Scratching Beneath the Surface: Linking Skin Pathology with Food Allergy

Marie Cherrier, Nadine Cerf-Bensussan

Scratching Beneath the Surface: Linking Skin Pathology with Food Allergy

Marie Cherrier[1,2] and Nadine Cerf-Bensussan[1,2]

[1] INSERM U1163 and Institut Imagine, Laboratory of Intestinal Immunity. 75015 Paris

[,2] Université de Paris. 75006 Paris

Correspondence to: N. Cerf-Bensussan, Laboratory of Intestinal Immunity INSERM U1163 and Institut Imagine, 24, boulevard du Montparnasse, 75015 Paris France. nadine.cerf-bensussan@inserm.fr

A link between atopic dermatitis and food allergy has long been suspected but remains elusive. In this issue, Leyva-Castillo et al show how mechanical injury of the skin initiates a cascade of events that stimulate the expansion of mucosal mast cells and promote food anaphylaxis.

Multiple reports have suggested that atopic dermatitis (AD) predisposes to food allergy. Data are however largely correlative (Robison and Singh, 2019). Moreover, the role of skin in food allergy is equivocal. Thus, epicutaneous application of allergens can induce IgE-dependent sensitization (Bartnikas et al., 2013). Conversely, it is also advocated as an efficacious method for desensitization (Esposito et al., 2018). In this issue of Immunity, Leyva-Castillo et al have expanded prior work indicating that epicutaneous delivery of a food allergen in the context of mechanical skin injury can lead to food anaphylaxis (Bartnikas et al., 2013). They now have shown that mechanical skin injury induced by tape stripping of the mouse back, (used as a surrogate of scratching in AD), could promote food anaphylaxis independently of cutaneous delivery of the antigen (Leyva-Castillo et al., 2019). They elegantly unravelled how cross talk between

interleukin-33 (IL-33) released by the injured skin and IL-25 produced by intestinal tuft cells could amplify intestinal type 2 responses to food allergens and promote food anaphylaxis. Their results thus provide a possible mechanism linking AD and food allergy.

Food allergy can manifest by digestive symptoms but also induce systemic anaphylaxis with circulatory collapse and or respiratory distress. Symptoms result from the degranulation of mast cells upon crosslinking of preformed membrane-bound IgE-FcεRI complexes by the allergen. Circulating food specific IgE do not accurately predict the risk of anaphylaxis (Robison and Singh, 2019). Leyva-Castillo et al proposed that one important factor is the size of the intestinal pool of mast cells, which has been correlated with the severity of oral anaphylaxis in mouse models (Vaali et al., 2012). Mast cells may notably increase gut permeability and thereby allow systemic exposure to food allergens, which is instrumental for food anaphylaxis (Strait et al., 2011). Pursuing their past unexpected observation that skin tape stripping alone can simultaneously drive mast cell expansion in the gut and predispose mice to severe passive IgE- dependent anaphylaxis, they have now deciphered the cascade of events that connect skin injury and food anaphylaxis.

Leyva-Castillo et al first confirmed that mechanical skin injury induced by tape stripping induced small intestinal accumulation of activated mast cells displaying enhanced binding of IgE and IL-13 mRNA expression. The authors next ascertained the crucial role of intestinal mast cells in driving both permeability to food proteins and oral anaphylaxis. Thus, the increase in serum horseradish peroxidase protein observed after tape stripping and oral feeding in wild type (WT) animals was absent in *c-Kit*$^{-/-}$ mice that are mast cell deficient and in *Itgb7*$^{-/-}$ mice, which selectively lack intestinal mast cells. Moreover, systemic anaphylaxis, as revealed by increased drop in body temperature after oral ovalbumin challenge of sensitized mice, was enhanced by tape stripping in WT but not in *Itgb7*$^{-/-}$ mice (Figure 1). Results were similar in mice actively sensitized by the intraperitoneal route or passively sensitized by intravenous administration of specific IgE, indicating that the effect of skin injury was not due to increased IgE production.

