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FcεR1 expressing nociceptors trigger allergic airway inflammation

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Abstract

Background.—Lung nociceptor neurons amplify immune cell activity and mucus metaplasia in response to an inhaled allergen challenge in sensitized mice.

Objective.—We now sought to identify the cellular mechanisms by which these sensory neurons are activated upon allergen exposure.

Methods.—We used calcium microscopy and electrophysiological recording to assess whether vagal neurons directly respond to the model allergen ovalbumin (OVA). Next, we generated the

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first nociceptor specific FcεR1γ knockdown (TRPV1^{Cre}::FcεR1γ$^{fl/fl}$) mice to assess whether this targeted invalidation would impact the severity of allergic inflammation in response to allergen challenges.

Results.—Lung-innervating jugular nodose complex ganglion (JNC) neurons express the high-affinity IgE receptor FcεR1 and the levels of this receptor increase in OVA-sensitized mice. FcεR1γ-expressing vagal nociceptor neurons respond directly to OVA complexed with IgE, with depolarization, action potential firing, calcium influx, and neuropeptide release. Activation of vagal neurons by IgE/allergen immune complexes, through the release of substance P (SP) from their peripheral terminals, directly amplifies T_H2 cell influx and polarization in the airways. Allergic airway inflammation is decreased in TRPV1^{cre}::FcεR1γ$^{fl/fl}$ mice or in bone marrow-transplanted FcsR1α$^{-/-}$ mice. Finally, increased *in vivo* circulating levels of IgE following allergen sensitization enhances the responsiveness of FcεR1 to immune complexes in both mouse JNC neurons and human iPSC-derived nociceptors.

Conclusions.—Allergen-sensitization triggers a feedforward inflammatory loop between IgE-producing plasma cells, FcεR1 expressing vagal sensory neurons, and T_H2 cells, which helps both initiate and amplify allergic airway inflammation. These data highlight a novel target for reducing allergy; FcεR1γ expressed by nociceptors.

Keywords

Neuro-immunity; nociceptor neurons; allergy; asthma; T_H2; FcεR1; TRPV1; allergen detection; vagal sensing; Substance P

Introduction.

Trigeminal and lumbar nociceptor somatosensory neurons express antibody-sensing Fc receptors (FcR) [1] for both immunoglobulin type G (IgG), FcγR; and immunoglobulin type E (IgE), FcεR [2, 3]. This phenomenon may explain why sensory neurons in a sensitized animal fire action potentials when exposed to an allergen [4,5]. Upon binding to the antigen for which they were raised, antibodies form immune complexes that activate FcR expressed by nociceptors. Such stimuli generate calcium flux, action potential firing, and neuropeptide release, triggering pain or itching [6,7,8]. However, whether vagal sensory neurons innervating visceral organs also sense immune complexes, and if they do, what the consequences are, is unknown. We set out to examine this question.

Nociceptor neurons respond to immune cell cues, such as cytokines, and drive immune responses [9–12,13]. For example, in the ovalbumin (OVA) and house dust mite (HDM) mouse models of allergic airway inflammation (AAI), sensory neurons innervating the lung drive $CD4^+$ T and ILC2 cell activation through a VIP-VPAC2 neuropeptide axis [14–16]. In turn, these immune cells release the cytokines IL-5 and IL-13, which activate lung sensory neurons as part of a feed-forward pro-inflammatory loop that amplifies the adaptive immune response to allergen exposure [14] and also drives mucus metaplasia [17]. However, the specific mechanisms by which an antigen initiates type 2 airway inflammation, and at what point nociceptors are engaged, remains to be determined. To address this issue, we have now used genetic approaches and allergic airway inflammation models to examine i) whether and how

jugular/nodose complex ganglion (JNC) nociceptor neurons sense allergens, ii) if they do, what the consequences on immune cells may be, and iii) whether allergen sensitization sets off an interaction between adaptive immune responses and nociceptor activity that triggers allergic airway inflammation.

Methods.

Detailed information on materials and methods are available in the supplementary Methods in this article's Online Repository (available at www.jacionline.org). In brief, all procedures were approved by the Institutional Animal Care and Use Committees of Boston Children's Hospital. Allergic airway inflammation was studied in an ovalbumin (OVA) based model [18]. On days 0 and 7, mice were sensitized by a 200 μl i.p. injections of a solution containing 1 mg/ml ovalbumin (Sigma, #A5503–25G) and 5 mg/ml aluminum hydroxide (Sigma, #239186–500G). On days 14–17 (10:00 am), mice were exposed to 6% OVA aerosol for 25 min or intranasal OVA instillation (50 μg /50 μl). Mice were sacrificed on days 0, 3, 6, 9, 13, 14, 15, 18, 20, 21 or 26; JNC neurons were harvested, cultured, and analyzed using calcium microscopy, electrophysiology [14], and single-cell qPCR [19]. Culture media were analyzed using ELISA [14]. BALF was harvested; cells isolated, counted, and immunophenotyped using FACS [14]. Alternatively, QX-314 (100 μM) and capsaicin (1 μM) were nebulized on day 14 and typically sacrificed on day 15. Another type 2 inflammation model was induced via house dust mites (HDM). Lightly anesthetized (isoflurane) mice were sensitized (day 1–5) and challenged (day 8–10) with house dust mites (CiteQ, #15J01; 20 μg/50μl, intranasal), sacrificed on day 11, BALF was harvested; cells were isolated, counted, and immunophenotyped by flow cytometry.

Results.

Vagal nociceptors initiate airway inflammation.

Type 2 allergic inflammation was induced in mice by an initial sensitization to ovalbumin (OVA) with aluminum hydroxide as an adjuvant (i.p. days 0 and 7), followed two weeks later by inhaled OVA challenges [14]. A single inhaled OVA exposure on day 14 after sensitization results in increased $CD45^+$ cells in bronchoalveolar lavage fluid (BALF) when tested 24 hours later (Figure 1A). To establish whether nociceptor activation at the time of the allergen exposure contributes to the inflammatory response, we tested if capsaicin (1 μM; intranasal (i.n.)), an exogenous TRPV1 ligand that activates lung afferents nociceptors, would modify the extent of the immune infiltrate. Capsaicin, given 3 hours before the initial allergen challenge, doubled the OVA-mediated influx of $CD45^+$ cell count in the BALF (Figure 1A).

Next, to specifically explore if nociceptors have a role in initiating the inflammation resulting from allergen exposure in sensitized mice, we used a strategy to selectively silence lung nociceptors before the allergen exposure. This approach uses non-selective large pore ion channels (TRPA1 and TRPV1) as cell-specific drug-entry ports to deliver a charged, membrane-impermeable form of lidocaine (QX-314) into pulmonary sensory fibers, to block sodium currents and thereby, action potentials [14]. A 3h pre-treatment with QX-314, in the presence of capsaicin as a TRPV1 channel opener, reduced the OVA-mediated

airway inflammatory response to the subsequent allergen inhalation (Figure 1A). QX-314 pre-treatment without capsaicin, which does not silence nociceptors, did not affect BALF $CD45^+$ cell numbers (Figure 1A).

To test for nociceptor activation by an allergen-challenge, we probed p-p38 MAPK activation levels as an activity marker in JNC neurons [20–24]. An acute inhaled OVA challenge in sensitized mice produced a time-dependent increase in phosphorylated p38 (p-p38) positive JNC neurons one-hour post-OVA challenges and continuing for 24hrs (Figure 1B–D). To determine whether this effect occurred in the airway-innervating neurons, $TRPV1^{Cre}$ mice were injected intranasally with AAV9-FLEX-ChR2-Tdtomato (10^9 titers, 50μL) virus two weeks prior to allergen sensitization. Using single-cell qPCR, we found that *p38* and *Tac1* transcript expression increased in airway-innervating neurons (Td-$tomato^+$) obtained from OVA-challenged mice (1h; Figure 1E). The finding that silencing sensory neurons before the first allergen exposure reduced the inflammatory response, while activating the nociceptors had the opposite effect, raises the possibility that vagal nociceptors might be directly engaged by the allergen challenge and that such activation may contribute to immune cell recruitment/activation.

