

Eosinophils and itch: Partners in crime or strange bedfellows?

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Itch is a noxious sensation that disturbs work and play during wakefulness and prevents rest and rejuvenation during sleep. Teleologically, itch must have conferred an evolutionary advantage. Etiologically, itch is not well understood. Itch has both similarities and differences to pain. An interesting difference is that pain can be superficial and deep, but itch is only superficial, affecting epithelial tissues and not solid organs. For example, cardiac, pancreatic, and renal pain are known, but cardiac, pancreatic, and renal itch are not. In addition to skin itch, mucosae can itch; patients with food allergy describe oral mucosal itching, and patients with yeast infection describe genital mucosal itching. In epithelial organs other than the skin and mucous membranes, “itch equivalents” exist; cough might be lung itch, dysphagia might be esophageal itch, and dysuria might be bladder itch. As our understanding of immunology has taken shape, itch often is found to be associated with T_H2 immunologic responses, which logically have their origins in parasite resistance. However, anyone who has had poison ivy–induced dermatitis, classical delayed-type hypersensitivity, will fervently attest to the severe itching that is associated with a T_H1 immunologic response.

After their discovery by Paul Ehrlich in 1879, eosinophils quickly were associated with parasitic diseases and asthma. The prominence of eosinophils in skin infected with an ectoparasite, *Sarcoptes scabiei*, is an example of a relationship between eosinophils and itch; scabies is derived from the Latin word “to scratch.” Later observations showed that eosinophil granule proteins, even in the absence of intact eosinophils, were extensively deposited in patients with edematous and pruritic skin afflictions, such as urticaria, atopic dermatitis, and prurigo nodularis, conditions in which aberrant neurophysiologic reactivity is known.¹

Lee et al,² in their article entitled “Eosinophil-dependent skin innervation and itching following contact toxicant exposure in

mice,” explore the relationship between eosinophils and itch. They use epicutaneously applied trimellitic anhydride (TMA) to induce cutaneous eosinophilia and eosinophil degranulation with accompanying edema and itching on the ears of BALB/cJ wild-type mice and compare the reaction histologically and phenotypically with that in eosinophil-deficient *PHIL* mice. They further characterize sensory innervation accompanying TMA reactions with and without eosinophils in wild-type and *PHIL* mice.

The investigators report key differences in the responses of *PHIL* and wild-type mice to TMA exposure. First, they demonstrate histologic differences in cellular infiltrates, with *PHIL* mice showing not only the expected absence of eosinophils but also decreases in levels of other cell infiltrates, including lymphocytes and mast cells. Furthermore, epithelial thickening is decreased, edema is less, and collagen deposition, as a marker of tissue remodeling, is reduced in *PHIL* mice compared with wild-type mice.

Second, through measurements of ear thickness, the authors show that edematous reactions in ear skin after contact exposure to TMA are greater in both *PHIL* and wild-type mice compared with those seen in ears of vehicle-treated (control) mice but that, similar to histologic comparisons, *PHIL* mice have less skin edema than wild-type mice.

Third, the investigators show that eosinophil degranulation is greater in TMA-treated ears of wild-type mice than in vehicle-treated ears, with essentially no eosinophil degranulation in either TMA- or vehicle-treated ears of *PHIL* mice.

Fourth, using 3-dimensional reconstructions of confocal microscopic images of whole ear mounts stained with antibodies to the panneuronal marker PGP 9.5 and to substance P, the authors assess TMA-induced nerve changes in relationship to eosinophil and extracellular eosinophil granule protein localization. They show that, although ear innervation is equivalent in wild-type and *PHIL* mice, TMA induces an increase in substance P–expressing nerves in wild-type mice over that seen in vehicle-treated ears and over that seen in both TMA- and vehicle-treated ears of *PHIL* mice.

Fifth and finally, analyses of time-lapse videography of scratching events reveal that the absence of eosinophils in *PHIL* mice is associated with a significant reduction in itch responses compared with those seen in wild-type mice. From these observations, the authors conclude that eosinophils are critical cells in the overall magnitude of the inflammatory reaction to TMA epicutaneous exposure with enhanced sensory nerve substance P expression and itching.

This report combines 2 sophisticated and intricate investigational approaches, mice without eosinophils (*PHIL* mice) and imaging of innervation and nerve expression, to analyze the physiologic response of itch, which has eluded basic understanding. For those who are eosinophilologists, for those who struggle to

