Eosinophil-dependent skin innervation and itching following contact toxicant exposure in mice

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Background: Contact toxicant reactions are accompanied by localized skin inflammation and concomitant increases in site-specific itch responses. The role(s) of eosinophils in these reactions is poorly understood. However, previous studies have suggested that localized eosinophil-nerve interactions at sites of inflammation significantly alter tissue innervation.

Objective: To define a potential mechanistic link between eosinophils and neurosensory responses in the skin leading to itching.

Methods: BALB/cJ mice were exposed to different contact toxicants, identifying trimellitic anhydride (TMA) for further study on the basis of inducing a robust eosinophilia accompanied by degranulation. Subsequent studies using TMA were performed with wild type versus eosinophil-deficient *PHIL* mice, assessing edematous responses and remodeling events such as sensory nerve innervation of the skin and induced pathophysiological responses (ie, itching).

Results: Exposure to TMA, but not dinitrofluorobenzene, resulted in a robust eosinophil skin infiltrate accompanied by significant levels of degranulation. Follow-up studies using TMA with wild type versus eosinophil-deficient *PHIL* mice showed that the induced edematous responses and histopathology were, in part, causatively linked with the presence of eosinophils. Significantly, these data also demonstrated that eosinophil-mediated events correlated with a significant increase in substance P content of the cutaneous nerves and an accompanying increase in itching, both of which were abolished in the absence of eosinophils.

Conclusions: Eosinophil-mediated events following TMA contact toxicant reactions increase skin sensory nerve substance P and, in turn, increase itching responses. Thus, eosinophil-nerve interactions provide a potential mechanistic link between eosinophil-mediated events and neurosensory responses following exposure to some contact toxicants. (J Allergy Clin Immunol 2015;135:477-87.)

Key words: *Contact hypersensitivity, eosinophil-deficient, sensory nerve, degranulation*

Chemically induced contact hypersensitivity responses induce inflammatory skin reactions with typical clinical features characterized by toxicant-specific inflammatory cell infiltrates,[1,2] poorly defined erythematous and edematous events, and skin remodeling (eg, dermal thickening) in chronic settings.[3] These contact responses, together with related diseases such as allergic contact sensitivities and atopic dermatitis, are typically linked with self-perpetuating scratch-itch cycles and common skin conditions associated with 7% to 10% of the population.[4] The underlying immunobiology of these responses is complex and also likely includes both genetic and environmental factors that affect immune responsiveness,[5] skin barrier function(s),[6] and the context of toxicant/allergen exposure.[6]

The specific character of the inflammatory responses to contact toxicant exposure defines the immune responses and, in turn, the symptoms/pathologies linked with a specific toxicant. Mixed T_H1/T_H17 and T_H2 cellular responses characterized by the production of IL-2, IFN-γ, and IL-17 are prevalent responses linked with the inflammation and injury associated with many toxicant contact hypersensitivity reactions.[7] For example, epicutaneous sensitization by haptens such as dinitrofluorobenzene[8] (DNFB), dinitrochlorobenzene[9] (DNCB), or certain metals (eg, nickel[10]) induce contact hypersensitivity reactions

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Abbreviations used
DNCB: Dinitrochlorobenzene
DNFB: Dinitrofluorobenzene
EAI: Eosinophil activity index
EPX-mAb: Eosinophil peroxidase-specific monoclonal antibody
PGP 9.5: Pan-neuronal antibody maker
PHIL: Eosinophil-deficient transgenic mice
TMA: Trimellitic anhydride (TMA)

characterized by acute inflammatory and T_H1 responses that are accompanied by skin inflammatory infiltrates often dominated by activated T lymphocytes, monocytes, and increased numbers of neutrophils. In contrast, other toxicants such as trimellitic anhydride[11] (TMA) represent non-classical contact allergens that elicit the production of T_H2 cytokines such as IL-4, IL-5, and IL-13. This cytokine profile is also linked with acquired humoral immune responses leading to antigen-specific IgE-immunoglobulin production that are similar to those associated with atopic dermatitis and contact allergic dermatitis.[12] Moreover, in contrast to DNFB- or DNCB-mediated toxicant hypersensitivity reactions, TMA-induced T_H2 contact responses invariably include a robust eosinophilia.[11] The contribution of these skin-infiltrating eosinophils to immune/inflammatory cascades, disease pathology, and ultimately symptoms, is often speculative.[13]

