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Mechanisms underlying the neuronal-based symptoms of allergy

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Persons with allergies present with symptoms that often are the result of alterations in the nervous system. Neuronally based symptoms depend on the organ in which the allergic reaction occurs but can include red itchy eyes, sneezing, nasal congestion, rhinorrhea, coughing, bronchoconstriction, airway mucus secretion, dysphagia, altered gastrointestinal motility, and itchy swollen skin. These symptoms occur because mediators released during an allergic reaction can interact with sensory nerves, change processing in the central nervous system, and alter transmission in sympathetic, parasympathetic, and enteric autonomic nerves. In addition, evidence supports the idea that in some subjects this neuromodulation is, for reasons poorly understood, upregulated such that the same degree of nerve stimulus causes a larger effect than seen in healthy subjects. There are distinctions in the mechanisms and nerve types involved in allergen-induced neuromodulation among different organ systems, but general principles have emerged. The products of activated mast cells, other inflammatory cells, and resident cells can overtly stimulate nerve endings, cause long-lasting changes in neuronal excitability, increase synaptic efficacy, and also change gene expression in nerves, resulting in phenotypically altered neurons. A better understanding of these processes might lead to novel therapeutic strategies aimed at limiting the suffering of those with allergies. (J Allergy Clin Immunol 2014;133:1521-34.)

Key words: *Allergy, allergen, mast cell, IgE, degranulation, nerve, afferent, efferent, autonomic, sensory, nociceptor, C-fiber, parasympathetic, sympathetic, enteric, myenteric, nucleus tractus solitarious, ion channel, depolarization, action potential, cough, itch, pain, reflex, motility, bronchospasm, dysfunction, inflammation, skin, lungs, airways, gut, eye, hypersecretion, anti-inflammatory, steroids, vagal, dorsal root, trigeminal, nodose, jugular, ganglia, mast cell-nerve interactions, symptoms, transient receptor potential channels, mast cell mediators, histamine, prostaglandin, leukotriene, cytokine, irritant, receptor, innervation, plasticity, critical periods, phenotypic switch*

Abbreviations used

CGRP:	Calcitonin gene–related peptide
CNS:	Central nervous system
GDNF:	Glial-derived growth factor
GFL:	Glial-derived neurotrophic factor family ligand
NGF:	Nerve growth factor
PGD_2:	Prostaglandin D_2
TRK:	Tyrosine kinase receptor
TRP:	Transient receptor potential
TRPA1:	Transient receptor potential ankyrin 1
TRPV1:	Transient receptor potential vanilloid 1

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Allergy is the consequence of an IgE-driven overreaction of the immune system to what would otherwise be a relatively innocuous stimulus. Clinically, allergy is characterized by symptoms that, by in large, are secondary to an altered nervous system. The panoply of neuronal symptoms depends on the organ in which the reaction occurs but can include itchy and red eyes; rhinorrhea, nasal congestion, and sneezing; urge to cough, dyspnea, airway mucus secretion, and episodic reflex bronchospasm; dysphagia, altered gastrointestinal motility, and discomfort; and cutaneous itching and flare responses. These events are either *in toto* or in part secondary to changes in neuronal activity. Therefore allergy can be characterized as an immune-neuronal disorder (Fig 1).

Immunologists predominate among those interested in investigating the mechanisms of allergy. Over the past few decades, scientists have made tremendous progress in untangling the complex web comprising the immunologic basis of allergy. This includes both the afferent (sensitization) and efferent (inflammatory cell recruitment and activation) limbs of the response. The outcomes from these investigations are filling pharmaceutical pipelines with rational, clever, and exciting therapeutic strategies aimed at quelling the inflammation associated with the allergic reaction.

However, one might argue that the immune-driven inflammation associated with allergic reactions might in some cases be trivial unless transduced into the neurogenic symptoms of suffering (eg, itch, cough, bronchospasm, motility disturbance, pain, sneeze, skin conditions). Yet although the anti-inflammatory pipeline in allergy therapeutics is teeming with activity, the antineuromodulatory pipeline is largely empty. This might be due to the less than appropriate attention given to the neuronal aspect of this immune-neuronal disorder.

Here we have attempted to review literature that provides a sense of how the nervous system is affected by an allergic

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Terms in boldface and italics are defined in the glossary on page 1522.

reaction. Space limitations preclude an exhaustive review of this literature, and therefore instead prime examples are selected to reveal some of the more fundamental principles of allergen-induced neuromodulation. We have not reviewed the clinical scientific literature that has investigated neuronal symptoms of allergy nor have we dealt with the issue of the role of higher brain centers (emotion and stress) on the allergic response. We have also largely avoided the related important aspects of mast cell–nerve interactions that occur independently of the immediate hypersensitivity response, as well as the literature that pertains to mechanisms by which the nervous system can modulate the immune response. Rather we have focused here on basic mechanistic investigations of allergy-induced neuromodulation that will give the reader a sense of the peripheral neurologic substrates of allergy.

GENERAL PRINCIPLES OF SENSORY-AUTONOMIC INNERVATION

Sensory (afferent) nerves sense the local tissue environment.[1] Their peripheral nerve terminals are "free" and do not synapse with other nerves. Instead, these peripheral sensory terminals synapse with the local tissue environment. Peripheral sensory terminals express various receptors and ion channels that transduce environmental signals into electrical signals (ie, ***action potentials***). In the visceral and somatosensory systems, these stimuli include touch and other mechanical perturbations, temperature, pH, osmolarity, and various types of chemical stimuli. The action potentials conduct along the axon centrally, past the cell body that resides in a specific peripheral ganglion (either dorsal root ganglia, vagal nodose ganglia, vagal jugular ganglia, or trigeminal ganglia), and into the central nervous system (CNS), where the signal is transposed into ***neurotransmitter*** release at the nerve's synapse with second-order central neurons.

Sensory nerves are heterogeneous with respect to sensitivity to stimuli (ie, receptor expression), size, myelination, conduction velocity, and neuropeptide and neurotransmitter content. In general, however, sensory nerves fall into 2 main categories: those that have been specifically adapted to detect routine physiologic stimuli (eg, touch, hearing, smell, mild temperature, changes in blood pressure, and osmolarity) and those that detect noxious or potentially noxious stimuli, such as physical damage, chemical irritants, and strong changes in pH.

Sherrington[2] was the first to specifically describe these latter types of nerves in his famous book, *The Integrative Action of*

GLOSSARY

ACTION POTENTIAL: A mammalian nerve fiber at rest is in a state of electronegativity because of concentration gradients of ions and membrane permeability for particular ions. An action potential involves brisk changes in the membrane potential that spread rapidly down the length of the nerve fiber membrane. A normal resting negative membrane potential changes suddenly (within a few 10,000ths of a second) to a positive potential (depolarization) and then back to a negative potential (repolarization). Events that cause an increase in the membrane potential from electronegativity toward the zero level trigger voltage-gated sodium channels to begin opening, causing a further increase in the membrane potential and more opening of voltage-gated sodium channels until all channels have been opened. Potassium-gated channels then begin to open and sodium channels close, leading to termination of the action potential.

CYSTEINYL LEUKOTRIENE D_4 (LTD_4): LTD_4 binds to cysteinyl leukotriene receptor (CysLT) 1 and CysLT2. CysLT1 promotes bronchial smooth muscle contraction and regulates various aspects of the immune system. Montelukast antagonizes CysLT1.

EICOSANOID FAMILY: A class of lipids derived from polyunsaturated fatty acids (eg, arachidonic acid) that mediate inflammation.

ENTERIC: A nervous system exclusive to the gastrointestinal tract. The enteric system contains approximately 100 million neurons, which is comparable to the number of neurons in the spinal cord. It contains an outer (myenteric) plexus and an inner (submucosal) plexus. Sympathetic and parasympathetic nerves connect with these 2 plexuses. Enteric nerves secrete a variety of neurotransmitters, including acetylcholine, norepinephrine, serotonin, dopamine, substance P, and vasoactive intestinal polypeptide.

