defective renal function, a former hepatitis B infection with no active virus load and a non-Hodgkin lymphoma in complete remission for almost 20 years. After excision of the tumour and a sentinel node biopsy in the right axilla, histopathology confirmed an ulcerated melanoma of 2-mm thickness with satellite metastases, as well as a positive sentinel lymph node. A second primary melanoma was excised at the same time from the neck revealing tumour thickness < 0.5 mm and mitoses < 1 per mm². Adjuvant treatment was declined by the patient, but regular follow-up examinations were