Received: 3 April 2019 | Revised: 21 August 2019 | Accepted: 26 August 2019

DOI: 10.1111/exd.14034

METHODS LETTER TO THE EDITORS

Experimental Dermatology WILEY

Interleukin-4 and interleukin-13 evoke scratching behaviour in mice

Abstract

Persistent and relapsing itch commonly manifests in inflammatory skin disorders such as atopic dermatitis (AD). AD pathogenesis is driven by interleukin-4 (IL-4) and interleukin-13 (IL-13). Dupilumab, a monoclonal antibody blocking the action of IL-4 and IL-13 effectively reduces the symptoms of AD and itch. Little is known whether IL-4 and IL-13 directly contribute to itch transduction. A recently published study (Oetjen et al, Cell, 2017, 171, 217) found IL-4 and IL-13 to directly activate itch-sensory neurons in vitro. Surprisingly, they found no significant increase in scratching after intradermally injecting high doses (2.5 ug/ml) of IL-4 and IL-13 into mice. Similar experiments in our lab, however, suggested that both IL-4 and IL-13 contribute to acute itch in vivo. We intradermally injected lower doses (1 ug/ml) of IL-4 and IL-13 into mice and found a significant increase of scratching bouts compared to vehicle. Interestingly, the combined treatment of IL-4 and IL-13 produced additive increase of scratching and acute pruritus at an earlier time point compared to each cytokine administered alone. In summary, our study shows a rapid and significant increase of scratching after intradermal injection of IL-4, IL-13 or combined IL-4/ IL-13 compared to vehicle in mice 5-10 minutes after injection. Our data suggest that IL-4 and IL-13 alone and combined directly act as potent acute pruritogens on sensory nerves. This finding expands our understanding of cytokines as pruritogens, how targeted anticytokine medications act in AD, and about neuroimmune communication in the skin.

1 | BACKGROUND

Molecular cross-talk between the immune system and the nervous system elicits evolutionary responses such as itch (pruritus) to protect the host from potential pathogens.[1,2] This neuroimmune, physiological response serves notably to remove pathogens from the skin.[1] Pruritus can also be associated with inflammatory disorders such as atopic dermatitis (AD).[3] Indeed, AD is a common skin disease in which IL-4 and IL-13 are key players in inflammation and neuroimmune dysfunction.[4] There is a growing body of evidence to show that IL-4 and IL-13 are sensible targets for AD therapy.[5,6] In March 2017, the US Food and Drug Administration (FDA) approved a human anti-interleukin-4 receptor alpha (IL-4Rα) monoclonal antibody known as Dupilumab/Dupixent for the treatment of moderate-to-severe AD.[7] Dupilumab targets the IL-4Rα subunit of IL-4 type I and IL-13 type II specific receptor complexes.[8] This leads to inhibition of the JAK-STAT signalling pathway known to participate in AD pathophysiology.[9] After subcutaneous injection of Dupilumab once a week for 12 weeks, AD symptoms were reduced by approximately 50%, including pruritus by 55.7%[10] suggesting that inhibition of IL-4 type I signalling plays a role in the reduction of pruritus. Little is known however about whether IL-4 or IL-13 directly contributes to pruritus.

2 | QUESTIONS ADDRESSED

Recently, Oetjen et al[11] found that both IL-4 and IL-13 are capable of directly activating itch sensory neurons in vitro. Thus, the authors intradermally injected wild-type mice with these cytokines and quantified scratching behaviour. Interestingly, the results were contrary to the researchers' hypothesis; "Based on our findings that type 2 cytokines directly activate itch sensory neurons, we hypothesized that intradermal (i.d.) administration of IL-4 and IL-13 would evoke acute itch. Surprisingly, in contrast to IL-31, high doses of either IL-4 or IL-13 did not elicit acute itch".[11] Similar experiments in our laboratory, however, have suggested that these cytokines do contribute to acute itch in vivo.