MECHANOSENSORS: A sensory receptor that detects mechanical compression or stretching of the receptor or adjacent tissues. Respiratory muscle mechanosensors provide afferent input to neurons in the medulla, as well as the sensory cortex.

MYELINATED: Nerve fibers with axons surrounded by a myelin sheath. Schwann cells envelop axons and rotate around the axon many times, creating layers of membrane containing the lipid substance sphingomyelin. Sphingomyelin acts as an electrical insulator and is capable of decreasing ion flow through the membrane approximately 5000-fold. Uninsulated junctions between Schwann cells are termed the node of Ranvier. Action potentials "jump" from node to node to increase the velocity of nerve transmission in myelinated fibers.

NEUROTRANSMITTER: A chemical substance secreted by neurons that causes signal transmission in CNS synapses. Most synapses involved in CNS signal transmission are chemical synapses. The neurotransmitter binds to membrane receptor proteins of the next neuron to excite, inhibit, or modify the sensitivity of the neuron. There are more than 40 neurotransmitters, including acetylcholine, norepinephrine, histamine, serotonin, and gamma-aminobutyric acid.

PARASYMPATHETIC: Nerve fibers of the autonomic nervous system that leave the CNS through cranial nerves III, VII, IX, and X, as well as spinal sacral nerves. Most parasympathetic nerves are in the vagus nerves (cranial nerve X). Parasympathetic nerves also contain preganglionic and postganglionic neurons. Most parasympathetic postganglionic neurons are cholinergic.

REACTIVE OXYGEN SPECIES (ROS): Substances typically generated at a low frequency during oxidative phosphorylation in the mitochondria, as well as in a variety of other cellular reactions. ROS can exert cellular damage by reacting with intracellular constituents, such as DNA and membrane lipids.

SYMPATHETIC: Nerve fibers of the autonomic nervous system that originate in the spinal cord between T1 and L2. They pass first into paravertebral chains of ganglia and then to tissues and organs. Each sympathetic nerve is composed of a preganglionic neuron and a postganglionic neuron. Preganglionic nerves can synapse in a paravertebral ganglion or a peripheral sympathetic ganglion (eg, celiac ganglion). Preganglionic nerves in both the sympathetic and parasympathetic systems are cholinergic (ie, secrete acetylcholine). Most sympathetic postganglionic neurons are adrenergic (ie, secrete norepinephrine).

TACHYKININS: A class of neuropeptides, which includes the sensory afferent neurotransmitter substance P.

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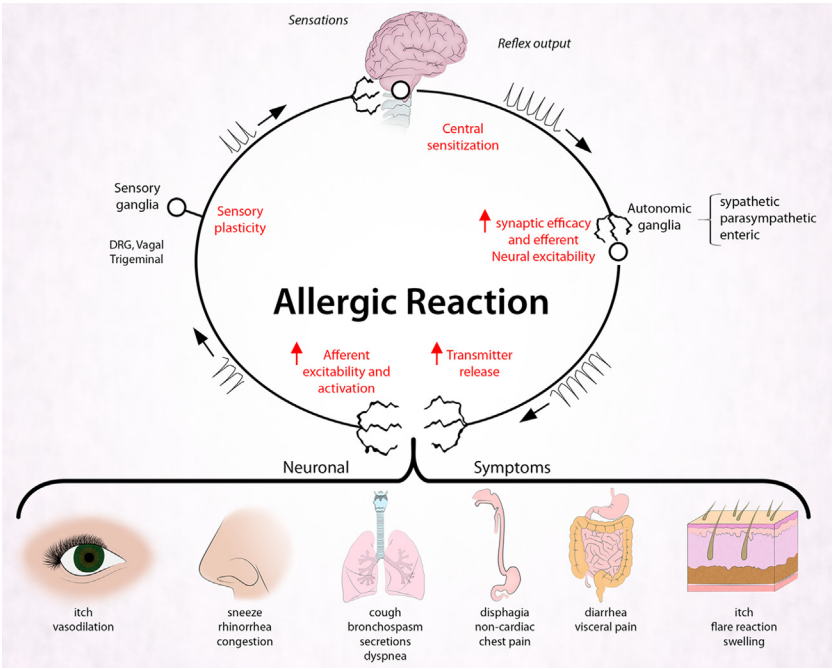

FIG 1. Neuromodulation during the allergic reaction. Experimental studies *in vivo* and *ex vivo* support the hypothesis that the allergenic response can involve neuromodulation along the sensory (afferent)–CNS–autonomic/enteric nerve axis (see text for details). The neuromodulation ultimately leads to many of the symptoms of allergic disease. *DRG*, Dorsal root ganglion.

the Nervous System.[2] He noted that a large number of sensory nerves in the skin were activated only by nonphysiologic stimulation of a noxious character. He termed these specialized sensory nerves "nociceptors" and suggested "…that under selective adaptation, they attach to the skin a so-to-say specific sense of its own injuries."[2] Generally, nociceptors are small-diameter, unmyelinated, slowly conducting nerves referred to as C-fibers (A- and B-fibers are ***myelinated***, faster-conducting nerves). It is now known that sensory nerves in visceral organs also comprise C-fibers that fit Sherrington's definition of nociceptors.

The signals (action potentials) arising from these primary afferent nerves are integrated in the CNS, where the ultimate consequence can be either conscious perception (eg, pain, cramping, itch, dyspnea, or urge to cough or sneeze) or a subconscious activation of preganglionic autonomic neurons, thereby initiating ***sympathetic***, ***parasympathetic***, and ***enteric*** reflexes. In the skin nociceptor activation leads to itching and pain. In the respiratory tract activation of nociceptors leads to sneezing, coughing, dyspnea, and reflex bronchospasm and secretions. In the gut nociceptor activation can lead to secretion, diarrhea, gastric discomfort, and visceral pain.[1,3-6] In other words activation of these nerves leads to strong sensations and/or reflexes aimed at avoidance of the stimulus. As we discuss in more detail below, nociceptors are the subtype of afferent nerve most susceptible to stimulation secondary to an acute allergic reaction.

The autonomic and enteric nervous system depends on synaptic communication between presynaptic and postsynaptic elements situated in the sympathetic, parasympathetic, and enteric ganglia. In most organs the efferent neural regulation is driven by presynaptic neurons arising from the CNS. The preganglionic nerve is stimulated within the CNS, and action potentials conduct along the preganglionic axon that ultimately form synapses with neurons in the autonomic ganglia. It should be kept in mind that these ganglia are not simple relay stations but sites where filtering and integration of the CNS input occurs. This might be relevant in allergy because mast cells are commonly associated with sympathetic, parasympathetic, and enteric ganglia (as we will discuss further below). In the gut, in particular, there is also autonomous efferent control that is independent of the CNS neural processing. In this case a sensory nerve that detects a stimulus in the local environment can transmit this information directly to nearby efferent enteric neurons through local afferent-efferent synapses. This is referred to as a local "peripheral reflex." The enteric ganglion neurons communicate through intraganglionic transmission. Peripheral reflexes can occur in other visceral organs, such as the airways and gall bladder, as well, although not to the extent seen in the gastrointestinal tract.[7,8]

In some organs neuropeptide-containing afferent C-fibers can directly regulate organ function independently of either the CNS or efferent autonomic or enteric neurons through local "axon reflexes."[9] In this case the action potential arising in a peripheral sensory nerve terminal is conducted centrally until it reaches a bifurcation, and then it antidromically conducts back to other peripheral terminals of the same nerve, where it evokes the release of sensory neuropeptides, such as substance P, neurokinin A, and calcitonin gene–related peptide (CGRP). The released peptides can lead to edema, vasodilation, smooth muscle contractions and relaxations, and immune cell recruitment and activation. The overall consequence of axon reflexes is often referred to as "neurogenic inflammation."[9] The extent to which neurogenic inflammation occurs is species and organ dependent. For example, in human subjects the evidence is stronger for neurogenic inflammation in the skin and nasal mucosa than in the lower airways.[10]