Vagal nociceptors express FcεR1

In-silico analysis of seven previously published expression profiling datasets [25] of $TRPV1^+$ neurons shows that, in addition to sensory neuron markers (TRPV1, TRPA1) and nociceptor neuropeptides (SP, VIP, NMU, CGRP), these afferents express the immunoglobulin receptors FcεR1, FcεR2, FcγR1, FcγR2, and FcγR3. In the case of FcεR1, we found higher relative expression levels than for the pattern recognition receptor Fpr1, or the P2Y purinoceptor 1 (P2YR1; Figure 2A); all of which were found to be functional on these neurons [11, 26]. Next, *ex vivo* JNC neurons from naïve and allergen-sensitized animals ($Tac1^{cre}$::$GCaMP6^{fl/wt}$ reporter) were co-cultured (1:1 mix). In this context, we found that 87% of all FcεR1γ transcript expressing neurons originated from allergen sensitized mice ($GCaMP6^+$, supplementary Figure 1A). We then measured the level of FcεR1 expression on vagal sensory neurons using fluorescent *in-situ* hybridization (Figure 2B–D), immunofluorescence (Figure 2E, supplementary Figure 1B–H), and qPCR performed on FACS-purified $TRPV1^+$ JNC neurons ($TRPV1^{cre}$::td-$tomato^{fl/wt}$ mouse, supplementary Figure 1I). As in the co-culture setting (supplementary Figure 1A), we found that FcεR1α/γ transcript and protein levels were expressed in naïve mouse JNC neurons, but that this level is further increased in neurons from allergen-sensitized mice (Figure 2B–E; supplementary Figure 1B–I).

FcεR1-expressing vagal nociceptors respond to immune complexes.

To assess if vagal sensory neurons directly respond to IgE-OVA complexes, we exposed primary JNC neuron cultures to a range of concentrations of IgE, ovalbumin and ovalbumin-IgE complex (IC) and tested calcium flux as a proxy of neuronal activation. ~12–15% of isolated neurons from a sensitized mouse responded to the allergen-antibody complex (>10% increase in Fura-2AM fluorescence), with a significantly lower response in non-sensitized JNC neurons (Figure 3A). Increased immune complex sensitivity of JNC neurons from isolated allergen-sensitized mice was confirmed in $Na_V1.8^{Cre}$::$GCaMP6^{fl/wt}$

and Fura-2AM-loaded neurons (supplementary Figure 2A). Immune complex-responsive neurons were mostly small-diameter AITC- and capsaicin-sensitive neurons (TRPA1 and TRPV1 responsive respectively; Figure 3B–D; supplementary Figure 2B). The identity of the responsive neurons as nociceptors was confirmed using cultured JNC neurons from $TRPV1^{Cre}$::$DTA^{fl/wt}$ or $Na_V1.8^{Cre}$::$DTA^{fl/wt}$ nociceptor depleted mice, where the response to IgE-OVA was absent in the latter and massively reduced in the former (Figure 3D). The IgE-OVA induced calcium flux was dose-dependent and higher than that produced by exposure to IgE or OVA alone (~1 – 4%; Figure 3E). Immune complex-stimulated allergen-sensitized JNC neurons release substance P (SP) and vasoactive intestinal peptides (VIP) but not calcitonin gene-related peptide (CGRP; Figure 3F). IgE or OVA exposure alone had no impact on SP secretion from allergen-sensitized mouse neurons (supplementary figure 2C).

Next, naïve and allergen-sensitized mice isolated neurons were co-cultured (1:1 mix) to directly compare their responsiveness to immune complex exposure in the same experimental conditions. In this setting, we found ~3 fold more IgE-OVA responsive neurons and higher amplitude of response in allergen-sensitized neurons from a reporter mouse ($TRPV1^{Cre}$::$Td\text{-}tomato^{fl/wt}$) than in non-sensitized (no reporter) naïve neurons (supplementary Figure 2D–E; supplementary movie 1 showing decay in unbound cytoplasmic Ca^{2+} in response to IC).

IgE controls FcεR1 expression.

Given the increased immune complex-mediated calcium flux in JNC neurons from sensitized mice, we set out to test what allergen-sensitization component might be responsible for this effect. To do this, we used unbiased single-cell qPCR to probe changes in transcript expression in $TRPV1^+$ JNC neurons (capsaicin-responsive) at various times during the asthma protocol. We found elevated FcεR1γ transcript expression as early as three days post-sensitization, as well as elevated IL-5R and TSLPR levels peaking on day 26 (Figure 3G). Given that in addition to immunologically “arming” mast cells to undergo antigen-mediated activation, IgE stabilizes and enhances FcεR1 expression on immune cells [27–29]; we posited that circulating IgE might be responsible for the neuronal expression of FcεR1. We tested whether immunoglobulins, interleukins, the adjuvant, the model allergen or a combination of these, induced the expression of functional FcεR1 in primary cultured JNC neurons. While KCl responses were similar across the various treatment groups, we found an increased response to the immune complex when JNC neurons were pre-exposed to IgE, IgE+OVA+AlOH, or BALF extracted from an asthmatic mouse (Figure 3H). As expected, allergen-sensitization prior to harvesting the neurons had a similar effect (Figure 3H). Interestingly, human iPSC-derived nociceptor neurons (iNoc) that were pre-exposed to IgE for 24h, also showed a calcium flux (11 %) in response to the immune complex, an effect that was absent in non-IgE exposed iNoc neurons (supplementary Figure 2F–G). These findings reveal the context-dependent expression of FcεRI on nociceptor neurons, which is modulated by IgE upon allergen-sensitization, highlighting a linkage between plasma B cells and vagal nociceptors.

Immune complex modulation of neuron excitability.

To probe whether the immune complex modulates nociceptor neurons' excitability, we first identified those allergen-sensitized cultured JNC neurons that respond to the immune complex with a calcium flux (supplementary Figure 3A). We then tested their electrophysiological response to the complex by whole-cell patch recording. While having similar baseline resting membrane potentials (~−60mV), the responder group became depolarized when re-exposed to the allergen-antibody complex (8/8) (Figure 4A), an effect virtually absent in the non-responder group (1/24) (Figure 4B). Furthermore, the depolarization was sufficient to evoke action potential firing in some allergen-antibody sensitive neurons (3/8; Figure 4C), an effect never observed when the cells were treated with OVA or IgE alone (Figure 4D).

To test whether allergen sensing by vagal nociceptors increases lung allergic responses, we first used whole nerve electrophysiological recording of vagal neurons to check whether immune complexes were sensed *in vivo.* Similar to the findings in an *in vitro* setting, allergen sensitized wildtype mice showed vagal compound action potential responses to the immune complex when injected intraperitoneally. Importantly, a similar effect was also found in allergen-sensitized mast cell-depleted mice ($Kit^{W\text{-}sh/W\text{-}sh}$, [30]), supporting a mast cell-independent effect. The immune complex had no effect in naïve wildtype mice or allergen-sensitized FcsR1$\gamma^{-/-}$ mice (Figure 4E; supplementary Figure 3B–E).

FcεR1-TRPC3 axis controls IC-induced calcium flux.

To specifically explore whether the activation of vagal nociceptors by the IgE-OVA complex occurred though FcεR1, we identified small diameter (≤25um) immune complex-responsive and non-responsive neurons in culture from sensitized and challenged mice (supplementary Figure 3A) and then profiled them using single-cell qPCR. The responsive neurons showed higher FcεR1 transcript expression than those in non-responsive neurons, even though both expressed similar transcript levels of TRPV1 and TRPA1 (Figure 4F). Next, we used FcεR1 a null mice to confirm the role of FcεR1 in sensing the immune complex. We then found that while cultured JNC neurons from FcεR1α$^{-/-}$ mice respond normally to KCl or capsaicin, they were virtually insensitive to the immune complex (supplementary Figure 4A–D). Similarly, two different FcεR1α blocking antibodies abolished the neuronal response to the immune complex, but not to KCl or capsaicin (MAR1 clone, supplementary Figure 4E; CRA1 clone, supplementary Figure 4F).

