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DR. PHILIPPE VASSEUR (Orcid ID : 0000-0002-5818-6042)

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Chronic Alcohol Consumption Exacerbates the Severity of Psoriasiform Dermatitis in Mice

Philippe Vasseur (philippe.vasseur@ghnv.fr)^{a,b}, Mathilde Pohin (mathilde.pohin@univ-poitiers.fr)^a, Coralie Gisclard (coraliegisclard@hotmail.fr)^{a,b}, Jean-François Jégou (jean.francois.jegou@univ-poitiers.fr)^a, Franck Morel (f.morel@univ-poitiers.fr)^a, Christine Silvain (c.silvain@chu-poitiers.fr)^{a,b,c}, and Jean-Claude Lecron (jean-claude.lecron@univ-poitiers.fr)^{a,b,c}

^aLaboratoire Inflammation Tissus Epithéliaux et Cytokines EA 4331, Université de Poitiers, Poitiers, France.

^bCentre Hospitalier Universitaire de Poitiers, Poitiers, France.

^cThese authors contributed equally

Corresponding author: Philippe Vasseur, MD, PhD, Laboratoire Inflammation Tissus Epithéliaux et Cytokines (EA 4331), Pôle Biologie Santé B36/B37, 1 rue Georges Bonnet, TSA 51106, 86073 Poitiers Cedex 9, France. Email: philippvasseur@gmail.com.

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Keywords: alcohol, psoriasis, imiquimod, IL-17A

Abstract

Background: A relationship between alcohol consumption and psoriasis has been reported, but it is unclear whether alcohol consumption aggravates psoriasis. Here, we studied the effect of chronic ethanol consumption in the murine model of imiquimod (IMQ)-induced psoriasiform dermatitis.

Methods: C57BL/6 mice received 5% ethanol in their drinking water for 10 weeks. Dermatitis was induced from weeks 9 to 10, by applying IMQ to shaved patch of skin on the back. Inflammation was characterized by histological and transcriptomic analyses.

Results: Ethanol consumption aggravated Aldara-induced dermatitis. The scales were more severe, epidermal thickening was more pronounced, and cutaneous expression of Th17-related cytokines was exacerbated. Control mice simply receiving ethanol displayed minimal cutaneous inflammation, characterized by epidermal infiltrates of T lymphocytes and the overexpression of IL-17A and the Th17-recruiting chemokine CCL20. *In vitro* studies showed that low concentrations of ethanol induce the expression of CCL20 by murine epidermal keratinocytes.

Conclusion:

Alcohol consumption leads to subliminal skin inflammation, which is revealed by the exacerbation of Aldara-induced experimental psoriasiform dermatitis, likely through Th17-type minimal skin inflammation. These results favor the systematic management of alcohol consumption in psoriatic patients.

Highlights:

- Experimental psoriasiform dermatitis is aggravated by alcohol consumption.
- Alcohol consumption induces minimal skin inflammation.
- Ethanol induces CCL20 chemokine production by murine keratinocytes.

Abbreviations

IL: interleukin, TNF: tumor necrosis factor, Th: T helper, IMQ: imiquimod

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1. Introduction

Psoriasis is a chronic skin disease which relies on the inappropriate activation of cutaneous T cells, particularly interleukin (IL)-17-, IL-22, and tumor necrosis factor (TNF)- α -secreting T helper (Th)17 lymphocytes (Lowes et al., 2015; Wilson et al., 2007). The efficacy of IL-17 antagonist therapies (Wu et al., 2017) illustrates the major contribution of IL-17A in psoriasis. IL-17A stimulates the secretion of various antimicrobial peptides by keratinocytes, leading to stimulation of the innate immune system (Lynde et al., 2014; Wilson et al., 2007). IL-17A also induces the production of multiple chemokines by keratinocytes, such as the Th-17-recruiting chemokine CCL20 (Guilloteau et al., 2010), amplifying the inflammatory loop of psoriasis. Beyond cutaneous plaques, psoriasis is a serious condition associated with systemic inflammation, increased cardiovascular risk (Miller et al., 2013), and finally, a reduced lifespan (Salahadeen et al., 2014). Thus, identifying aggravating factors of psoriasis is of importance. Alcohol consumption is greater in psoriasis patients than in the general population (Brenaut et al., 2013, Al Jefri et al BJD 2017), but systematic reviews of the literature fail to show a significant correlation between alcohol consumption and the severity of the disease due to the inappropriate methodology generally used in clinical studies (M. A. Richard et al., 2013). In this context, an experimental approach could be a simple and direct method to address this issue. We used the imiquimod (IMQ)-induced murine model of dermatitis (van der Fits et al., 2009), which relies on the IL-17 axis and recapitulates the central features of psoriasis (Flutter and Nestle, 2013). Our aim was to study the impact of chronic ethanol consumption (Gäbele et al., 2011) on the severity of murine psoriasiform dermatitis induced by the topical application of IMQ.