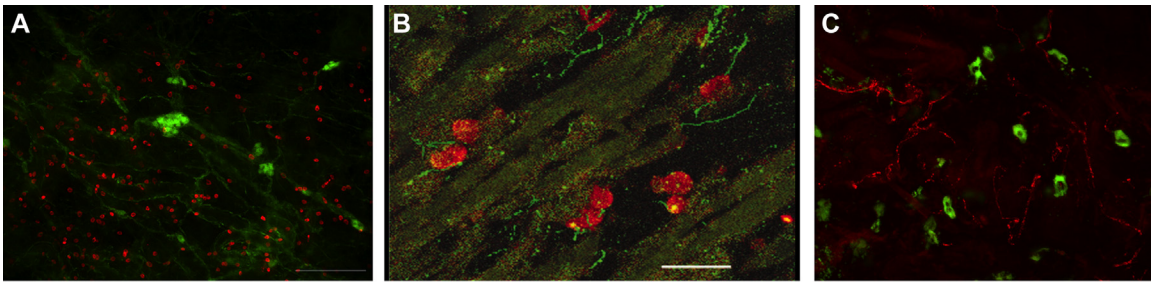

FIG 2. Mast cells are found in close proximity to nerves in virtually all organs. **A,** Mast cell tryptase–positive cells *(red)* near PGP9.5-positive nerves *(green)* in human intestinal submucosal plexus.[11] **B,** Mast cells *(red)* near synapsin-positive neurons *(green)* in rat cardiac ventricle.[12] **C,** Mast cells *(purple)* near MrgA3 expressing "afferent itch nerves" *(orange)* in mouse skin (personal observation).

BASIC MECHANISMS OF ALLERGEN-INDUCED NEUROMODULATION

The allergic response comprises changes at all 3 levels of the neural arc: sensory nerve function, CNS integration, and autonomic/enteric neuroeffector cell function (Fig 1). These changes can be subdivided into acute changes (overt activation of nerves that lasts only as long as the stimulus is present), longer-lasting changes in *neuroexcitability* that can outlast the stimulus by hours or days, and the even more persistent phenotypic changes that can last for weeks and perhaps, when one considers the idea of developmental "critical periods," for years.

Morphologic considerations

The allergic reaction seems particularly adept at altering neuronal function in the skin and visceral tissues. This is likely due to the close association between nerve fibers and mast cells (Fig 2).[11,12] Mast cells have been anatomically associated with nerves in virtually all organ systems in laboratory animals and human subjects.[11,13-17] The percentage of mast cells making meaningful associations with nerves depends on the distance that one considers meaningful. In the bladder, for example, it has been argued that greater than 75% of mast cells are close enough to nerve fibers for meaningful bidirectional communication,[18] and in the human gastrointestinal tract 50% to 70% of mast cells were considered to be in close apposition to neurites.[19] There is good evidence that mast cells actually make synaptic-like contacts with nerves, but this holds true for only a small subset of mast cells.[20,21] The intimate synaptic-like nerve–mast cell contacts can be induced or enhanced by specific adhesion molecules, such as cell adhesion molecule 1.[22] In experimental systems of mast cell–sensory nerve contacts, nerve–mast cell communication was inhibited by blocking the heterophilic binding between mast cell–expressed cell adhesion molecule 1 and nectin 3 localized to the nerve.[23]

More often, mast cells are situated close to nerves without forming true contacts. The anatomic association between mast cells and nerves becomes even more prevalent at sites of inflammation.[20,24,25] Mechanisms have been proposed to explain the propensity for mast cell–nerve associations, including the release of neurotrophic factors and cytokines by mast cells that promote nerve elongation into the vicinity of mast cells.[26] With respect to allergic inflammation, the infiltrating eosinophil is also often associated with nerves.[27] In the airways eosinophils might be attracted to autonomic cholinergic neurons as a consequence of neuronal vascular cell adhesion protein 1 and intercellular adhesion molecule 1 expression.[28] On the other hand, a careful morphometric analysis revealed that fine nerve axons and terminals in various tissues are also commonly associated not just with mast cells and eosinophils but also with other bone marrow–derived cells, such as plasma cells, and these "associations" do not appear to favor one cell type over the other.[13] This type of analysis leads to the conclusion that fine nerve branches, mast cells, and other bone marrow–derived cells are often concentrated in the same tissue region, making it all but certain they will, in a sense, "colocalize" by random chance. Mast cells, along with autonomic and sensory nerves, are frequently found in close proximity of the microvasculature, in the mucosa and submucosa of visceral tissues, near smooth muscle, and throughout the dermis.

Regardless of the mechanisms, anatomic investigations, especially when evaluating whole mounts of tissue, often reveal a spectacular display of mast cells residing along afferent, autonomic, and enteric nerve branches (Fig 2). These images leave little doubt that many, if not most, nerve fibers in tissues are within the sphere of influence of mediators released from mast cells. Just what this influence might be is discussed below.

Allergen-induced neuromodulation of afferent (sensory) nerves

Action potential discharge. Allergen challenge is often associated with the overt activation of afferent nerve terminals leading to action potential discharge (Fig 3).[29,30] Generally, it is thought that the types of sensory nerves most susceptible to direct activation through allergic mediators are polymodal C-fibers. This is because afferent C-fibers are known to express receptors for many chemical mediators present in the allergically inflamed tissue. Often, these nerves can be accurately categorized as nociceptors.

A sensory nerve terminal is activated when a specific stimulus acts on specific receptors or ion channels in the nerve membrane, leading to membrane depolarization. This depolarization is