Immune complex-induced calcium flux was absent when allergen-sensitized neurons were cultured in the absence of extracellular calcium (supplementary Figure 4G–H) and when treated with the non-selective TRP channel blocker ruthenium red (supplementary Figure 4I) or with a TRPC3 antagonist (supplementary Figure 4J–K). Similar to the IgG-antigen complex activation of lumbar DRG neurons [31] and in accordance with their co-expression in a subset of peptidergic neurons (supplementary Figure 5) [32–34], our data indicate that the allergen-IgE complex produces a calcium influx through an FcεR1-TRPC3 axis.

Neuron-sensing allergen helps prime T_H2 cell activity,

Although detection of allergen immune complexes by vagal neurons constitute a novel feature of danger detection and may elicit sensations and reflexes, we tested whether such early sensing also impacts local immune responses. To do this, we focused on ILC2 and T_H2 cells, which are early drivers of allergic airway inflammation that are modulated by sensory neurons [14, 35, 36]. T_H2 (Figure 5A; supplementary Figure 6A, B) or BALF CD4 T (Figure 5B) cells harvested from OVA-challenged mice showed increased *Tacrl* transcript expression compared to those from naïve mice. Therefore, these cells are poised to respond to nociceptor cues.

Splenocyte-harvested naïve CD4+ T cells were polarized into T_H2 cells. In the presence of peptidase inhibitor, the cells were then either co-cultured with JNC neurons from allergen-sensitized mice JNC neurons, stimulated with conditioned media harvested from IC-stimulated allergen-sensitized JNC neurons (1:2 dilution) or exposed to substance P (1μM) and T_H2 cell activation, then profiled by flow cytometry. In comparison to IgE-OVA stimulated T_H2 cells, T_H2 cells co-cultured with IgE-OVA-stimulated neurons, T_H2 cells exposed to IgE-OVA- induced neuron conditioned media or T_H2 cells exposed to substance P shows increased levels of IL-5^+ (Figure 5C), IL-13^+ (Figure 5D), and IL-5^+IL-13^+ (Figure 5E). Also, substance P or JNC neuron co-culture increased T_H2 expression of IL-4^+IL-13^+ and IL-4^+IL-5^+ (supplementary Figure 6C, D; supplementary Figure 7). These treatments had no impact on the proportion of GATA3^+ and IL-4^+ in $CD4^+$ T cells (supplementary Figure 6E, F; supplementary Figure 7). T_H2 cells polarization was also not impacted when co-cultured with naive JNC neurons (supplementary Figure 8). While ILC2 also express various neuropeptide receptors (supplementary Figure 9A), IL-7 stimulated lung ILC2 cells were insensitive both to IgE-OVA triggered neuron conditioned media or substance P (supplementary Figure 9B–D).

Allergen sensing by vagal nociceptors amplifies allergic lung inflammation.

To assess the impact of vagal neuronal sensing of allergens, we transplanted C57BL6 bone marrow into whole-body irradiated C57BL6 (BL6→BL6) and FcεR1a$^{-/-}$ (BL6→FcsR1a$^{-/-}$) mice. We found that spleen CD45+ immunocytes had a similar expression of FcεR1α (supplementary Figure 10A) in allergen-sensitized BL6→FcεR1α$^{-/-}$ chimeric mice, and the JNC neurons were insensitive to immune complex (supplementary Figure 10B). We also found that one day after an acute OVA-challenge, allergen-sensitized BL6→FcεR1α$^{-/-}$ chimeric mice had reduced numbers of BALF-infiltrating $CD45^+$, total $CD4^+$, and $CD4^+$IL-5^+ cells, in comparison to control chimeric mice (Figure 6A). These findings support a key upstream role for FcεR1$^+$ non-hematopoietic cells (e.g., nociceptors) in inducing allergic airway inflammation.

To directly pinpoint a role for Fcε1γ$^+$ nociceptors from radio-resistant hematopoietic cells, we used cre/lox technology to generate nociceptor specific Fcε1γ knockdown (TRPV1^{Cre}::FcεR1γ$^{fl/fl}$) mice. The TRPV1^{Cre}::FcεR1γ $^{fl/fl}$ mice showed a 3.2-fold decrease in HDM-induced AAI (BALF numbers of $CD45^+$, eosinophils, total and IL-5^+ $CD4^+$ T cells; Figure 6B; supplementary figure 12, supplementary figure 13), and BALF cytokines levels (IL-13; supplementary Figure 11A) when compared to littermate control

mice (TRPV1^{wt}::FcεR1γ$^{fl/fl}$). Heterozygote knockout mice (TRPV1^{Cre}::FcεR1γ$^{fl/wt}$) also showed a significant, albeit lower, reduction in HDM-induced AAI (~1.58-fold) and OVA-induced AAI (~1.65-fold; supplementary Figure 11B, C). We then tested whether the TRPV1^{cre} mice nocifensive behavior remained intact. We confirmed that cre recombinase expression in TRPV1^{+} neurons had no impact on the mice's thermal nociceptive threshold nor on neurons' sensitivity to noxious stimuli (supplementary figure 14).

An *in-silico* analysis of five mouse expression profiling datasets (single-cell RNA-sequencing, bulk RNA sequencing, microarray; supplementary Figure 15–18) revealed that TRPV1 is not expressed by airway stromal or immune cells (including T_H2 and mast cells). In addition, TRPV1^{cre}FCεR1γ$^{fl/fl}$ peritoneal mast cells degranulate normally in response to IgE-OVA (supplementary Figure 18C). TRPV1^{cre}DTA$^{fl/wt}$ mice have the same proportion of peritoneal mast cells as controls (supplementary Figure 18D; supplementary figure 19), whereas flow cytometry analysis of peritoneal mast cells from TRPV1^{cre}Td-tomato$^{/wt}$ mice showed no cre expression (supplementary Figure 18E; supplementary figure 20). While TRPV1^{cre}FCεR1γ$^{fl/fl}$ mouse T_H2 and mast cells are intact, their JNC neurons do not express FCεR1γ following *in vivo* allergen-sensitization (supplementary Figure 21A–D), nor do they respond to IgE-OVA (supplementary Figure 21E). However, capsaicin-induced calcium flux responses are still intact (supplementary Figure 21F).

The reduction in the influx of lung immunocytes in TRPV1^{Cre}::FCεR1γ$^{fl/fl}$ mice was accompanied by similar levels of IgE (not shown), suggesting that while the capacity to mount an allergic antibody response is present in these mice, allergic airway inflammation is reduced. The diminished inflammatory phenotype in the TRPV1^{Cre}::FcεR1γ$^{fl/fl}$ mice was phenocopied by mice whose TRPV1^{+} sensory neurons were genetically ablated (TRPV1^{Cre}::DTA$^{fl/wt}$; supplementary Figure 22A–C). While the specific knockdown of FcεR1γ on TRPV1^{+} sensory neurons did not prevent OVA- and HDM-influx and polarization of ILC2 cells (not shown), the genetic ablation of TRPV1^{+} neurons did (supplementary Figure 22D–F). Finally, co-exposing HDM-challenged mice on days 8 and 9 to neuron-conditioned media (supernatant from immune complex-stimulated allergen-sensitized JNC neurons), further augmented the HDM evoked increases in BALF CD45^{+}, CD4^{+}, eosinophil, and neutrophil cell numbers (Figure 6C).

Discussion.