Our studies defining the potential role(s) of eosinophil-mediated activities during inflammatory diseases have recently identified a previously underappreciated link between eosinophils and the nerves that innervate the skin in biopsies from patients with atopic dermatitis.[14] We were able to extend these observations *in vivo* using a keratinocyte-specific IL-5 overexpressing transgenic line of mice that elicits an eosinophilic dermatitis. In both cases, the induced dermal eosinophilia occurring in these mice correlated with a corresponding increase in nerve axon length and branching. We also co-cultured eosinophils with dorsal root ganglia cells (ie, sensory neurons) and showed that this alone increased axon growth. Provocatively, these studies further demonstrated that eosinophil-mediated effects on nerves were complex and occurred through the secretion of one or more factors beyond obvious explanations such as the release of nerve growth factor.[14]

The potential consequences of eosinophil-nerve interactions in the skin were examined in this report of chemically induced contact toxicant reactions using an array of eosinophil-specific reagents and mouse models we have developed and characterized. In particular, we showed that eosinophil infiltration and eosinophil degranulation within the skin are prominent features of TMA contact responses but not DNFB contact hypersensitivity reactions. We further demonstrated that edematous inflammatory responses following TMA exposure were dependent on the presence of the induced eosinophil infiltrate as were remodeling events linked with dermal innervation by sensory nerves. Significantly, TMA-induced pathophysiological responses such as itch were reduced in eosinophil-deficient mice exposed to TMA, suggesting that eosinophil-mediated effects on dermal sensory nerve innervation may represent a mechanistic link between eosinophils and TMA-induced itching.

METHODS

Mice

All studies were performed with 6- to 14-week-old mice on a BALB/cJ background. Eosinophil-deficient *PHIL* mice[15] were bred in-house, continually backcrossing to BALB/cJ mice (n > 15 generations). BALB/cJ control mice were purchased directly from Jackson Laboratories (Jackson Research Laboratories, Bar Harbor, Me). Mice in these studies were maintained in ventilated micro-isolator cages housed in the specific pathogen-free animal facility at the Mayo Clinic in Arizona. All protocols and studies involving animals were performed in accordance with National Institutes of Health and Mayo Foundation institutional guidelines.

Induction of toxicant contact responses to DNFB and TMA exposure

DNFB and TMA sensitization and exposure were performed as described by O'Leary et al[8] and Schneider et al[11] (Fig 1, *A* and *B*).

Assessments of toxicant-induced ear swelling and collection of biopsies for histology

The region of the ear below the first cartilage ridge was measured using a Digimatic Caliper (Mitutoyo Corporation, Aurora, Ill) as an inflammatory marker of TMA toxicant contact exposure. The change in ear thickness in these studies was determined by calculating the absolute increase in thickness of experimental and control ears (day 15) from the average thickness measured at baseline (day 6).

Assessments of TMA-mediated skin histopathology: Structural tissue changes, eosinophil infiltration/degranulation, and collagen deposition/fibrosis

Ear biopsies were collected and processed for histopathology and immunohistochemistry using eosinophil-specific antibodies, as described previously.[16,17] Six transverse serial sections across the midline of the anterior-posterior axis of each ear were obtained and numbered 1, 2, 3, 4, 5, and 6 (A-P). Slide 3 of each set was stained with hematoxylin-eosin (H&E; general histological assessments). Slides 1 and 2 were used for immunohistochemistry with an eosinophil-specific mouse anti-mouse EPX monoclonal antibody (*EPX-mAb*, slide 2) and a negative isotype control antibody (slide 1) as previously described.[18] Tissue infiltrating eosinophils and eosinophil degranulation were evaluated as described in earlier studies,[18,19] with modifications noted in this report. Slides 4 and 5 were reserved for qualitative and quantitative assessments of remodeling events, staining slide 4 with Masson trichrome (collagen deposition/fibrosis) and slide 5 with picrosirius red (collagen deposition/fibrosis), respectively. Quantitative morphometric assessments of subepithelial fibrosis occurring in each experimental cohort of mice were determined from picrosirius red–stained ear sections evaluated under polarized light, as described previously.[20] Slide 6 was used for additional histology evaluations, including assessments of mast cell numbers and tryptase release by activated mast cells.[21]

Imaging of epidermal ear innervation

Immunofluorescence staining/epidermal nerve imaging was performed on whole mounts of microdissected skin obtained from the base of control (vehicle-alone treated) and toxicant-exposed ears. The concave half of the ear was initially separated from the convex half and intervening cartilage using sharp microdissection scissors. The base of the concave half of ear skin was manually plucked free of large hairs using forceps. Afterward, a rectangular strip of skin that encompassed the full width of the tissue section was isolated, extending from the base of the ear up 1 cm. The tissue was then flipped epidermis-side down and secured with dissecting pins for removal of deeper dermis layers using forceps under a dissecting