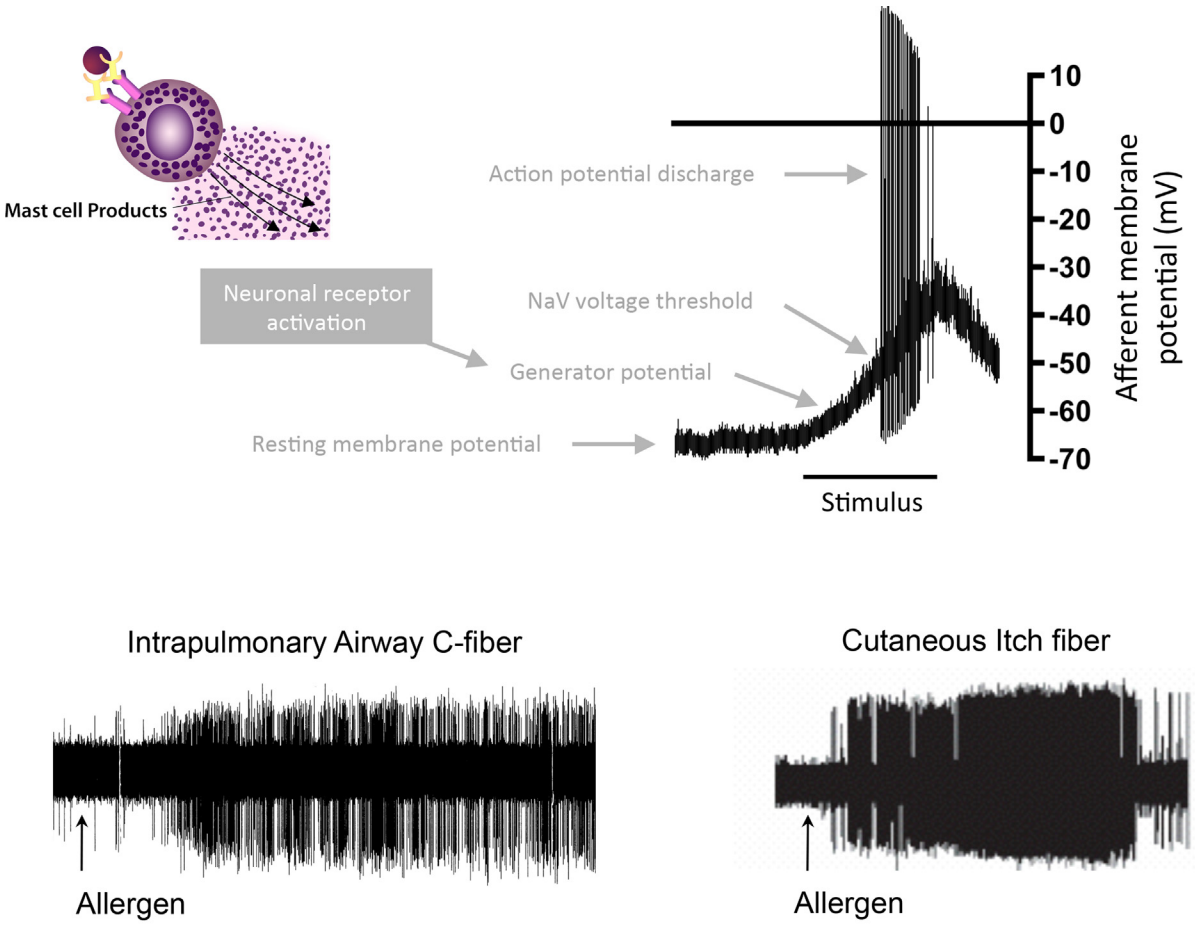

FIG 3. Allergen challenge overtly activates afferent C-fibers. *Top,* Hypothetical effect of allergenic activation of mast cells on afferent nerve terminal action potential discharge. *Bottom,* Examples of allergen-induced activation of afferent nociceptors: *left,* allergen (ovalbumin) evokes strong activation of vagal jugular C-fiber innervating the lung (action potentials recorded in vagal sensory ganglion)[30]; *right,* allergen (mosquito extract) induces the activation of a somatosensory itch fiber in the skin (action potentials recorded in the dorsal horn).[29]

termed a "generator potential." If the generator potential is of sufficient magnitude, it will activate voltage-gated sodium channels that, in turn, lead to action potential discharge (Fig 3, top). The frequency of the "all-or-none" action potentials is proportional to the amplitude of the generator potential.

Afferent C-fibers commonly express members of the transient receptor potential (TRP) family of ion channels. Two TRP channels that appear to be important in allergic responses are transient receptor potential vanilloid 1 (TRPV1) and transient receptor potential ankyrin repeat 1 (TRPA1). When these channels open, they provide a pathway for the influx of cations (sodium and calcium), which leads to generator potentials of magnitudes sufficient to reach the threshold for action potential discharge.[31,32] TRPV1 is stimulated by several mechanisms germane to the allergic reaction. These stimuli include agonists of Gq-coupled GPCRs, such as bradykinin 2 receptor and histamine H1 receptor; acidification of the local environment of the nerve endings; and certain lipoxygenase products of the ***eicosanoid family*** of inflammatory mediators.[33,34] TRPA1 is also activated by mechanisms relevant to allergy. C-fibers in the airways are stimulated through TRPA1 by a variety of mediators of oxidative stress, including ***reactive oxygen species*** (eg, H_2O_2) and lipid products of peroxidation (eg, 4-hydroxynonenal and 4-oxononenal) and nitration (9-nitrooleate).[35] Metabolites of the mast cell prostaglandin D_2 (PGD_2) can also strongly activate C-fibers secondary to gating TRPA1.[36]

In addition to TRPV1 and TRPA1 channels that can integrate many disparate stimuli, afferent C-fibers also express receptors for specific mediators associated with the allergic response, including ATP receptors (P2X2 and P2X3), adenosine receptors (A1 and A2A), 5-HT receptors (5HT-3 and 5HT2), cysteinyl leukotriene receptors (CysLT1), tryptase, and trypsin (protease-activated receptor 2 and 1).[37-43]

On the basis of the basic pharmacology of polymodal afferent nerve fibers (usually but not exclusively C-fibers), it should not come as a surprise that activation of mast cells will lead to their overt activation. This has been observed in many organs, including the airways, skin, gastrointestinal tract, and bladder.[24,29,30,43,44] Examples of C-fiber action potential discharge in response to an acute allergen challenge are exemplified in Fig 3. One can rationally assume that C-fibers will also be stimulated by mediators arriving during the later phase of the allergic reactions, when the cellular inflammatory response sets in.

Changes in sensory excitability. Sensory nerves can be excited independently of stimuli that evoke generator potentials. Opening or closing of various ion channels (typically leak channels or voltage-gated channels) can cause electrophysiologic

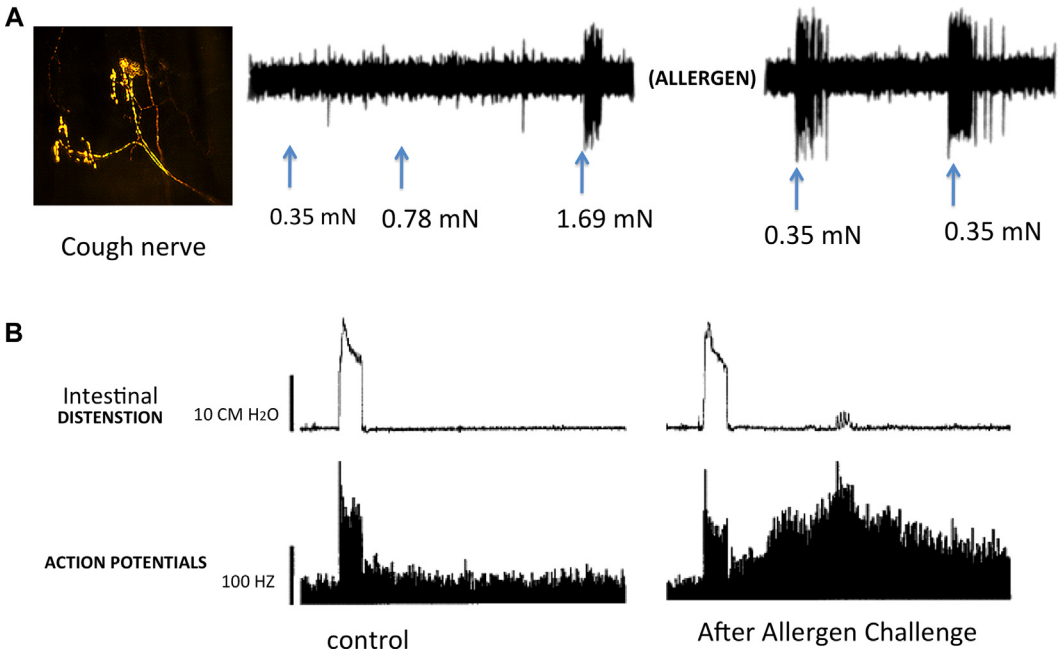

FIG 4. Allergen-induced increase in sensory nerve excitability. **A,** Nodose Aδ "cough" fiber terminals in the guinea pig trachea; increased sensitivity to mechanical stimulation of this fiber type after allergen (ovalbumin) challenge.[47] **B,** Multiunit recording of intestinal afferent nerves demonstrating markedly enhanced (prolonged) response to distension after allergen (ovalbumin) challenge.[48]

effects that render a nerve hyperexcitable to an activating stimulus. In the vernacular of immunology, we might say that the nerve endings are primed by certain inflammatory mediators. Allergy-associated mediators, such as histamine, PGD_2, ***cysteinyl leukotriene D_4***, serotonin, and bradykinin, can decrease the activity of certain potassium channels, leading to an increase in the resistance of the afferent nerve and a subsequent increase in electrical excitability.[39,45,46] Cysteinyl leukotriene D_4 stimulating cysteinyl leukotriene 1 receptors in C-fiber neurons of the trigeminal ganglia does not overtly activate the nerve but increases its excitability such the action potential discharge frequency in response to another activating stimulus is substantially enhanced.[39]

Allergen challenge and mast cell mediator release in a sensitized trachea *ex vivo* does not overtly activate nociceptive Aδ cough nerves but instead lowers their activation threshold to a mechanical stimulus (Fig 4).[47,48] In the lower airways allergic mediators, including histamine, can increase the excitability of certain C-fibers to the point that the mechanical perturbation of eupneic breathing leads to their activation.[49,50] Immunologic activation of mast cells in the intestine increases the excitability of spinal afferent nerves such that the same short-lived mechanical distention results in a much more prolonged afferent nerve discharge (Fig 4).[48] Likewise, mast cell activation leads to a pronounced increase in the mechanical sensitivity of vagal C-fibers in the esophagus.[51] The kinetics of the increase in afferent nerve excitability secondary to allergen activation of mast cells can be quite persistent, lasting for several hours after acute mast cell activation. Products from activated eosinophils might also contribute to increases in afferent nerve excitability at sites of allergic inflammation.[52]

The activation and/or increase in afferent nerve terminal excitability occurs so commonly in disparate tissues and experimental systems that it might be considered a principle result of activation of tissue mast cells. In general terms, overt activation of C-fiber leads to sensations and reflexes consistent with the organ sensing a danger stimulus and responding in a manner that attempts to reduce the exposure of this stimulus. As discussed above, depending on the tissue, activation of nociceptors can ultimately lead to urge to cough or sneeze, reflex bronchospasm, secretions, itch, neurogenic inflammatory responses that contribute to wheal-and-flare reactions, visceral discomfort, and changes in gastrointestinal and bladder motility. Furthermore, if the sensory nerve has been made hyperexcitable by allergic mediators, then subthreshold stimuli and even nonnoxious routine stimuli might evoke these nociceptor-associated reflexes.