2. Methods

2.1 Animals

Eight- to 10-week old C57BL/6 male mice received 5% alcohol in their drinking water (EtOH mice) for 10 weeks *ad libitum*, as published elsewhere (Gäbele et al., 2011). Control mice received water (H₂O mice). From weeks 9 to 10, the mice were randomly divided into two groups: mice receiving 3 mg IMQ (Aldara 5%, Meda, Sweden) on depilated skin on the back every other day and those receiving Vaseline (VAS) control cream. All animal procedures were approved by the local ethics committee (protocol 2012-CE) and were in accordance with the French laws and institution's guidelines for animal welfare.

2.2 Scoring severity of experimental skin inflammation

Skin inflammation was evaluated with a score adapted from the clinical PASI as described elsewhere (Vasseur et al., 2016). The cumulative score (0-12) was the sum of three criteria (erythema, scaling, and thickening), ranging from 0 to 4: 0, none; 1, slight; 2, moderate; 3, marked; 4, very marked.

2.3 Immunofluorescence

Frozen 8- μ m tissue sections were fixed in paraformaldehyde and incubated for 2 h at room temperature with hamster anti-mouse CD3 ϵ mAb (1:100, clone 145-2C11, BD Biosciences). After washing, slides were incubated for 60 min at room temperature with RRX-conjugated goat-anti-hamster (1:200, Invitrogen, Carlsbad, CA). Nuclei were stained with To-Pro3 (1:800, Invitrogen). Confocal microscopy was carried out on an Olympus FV1000 (ImageUP microscopy platform, University of Poitiers).

2.4 Real-time quantitative PCR analysis

Skin mRNA was isolated using a NucleoSpin RNA II kit (Macherey-Nagel, Hoerd, France) and reverse-transcribed with SuperScript II reverse transcriptase (Invitrogen) according to the manufacturer's instructions. Real-time quantitative PCR (RT-qPCR) was carried using the LightCycler-FastStart DNA Master^{Plus} SYBRGreen I kit on a LightCycler 480 thermocycler

(Roche Diagnostics, Meylan, France). The reaction components were 1X DNA Master Mix, and 0.5 μ M HPLC-purified sense and antisense oligonucleotides purchased from Eurogentec (Angers, France). Primers were designed using Primer3 software. Gene expression in samples was normalized to that of the *GAPDH* housekeeping gene.

2.5 Keratinocyte culture and stimulation

Normal murine epidermal keratinocytes (NMEKs) were harvested from pooled newborn C57BL/6 mouse epidermis and cultured as published elsewhere (Pohin et al., 2016). NMEKs were stimulated for 6 h with two different concentrations of ethanol (0.002 g/L, 0.02 g/L) (Cooper, Melun, France).

2.6 Statistical analysis

Results are presented as the mean \pm S.E.M. Non parametric Kruskal-Wallis or Mann-Whitney tests were used for the statistical evaluation (GraphPad Prism, version 5.01). The p values were as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3. Results