Sensory neurons' involvement in allergic airway inflammation was first suggested by the finding that TRPA1 genetic ablation reversed OVA-induced AAI [37]. How are these neurons activated? IL-5, a type II effector cytokine produced by multiple immune cells during allergy, directly activates airway sensory neurons, leading to the secretion of the neuropeptide VIP. VIP, in turn, acts on ILC2 and CD4+ cells to induce cytokine production, including IL-5 and IL-13, to initiate a positive feedforward pro-inflammatory cycle[14, 15, 38] In parallel, neuromedin U (NMU), the ligand of NMUR1, activates ILC2s. Acting with IL-25, it also amplifies allergic inflammation [35, 36, 39]. In the airway, NMU is found exclusively in lung afferent DRG neurons, and a loss of NMU-NMUR1 signaling reduces ILC2 function by altering transcriptional programs following an allergen-challenge *in vivo,* and this signaling loss prevents the development of allergic airway inflammation [35].

What sets off the neuro-immune cycle in the setting of allergic airway inflammation? Does it begin with the activation of immune cells or nociceptors by allergen exposure in sensitized animals? The data presented here point to an upstream detection role of allergen-IgE complexes by nociceptors in driving T_H2 cell activation and downstream allergic inflammation. Following allergen sensitization, immune-complex-sensing by FcεR1γ$^+$ nociceptors sets off the airway neuro-immune interactions that trigger and maintain allergic airway inflammation.

JNC neurons express FcεR1.

We identified a subset of mouse JNC neurons that express functional FcεR1. Studies have reported neuronal-Ig interactions in Alzheimer's disease, chronic pain, arthritis-induced hypersensitivity, allergy, and antigen-specific autoimmune diseases [2, 3, 6, 7, 40–46]. IgG and IgE activate motor neuron release of neurotransmitters and help control antigen trafficking in lymph nodes [45, 47–50]. These findings are in line with the expression by neurons of receptors typically expressed by immune cells, such as MHCI on CNS neurons, as well as nociceptor expression of the immune checkpoint receptor PD1 [51, 52].

IgE drives neuronal expression of FcεR1.

Sensory neuron responsiveness is highly tunable, changing in response to diverse insults [15, 53]. Similar to FcεR1 expression on hematopoietic cells [54, 27–29], we now reveal that IgE drives FcεR1 overexpression in cultured murine JNC nociceptors and in human iPSC-derived nociceptors. FcεR1 is also upregulated in the abdominal vagus in food-allergic mice [49]; in trigeminal ganglion neurons of allergen-sensitized mice [8], and in lumbar DRG neurons following exposure to ragweed pollens or during cutaneous anaphylaxis [43]. Overall, these data support a mechanistically conserved mechanism for the neuronal sensing of immune complexes during allergy.

TRPC3 mediates IC-FcεR1-induced calcium influx.

Immune complex sensing leads to TRPC3-dependant calcium influx and the subsequent release of neuropeptides. TRPV1, TRPC3, and FcεR1 are co-expressed in nociceptors, as revealed by the single-cell RNA-sequencing of sciatic nerve crushed mouse peptidergic neurons (cluster 2) [55]. The FcγR-syk-TRPC3 axis is also responsible for IgG-IC induced hyperalgesia [31].

Nociceptor-ILC2 crosstalk.

ST2$^+$ ILC2 cells express transcripts for *Rampl, Nmurl,* and *Tacrl.* Previous reports have shown that vagal neurons produce VIP [14, 56] and that lumbar neurons release NMU, both of which control ILC2 activation [35, 36, 39]. In addition, while CGRP supports ILC2 production of IL-5 [57], it also inhibits alarmin-driven type 2 cytokine production, constrains IL-13 expression, and blocks ILC2 proliferation [57–59]. We found here that the complete ablation of peptidergic neurons (in TRPV1^{Cre}::DTA$^{fl/wt}$ mice) prevents type 2 cytokine production and infiltration of ILC2. Such findings phenocopy our earlier data obtained with ablation of all nociceptors in $Na_V1.8^{Cre}$::DTA$^{fl/wt}$ mice or nociceptor silencing pharmacologically with QX-314 [14]. While immune-complex-sensing promotes SP and VIP (but not CGRP)

production, IgE-allergen complex-stimulated JNC neurons fail to modulate ILC2 cell function. Immune complex-FcεR1-induced VIP release (0.1 ng/ml) is, however, lower than that produced by IL-5 (0.8 ng/ml) or capsaicin (40 ng/ml). In addition, the ILC2 cells used here were co-stimulated with IL-7, paralleling some studies that found a broader co-stimulation, including IL-25 and IL-33, is necessary for these cells to respond to CGRP and NMU. This result suggests that neuron-ILC2 crosstalk requires the maximal engagement of multiple peptidergic neurons to trigger a broad neuropeptide release, including NMU and CGRP, for their activation, and such a pattern of neuropeptide release may not occur upon allergen sensing.

Neuropeptides drive T_H2 polarization.

Conditioned media harvested from IgE-OVA-stimulated JNC neurons obtained from allergen-sensitized mice drives T_H2 cell polarization *in vitro* and enhances HDM-mediated airway inflammation with increased BALF influx of $CD45^+$, $CD4^+$, eosinophils *in vivo.* The neuronal detection of immune complexes leads to the release of SP and VIP in the mucosa [60, 61], which can facilitate the local influx and the polarization of these immune cells. Indeed, T_H2 cells and OVA-exposed BALF $CD4^+$ T cells express the receptors for these peptides, *Vipr2* and *Tacrl*. While we previously found that recombinant VIP increased T_H2 cell activation and polarization, we now show that the SP-Tacr1 axis increases the production of $IL\text{-}5^+$, $IL\text{-}13^+$, and $IL\text{-}5^+IL\text{-}13^+$ T_H2 cells *in vitro.* In addition, we also have found that SP contributes to mucus hyperplasia, mucin imbalance (Muc5AC/Muc5B), and metaplasia [17]. Overall, all our current and past data indicate that antigen detection by lung nociceptors sets off systemic defensive reflex responses like cough or mucus secretion[17] and, by mobilizing T_H2 cells, prompts the engagement of the adaptive immune system.

Neurons control allergic airway inflammation.

Fcerlγ acts as an ITAM adapter protein transducing signals of immunoreceptors like FcRs and IL-3R [62, 63]. Consistent with the immune complex-sensing by FcεR1-nociceptors as a major upstream driver of T_H2-mediated allergic airway inflammation, we found that nociceptors lacking FcεR1γ ($TRPV1^{cre}$::$FcεR1γ^{fl/fl}$) are protected from IgE-OVA-induced calcium flux. In addition, mice whose nociceptors lack FcεR1γ are protected from airway inflammation induced by ovalbumin, a classic mouse model of allergic asthma, and house dust mites, a more clinically relevant model of patient allergy. These effects phenocopy the findings we observed in irradiated bone- marrow transplanted FcsR1a knockout mice, a model resistant to IgE-induced systemic anaphylaxis. In addition, the detection of allergens by nociceptors preceded an immune response because silencing nociceptors prior to the allergen challenge reduced the inflammation. *In vivo* immune-complex challenges in sensitized mice, triggered action potentials in vagal neurons within 5 minutes of exposure, an effect independent of mast cells. These findings are similar to the role of IL-4R+ skin nociceptors in atopic dermatitis-induced skin inflammation via infiltration of T_H2 cells and basophils [9].

TRPV1 does not impact mast cell function.

Along with the irradiated bone-marrow transplanted mice, we generated nociceptors lacking FcεR1γ using a well-characterized $TRPV1^{cre}$ mouse line [64,65,66,15,37,68,69,70]. While

immortalized human mast cells (from mast cell leukemia) might bear TRPV1, detailed genetic findings reveal that mouse mast cells express do not express this ion channel in functional assays [71] and that mouse mast cells do not express either the TRPV1 transcript or protein [72,73,74,65], and do not degranulate in response to heat [73] or capsaicin [73,75] and are insensitive to resiniferatoxin chemo-ablation, which occurs through TRPV1 activation [72].