Phenotypic changes. The term phenotypic change is loosely defined, but in the present context we are referring to neuromodulation that takes place at the level of gene expression.

In an adult the afferent nerve terminals in the large airways are more than 10,000 cell diameters away from the cell body and nucleus. Yet it is clear that local allergic reactions in the airways can lead to changes in gene expression in the distant vagal afferent neuronal cell bodies.[53,54] The most likely explanation for this is that the allergic response can lead to production and release of neurotrophic factors in the local environment of the nerve terminals. There is strong evidence that increased neurotrophin production occurs during the allergic response in all tissues.[55,56] Allergic reactions can lead directly to the release of neurotrophic factors from mast cells and also secondary to the stimulation of other cells in the tissue, such as the airway epithelium.

Neurotrophic factors, typified by nerve growth factor (NGF), can bind with high affinity to its cognate receptors (eg, tyrosine kinase receptor [TRK] A) on nerve terminal membranes. This leads to an internalization of the NGF-TRKA complex that is then transported through axonal transport mechanisms the long distance back to the cell body, where it influences gene

expression. NGF is a member of the neurotrophin family of neurotrophic factors that also comprise brain-derived neurotrophic factor and neurotrophin 3 and 4.[57] In addition to neurotrophins, another family of neurotrophic factors referred to as the glial-derived neurotrophic factor (GDNF) family ligands (GFLs) might also be relevant to the allergic reaction.[54] The GFLs comprise GDNF, neurturin, artemin, and persephin.

The effects of neurotrophic factors on phenotypic changes in afferent neurons innervating the airways will depend on the repertoire of neurotrophic factor receptors expressed by the nerves. For example, nearly all the airway afferent nerves express GFRa1 and Ret, the receptors for GDNF. Vagal nodose neurons express TRKB, the TRK for brain-derived growth factor and neurotrophin 4, whereas spinal C-fibers and vagal jugular C-fibers commonly express TRKA, the receptor for NGF.[58,59]

Inhalation of allergen leads to increased expression of genes involved in the production of ***tachykinins***, such as substance P and CGRP.[53] Interestingly, after allergen challenge, large-diameter myelinated A-fiber nonnociceptor neurons that normally do not express these potent neuropeptides begin to synthesize and transport the peptides to their central and peripheral terminals.[60,61] In other words, there is a "phenotypic switch" in the neuropeptide innervation of the allergically inflamed tissue. This effect can be mimicked with exogenous neurotrophins. This type of phenotypic switch has also been observed in somatosensory nerves after painful inflammation.[62]

There is also a phenotypic switch when it comes to the expression of TRP channels in airway sensory nerves. The expression of TRPV1, the capsaicin receptor discussed above, is limited to nociceptive C-fibers in healthy tissues. Inhalation of allergen by rats or guinea pigs leads to the expression of TRPV1 in Aδ cough nerves as well as in Aβ low-threshold ***mechanosensors*** (both rapidly adapting and slowly adapting receptor subtypes, Fig 5).[54,63] More investigation in this area is needed to determine the molecular mechanisms underlying allergen-induced phenotypic changes, but early work supports the potential involvement of both neurotrophins and GFLs in the response.[54]

Overall, the studies on allergen-induced sensory modulation support the hypothesis that at sites of an allergic reaction, there will be an intense quantitative increase in action potential volleys arising at the central terminals of afferent C-fibers in the CNS (and within myenteric ganglia). This will occur secondary to overt activation of C-fiber terminals, as well as a generalized "priming" effect of increases in their electrical excitability. In addition, there will also be relevant qualitative changes in the activation profile of A-fibers, and because these nerves begin to express sensory neuropeptides, this can change the reflex consequences of their activation. These allergen–sensory nerve interactions can be acute and short lived but can also persist long after the initial allergic response.

Allergen-induced modulation of CNS neurons

Mast cells are found in the brains of all species that have been evaluated,[64] but evidence is lacking for direct allergen-induced activation of these cells. It would seem more likely that allergic reactions modulate CNS neurons indirectly by creating a situation in which there is an intense and in some cases sustained increase in nociceptor activity, occurring as a consequence of overt activation, and increases in excitability, as described above. This leads to increases in neurotransmitter/neuropeptide release from the central terminal of the afferent nerves into the synapse of the second-order CNS neurons situated in the dorsal horn of the spinal cord for spinal afferent nerves and in the brainstem within the nucleus of the solitary tract for vagal afferent nerves.[65] In the case of phenotypic changes, the CNS neurons can be modulated by peptides, such as CGRP and substance P, released from nonnociceptive nerves. In either case peptides and transmitter releases from the central terminals of the afferent nerves can be set in motion events that lead to increases in the synaptic efficacy of the CNS neurons, a process often referred to as "central sensitization."[66]

When the CNS neurons become sensitized, the consequence of a given amount of afferent input can be enhanced and even qualitatively changed. Evidence supports the concept that central sensitization might contribute to the phenomenon of allodynia (in which a normally nonpainful stimulus, such as gentle brushing of the hair and the activation of touch-sensitive Aβ-fibers, leads to inappropriate pain).[66] Analogously, many with allergy to aeroallergens experience what might be termed "allotussivity," in which the sensation of a persistent urge to cough is present, even when there is nothing in the airways to cough up, or the persistent urge to sneeze is present when nothing physical is present in the nasal passage. In experimental animals it has been reported that activation of airway vagal C-fibers (which can occur with mast cell activation) leads to a situation in which stimulation of tracheal A-fibers causes coughing with stimulus intensities that are normally far less than the normal cough threshold.[67] Similarly, stimulation of C-fibers can, through central sensitizing interactions, increase the parasympathetic drive to the respiratory tract.[68] In both cases this is due to transmitters released from the central terminals of C-fibers, increasing the efficacy of neurons innervated by the A-fibers (convergence of A-fibers and C-fibers onto second-order neurons). A clinically relevant aspect of central sensitization is that it provides a mechanism whereby an allergic reaction in one location can influence the physiology of a disparate location. For example, stimulation of C-fibers in the larynx can enhance parasympathetic drive to the peripheral airways. C-fiber activation in the esophagus (eg, during acid reflux) can lead to the urge to cough through enhancing synaptic activity of A-fibers in the trachea. Allergen challenge in the nose can lead to central sensitization of lower airway cough nerves.[69] This raises the possibility that cough associated with gastroesophageal reflux is not secondary to microaspiration of substances into the airways and that cough associated with nasal allergy is not necessarily secondary to "postnasal drip" and the direct activation of cough nerves, as much as it is due to central sensitization of the cough pathway by converging esophageal and nasal nociceptors.