In the second part of the article, Leyva-Castillo et al untied the mechanisms that connect mechanical skin injury to the remote accumulation and activation of mast cells in the small intestine. They searched first for the soluble factors that may transmit the signal from the locally injured skin into the intestine. Three alarmins, IL-33, IL-25 and thymic stromal lymphopoietin

(TSLP), are induced by danger signals in tissues and play a key role in initiating T helper-2 (Th2) cell responses associated with IgE production and mast cell activation (Hammad and Lambrecht, 2015). Confirming and extending their past results (Galand et al., 2016), the authors showed that skin tape stripping induced IL-33 production by keratinocytes followed by its release into the serum (Figure 1). Keratinocytes were likely the crucial source of IL-33 for intestinal mast cell accumulation, as the latter did not occur when IL-33 was specifically inactivated in K14 expressing cells. TLSP but not IL-25 was also induced in the skin. Strikingly however, mast cell expansion was preserved in TLSP deficient mice but abolished in mice lacking IL-17RB, eliminating a contribution of TSLP and, alternatively, uncovering a key role for IL-25. The source of IL-25 was likely the epithelial tuft cells, a well-known source of IL-25 in the intestine. Thus, tuft cell number increased in the jejunum after tape stripping while mucosal mast cells failed to accumulate when IL-25 was selectively inactivated in villin-expressing epithelial cells. Overall these data indicate that both skin-derived IL-33 and IL-25 produced by intestinal epithelial cells are necessary to drive the expansion of mast cells.

Interestingly, it was previously shown that systemic injection of IL-33 induces the expansion of tuft cells and their production of IL-25. IL-33 did not act directly on tuft cells, but stimulated the activation of group 2 innate lymphoid cells (ILC2) that produced IL-13. In turn, IL-13 acted on epithelial cell progenitors to drive their differentiation into tuft cells (von Moltke et al., 2016). Data obtained by Leyva-Castillo et al confirmed this scenario and further indicate that ILC2 activated by both IL-33 and IL-25 are necessary to drive the expansion of mast cells. Demonstrating a key role of ILC2, they first established that intestinal mast cell expansion was induced by skin tape stripping in *Rag2*$^{-/-}$ mice but neither in *Rag2*$^{-/-}\gamma_c^{-/-}$ nor in hematopoietic chimeras reconstituted with bone marrow cells deficient in RORα, a transcription factor indispensable for ILC2 differentiation. They next showed that, after skin tape stripping, there was a 2 to 3 fold expansion of ILC2 expressing IL-13 and IL-4 mRNA in the small intestine that was abolished upon selective inactivation of either IL33 or IL25 receptors in RORα^{+} cells. Possibly explaining why ILC2 expanded exclusively in the small intestine after skin injury, they noted that, in contrast to the IL33 receptor that is present on all ILC2, the IL-25 receptor was preferentially expressed by intestinal ILC2. Leyva-Castillo et al finally explained how ILC2 could drive mast cell expansion. They showed that ILC2 were the main source of IL-4 and IL-13 after skin tape stripping and that the two cytokines played essential and non redundant roles in mast cell expansion (Figure 1). Accordingly,

mast cell expansion was not observed in mice treated by antibodies blocking IL-4 or IL-13 or when these cytokines were selectively inactivated in RORα^{+} cells. In keeping with previous evidence that ILC2 and tuft cells contribute to a feed forward loop via their production of IL-13 and IL-25 respectively (von Moltke et al., 2016), tuft cell expansion was also absent in the latter mice. Along the same line, expansion of tuft cells, ILC2 and mast cells after systemic injection of IL-33 was significantly reduced in mice lacking the IL-25 receptor. Conversely IL-25 injection induced the expansion of mast cells in the absence of IL-33 receptor but not of IL25 receptor.

