

Allergens TRP a swITCH to Initiate Type 2 Immunity

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Allergens induce type-2 immunity, but unresolved questions remain about initiation of this response. In this issue, Perner et al. propose that cutaneous activation of TRPV1⁺ sensory neurons by protease allergens stimulates release of substance P to induce migration of Th2-skewing CD301b⁺ DC to draining lymph nodes.

Dendritic cells (DCs) are sentinels, continuously sampling their environment. Upon sensing pathogens they become activated, migrate to draining lymph nodes (dLN) and present antigen to naive T cells to commonly drive anti-microbial T helper-1 (Th1) and helper-17 (Th17) cell responses. In contrast allergens and helminths preferentially stimulate Th2 cell-mediated immunity. However, how allergens activate the innate immune system is not fully understood, representing a gap in our understanding of allergic disease and thus its prevention and treatment. Specific properties of allergens such as enzymatic activity, lipid content or contaminants, and their route of entry into the body, commonly through inhalation or the skin, may be relevant. Here, Perner et al. (2020) employ murine models to reveal that following cutaneous allergen exposure, sensory TRPV1⁺ neurons release the neuropeptide, substance P (SP), which induces migration of CD301b⁺ DCs to the dLN and subsequent Th2 cell differentiation (Figure 1).

CD301b⁺ DCs, a dermal subset of conventional DCs, are implicated in Th2 cell differentiation following exposure to allergen or helminth parasites in mice. How they detect and migrate in response to type 2 immunogens remains unclear since CD301b⁺ DCs do not upregulate the chemokine receptor CCR7 upon allergen exposure (Sokol et al., 2018), or induce Th2 cell differentiation following *in vitro* allergen stimulation (Kumamoto et al., 2013). The hypothesis that an additional cell in the dermis, acting as the primary sensor of allergen and relaying information to CD301b⁺ DCs to induce migration to dLNs was investigated here.

Cutaneous sensory neurons, the cell bodies of which are contained in the dorsal root ganglia (DRG), are one such candidate. They are reported to play a role in fungal infection-induced IL-17 synthesis by $\gamma\delta$ T cells (Kashem et al., 2015) and HDM-stimulated mast cell degranulation (Serhan et al., 2019) via calcitonin gene-related peptide (CGRP) and SP release, respectively. Perner et al. (2020) asked whether this axis is important in DC activation following allergen stimulation.

Using the model allergen papain, a cysteine protease, Perner et al. (2020) investigated a two-step model of DC activation, where allergen activates sensory neurons, releasing neuropeptides that act on local DCs to induce maturation and migration to the dLN. The authors first confirmed that intra-dermal injection of papain induced a TRPV1-dependent sensory response of itch and pain in mice. This was inhibited by blocking the activation of TRPV1⁺ neurons with a sodium channel blocker, QX314, or through selective deletion of TRPV1⁺ neurons in *Trpv1*^{DTR} mice.

The authors next confirmed that CD301b⁺ DCs do not respond directly to stimulation with papain *in vitro*, although they did respond to LPS. In contrast, *in vivo* immunization with papain induced CD301b⁺ DC activation and chemotaxis, suggesting that CD301b⁺ DCs require additional signals to respond to allergen. In support of their hypothesis that TRPV1⁺ neurons were involved the authors demonstrated that sensory neurons and CD301b⁺ DCs lie in close proximity within murine skin.

Kaede mice, expressing a photoconvertible green to red fluorescent protein,

were utilized to confirm that papain immunization induced migration of CD301b⁺ DCs from the photoconverted skin to the dLN, which could be blocked with co-injection of QX314. Blocking of TRPV1⁺ neurons did not affect dermal CD103⁺ DC (which include cDC1 and Th17-skewing cDC2s) migration in response to OVA plus LPS, suggesting specificity to allergen-induced CD301b⁺ DC migration. The requirement for TRPV1⁺ sensory neurons in CD301b⁺ DC migration was further confirmed using kaede x *Trpv1*^{DTR} mice. These data suggest the capacity of sensory neurons to sense allergen and thus direct CD301b⁺ DC migration to the dLN.

SP and CGRP were potential candidates by which TRPV1⁺ neurons induce migration of CD301b⁺ DCs since they are released from activated TRPV1⁺ neurons in response to allergen versus bacterial or fungal exposure, respectively (Kashem et al., 2015; Serhan et al., 2019). Papain immunization induced SP, but inhibited CGRP release from skin explants, an effect that was abrogated by QX314 co-injection. SP was also released by DRG cultures following papain stimulation and also by house dust mite (HDM) and *Alternaria* – alternative immunogens with protease activity. Injection of OVA and SP, but not OVA and CGRP, in the photoconverted skin of kaede mice induced the migration of kaede^{red} CD301b⁺ DCs to the dLN, with no effect on CD103⁺ dermal DCs. Further experiments indicated that mast cells were not required for SP release or DC migration following papain immunization. A role for mast cells cannot however be excluded, as SP is reported to stimulate their degranulation resulting in skin inflammation (Serhan et al., 2019).