3 | EXPERIMENTAL DESIGN

Ten ul of recombinant mouse (rm) IL-4 (1 μg), IL-13 (1 μg) alone or in combination, histamine (50 μg) or vehicle (0.1% BSA in PBS) was intradermally injected, into the right cheek of wild-type C57/Bl6 mice (male, aged 12 weeks, n = 9/group). Mice were video-recorded immediately after injection for 30 minutes. Scratching behaviour was measured as "bouts" in 5 minutes time bins for 30 minutes. One bout of scratching was defined as beginning when the hind paw was lifted from the floor to the right cheek and ending when it returned to the floor or to the mouth.[12]

4 | RESULTS

Scratching behaviour analysis was carried out in a blinded fashion. After injection of rmIL-4, there was a significant increase of scratching bouts compared with vehicle and an overall significant effect of treatment ($P < .0001$) (Figure 1A). rmIL-13 also induced a

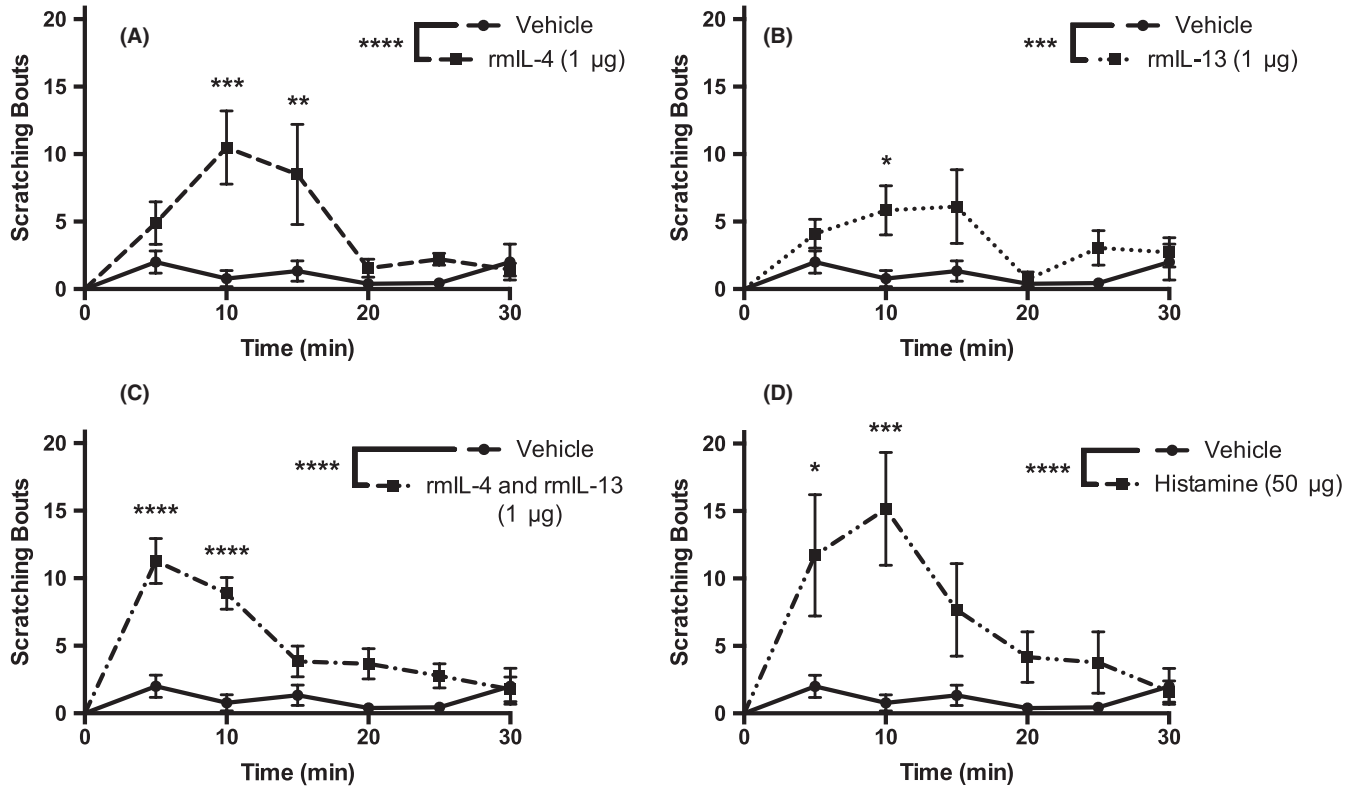

FIGURE 1 IL-4 and IL-13 Induce Acute Scratching Behaviour in Mice. Number of scratching bouts over the time after injection of rmIL-4 alone (A), IL-13 alone (B), IL-4 and IL-13 (C) and histamine (D). Statistical significance was found at 10 min ($P < .001$) and 15 min ($P < .01$) (A), 10 min ($P < .05$) (B), 5 min ($P < .0001$) and 10 min ($P < .0001$) (C), 5 min ($P < .05$) and 10 min ($P < .001$) (D). Data in figures represent mean ± standard error of the mean (SEM). Statistical significance was determined by two-way ANOVA with Bonferroni's multiple comparison test. Statistical analyses were performed using Prism 7 (GraphPad Software). Two-way ANOVA significance of treatment is labelled as: ****$P < .0001$ and ***$P < .001$, and Bonferroni's multiple comparison test significance of time is labelled as: ****$P < .0001$, ***$P < .001$, **$P < .01$ and *$P < .05$. n = 9/group