The idea of central sensitization in allergy is supported by elegant studies with young adult rhesus monkeys.[70] After sensitization to house dust mite, monkeys were repeatedly challenged with house dust mite–containing aerosol. Approximately 5 to 6 months later, the electrical excitability of brainstem neurons in the nucleus of the solitary tract were evaluated and found to be strongly upregulated; their responsiveness to a given input stimulus was much stronger than that seen with similar neurons from nonallergic monkeys (Fig 6).[70]

The transmitters and molecular mechanisms underlying central sensitization are multifarious and beyond the scope of this review. How long the central sensitization persists beyond the inflammatory reaction is open to debate. Nevertheless, it would

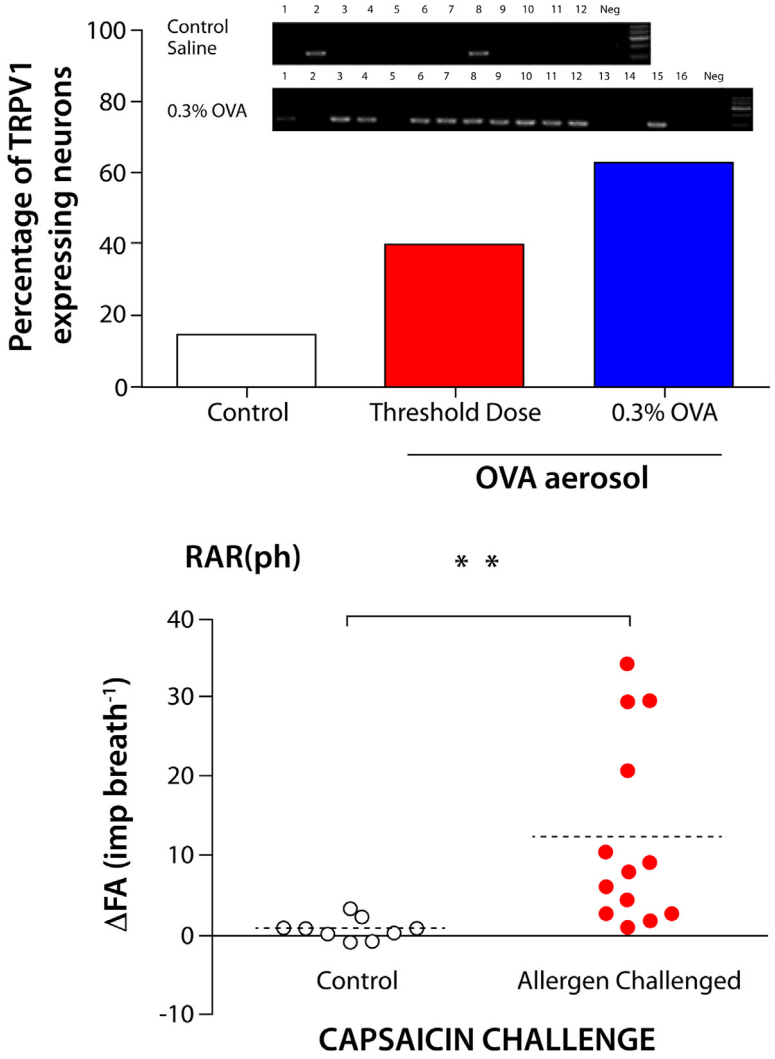

FIG 5. Examples of allergen-induced sensory neuroplasticity. *Top,* RT-PCR from individual neurons retrogradely traced from the trachea. The example shows 12 neurons from control-treated guinea pigs and 16 neurons from ovalbumin *(OVA)*–treated guinea pigs. The histograms show the percentage of trachea-specific nodose neurons in each treatment group that express TRPV1 mRNA. Allergen (ovalbumin) challenge induces *de novo* expression of TRPV1 in nodose Aδ "cough" fibers innervating the trachea (from Lieu et al[54]). *Bottom,* Allergen challenge induces *de novo* capsaicin sensitivity in lung rapidly adapting receptor *(RAR)* fibers that have been characterized by their phasic response during respiration *(RARph). ΔFA,* Difference in firing activity. Used with permission from Zhang et al.[63] $^{**}P < .01$.

seem rational to infer, on the basis of available evidence, that the allergic reaction can lead to nociceptor-based input into the CNS (brainstem or dorsal horn), which sensitizes the synaptic transmission therein. This could lead to sensations and reflexes that are grossly inappropriately matched to the insult present in the peripheral allergically inflamed tissues.

Allergic modulation of efferent (autonomic and enteric) nerves

Activation and changes in synaptic efficacy. Mast cells are commonly observed to be close to neurons within enteric, sympathetic, and parasympathetic ganglia.[11,71,72] Neurons within these ganglia receive synaptic input from preganglionic autonomic neurons with cell bodies in the CNS. The enteric ganglion in particular can also function autonomously through intraganglionic synaptic transmission. When mast cells situated close to the autonomic ganglia are stimulated, this leads to substantial increases in "synaptic efficacy." That is to say that the postganglionic output increases relative to a given preganglionic input. These ganglia are the first sites of peripheral integration of efferent information arising from the CNS. Therefore changes in synaptic efficacy at these sites can exert powerful modulation of the CNS control over organ function. In addition, changes in activity of the neuron within the enteric ganglia can also alter the characteristics of the autonomous functioning of the gastrointestinal tract.[73]

Allergen-induced increase in synaptic efficacy can be seen electrophysiologically in bronchial parasympathetic ganglia that are located mainly in the trachea and larger bronchi. Input from the CNS often leads to excitatory potentials that are subthreshold for action potential discharge in the postganglionic neurons.

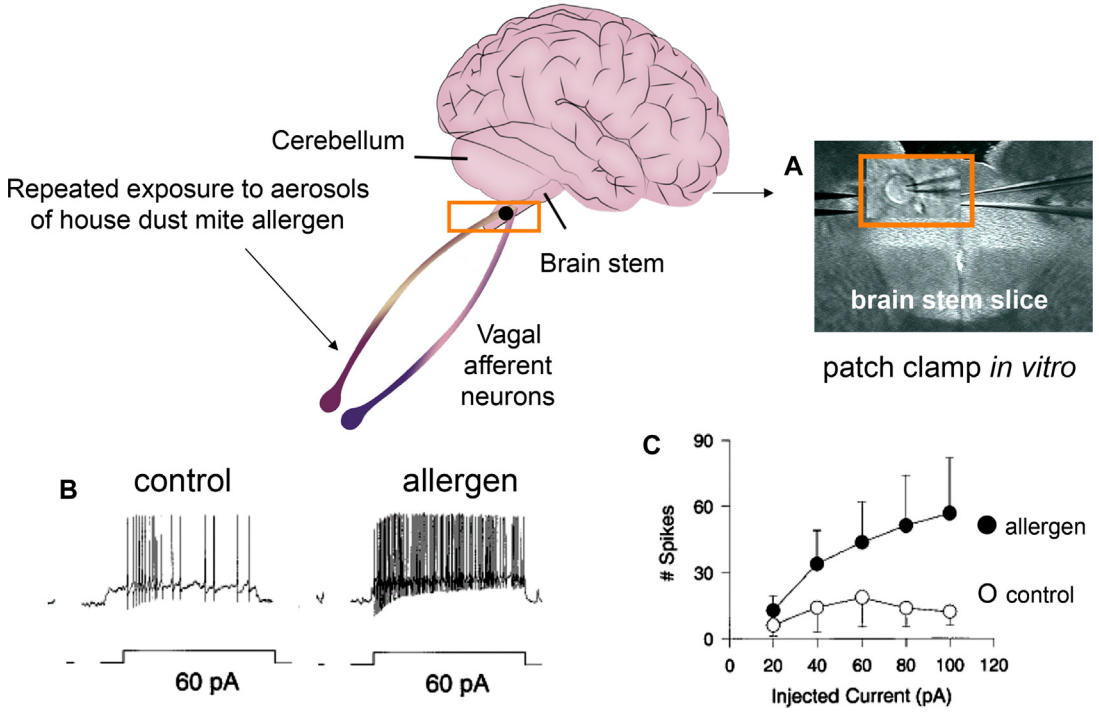

FIG 6. Allergen-induced increases in electrical excitability in CNS neurons. *Top,* Brainstem location of the nucleus of the solitary tract. **A,** Photomicrograph of a patch-clamped neuron in a brain slice from the caudomedial nucleus of the solitary tract, which is where the vagal sensory afferents terminate. **B,** Whole-cell patch-clamp recordings of depolarizing current pulses applied to individual nucleus of the solitary tract neurons from naive or allergen-challenged (dust mite) rhesus monkeys. **C,** Increased action potential discharge in response to a depolarizing stimulus in neurons isolated from allergic monkeys.[70]

