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The microbiota is important for IL-17A expression and neutrophil infiltration in lesional skin of *Flg^{fl/fl}* mice

Sabine Hoff¹,

Division of Immunology, Boston Children's Hospital, Boston, MA, USA

Michiko K. Oyoshi,

Division of Immunology, Boston Children's Hospital, Boston, MA, USA

Andrew Macpherson, and

Division of Gastroenterology and Mucosal, Immunology, University of Bern, Bern, Switzerland

Raif S. Geha

Division of Immunology, Boston Children's Hospital, Boston, MA, USA

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To the Editor

Atopic dermatitis (AD) is a common skin disease affecting about 20% of children and 6% of adults in developed countries. A hallmark of AD is an altered skin barrier, leading to increased water loss through the epidermis and dry skin. Filaggrin (FLG) plays a crucial role in the formation and maintenance of a protective skin barrier, and mutations in the *FLG* gene are strong predisposing factors for the development of AD [1].

Filaggrin-deficient flaky tail (*Flg^{fl/fl}*) mice carry a loss-of-function mutation in the *Flg* gene and have been described as a spontaneous model for both ichthyosis vulgaris and AD [2,3]. Filaggrin knock-out mice display impaired barrier function of the skin [4]. *Flg^{fl/fl}* mice on a mixed background develop spontaneous skin lesions, which are characterized by T_H17-dominated skin inflammation, starting at 28 weeks of age [5]. The onset of skin lesions in these mice is dependent on the adaptive immune system, as evidenced by the absence of skin lesions in Rag2 deficient *Flg^{fl/fl}* mice [6]. The microbiota is thought to play a complex role in AD. Bacterial colonization of the skin, especially with *S. aureus*, is associated with eczema flares in AD patients [7]. By contrast, increased hygiene standards resulting in reduced exposure to bacteria from the environment, are associated with increased prevalence of AD in developed countries [8]. We sought to identify a possible role for the microbiota in the development of skin lesions in *Flg^{fl/fl}* mice.

Corresponding author at: Division of Immunology, Children's Hospital, Karp Building 10th floor, One Blackfan Circle, Boston, MA 02115, USA. Fax: +1 617 730 0528. raif.geha@childrens.harvard.edu.

Current address: Department of Immunotherapy and Antibody Conjugates, Bayer Pharma AG, Berlin, Germany.

To this end, *Flg^{fl/fl}* mice bred onto BALB/c background for 10 generations were derived and housed under germ-free (GF) conditions or kept under specific pathogen-free (SPF) conditions. Weekly observation over 32 weeks revealed that 91% (10 out of 11) *Flg^{fl/fl}* mice kept under GF conditions developed spontaneous skin lesions by the end of the observation period (Fig. 1a, b). This frequency is comparable to the 92% frequency of lesion development in *Flg^{fl/fl}* mice kept under SPF conditions (11 out of 12 mice). The development of skin lesions was significantly accelerated between 18 and 22 weeks of age in GF *Flg^{fl/fl}* mice compared to SPF *Flg^{fl/fl}* mice. In fact, a log-rank test revealed that the distribution of time to the development of spontaneous skin lesions differed significantly between GF and SPF *Flg^{fl/fl}* mice from birth to 18, 20 and 22 weeks of age, respectively (Fig. 1b). The development of skin lesions was completed by 18 weeks in GF *Flg^{fl/fl}* mice compared to 26 weeks in SPF *Flg^{fl/fl}* mice. No skin lesions developed in WT BALB/c mice kept under GF (n = 11) and SPF conditions (n = 10), respectively.