Overall, the data obtained by Leyva-Castillo et al in the mouse model indicated that IL-33 was induced in injured keratinocytes and cooperated with IL-25 produced by tuft cells to promote the accumulation and activation of tissue resident ILC2. Via their production of IL4 and IL-13, ILC2 stimulated the expansion of activated mast cells in the small intestine. In turn, mast cells increased gut permeability to food antigens and promoted anaphylaxis (Figure 1). Whether this scenario provides the missing link between AD and food allergy in humans remains to be established. In order to support this hypothesis, the authors compared the number of mast cells in intestinal biopsies of 8 age-matched children with or without AD and or food allergy or eosinophilic oesophagitis. They observed that the numbers of duodenal mast cells were significantly increased in the 4 patients with active AD and skin scratching compared to children without AD and this even in the absence of high serum IgE concentration. The results are too limited to draw any definitive conclusion inasmuch as AD and food allergy did not strictly overlap, but they encourage further analysis. It would notably be interesting to define whether IL-33 is increased in the serum of patients with AD with food allergy. Whether this increase can be correlated with an increase in IL25, ILC2 and mast cells in intestinal biopsies may however be difficult to demonstrate as such biopsies are rarely performed in children with immediate IgE-dependent food allergy. The skin-to-gut connection unraveled in this study however supports clinical observations suggesting that the risk of food anaphylaxis is alleviated after effective topical treatment of AD (Thompson and Hanifin, 2005). This work also suggests that interventions to decrease scratching may be useful for dampening the severity of food allergy. Finally, one puzzling question concerns the physiological role of this skin-to-gut connection. The authors proposed that the cross-talk may have evolved as part of a defense mechanism to alert the gut to react against helminths that have breached the skin barrier. It may prove very interesting to scratch beneath the surface of this attractive hypothesis……

The authors declare no conflict of interest.

REFERENCES

Bartnikas, L.M., Gurish, M.F., Burton, O.T., Leisten, S., Janssen, E., Oettgen, H.C., Beaupre, J., Lewis, C.N., Austen, K.F., Schulte, S.*, et al.* (2013). Epicutaneous sensitization results in IgE-dependent intestinal mast cell expansion and food-induced anaphylaxis. J. Allergy Clin. Immunol. *131*, 451-460 e451-456.

Esposito, S., Isidori, C., Pacitto, A., Salvatori, C., Sensi, L., Frati, F., Di Cara, G., and Marcucci, F. (2018). Epicutaneous immunotherapy in rhino-conjunctivitis and food allergies: a review of the literature. J Transl Med *16*, 329.

Galand, C., Leyva-Castillo, J.M., Yoon, J., Han, A., Lee, M.S., McKenzie, A.N.J., Stassen, M., Oyoshi, M.K., Finkelman, F.D., and Geha, R.S. (2016). IL-33 promotes food anaphylaxis in epicutaneously sensitized mice by targeting mast cells. J. Allergy Clin. Immunol. *138*, 1356-1366.

Hammad, H., and Lambrecht, B.N. (2015). Barrier Epithelial Cells and the Control of Type 2 Immunity. Immunity *43*, 29-40.

Leyva-Castillo, J., Galand, C., Kam, C., Bunton, O., Gurish, M., Musser, M., Goldsmith, J., Hait, E., Nurko, S., Brombacher, F.*, et al.* (2019). Mechanical injury promotes food anaphylaxiis by driving mast cell expansion. Immunity.

Robison, R.G., and Singh, A.M. (2019). Controversies in Allergy: Food Testing and Dietary Avoidance in Atopic Dermatitis. J Allergy Clin Immunol Pract *7*, 35-39.

Strait, R.T., Mahler, A., Hogan, S., Khodoun, M., Shibuya, A., and Finkelman, F.D. (2011). Ingested allergens must be absorbed systemically to induce systemic anaphylaxis. J. Allergy Clin. Immunol.*127*, 982-989 e981.

Thompson, M.M., and Hanifin, J.M. (2005). Effective therapy of childhood atopic dermatitis allays food allergy concerns. J Am Acad Dermatol *53*, S214-219.