From five unbiased expression profiling datasets (microarray, bulk RNA sequencing, and single-cell RNA- sequencing), we now demonstrate that mouse mast cell populations do not express TRPV1. In addition, peritoneal mast cells from TRPV1^{cre}FCεR1γ$^{fl/fl}$ mice degranulate normally in response to IgE-OVA, and TRPV1^{cre}DTA$^{fl/wt}$ mice have the same proportion of peritoneal mast cells as controls, while flow cytometry analysis of peritoneal mast cell from TRPV1^{cre}Td-tomato$^{fl/wt}$ mouse shows no Cre expression. Overall, while an indirect activation of nociceptors by mast cell-produced histamine is likely[76], our data suggest that the earliest activation of nociceptors by allergens is actually direct - via the immune-complex. Both IgE-OVA and capsaicin (TRPV1 agonist) induces vagal nerve compound action potentials *in vivo,* and this activity is present in mast cell-depleted mice (Kit$^{W\text{-}sh/W\text{-}sh}$) [30] but absent in the FCεR1γ$^{-/-}$ mouse.

Immunological sensitization drives B cells to initiate antibody class switching, the generation of memory B cells, and the release of specific immunoglobulins [77]. Upon antigen recall, the specific antibodies bind to Fc receptors on innate immune cells allowing for a swift and precise response [78]. We have now uncovered a novel aspect of allergen detection, that by vagal nociceptors expressing FcεR1, which detect allergens complexed to IgE, and show that this detection leads to the activation of lung T_H2 cells. Preventing immune-complex sensing by FcεR1-nociceptors reduces antigen-induced airway inflammation. These findings uncover a novel potential therapeutic avenue for the early treatment or prevention of allergic airway disease, one that targets direct sensory neuron activation by allergens in an immune complex.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations.

AAI	allergic airway inflammation
AITC	mustard oil

BALF	bronchoalveolar lavage fluid
BL6	C57BL6
CGRP	calcitonin gene-related peptide
DTA	Diptheria toxin
FcR	Fc receptors
HDM	house dust mite
IC	ovalbumin-IgE complex
IgE	immunoglobulin type E
IL	Interleukin
ILC2	innate lymphoid type 2 cells
i.n.	intranasal
JNC	nodose complex ganglion
KCl	potassium chloride
Muc5AC	Mucin 5AC
NMU	neuromedin U
OVA	ovalbumin
QX-314	membrane-impermeable form of lidocaine
SP	substance P
TRPC3	Transient Receptor Potential Cation Channel Subfamily C Member 3
TRPV1	transient receptor potential vanilloid subtype 1
VIP	vasoactive intestinal peptide

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Key Messages

- Vagal nociceptor neurons overexpress FcεR1γ during allergy
- FcεR1γ-expressing neurons sense invading allergens which initiate allergic inflammation
- Nociceptor neurons released Substance P drive T_H2 cell polarization.

Capsule summary

Airway-innervating vagal nociceptor neurons sense invading allergens, which, in turn, drives T_H2 cells polarization. Fcε1γ$^+$ neurons, therefore, constitute a novel target to prevent the development of allergy.

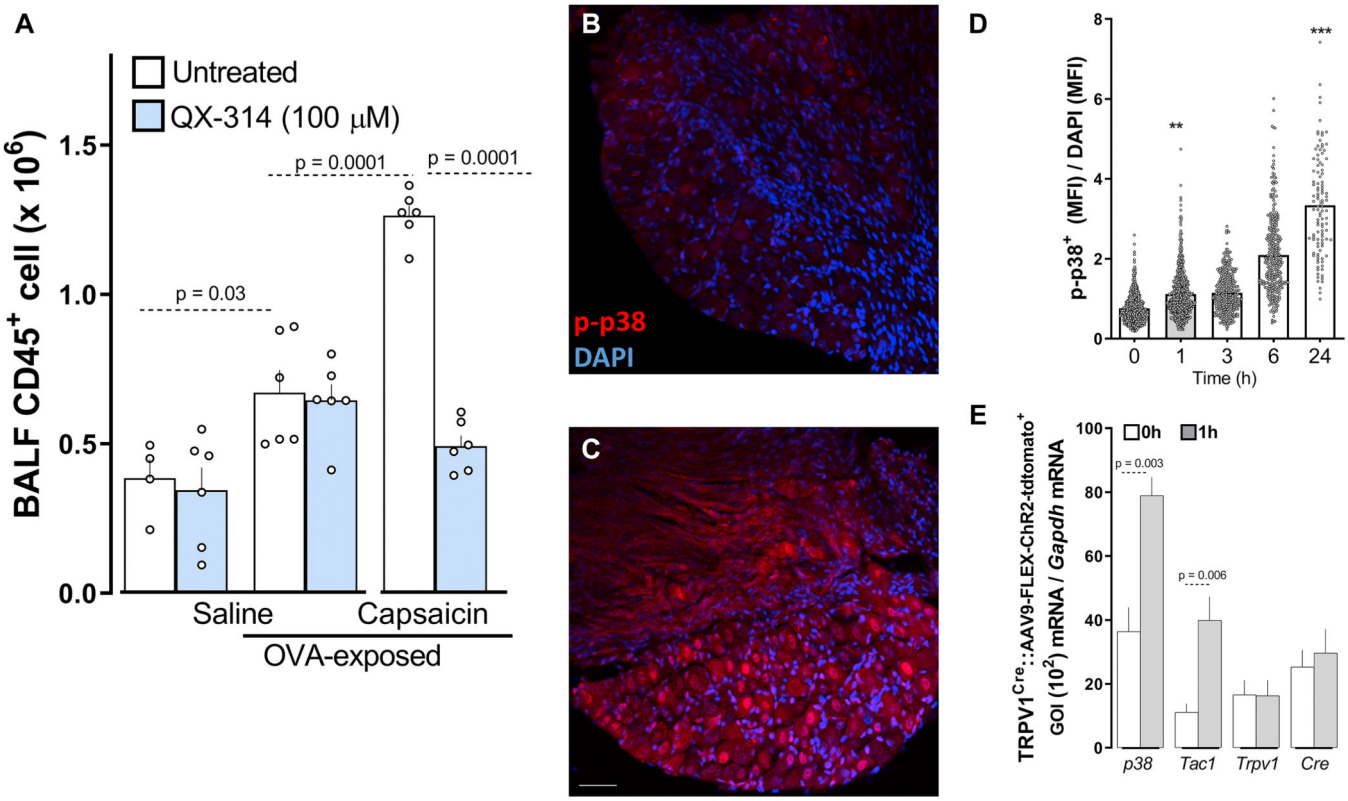

Figure 1: Vagal nociceptors initiate airway inflammation.

An acute inhaled ovalbumin challenge (day 14) increased CD45$^+$ cells in the BALF of previously sensitized mice. A 3h pre-treatment with intranasal 1μM capsaicin led to a further increase in CD45$^+$ cells over OVA treatment alone. QX-314 treatment 3h before the first OVA challenge did not alter the allergen-induced inflammation, but co-treatment with capsaicin to open TRP channels, and allowed entry of QX-314 in airway nociceptors to silence their electrical activity, prevented the onset of allergic airway inflammation (A). Allergen challenge (day 14) with ovalbumin time-dependently increased the number of neurons that were positive for p-p38, an activity marker in JNC neurons (B-D). To track airway-innervating neurons, a cre-dependent AAV9-FLEX-Tdtomato virus (10^9 titers, 50 μL) was injected intranasally two weeks prior to sensitization. Using single-cell qPCR, *p38* and *Tac1* transcript expression were increased in airway-innervating neurons (Td-tomato$^+$) from OVA-challenged mice (1h). TRPV1 and cre expression were not impacted (E). *Mean ± S.E.M; One-way ANOVA post-hoc Bonferroni (A); two-tailed unpaired Student's t-test (D-E); n = 4 – 10 animals/group, 1–3 cohorts. In (B-C) p-p38 are labeled in red, and nuclei are labeled in blue; scale bar = 50 ym.*