After allergen activation of nearby mast cells, the synaptic efficacy is enhanced and the filtering capacity of the ganglion is lost (Fig 7).[71,74-76] The postganglionic neurons in these ganglia provide the dominant control of smooth muscle tension not only in the larger airways but also all the way down the airway tree to the small bronchioles. Therefore changes in filtering that can occur secondary to mast cell activation in the larger airways could have a major effect on airway caliber throughout the bronchial tree. In mice this mast cell–parasympathetic cholinergic nerve interaction is responsible for almost all of the allergen-induced airway constriction.[77,78]

Unlike parasympathetic ganglia, sympathetic ganglia are large and very amenable for the study of synaptic transmission. Within minutes of allergen-induced activation of mast cells in the superior cervical ganglion or celiac ganglion, there is a pronounced increase in the synaptic efficacy, often leading to a doubling of the postganglionic output.[75,79] The potentiation of synaptic efficacy by mast cell activation is not a short-lived event. As with the increase in excitability of afferent nerve terminals, allergen-induced synaptic potentiation can persist for many hours after acute activation of the mast cells. An example of the difference in duration of the increase in synaptic efficacy versus histamine release in the sympathetic ganglion is shown in Fig 7.

Of particular relevance to food allergy, immunologic activation of mast cells in the gut is commonly associated with alterations in neurotransmission within the enteric ganglia, thereby altering the activity of submucosal secretomotor neurons.[4,6] Mast cells are particularly rich in the human submucosal plexus compared with the myenteric plexus.[11] Mediators released from IgE-dependent activation of human intestinal mast cells directly excites enteric neurons in the submucosa of guinea pigs and human subjects (Fig 8).[80,81] A guinea pig model of food (milk) allergy elegantly reveals that allergenic activation of mast cells increases and modulates the synaptic efficacy of submucosal neurons in the colon and small intestine (Fig 8).[81,82]

The mediators and ionic mechanisms underlying the increase in autonomic and enteric synaptic activity are numerous and dependent on which specific ganglion is studied. Histamine is a particularly promiscuous mediator when it comes to modulating synaptic activity in the ganglia, with all 4 histamine receptors potentially being involved. However, it is clear that histamine is not the sole culprit. Many allergy-associated mediators have been shown to alter autonomic and enteric transmission, including mast cell mediators, such as serotonin, PGD_2, platelet-activating factor, and tryptase.[6,17,73,79,83,84] Allergen-induced increase in synaptic transmission can occur through both presynaptic and postsynaptic mechanisms of action. There is evidence that mast cell activation leads to more neurotransmitter (typically acetylcholine) released from the presynaptic terminals per given amount of stimulus.[85] The evidence also supports the hypothesis that the neurotransmitter in the synapse stimulates a more excitable postsynaptic neuron because of a general increase in the neuron's resistance and electrical excitability state.[4,76,85] Irrespective of the ionic mechanisms, the relatively consistent finding in studies on synaptic transmission is one in which the action potential discharge for a given amount of presynaptic input is persistently enhanced. In susceptible subjects this may be heightened to the extent that serious symptoms occur (eg, severe bronchospasm, mucus secretion, gastrointestinal cramping and pain, diarrhea, and dysregulation of motility).

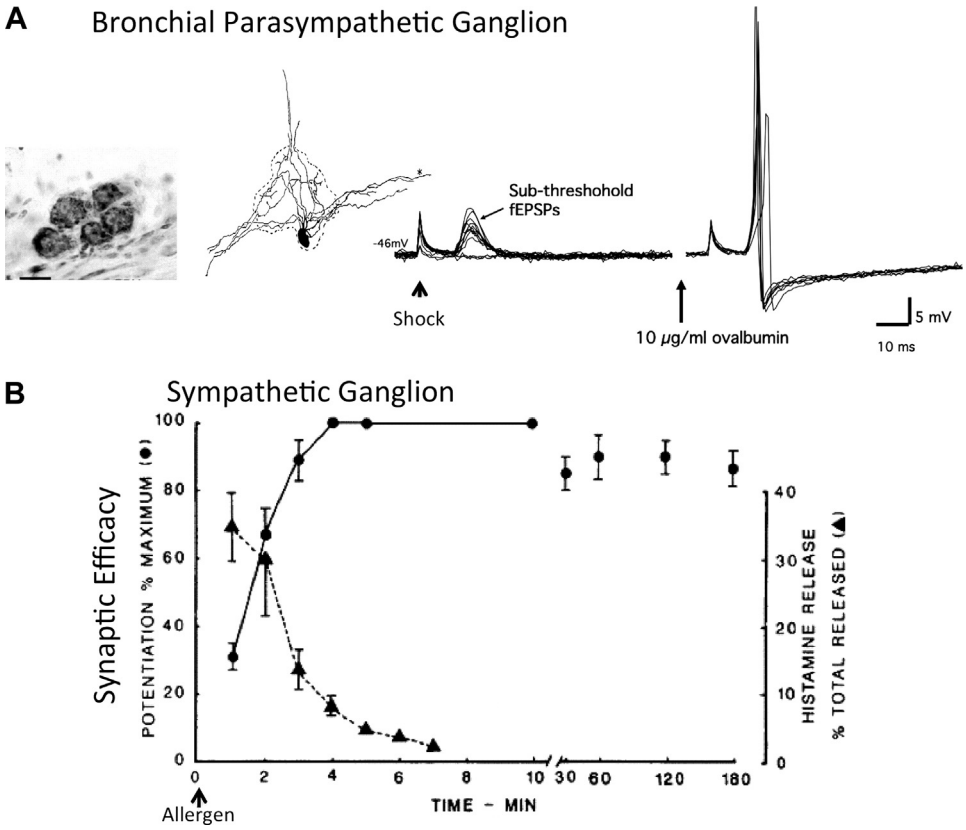

FIG 7. Allergen-induced increases in synaptic efficacy in autonomic ganglia. **A,** Small parasympathetic bronchial ganglion in the guinea pig bronchus. A single neuron filled with horseradish peroxidase and drawn by using the camera lucida technique is shown. The parasympathetic neuron's synaptic efficacy in response to stimulation of preganglionic nerves (shock artifact) is greatly increased after allergen (ovalbumin) exposure.[76] *fEPSPs,* Fast excitatory postsynaptic potentials. **B,** Time course of histamine release and superior cervical ganglia synaptic efficacy (postganglionic compound action potential magnitude) during allergen (ovalbumin) challenge.[75]

Allergen challenge can also lead to an increase in autonomic neurotransmitter release from the postganglionic peripheral terminals per a given amount of stimulus. This has been observed with postganglionic parasympathetic cholinergic nerve terminals innervating airway smooth muscle and has been attributed to an eosinophil-mediated decrease in the muscarinic M2 receptor function on the nerve terminals.[86,87] These M2 receptors are inhibitory "autoreceptors" and serve to limit the release of acetylcholine through a negative feedback loop.

Phenotypic changes in autonomic nerves. As with the primary afferent nerves, there is evidence of allergen-induced phenotypic changes in autonomic neurons. As discussed above, autonomic nerves innervating the airways are mainly parasympathetic. The parasympathetic branch supplies both contractile (cholinergic) control of airway smooth muscle and relaxant (nitrergic/vasoactive intestinal peptide) control. In guinea pigs repeatedly challenged with allergen inhalation, there was a phenotypic switch, such that the vasoactive intestinal peptide/nitric oxide neurons began to change to a cholinergic phenotype synthesis.[88] In other words, the balance between contractile versus relaxant innervation was being altered in favor of the former. This is likely explained by the allergen-induced production of neurotrophins in the airways, with neurotrophin 3 being a potential culprit.