3.1 Chronic ethanol administration exacerbates the severity of Aldara-induced dermatitis

Mice received plain water or water supplemented with 5% ethanol during the study. The mean daily water consumption was similar between H₂O and EtOH mice (5.1 ± 0.3 and 5.1 ± 0.2 ml respectively, $p = 0.9$), daily ethanol consumption was 0.26g per mice. From weeks 9 to 10, IMQ was applied every other day. Mice of the control group who did not receive alcohol, and treated with IMQ (H₂O IMQ), displayed dermatitis at week 10 with erythematous and thickened skin relative to mice treated with control cream (H₂O VAS) (Figure 1A). In comparison, mice receiving both ethanol and IMQ (EtOH IMQ) had more severe skin inflammation than H₂O IMQ mice, characterized by the formation of scales and more intense erythema (clinical score 5.4 vs 6.5, $p = 0.002$) (Figures 1A and 1B). Histological analysis showed that EtOH IMQ mice displayed more prominent epidermal thickening than H₂O IMQ mice (70 vs $55\mu\text{m}$, $p < 0.001$) (Figures 1A and 1C). Moreover, downward elongation of rete ridges, a histopathological feature of psoriasis, was more conspicuous in EtOH IMQ mice (Figure 1A). Relative to H₂O IMQ mice, EtOH IMQ mice also showed greater expression of Th17-related cytokines known to be involved in the pathogenesis of Aldara-induced dermatitis, *i.e.* IL-17A, IL-22 and IL-23 (Figure 1D). Consistent with these results, EtOH IMQ mice showed stronger expression of S100A9, an antimicrobial peptide mainly produced by activated keratinocytes (Figure 1D).

3.2 Mice receiving ethanol display minimal skin inflammation

Since ethanol administration exacerbated the severity of dermatitis, we next explored whether EtOH mice in the absence of IMQ treatment displayed a minimal skin inflammation. As compared to the H₂O control group, mice receiving EtOH and treated with control cream (EtOH VAS) did not show macroscopic nor histological signs of inflammation (Figure 2A). Nevertheless, immunostaining analysis showed a greater number of T lymphocytes (CD3 ϵ^+) in the skin of EtOH VAS mice than that of the H₂O control group (6 ± 1 vs 2 ± 1 CD3 ϵ^+ cells per field respectively, $p=0.03$), largely localized in the epidermal layer (Figures 2A and 2B). EtOH mice did not display skin infiltrates of macrophages (F4/80 $^+$ cells) or granulocytes (Gr1 $^+$ cells) (data not shown). Along with the T-lymphocyte infiltrates, EtOH mice also showed a two-fold increase in skin expression of the Th17-related cytokines IL-17A and TNF- α and of the Th17-recruiting chemokine CCL20

(Figure 2C). In contrast, there were no changes in the expression of the Th1-related factors interferon- γ or T-bet (data not shown).

At a systemic level, EtOH did not induce any weight variation between the EtOH mice and H₂O mice (+19% vs +16%, respectively, $p = 0.37$), with a daily energetic intake which did not differ between the two groups (25 vs 28 Kcal, respectively, $p = 0.31$). EtOH mice did not display any liver inflammation at the histological nor transcriptional level (data on IL-6 and TNF- α mRNA expression not shown). We did not observe any significant variations for spleen weight in H₂O VAS mice as compared to EtOH VAS mice (82 ± 19 vs 91 ± 20 mg respectively, $p = 0.26$), nor in H₂O IMQ mice as compared to EtOH IMQ mice (190 ± 47 vs 204 ± 51 mg respectively, $p = 0.51$).

3.3 Ethanol induces keratinocyte expression of CCL20

We further explored whether the minimal skin inflammation of the EtOH VAS mice is a consequence of the direct interaction of ethanol with keratinocytes, as C57BL/6 mice fed alcohol for several weeks have an ethanol concentration in the skin ranging from 5 to 12% of their blood concentrations (Brand et al., 2015). Consistent with a 0.19 g/L blood alcohol concentration in our model (Gäbele et al., 2011), we cultured normal murine epidermal keratinocytes in media containing ethanol at a concentration of 0.02 g/L (the expected ethanol concentration in the skin) or 0.002 g/L. Ethanol induced a three-fold increase in CCL20 expression by keratinocytes after 4 h of exposure (Figure 2C), whereas the effect was no longer detectable after 24 h (data not shown).