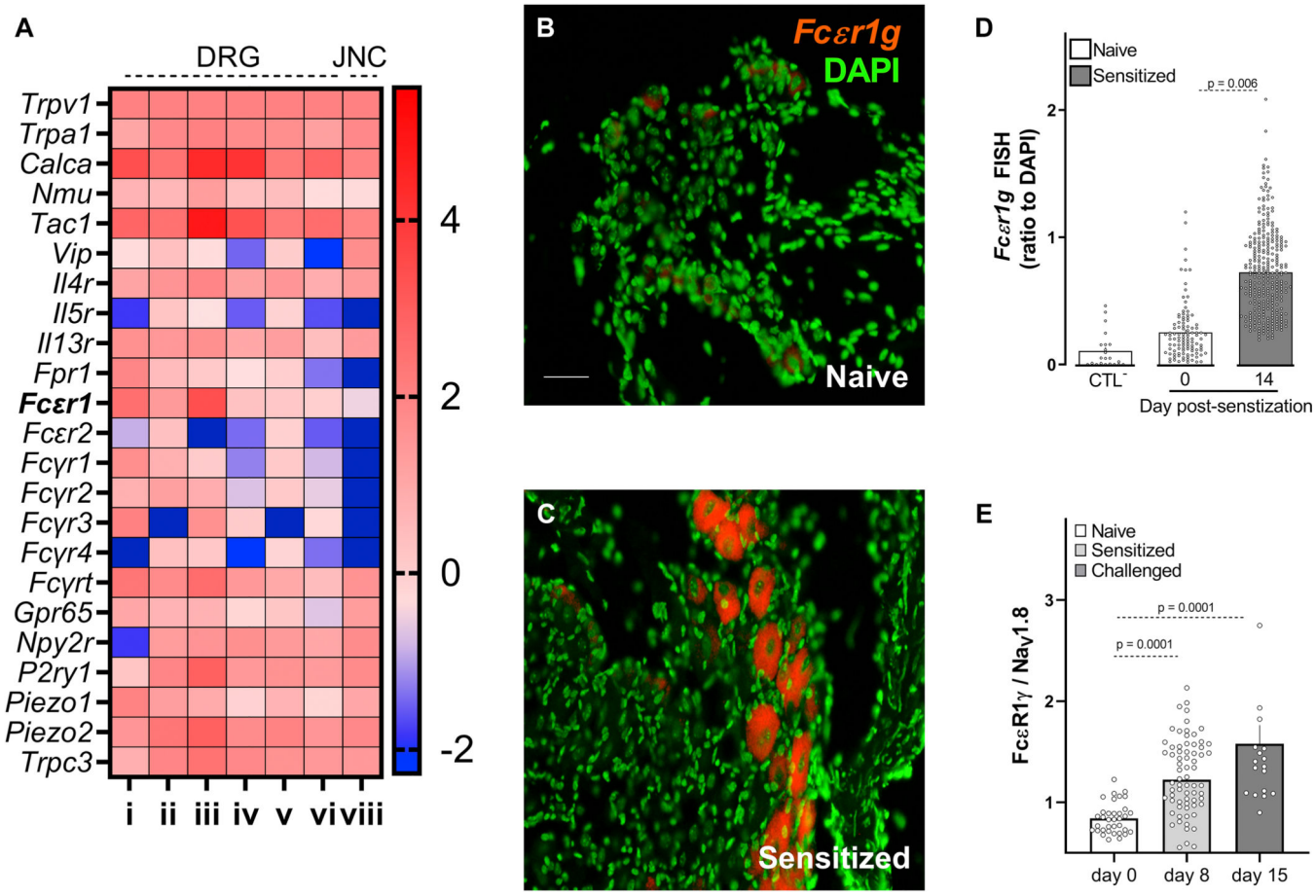

Figure 2: Vagal nociceptors express FctR1.

A meta-analysis of seven published nociceptor expression profiling datasets[79] showed basal expression of sensory neuron markers (TRPV1, TRPA1), neuropeptides (SP, VIP, NMU, CGRP), asthma-driving cytokine receptors (IL-4R, IL-5R, IL-13R), and the immunoglobulin receptor FcεR1 (A). Fluorescent in situ hybridization and immunohistochemistry was used to analyze the levels of FcεR1 transcript (B-D) and protein (E) expression in JNC neurons (day 0 and 14 (B-D); day 0, 8, and 15 (E)). The data reveal that these levels increased in allergen-sensitized mice neurons relative to those in naïve mice (B-E). *Mean ± S.E.M; Two-tailed unpaired Student's t-test (D-E); n = 8 animals/group, 2 cohorts. i) RNA-sequencing of human lumbar neurons*[80]*; ii) microarrays of mouse FACS-sorted* $Na_V1.8^+$ *neurons*[81]*; iii) and iv) single-cell RNA- sequencing of mouse lumbar neurons*[2,83]*; v) refers to microarray profiling of mouse whole DRG*[8] *; vi) refers to performed RNA-sequencing of mouse* $TRPV1^+$ *neurons*[84]*; vii) single-cell RNA sequencing of mouse vagal ganglia*[85]*. Expression across datasets was ratioed over Trpv1, multiplied by 100, and the* Log_{10} *of these values were presented as a heatmap (A). FceR1 are labeled in red, and nuclei are labeled in green (B-C); scale bar = 50 μm.*