significant increase of scratching compared with vehicle ($P < .001$) (Figure 1B). After injection of the combination treatment rmIL-4 and rmIL-13, there was a significant increase of scratching compared with vehicle ($P < .0001$). Interestingly, the combination treatment of rmIL-4 and rmIL-13 produced acute pruritus at an earlier time point than the two treatments administered alone (Figure 1C). Two-way ANOVA Bonferroni's multiple comparison test indicated a significant increase of scratching bouts in mice i.d. injected with rmIL-4 at 10 minutes ($P < .001$) and 15 minutes ($P < .01$), rmIL-13 at 10 minutes ($P < .05$) and rmIL-4 and rmIL-13 in combination at 5 minutes ($P < .0001$) and 10 minutes ($P < .0001$). The combined activation of IL-4 type I and IL-13 type II specific receptor complexes could account for the marked increase in acute pruritus. rmIL-4 showed a similar scratching behaviour profile to the positive control group, histamine (Figure 2).

5 | CONCLUSIONS

Our results show that IL-4 and IL-13 produce a direct, acute pruritic effect immediately after intradermal injection in mice. Our data show comparable direct acute effects of IL-4 and -13, as observed for IL-31 which is considered as a direct acute pruritogen in

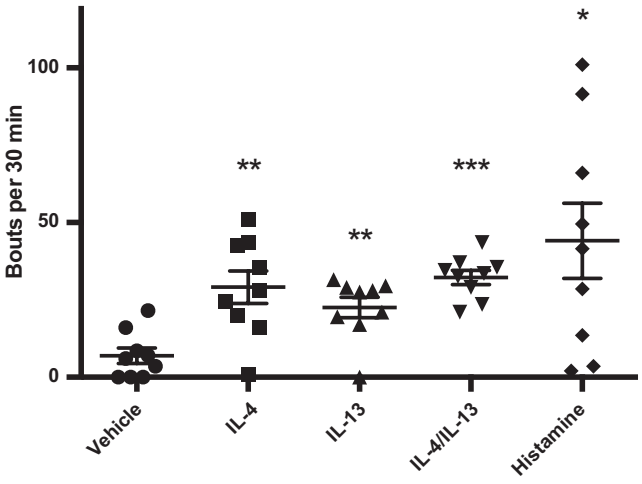

FIGURE 2 Low Dose of IL-4 and IL-13 Induces Acute Pruritus in Mice. Number of scratching bouts measured in response to intradermal (i.d.) cheek injection of vehicle (0.1% BSA in PBS, 10 mL), rmIL-4, rmIL-13, rmIL-4 and rmIL-13 in combination (all 1 µg/10 µL) or histamine (50 µg/10 µL). Data in figures represent mean ± standard error of the mean (SEM). Statistical significance was determined by unpaired Student's *t* test with Mann-Whitney *U* test. Statistical analyses were performed using Prism 7 (GraphPad Software). Significance is labelled as: ***$P < .001$, **$P < .01$ and *$P < .05$. n = 9/group