Concept of modulation in critical periods

Up to this point, the preclinical research in support of our discussion has been carried out in adult or young adult animals. The allergen-induced neuromodulation is often quite profound but would be expected to last in the timeframe of minutes to weeks. At the risk of ending this review on an overly speculative note, it is worth considering a potential role of "critical periods" in allergen-induced neuromodulation because these changes can persist for years or even a lifetime.

It is well established that the development of sensory systems often requires use-dependent activity early in life.[89] During postnatal sensory nerve development, there is a defined window of time during which the nerves are susceptible to this experience-dependent plasticity. Before or after the defined window of time, the same experience does not alter neuronal development. Thus, for example, if a young animal is deprived of vision by lid closure (or various other techniques), changes occur in the neural circuitry of the visual cortex, leading to severe and permanent loss in visual acuity.[90] This only occurs if the vision deprivation occurs during a critical period of time. Even prolonged vision deprivation after the critical period is without an effect on visual acuity. The exact time of the critical period depends on the system studied. Since these pioneering studies, critical periods have been defined in audio and somatosensory

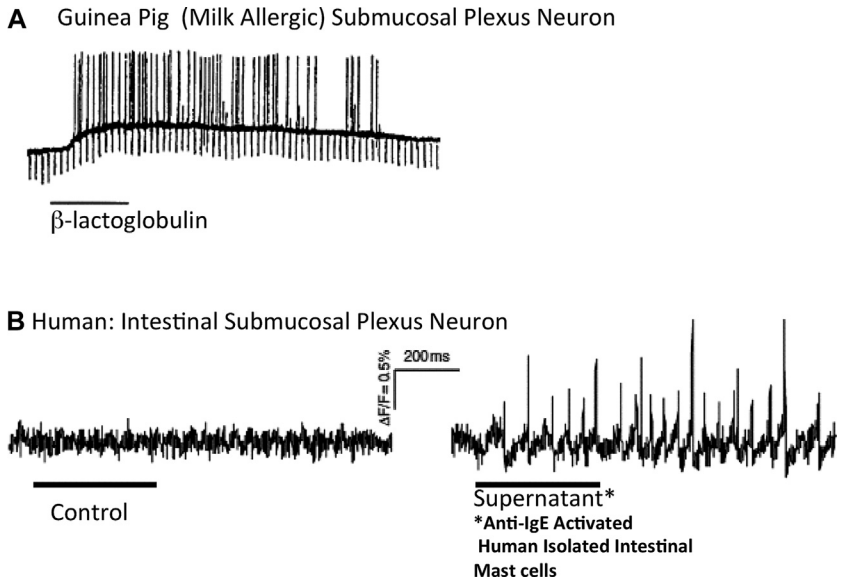

FIG 8. Allergen-induced stimulation of enteric neurons. **A,** Intracellular recording of a neuron in the submucosal plexus isolated from a guinea pig shows neuronal depolarization to action potential threshold after milk allergen challenge.[81] **B,** Action potential recording from a neuron in the submucosal plexus of the human intestine. Action potentials were evoked in response to treatment with the supernatant solution of anti-IgE–activated human intestinal mast cells. Used with permission from Schemann et al.[80]

systems and have been noted in virtually all species from human subjects to songbirds to *Drosophila*.[89]

Recent reports are supporting the concept that inflammation-dependent sensory nerve plasticity during early-life critical periods can lead to persistent changes in somatosensory and vagal-sensory neural circuits. Ruda et al[91] inflamed one hind paw of rat pups using the complete Freund adjuvant model. The pups exhibited stereotypical behavior, indicating pain in the paw. More importantly, there was a substantial increase in the density of primary afferent nerves in the ipsilateral dorsal horn of the spinal cord. This change in the density of neuronal circuits in the CNS persisted beyond the inflammation and lasted into adulthood. Behavioral studies revealed that adult rats that experienced paw inflammation as neonates were significantly more hyperalgesic in response to subsequent inflammatory stimuli than control rats. The authors concluded that "peripheral inflammation experienced during neonatal periods has long-standing consequences on nociceptive neuronal circuitry."

Similar findings are being made with respect to visceral neural hypersensitivity. It has long been known that inflammation of the colons of laboratory animals leads to a neuronal hypersensitivity and an exaggerated and abnormal reflex physiology of the gut (somewhat analogous to the airway hyperreactivity of asthma). When the colons of rats were chemically or mechanically irritated 8 to 21 days postnatally, an inflammatory response was evoked that led to afferent nerve hyperexcitability and a state of heightened visceral reflexia. These changes in sensory nerve hyperexcitability persisted beyond the gut inflammation, lasting for at least 3 months (the longest time point analyzed). By contrast, if the colons of rats were inflamed after postnatal day 21 (beyond the critical period), the hyperreflexia was not persistent.[92] Likewise, exposure of animals to cigarette smoke during a postnatal critical period is associated with persistent increases in airway hyperreactivity and sensory nerve density.[93] Thus in both the somatosensory pain model and the visceral-sensory model of hyperreflexia, inflammation during a critical period in postnatal development caused abnormalities in the sensory neurocircuitry that persisted perhaps into adulthood.

The reason this might be germane to allergic inflammation in early life is that neurotrophins have been implicated in several models of allergic inflammation (as well as viral infections) and that critical period sensory nerve plasticity is dependent on the production of neurotrophic factors.[94,95] Allergic (or infectious) inflammation in critical periods therefore raises the possibility that the inflammatory response might leave behind a nervous system that is subtly altered many years later, such that a mild inflammatory insult could lead to overly exaggerated responses.

CONCLUSIONS

Among the constellation of symptoms that characterize the allergic reaction, many, if not most, are secondary to changes in the nervous system. Depending on the organ in which the reaction occurs, these neuron-based symptoms, include tearing, ocular irritation and vasodilation, rhinorrhea, nasal congestion, sneezing, persistent urge to cough, chest tightness, bronchospasm, airway secretions, dysphagia, changes in gastrointestinal motility, itching, and wheal-and-flare reactions. In this sense allergy is an immune-neuronal disorder.

Activation of mast cells and the consequent eosinophilic T_H2-driven inflammation can lead to profound alterations in the function of afferent neurons, neurons within the CNS, and neurons in sympathetic, parasympathetic, and enteric ganglia. These alterations comprise acute overt activation of nerves, long-lasting increases in their excitability, and even longer-lasting phenotypic changes in the nervous system.

More than the fact that those with allergy produce neuroactive mediators at sites of allergic inflammation, it would appear that the nervous system itself is altered in allergic disease. Whether because of events occurring during critical periods in neuronal

development or simply because of persistent nerve activation, the nervous system is rendered hyperactive in many patients with allergic disease. In a clinical setting this altered neuronal state is readily observed when a concentration of a neuroactive mediator that normally has no effect in healthy subjects leads to sensations and reflexes in those with allergy. This is readily apparent, for example, in patients with allergic rhinitis: a concentration of a C-fiber stimulant (eg, bradykinin or capsaicin) that has no effect when applied to the nasal mucosa of a healthy subject causes strong sneezing and reflex parasympathetic cholinergic contractions when applied to someone with allergic rhinitis.[96] Shusterman et al[97] found that nasal exposure to low levels of chlorine gas leads to stronger responses in patients with seasonal allergic rhinitis than in healthy control subjects. These data support the clinical observations that patients with allergic rhinitis commonly complain of exaggerated nasal symptoms induced by various strong odors and smoke, as well as by changes in atmospheric conditions. In a questionnaire delivered as part of a screening protocol in 350 patients with allergic rhinitis, 60% listed smoke as one of the triggers of their nasal symptoms, 58% listed other irritants, and 47% listed cold air. These percentages were even higher in the volunteers with perennial versus seasonal allergic rhinitis.[98]

At present, our understanding of the specific mediators, ion channels, and signaling pathways involved in allergen-induced neuromodulation remains at a relatively rudimentary state. It can be anticipated that as our understanding of these basic mechanisms continues to evolve, new therapeutic strategies that target the nervous system will continue to emerge that, by working synergistically with anti-inflammatory strategies, will serve to quell the suffering of those with the immune-neuronal disorder we refer to as allergy.

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