We previously reported that lesional skin in *Flg^{fl/fl}* mice on BALB/c background raised in SPF conditions is characterized by significantly increased epidermal thickness, dermal infiltration with eosinophils and neutrophils, and *Il17a*, but not *Il4* or *Ifn γ* , mRNA expression [6]. Histological analysis at 32 weeks revealed a significant and comparable increase in epidermal thickness in lesional skin compared to non-lesional skin in GF *Flg^{fl/fl}* and in SPF *Flg^{fl/fl}* mice (Fig. 1c, d), suggesting that endogenous factors drive the development of epidermal thickness in the lesional skin of these mice. As previously reported [6], the numbers of dermal eosinophils and neutrophils were significantly increased in lesional skin compared to non-lesional skin in SPF *Flg^{fl/fl}* mice (Fig. 1e, f). By contrast, there was no significant increase in the numbers of dermal eosinophils or neutrophils in lesional skin of GF *Flg^{fl/fl}* mice. Furthermore, the numbers of dermal eosinophils and neutrophils in lesional skin were significantly lower in GF *Flg^{fl/fl}* mice compared to SPF *Flg^{fl/fl}* mice. These results suggest that the microbiota drives dermal infiltration of neutrophils and eosinophils in *Flg^{fl/fl}* mice. The histological appearance of non-lesional skin was comparable in *Flg^{fl/fl}* mice and WT BALB/c mice, regardless of the housing conditions (Fig. 1c and data not shown). As previously reported [6], *Il17a* mRNA expression was significantly increased in lesional skin compared to non-lesional skin of SPF *Flg^{fl/fl}* mice (Fig. 1g). By contrast, *Il17a* mRNA expression was not significantly increased in lesional skin of GF *Flg^{fl/fl}* mice. Furthermore, *Il17a* mRNA expression in lesional skin was significantly lower in GF *Flg^{fl/fl}* mice compared to SPF *Flg^{fl/fl}* mice. There was no significant increase in *Il4*, *Il13* or *Ifn γ* mRNA expression in lesional versus non-lesional skin in either SPF *Flg^{fl/fl}* or GF *Flg^{fl/fl}* mice (Fig. 1h–j). These results suggest that increased *Il17a* expression in the lesional skin of *Flg^{fl/fl}* mice is driven by the microbiota, but is not necessary for the development of skin lesions in these mice. The latter conclusion is supported by our observation that there is no significant difference in the development of skin lesions between *Flg^{fl/fl}* mice and *Flg^{fl/fl}/Il17a^{-/-}* mice raised in SPF conditions (data not shown). Impaired *Il17a* mRNA expression could explain the decreased dermal infiltration by neutrophils and eosinophils into lesional skin of GF *Flg^{fl/fl}* mice, as IL-17A drives the expression of neutrophil chemoattractants by tissue resident cells and is important for eosinophil extravasation from the blood into the lung [9].

Flg^{ft/ft} mice harbor a second mutation in the closely linked *matted* gene, coding for the Tmem79/Matt protein [10]. Both filaggrin and Tmem79/Matt are important for skin barrier function. Mice carrying the *matted* mutation develop spontaneous dermatitis, while *Flg* null mice only display dry skin and epidermal thickening [4,10]. Spontaneous skin lesions have been reported to be comparable in *Flg^{ft/ft}* mice and *Tmem79/Matt^{ma/ma}* homozygous mice [10]. This suggests that the microbiota may be important for increased *Il17a* expression and dermal infiltration by neutrophils and eosinophils in the skin lesions of *Tmem79/Matt^{ma/ma}* mice.

In summary, microbiota-derived signals are essential to promote cutaneous *Il17a* expression and dermal infiltration by eosinophils and neutrophils in mice with a disrupted skin barrier, but may be dispensable for the development of skin lesions.

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Abbreviations

AD	atopic dermatitis
FLG/Flg	filaggrin (human/mouse)
Ft	flaky tail
GF	germ-free
SPF	specific pathogen-free
WT	wild type

Figure 1.

Despite similar development of skin lesions in GF and SPF *Flg^{fl/fl}* mice, respectively, GF *Flg^{fl/fl}* mice display decreased numbers of inflammatory cells into the dermis and decreased *Il17a* mRNA expression in their skin lesions. a: Representative pictures of GF and SPF *Flg^{fl/fl}* mice at 32 weeks of age show lesions on the back (arrows). b: Kaplan–Meier plot of lesion development over 32 weeks (n = 11–12/group). * p < 0.05 as determined by log-rank test of the curves from birth to 18, 20, and 22 weeks, respectively, indicated by arrows. c: Representative photomicrographs of non-lesional and lesional skin (scale bar: 100 μm). d: Epidermal thickness at 32 weeks of age (n = 4–6/group). e: Dermal eosinophils, and f: neutrophils per HPF (400×, n = 4–6/group). g–j: Skin cytokine mRNA expression of *Il17a*, *Il4*, *Il13* and *Ifnγ* at 32 weeks of age (n = 4–6/group). Bars represent mean ± SEM. GF — germ-free; HPF — High-powered field; SPF — specific pathogen-free. n.s. — not significant. * p < 0.05, ** p < 0.01, *** p < 0.001 by unpaired 2-tailed Student's *t*-test.