mice.[13] The observation of a direct and acute effect is supported by expression of IL-4 and IL-13 receptors on sensory neurons (dorsal root ganglion neurons (DRG) and trigeminal ganglia) in both mice and humans[11] and calcium-imaging trace studies where IL-4 and IL-13 receptor-positive mouse DRG directly and rapidly release Ca^{2+} in response to IL-4 or IL-13 stimulation.[11] However, our findings in parts are in contradiction to results obtained by Oetjen et al[11] where IL-4 is described as a chronic itch mediator in mice and humans without acute pruritic effects. A number of hypotheses could explain these differences. First, the vehicle group in Oetjen's work[11] showed an unexpected high number of scratching bouts (approx. 25 bouts in 30 minutes) similar to treatment groups. As a result, their study finds no significant difference of scratching between vehicle and treatment. Second, a possible explanation for disparity could be that Oetjen et al[11] used 2.5 μg rmIL-4 and 2.5 μg rmIL-13 treatment concentrations. Maintaining equilibrium, higher concentrations in healthy mice could stimulate negative feedback regulation. Negative feedback dysregulation of the JAK-STAT pathway has been implicated in inflammatory diseases.[14] Suppressor of cytokine signalling proteins (SOCS) is involved in the negative regulation of cytokine signalling.[15] Suppressor of cytokine signalling 1 (SOCS1) and suppressor of cytokine signalling 5 (SOCS5) have negative feedback activity on the JAK-STAT pathway of IL-4 signalling.[16] Also, IL-13Rα2 is known to employ a negative feedback system by blocking the signalling of IL-4 and IL-13.[17–19] In healthy wild-type mice, it is possible that higher concentrations may be saturating IL-4 and IL-13 receptors, stimulating the expression of SOCS1, SOCS5 and IL-13Rα2, inhibiting JAK-STAT signalling of IL-4 type I and type II receptors, blocking the activation of sensory neurons and in turn, producing a lower scratch response compared with lower concentrations. Interestingly, the combined treatment of IL-4 and IL-13 produced increased scratching behaviour compared with treatment alone. Transgenic mice overexpressing IL-13 produce similar pruritic effects and have increased levels of IL-4 and IL-13 in pruritic lesions.[20] IL-4 and IL-13 are also found to be upregulated in the pruritic AD lesions of canines[21] and also humans.[22] It is possible that combined activation of IL-4 and IL-13 receptors on itch sensory neurons produce an amplified pruritic response compared with activation alone.

Whether IL-4 and IL-13 recapitulate the acute pruritic effects in humans in clear distinction to the pruritic effects of IL-31 which acts as an acute pruritogen in mice[11,13] and a late onset pruritogen in humans[23] needs to be addressed in detailed future studies.

In summary, our study shows a significant increase of scratching after intradermal injection of rmIL-4, rmIL-13 or rmIL-4 and rmIL-13 in combination compared with vehicle in mice after 5-10 minutes. Our data suggest that IL-4 and IL-13 act as acute (immediate) pruritogens. To support these results, it would be interesting to further examine the expression of SOCS proteins in response to IL-4 and IL-13 in a dose-dependent manner and using genetically altered IL-4 and IL-13 mice. Underpinning the molecular pruritic profile would complement and more clearly elucidate the role of IL-4 and IL-13 in acute pruritus. Thus, IL-4 and IL-13 directly induce scratching behaviour in mice independently and exert an additional effect when applied simultaneously thereby being direct targets for itch therapy. Clarifying the role of cytokines directly on nerves is important to better understand their role in human disease and to interpret the effects of anti-cytokine therapies with respect to blocking inflammation and/or pruritus, respectively.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTION

Ms Michelle Campion performed the research, analysed the data and wrote the manuscript. Ms Leila Smith and Dr Solène Gatault aided in the research and analysed the data. Dr Charles Métais analysed the data. Dr Buddenkotte aided in the itch mouse model and wrote manuscript. Prof. Dr Dr Martin Steinhoff designed the research study, analysed the data and wrote manuscript. Study was funded by Science Foundation Ireland (IVP award to MS) and Qatar National Research Award (QNRF) (to MS). All authors have read and approved the final manuscript.

Michelle Campion[1,2]
Leila Smith[1]
Solène Gatault[1]
Charles Métais[1]
Jörg Buddenkotte[3,4]
Martin Steinhoff[3,4,5,6]

[1]*Charles Institute of Dermatology, University College Dublin, Dublin, Ireland*
[2]*Conway Institute, University College Dublin, Dublin, Ireland*
[3]*Department of Dermatology and Venereology, Hamad Medical Corporation, Doha, Qatar*
[4]*Translational Research Institute, Academic Health System, Hamad Medical Corporation, Doha, Qatar*
[5]*Department of Dermatology, Weill Cornell University New York and Weill Cornell Medicine-Qatar, Doha, Qatar*
[6]*Medical School Doha, Qatar University, Doha, Qatar*

Correspondence
Michelle Campion, Neurotherapeutics Research Group, School of Medicine, Conway Institute, University College Dublin, Dublin, Ireland.
Email: michelle.campion@ucdconnect.ie
Martin Steinhoff, Department of Dermatology and Venereology, Medical School, Hamad Medical Corporation, Weill Cornell University-Qatar, Qatar University, Doha, Qatar.
Email: msteinhoff@hamad.qa