Discussion and conclusion

Here, we show that psoriasisiform dermatitis is aggravated by feeding mice ethanol. In mice treated with IMQ, ethanol exacerbated the Th17-type response, inducing clinically and histologically more severe phenotype. This model of “acute” psoriasis has been extensively used since 2009 (van der Fits et al., 2009), as it recapitulates central features of the disease, such as the scaly aspect of the plaques, epidermal hyperplasia, and dependence on the IL-23/IL-17 axis (Flutter and Nestle, 2013). We confirm in this animal model the assumption raised by clinicians that alcohol is an aggravating factor for psoriasis (Adamzik et al., 2013; M.-A. Richard et al., 2013), which should encourage patients to avoid or reduce alcohol consumption to improve skin lesions and limit the use of systemic therapies. Moreover, the IL-17-biased skin response in EtOH mice may also explain the resistance of some patients to biotherapies that target the IL-17 axis. We investigated

the effect of chronic alcohol consumption by giving mice drinking water supplemented with 5% ethanol to mice, a level that reproduces the habits of psoriatic patients, whose average daily consumption is estimated to be between 9 and 40 g/day (Brenaut et al., 2013). This liver-sparing model does not alter peripheral immunity nor hemato/lymphopoiesis (Cook et al., 2007), suggesting that the direct interaction of ethanol with keratinocytes could be responsible for the Th17 skin phenotype. Indeed, ethanol has been reported to target keratinocytes and induce the proliferation of HaCaT cells (Farkas et al., 2003). Keratinocytes are a major cutaneous source of CCL20, a chemokine that interacts with its receptor CCR6, expressed by T cells and dendritic cells. CCR6 is considered a representative marker for circulating Th17 cells, and CCR6/CCL20 interaction plays a crucial role in the recruitment of Th17 cells to local tissues (Maecker et al., 2012). We observed that ethanol induces the expression of CCL20 by keratinocytes. This observation may partially explain the epidermal infiltration of T lymphocytes in mice fed ethanol, along with their specific cutaneous transcriptomic profile, characterized by the overexpression of Th17-related cytokines. We suggest that the cutaneous overexpression of IL-17A induced by ethanol consumption may account for the exacerbation of psoriasiform dermatitis in accordance with our previous report showing that the cutaneous administration of rIL-17A exacerbates Aldara-induced dermatitis to the same extent as that observed in EtOH IMQ mice (Vasseur et al., 2016).

In conclusion, our experiments show that chronic ethanol consumption exacerbates Aldara-induced psoriasiform dermatitis, potentially through the upregulation of the IL-23/IL-17A axis subsequent to the interaction of ethanol with keratinocytes. These results favor the systematic management of alcohol consumption in psoriatic patients.

Author disclosures

Role of funding source: nothing to declare

Contributors

P.V. conceived the experiments, acquired, analyzed and interpreted the data, and wrote the manuscript. M.P. and JFJ acquired and analyzed the data and revised the manuscript. C.G. and L.S. acquired and analyzed the data. F.M. revised the manuscript. C.S. and J.-C.L. conceived the experiments, interpreted the data, and revised the manuscript.