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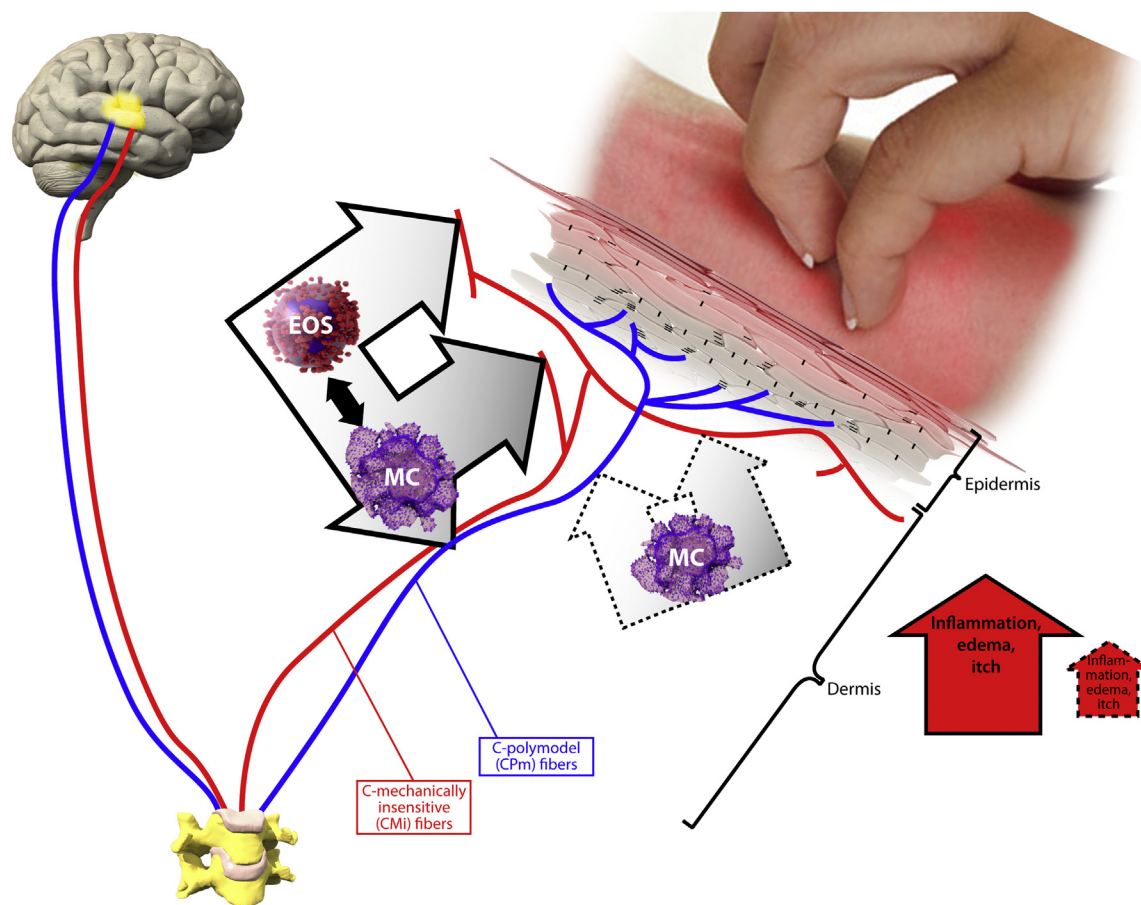


FIG 1. Eosinophils, mast cells and itch. Eosinophils (*Eos*) and mast cells (*MC*) both have described associations with C-type nerve fibers implicated with itch. C-polymodal (CPm) fibers are activated in the epidermis through protease-activated receptor 2 (PAR-2), of which mast cell tryptase is an agonist; PAR-2 appears to mediate cutaneous itch directly through a histamine-independent mechanism. Furthermore, histamine released by mast cells activates C-mechanically insensitive (CMI) fibers that innervate widespread areas of the skin and, on activation, release proinflammatory mediators that lead to vasodilatation. Therefore activated mast cells exhibit effects on both types of C-fibers that contribute to itch and inflammation.¹¹ Relationships between eosinophils and nerves are broadly known, but the effects of eosinophils on nerve activation related to itch are not as directly understood as are the effects of mast cells. Eosinophils synthesize neurotrophins, including nerve growth factor, brain-derived neurotrophic factor, and neurotrophin 3. Eosinophils localize to the skin in mouse models near increased nerves, and eosinophils in culture with nerves cause branching of sensory neurons. Eosinophil granule proteins block neuronal M2 muscarinic receptors, possibly contributing to airways hypersensitivity in asthmatic patients. Eosinophils and mast cells interact to enhance nerve activation, perhaps synergistically both through effects on nerves and effects on each other. If mast cell numbers, activation state, or both are decreased in the absence of eosinophils, then the mast cell contribution to itch and inflammation (and edema and tissue remodeling) will be decreased. The relative contributions of eosinophils and mast cells in this situation cannot be assessed specifically unless mast cells are taken out of the picture. Indirectly, they could be assessed if mast cell numbers and activation products were compared with the reaction parameters both in the presence and absence of eosinophils.

treat itch, and, perhaps even more so, for those who are afflicted by itch, it is an exciting report that adds important elements to the investigative landscape. This report also is tantalizing in several other respects.