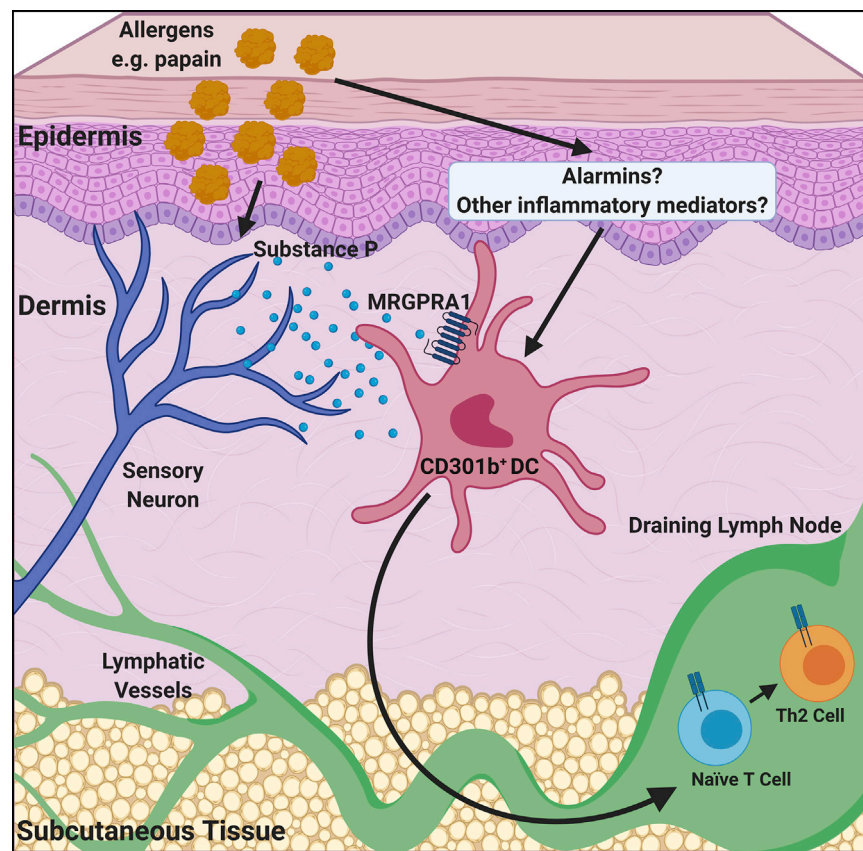


Figure 1. Allergen-Induced Substance P Release by TRPV1⁺ Sensory Neurons Triggers Th2 Skewing

A number of dendritic cell subsets reside within the skin, sensing their local environment and responding to insults such as pathogens, allergens and cellular damage. CD301b⁺ DCs initiate type 2 immunity in response to allergen, helminth and some fungal antigens. CD301b⁺ DCs reside close to TRPV1⁺ sensory neurons. Here, allergens such as the cysteine protease papain are proposed to directly activate these sensory neurons, to release substance P (SP). [Perner et al. \(2020\)](#) show that CD301b⁺ DCs require the expression of the SP receptor, MRGPRA1, which triggers their migration to draining lymph nodes, whereupon they drive Th2 cytokine responses. Since in a previous study [Sokol et al. \(2018\)](#) report that CD301b⁺ DCs require CCR7 and CCR8 expression to migrate and enter the dLN, SP is proposed to provide an additional trigger for this process. Since SP injection alone is insufficient to support Th2 responses, this model does not exclude a role for alarmins and other local endogenous signals ([Hammad et al., 2009](#)). Figure created with [BioRender.com](#).

These data suggested that SP released from sensory neurons is required for allergen-induced CD301b⁺ DC migration. CD301b⁺ DCs, but not CD103⁺ DCs, were shown to express the gene for the SP receptor *Mrgpra1*, but not other SP receptors. Experiments using chemical antagonists to block the various SP receptors suggested that SP acts through MRGPRA1 on CD301b⁺ DCs to induce migration, independently of CCR7 upregulation. This was further investigated using mixed bone marrow (BM) chimeras from CD301b^{WT} (*Mrgpra1* sufficient CD301b⁺ DCs) or CD301b^{*Mrgpra1*−/−} (*Mrgpra1* deficient CD301b⁺ DCs) mice. CD301b^{*Mrgpra1*−/−} chimeras lost papain-

induced CD301b⁺ DC migration. Following papain immunization, WT DCs displayed an advantage in migration to the dLN, thereby indicating that MRGPRA1 is required for allergen-induced migration of CD301b⁺ DCs. Ablation of *Mrgpra1* expression in CD301b⁺ DCs would provide further confirmation of this model.