Conflict of interest: no conflict declared

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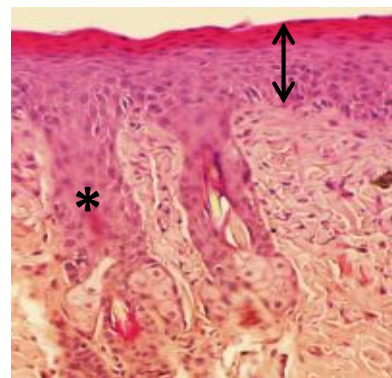
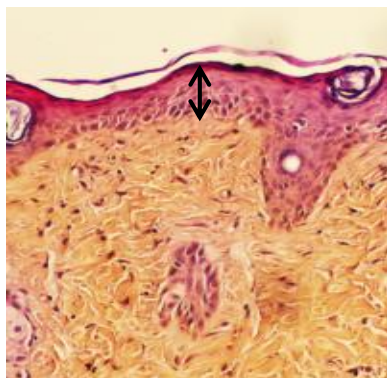
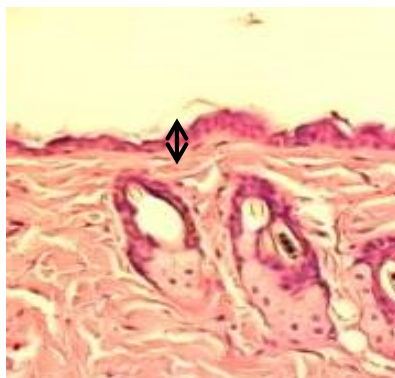
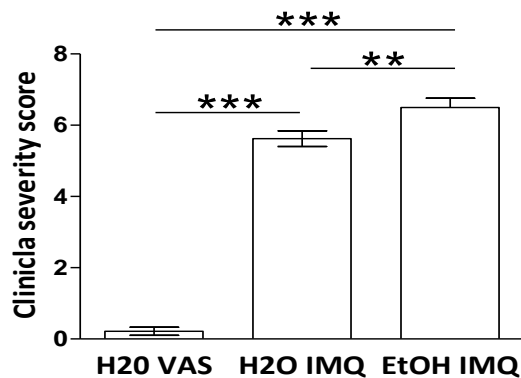
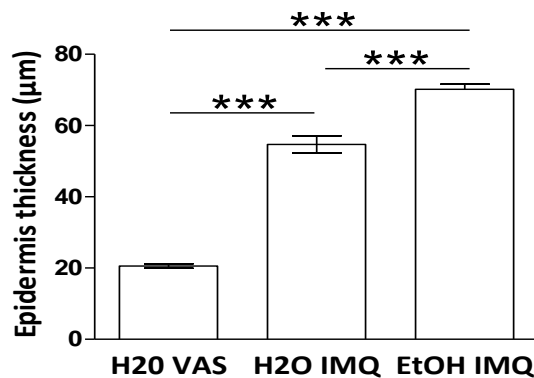
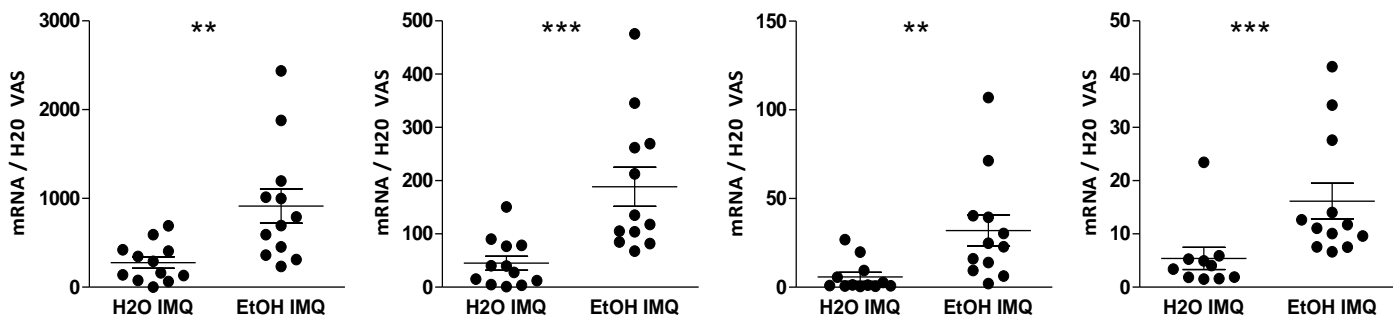
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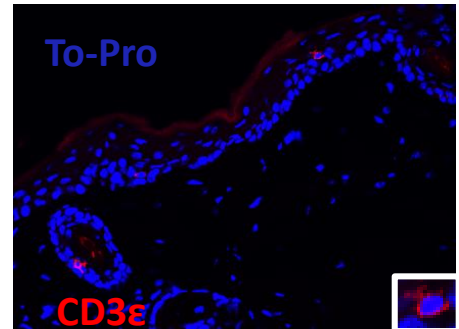
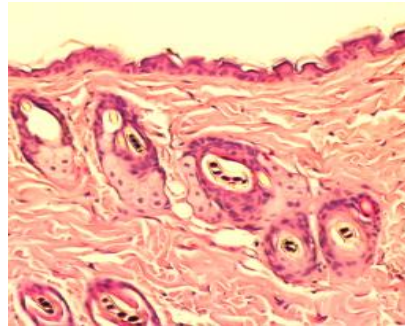
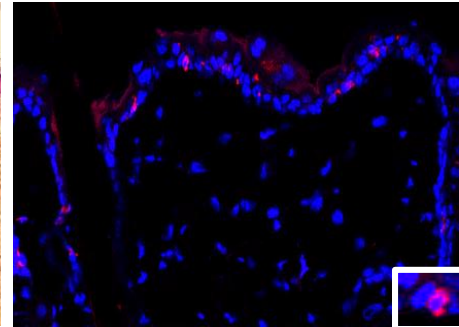
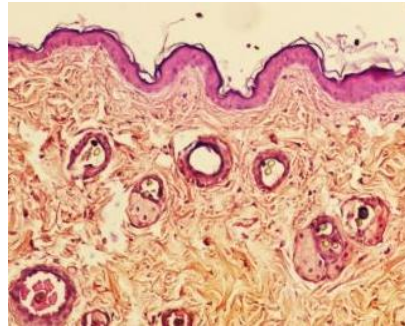
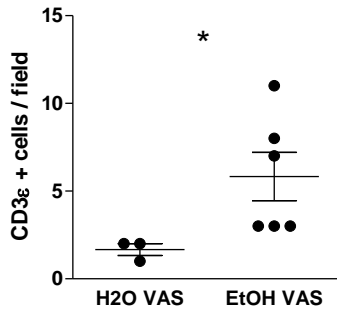
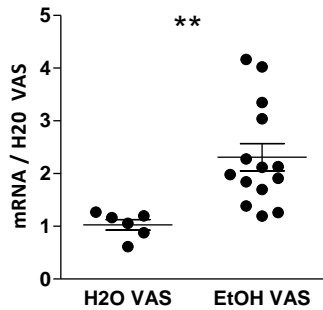
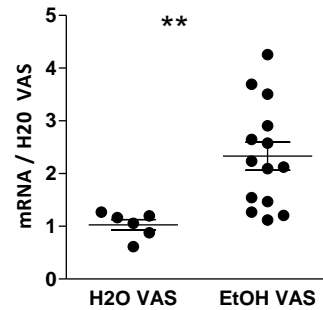
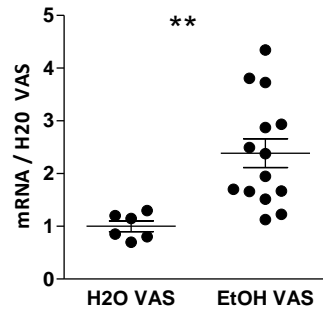
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Figure legends