The authors begin their studies by establishing that contact with TMA results in robust eosinophil skin infiltration that they contrast to a lack of an eosinophil response to dinitrofluorobenzene contact exposure in mouse ear skin. However, as mapped on the timeline in Lee et al's Fig 1,² the exposure algorithms are very different for the 2 contactants both in frequency and number of challenge days. Notably, reactions in mice subjected to repeated

TMA challenges show different response profiles than in those undergoing a single exposure after sensitization.³ What is interesting is that this is a model of epicutaneous exposure that predominantly induces eosinophils and itch, and therefore the immunologic mechanisms are important. However, current nomenclature regarding "contact hypersensitivity" does not explicitly discriminate reaction types, such as T_H2 -polarized contact hypersensitivity versus classical T_H1 -polarized contact hypersensitivity. Exposure timing is critical to expression along with probable genetic and epigenetic influences for which key mechanistic differences are at play.⁴ Subsets of drug

hypersensitivity have been characterized by immunologic responses and pathologic characteristics,⁵ but subsets of contact hypersensitivity are referred to by using various labels in this article. For the purposes of this investigational study, the comparison between dinitrofluorobenzene and TMA is fundamentally extraneous but highlights this important issue.

TMA is a sensitizer that is widely used industrially in the manufacture of plasticizers as a curing agent for epoxy resins and in surface-coating resins. TMA readily reacts with proteins and functions as an immunogen to induce all hypersensitivity reaction types⁶ including IgE-mediated allergic reactions in human subjects manifesting as occupational asthma, rhinitis, and conjunctivitis.⁶ In mouse models acute, subacute, and chronic contact hypersensitivity are described,³ as is a late phase of contact hypersensitivity⁷; both the chronic and late-phase reactions result from repeated TMA challenges, and both are associated with a T_H2 response. In the chronic model as represented in Lee et al's Fig 1² based on methods in Schneider et al,³ analyses show about a 10-fold increase in IgE levels.³ In the late-phase mouse model the inflammatory responses are reduced with anti-IgE administration.⁷ Thus TMA does not exclusively induce a T_H2 hypersensitivity response, although for the studies in this article, it is likely that the TMA reaction is an IgE-mediated reaction.^{3,7} Explaining the immunologic mechanisms of this model including relative changes in IgE and cytokine levels would have been helpful rather than adopting terms such as contact toxicant hypersensitivity reaction and nonclassical contact allergen reaction, which muddy the issue.

More generally, an expanded uniform nomenclature for types of contact hypersensitivity reactions that confers an understanding of the prevalent immunologic mechanisms is desirable. Such understanding will be even more relevant as the contributions from type 2 innate lymphoid cells (ILC2) become better defined. Tissue ILC2s regulate basal eosinophilopoiesis and tissue eosinophil accumulation through constitutive and stimulated cytokine expression. ILC2s are present in the skin both in mice and humans.⁸ Studies of the interactions of skin ILCs with other cell types demonstrate that dermal ILCs interact selectively and strongly with mast cells. Type 2 innate lymphocytes have been designated part of the "T_H2 franchise,"⁹ and a broad role for ILC2s is emerging for eosinophils and metabolic homeostasis, such as in adipose tissue, as well as in patients with allergic respiratory disease, including associated tissue remodeling, and atopic dermatitis. Although reference to ILC2s is not made in this article, it is reasonable to consider that innate immunity through ILC2s and acquired immunity through T_H2 together make eosinophils and nerves partners in the crime of itch.

Overall, the authors' data appear to support their study conclusions, at least until the information in Fig E1 is appraised. The legend for Fig E1 states that, "...reactions...in *PHIL* mice...following exposure to TMA are accompanied by lower numbers of 'resident' mast cells" and appears to suggest that there are fewer "resident" mast cells in *PHIL* mice compared with wild-type mice. In turn, this raises the question as to whether

PHIL mice have an alteration of mast cells. Unfortunately, no comparison between pre-exposure or vehicle-exposed sites and TMA-exposed sites on *PHIL* mice is presented. Do *PHIL* and wild-type mice start out with the same numbers and activation state of mast cells? Does the legend really mean that TMA induces fewer mast cells in *PHIL* mice, or are mast cells depleted in *PHIL* mice after TMA exposure? Do *PHIL* and wild-type mice show comparable increases in IgE levels?³ Is there a relationship between IgE levels (and IgE antibodies to TMA) and mast cell degranulation? Relationships between mast cells and nerves have long been known.¹⁰ With the extensive degree of mast cell infiltration and degranulation revealed in Fig E1, how can one be certain that the main effects or at least significant contributory effects on the overall inflammatory response and on nerves are not through mast cells? If the TMA reaction is IgE dependent and presumably mast cell dependent as well and if the TMA-induced reaction in *PHIL* shows fewer mast cells, then the findings suggest that Lee et al² have discovered a new feature of *PHIL*: a reduction in mast cell function.

Although the investigative approach is elegant and enticing and the authors' conclusion that "eosinophil-mediated events following TMA contact toxicant reactions increase skin sensory nerve substance P and, in turn, increase itching responses" is something that I wish to believe, I am concerned that the picture is incomplete and the conclusion not wholly valid. Perhaps eosinophils and itch have a serendipitous relationship in these studies through IgE and mast cells (Fig 1),¹¹ such that eosinophils are merely strange bedfellows in this immunopathology.

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