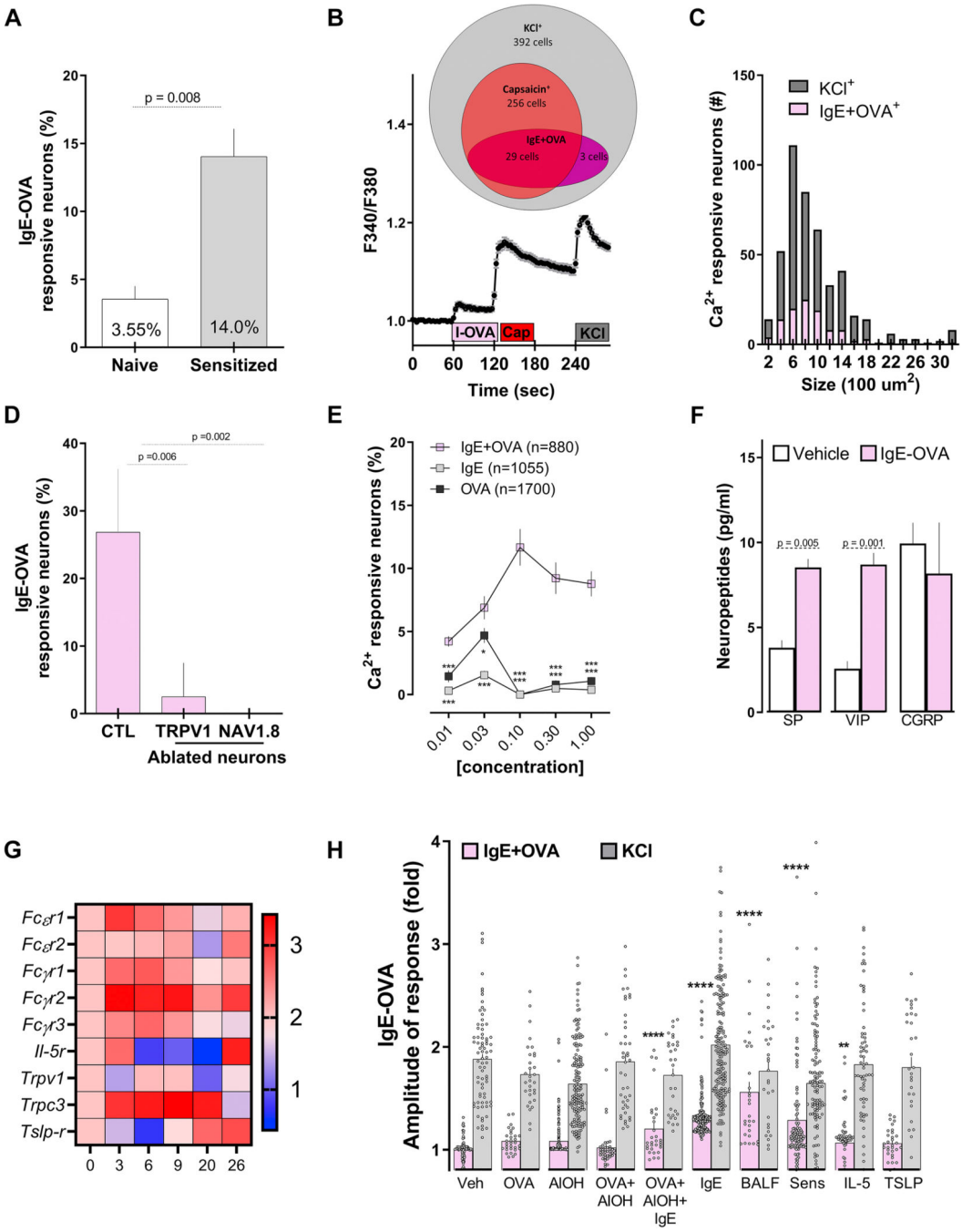

Figure 3: IgE-Ovalbumin complex evokes calcium flux in vagal nociceptors.

Ultra-pure ovalbumin (1%), when complexed with mouse recombinant immunoglobulin E (IgE, 0.001%), triggered larger calcium transients in cultured JNC neurons obtained from allergen-sensitized (day 14) than from naïve mice (A). Shown is an example of the response evoked in capsaicin-sensitive neurons (B). IgE-OVA-induced calcium influx was mostly observed in small-sized neurons (C) and capsaicin-sensitive neurons and absent in cultured neurons obtained from allergen-sensitized (day 14) TRPV1 ($TRPV1^{cre}DTA^{fl/wt}$) or $Na_V1.8$ ($Na_V1.8^{cre}DTA^{fl/wt}$) nociceptor ablated mice. (D). The IgE-OVA complex dose-dependently

evoked a calcium response greater than that for IgE or OVA alone (E) and triggered the release of substance P (SP) and vasoactive intestinal peptide (VIP; F). Capsaicin- responsive neurons were picked at various time points of the allergy-induction protocol and analyzed by single-cell qPCR. Allergen sensitization transiently drove the expression of FcsR1, which peaked on day 3 (G). Compared with naïve vagal neurons, we found an increased number of immune complex-responsive neurons in cells pre-treated (24h) with IgE, OVA+AlOH+IgE, IL-5, or asthmatic BALF or harvested from allergen-sensitized (day 14) mice (H). *Mean ± S.E.M; Two-tailed unpaired Student's t-test (A, D, F); two-tailed Mann-Whitney U test (H), one-way ANOVA post-hoc Bonferroni (E); n = 3–8 animals/group, 2–3 cohorts. In (B), IgE-OVA (1%+0.001%; 60–75 sec) is represented by the pink box, capsaicin (1 jM; 120–135 sec) is represented by the red box, and the gray box represents KCl (40 mM; 240–255 sec). Relative gene expression was ratioed over day 0, multiplied by 1000, and the Log10 of these values were presented as a heatmap (G).*

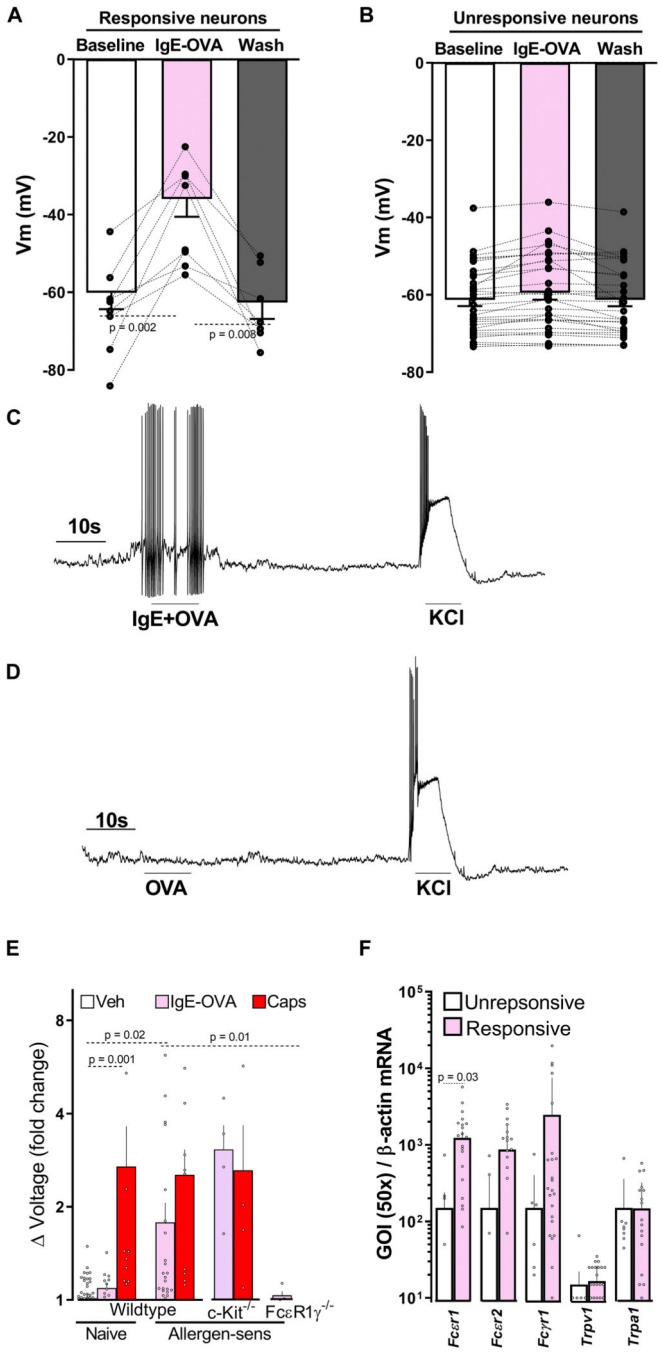

Figure 4: Direct detection of allergens by nociceptors.

In comparison to IC-unresponsive neurons, IgE-OVA sensitive neurons (day 14) showed increased depolarization of the membrane potential (A-B), and action potential firing following IgE-OVA re-exposure (C). OVA alone did not induce action potential firing (D). Whole- nerve electrophysiology was used to assess *in vivo* allergen sensing by vagal neurons and revealed that allergen-sensitized wild type and mast cell-depleted mice (c-Kit$^{-/-}$) demonstrated compound action potential when exposed to IgE-OVA (1%, i.p.), effects that were absent in naïve or allergen-sensitized FcεR1γ$^{-/-}$ mice. Capsaicin (1 μM)

showed compound action potential in all tested mice lines (E). IgE-OVA responsive neurons overexpressed FcεR1 transcript, as evidenced by single-cell qPCR (F). *Mean ± S.E.M; Two-tailed paired (A, B), or unpaired (F) Student's t-test; one-way ANOVA post-hoc Bonferroni (E); n = 8–24 animals/group, 2–8 cohorts.*