Figure 1. Ethanol consumption aggravates imiquimod-induced psoriasiform dermatitis. Mice received 5% ethanol for 10 weeks in their drinking water and were topically treated with imiquimod (IMQ) or control cream (VAS) during the last week. (A) Macroscopic and histological aspects of mice back skin at week 10. Magnification is 100x. Double-headed arrows indicate the epidermis and the asterisk indicates epidermal psoriasiform hyperplasia. (B) Clinical severity score at week 10. (C) Histological analysis of epidermal thickness. Measures were made every 50 μm on four random sections per mouse. (D) Relative mRNA expression of the skin of the back at week 10 of IL-17A, IL-22, IL-23, and S100A9 normalized to that of GAPDH and expressed as the fold increase relative to the expression in control mice. $n = 6$ mice / group from two independent experiments. $**p < 0.01$, $***p < 0.001$.

Figure 2. Ethanol induces minimal skin inflammation. (A) Macroscopic aspect, histological analysis, and CD3 ϵ immunostaining of mice skin at week 10. Mice received 5% ethanol or only water and were treated with control cream (VAS). Stained T lymphocytes are shown in the inset. (B) Quantification of CD3 ϵ -stained cells per microscopic field. $n = 3$ to 6 mice/group. (C) Relative mRNA expression of the skin of the back at week 10 of IL-17A, CCL20, and TNF- α normalized to that of GAPDH and expressed as the fold increase relative to the expression in control mice. $n = 3$ to 7 mice/group from two independent experiments. (D) Relative expression of CCL20 by normal murine epidermal keratinocytes incubated for 4 h in 0.02 or 0.002 g/L ethanol. Results are normalized to the expression of GAPDH and expressed as the fold increase relative to samples incubated in the control medium. $n = 3$ samples/group from two independent experiments. $*p < 0.05$, $**p < 0.01$.

A**H2O VAS****H2O IMQ****EtOH IMQ****Back skin****HES (100x)****B****Clinical severity****C****Epidermis thickness****D****S100A9****IL-17A****IL-22****IL-23**

A**Back skin****Back skin H&E****CD3 ϵ** **H2O
VAS****EtOH
VAS****B****CD3 ϵ ⁺ cells****C****IL-17A****TNF- α** **CCL20****D****CCL20**