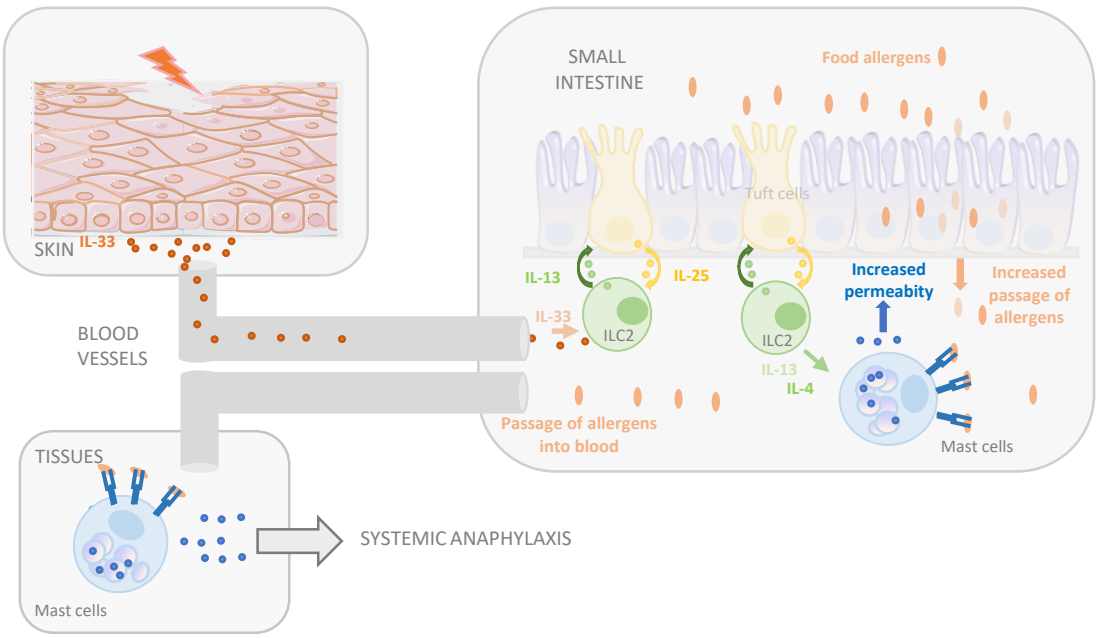
Vaali, K., Lappalainen, J., Lin, A.H., Mayranpaa, M.I., Kovanen, P.T., Berstad, A., and Eklund, K.K. (2012). Imatinib mesylate alleviates diarrhea in a mouse model of intestinal allergy. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society *24*, e325-335.

von Moltke, J., Ji, M., Liang, H.E., and Locksley, R.M. (2016). Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. Nature *529*, 221-225.

Legend of Figure 1

Keratinocytes injured by tape stripping (or scratching) release IL-33, which diffuses through blood toward the small intestine, where it acts in synergy with tuft cell-derived IL-25 to stimulates type 2 innate lymphoid cells (ILC2) that produce IL4- and IL-13. IL-13 stimulates the expansion of tuft cells, which produce IL-25, resulting in a feedback loop which further enhances the expansion and activation of ILC2. Via the production of IL-4 and IL-13, ILC2 stimulate the expansion and activation of mast cells. Cross-linking by the food allergen of IgE-FcεRI complexes preformed at the surface of intestinal mast cells stimulates the release of mediators that induce digestive symptoms but also increase the transepithelial passage of food allergens. These allergens can then diffuse into the blood and reach peripheral mast cells, triggering the release of mediators that act on the cardiovascular system and or the bronchi and induce the characteristic symptoms of anaphylaxis.

Figure 1


SMALL
INTESTINE
Food allergens
Tuft cells
SKIN
IL-33
IL-13
IL-25
IL-33
ILC2
ILC2
Increased
permeabity
Increased
passage of
allergens
IL-13
IL-4
BLOOD
VESSELS
Passage of allergens
into blood
Mast cells
TISSUES
SYSTEMIC ANAPHYLAXIS
Mast cells

PAF