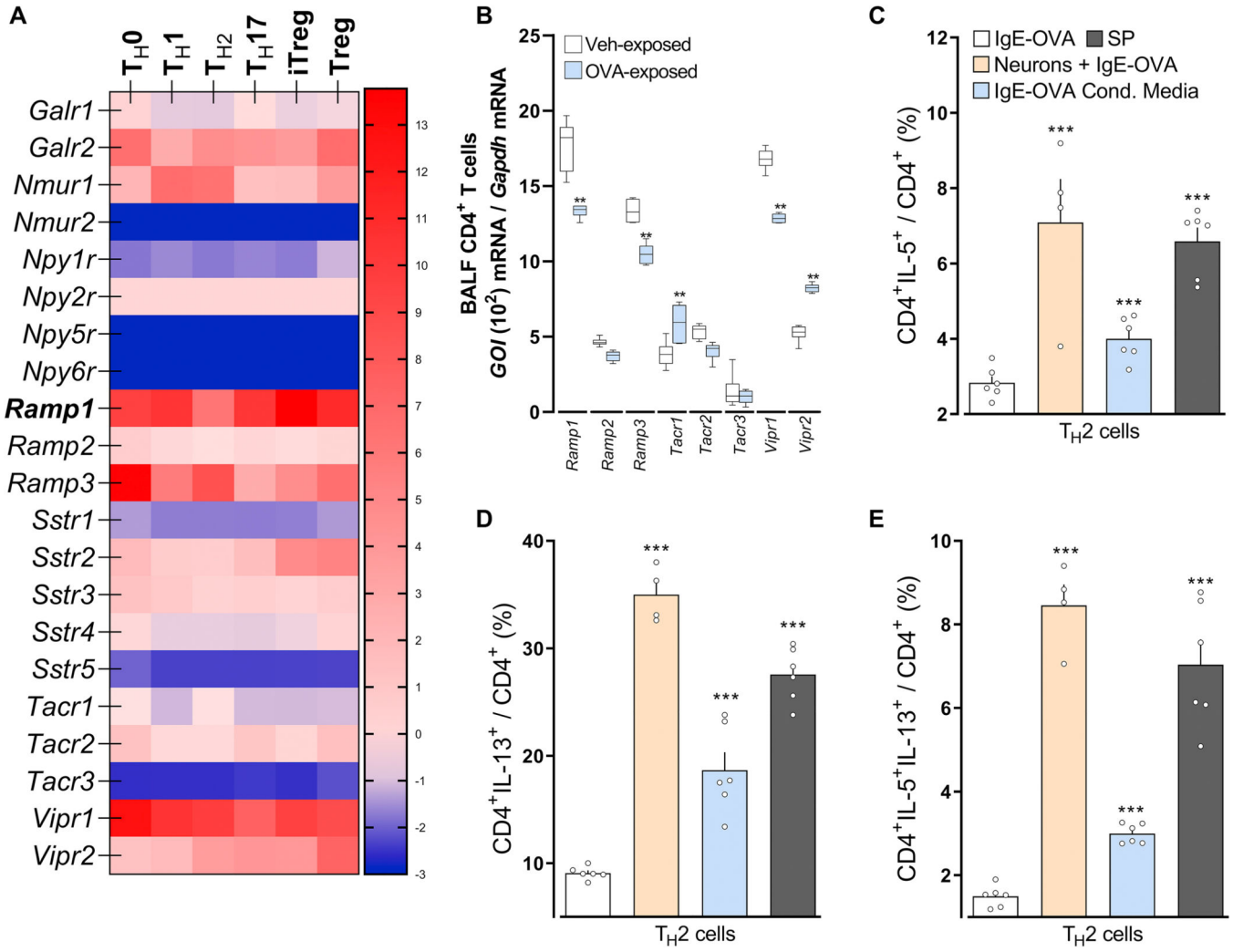

Figure 5: Following allergen sensing, FctR1$^+$ nociceptor neurons drive T_H2 cell polarization.

In comparison to naïve, T_H1, T_H17, iTreg and Treg (A), or naïve CD4 T cells (B), T_H2 cells (A; *In-silico analysis of Immgen's RNA sequencing data)* or BALF CD4 T cells harvested from OVA-challenged mice (day 15; B) showed increased *Tacrl* transcript expression (A, B). Purified naïve CD4 spleen cells were polarized into T_H2 cells and then either: co-cultured with allergen-sensitized mice JNC neurons (beige bar), stimulated with conditioned media harvested from IC-stimulated allergen-sensitized JNC neurons (blue bar) or with Substance P (C-E; gray bar). In comparison to IgE-OVA-stimulated T_H2 cells, T_H2 cells co-cultured with neurons then stimulated with neuron-conditioned media or Substance P, showed increased levels of IL-5$^+$ (C), IL-13$^+$ (D), and IL-5+IL-13$^+$ (E). *Mean ± S.E.M; Two-tailed unpaired Student's t-test (B-E). Transcript expression are shown as DESeq2 normalized counts (A). Stimulation and co-culture were done in the presence of a cocktail of protease inhibitors (1/1000; C-E); n = 5–6 animals/group, 2 cohorts.*

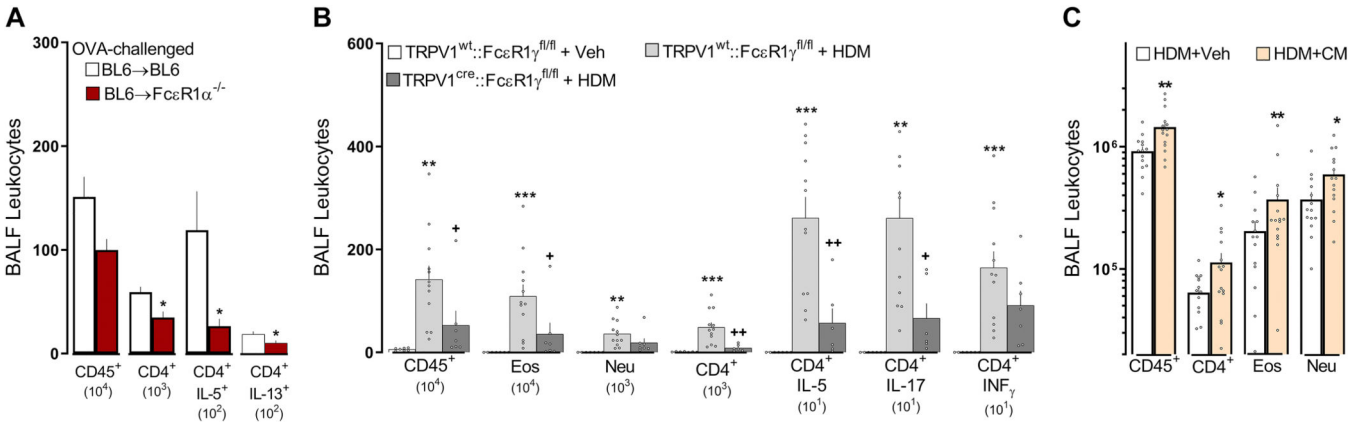

Figure 6: FcεR1+ nociceptor neurons initiate type 2 airway inflammation.

One day after an acute OVA challenge, allergen-sensitized FcεR1α$^{-/-}$ chimeric mice (BL6⟶FcεR1α$^{-/-}$) displayed a reduced level of AAI (lowered numbers of BALF-infiltrating leukocytes, CD45$^+$, CD4$^+$) and polarization (IL-5$^+$, IL-13$^+$) in comparison with allergen-sensitized control (BL6⟶BL6) mice (A). Next, we used the cre/lox toolbox to generate the first cell- specific FcεR1γ knockdown (TRPV1^{Cre}::FcεR1γ$^{fl/fl}$) and littermate control (TRPV1^{wt}::FcεR1γ$^{fl/fl}$) mice. TRPV1^{Cre}::FcεR1γ$^{fl/fl}$ mice were protected from HDM-challenge-induced BALF infiltration of CD45$^+$, eosinophils, and CD4$^+$ as well as CD4 polarization (IL-5, IL-17; B). HDM-challenged mice exposed to neuron-conditioned media harvested from IgE-OVA-stimulated allergen-sensitized JNC neurons demonstrated increased AAI (BALF influx of CD45+, CD4+, eosinophils, neutrophils; C). *Mean ± S.E.M; Two-tailed unpaired Student's t-test (A, C); one-way ANOVA post-hoc Bonferroni (B); n = 5–14 animals/group, 2 cohorts.*