Allergen or SP alone do not increase CCR7 expression by CD301b⁺ DCs ([Perner et al., 2020](#); [Sokol et al., 2018](#)). Thus, how CD301b⁺ DCs are signaled to migrate to the dLN upon allergen stimulation remained unclear. SP or a MRGPRA1-agonist also induced chemokinesis (in the absence of a chemokine gradient) and chemotaxis (toward a

CCL21 gradient) in bone marrow-derived DC (BMDC) cultures. This effect was lost in *Mrgpra1*^{−/−} BMDC cultures, indicating that SP signaling through MRGPRA1 is required for DC migration *in vitro*. The authors speculate that the SP signal, acting through MRGPRA1, induces CD301b⁺ DCs to release contacts with the dermis. The study does not directly address the importance of the proteolytic activity of papain, however, could this contribute to CD301b⁺ DCs releasing contacts with the dermis? The authors suggest that CD301b⁺ DC then use their baseline expression of CCR7 to migrate toward the CCL21-rich dLN. The CCR8-CCL8 axis may also act in synergy with CCL21 to promote CD301b⁺ DC migration ([Sokol et al., 2018](#)). Future studies are needed to unravel this further.

If CD301b⁺ DCs are depleted, or their migration to the dLN is blocked, the Th2 cell response to papain is lost ([Kumamoto et al., 2013](#); [Sokol et al., 2018](#)). Here the authors showed that blocking or depletion of TRPV1⁺ neurons also resulted in decreased Th2 cell differentiation in response to papain. Although SP controlled migration, SP injection alone was unable to induce a Th2 cell response. These data suggest a crucial role for TRPV1⁺ neurons in initiating the migration of CD301b⁺ DCs to dLNs where the DC then promote Th2 cell responses, but additional signal(s), such as alarmins, are likely to be required ([Figure 1](#)). These data build on earlier scientific publications and raise further interesting questions.

The data imply a 2-signal model for cutaneous sensitization to allergen; activation of sensory neurons and their subsequent release of neuropeptides (signal-1), which then signal Th2-skewing DC migration to the dLN (signal-2). A distinct 2-signal model exists for aeroallergen sensitization in mice. Activation of airway barrier epithelium by HDM and subsequent alarmin release signals mucosal DC and lymphocytes to promote allergic airway disease ([Hammad et al., 2009](#)). The present study does not directly address, or preclude, similar mechanisms in the skin, where structural and innate cells e.g., keratinocytes may be relevant. Whether the current model of dermal sensitization involving TRPV1⁺ sensory neurons promotes allergic sensitization via the airways remains to be investigated, although TRP-signaling is implicated in cough,

bronchospasm and airway inflammation in response to airway irritants (e.g., diesel exhaust, capsaicin) and allergens (Belvisi and Birrell, 2017).

Neuropeptide-signaling in the skin is not unique for type-2 priming since TRPV1⁺ neuron-derived CGRP primes CD301b⁺ DC to induce IL-17 anti-fungal responses (Kashem et al., 2015; Cohen et al., 2019). The authors discuss the therapeutic potential of exploiting neuropeptide specificity for induction of Type-1, Type-2 or Type-17 immune responses to prevent allergy. Additional work is required to elucidate the way in which TRPV1⁺ neurons are able to differentiate between stimuli type, release unique neuropeptide patterns and as such, initiate diverse immune responses. It is unclear whether ultimately such immune deviation would be beneficial or might conceivably be detrimental, requiring further dissection in murine and human models.

Proteolytically active allergens have been previously proposed to promote allergic responses. Their enzymatic activity can damage airway tight junctions to facilitate allergen entry, exposing underlying DC to allergen. Barrier epithelial cells, DC, mast cells and other cells express protease activated receptors which enhance

cellular activation (Reed and Kita, 2004). The present study adds a new dimension to the action of protease allergens.

In summary, Perner et al. (2020) provide evidence that CD301b⁺ dermal DCs are indirectly activated in response to protease allergens such as papain. The study reports that TRPV1⁺ sensory neurons act as a primary sensor of papain, releasing SP that signals CD301b⁺ DC migration to the dLN to induce type 2 immune responses.

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Memory Lapses—Winning the Slow Race

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Pre-existing humoral immunity can impact immune responses to heterologous infections. In this issue of *Immunity*, Wong et al. reveal that memory B cells, while failing to re-enter the germinal center, create an affinity-restricted, diversified B cell repertoire for rapid plasma blast responses. Their findings have important implications to vaccine design.

Immunological memory is currently on the minds of many, as we are watching the unfolding of the COVID-19 pandemic. Natural and vaccine-induced pre-existing immunity to viral infections is provided by long-lived antibody-producing plasma cells (LLPCs) and quiescent, antigen-

reactive memory B cell (MBC) pools. Yet, the presence of pathogen-reactive antibodies and B cell memory may not always be a good thing. In fact, pre-existing immunity to previously circulating strains of influenza virus has been labeled “original antigenic sin” (Fazekas de St. Groth and

Webster, 1966), as it has been associated with reduced antibody responses to novel epitopes on mutated influenza viruses. In areas endemic for Dengue virus, a flavivirus of which multiple serotypes circulate, the presence of cross-reactive, non-neutralizing antibodies has been linked