ORCID

Michelle Campion https://orcid.org/0000-0002-6608-525X

REFERENCES

[1] R. Paus, M. Schmelz, T. Bíró, M. Steinhoff, *J. Clin. Invest.* **2006**, *116*, 1174.
[2] M. Steinhoff, M. Schmelz, I. L. Szabó, A. L. Oaklander, *Lancet Neurol.* **2018**, *17*, 709.
[3] J. Hong, J. Buddenkotte, T. G. Berger, M. Steinhoff, *Semin. Cutan. Med. Surg.* **2011**, *30*, 71.
[4] M. H. Oh, S. Y. Oh, J. Lu, H. Lou, A. C. Myers, Z. Zhu, T. Zheng, *J. Immunol.* **2013**, *191*, 5371.
[5] N. A. Gandhi, G. Pirozzi, N. M. H. Graham, *Expert Rev. Clin. Immunol.* **2017**, *13*, 425.
[6] M. C. Matsunaga, P. S. Yamauchi, *J. Drugs Dermatol.* **2016**, *15*, 925.
[7] D. M. Paton, *Drugs Today* **2017**, *53*, 477.
[8] A. Vatrella, I. Fabozzi, C. Calabrese, R. Maselli, G. Pelaia, *J. Asthma Allergy* **2014**, *7*, 123.
[9] L. Bao, H. Zhang, L. S. Chan, *JAKSTAT* **2013**, *2*, e24137.
[10] L. A. Beck, D. Thaçi, J. D. Hamilton, N. M. Graham, T. Bieber, R. Rocklin, J. E. Ming, H. Ren, R. Kao, E. Simpson, M. Ardeleanu, S. P. Weinstein, G. Pirozzi, E. Guttman-Yassky, M. Suárez-Fariñas, M. D. Hager, N. Stahl, G. D. Yancopoulos, A. R. Radin, *N. Engl. J. Med.* **2014**, *371*, 130.
[11] L. K. Oetjen, M. R. Mack, J. Feng, T. M. Whelan, H. Niu, C. J. Guo, S. Chen, A. M. Trier, A. Z. Xu, S. V. Tripathi, J. Luo, X. Gao, L. Yang, S. L. Hamilton, P. L. Wang, J. R. Brestoff, M. L. Council, R. Brasington, A. Schaffer, F. Brombacher, C. S. Hsieh, R. W. Gereau, M. J. Miller, Z. F. Chen, H. Hu, S. Davidson, Q. Liu, B. S. Kim, *Cell* **2017**, *171*, 217.
[12] S. G. Shimada, R. H. LaMotte, *Pain* **2008**, *139*, 681.
[13] F. Cevikbas, X. Wang, T. Akiyama, C. Kempkes, T. Savinko, A. Antal, G. Kukova, T. Buhl, A. Ikoma, J. Buddenkotte, V. Soumelis, M. Feld, H. Alenius, S. R. Dillon, E. Carstens, B. Homey, A. Basbaum, M. Steinhoff, *J. Allergy Clin. Immunol.* **2014**, *133*, 448.
[14] L. Larsen, C. Röpke, *APMIS* **2002**, *110*, 833.
[15] D. L. Krebs, D. J. Hilton, *Stem Cells* **2001**, *19*, 378.
[16] B. A. Croker, H. Kiu, S. E. Nicholson, *Semin. Cell Dev. Biol.* **2008**, *19*, 414.
[17] S. M. McCormick, N. M. Heller, *Cytokine* **2015**, *75*, 38.
[18] G. K. Hershey, *J. Allergy Clin. Immunol.* **2003**, *111*, 677.
[19] M. O. Daines, Y. Tabata, B. A. Walker, W. Chen, M. R. Warrier, S. Basu, G. K. Hershey, *J. Immunol.* **2006**, *176*, 7495.
[20] T. Zheng, M. H. Oh, S. Y. Oh, J. T. Schroeder, A. B. Glick, Z. Zhu, *J. Invest. Dermatol.* **2009**, *129*, 742.
[21] T. Olivry, D. Mayhew, J. S. Paps, K. E. Linder, C. Peredo, D. Rajpal, H. Hofland, J. Cote-Sierra, *J. Invest. Dermatol.* **2016**, *136*, 1961.
[22] T. Czarnowicki, J. G. Krueger, E. Guttman-Yassky, *J. Allergy Clin. Immunol. Pract.* **2014**, *2*, 371.
[23] T. Hawro, R. Saluja, K. Weller, S. Altrichter, M. Metz, M. Maurer, *Allergy* **2014**, *69*, 113.

How to cite this article: Campion M, Smith L, Gatault S, Métais C, Buddenkotte J, Steinhoff M. Interleukin-4 and interleukin-13 evoke scratching behaviour in mice. *Exp Dermatol.* 2019;28:1501–1504. https://doi.org/10.1111/exd.14034