

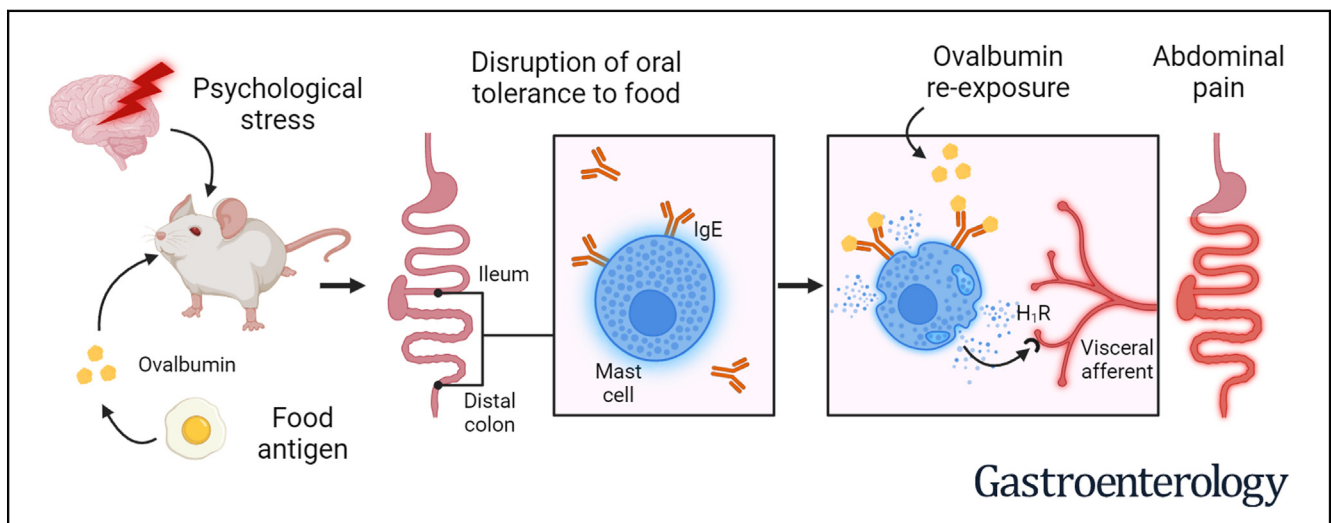
INTESTINAL INFLAMMATION

Psychological Stress-Induced Local Immune Response to Food Antigens Increases Pain Signaling Across the Gut in Mice



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BACKGROUND & AIMS: We recently showed that a bacterial infection can break oral tolerance to food and lead to immunoglobulin E (IgE)-dependent mast cell activation and food-induced abdominal pain, which could constitute an important pathogenic mechanism in postinfectious irritable bowel syndrome (IBS). Here, we investigated whether similar immune mechanisms in response to psychological stress lead to food-evoked pain signaling, and thus potentially explain the pathophysiology in a larger group of patients with IBS. **METHODS:** Mice were exposed to ovalbumin (OVA) during water avoidance stress (WAS) and re-exposed to OVA 5 weeks later. Nociception was evaluated by visceromotor responses and afferent nerve recordings to intestinal distension, and patch-clamp recordings of sensory neurons incubated with intestinal supernatants. The role of IgE and type 2 immunity was evaluated using pharmacologic and genetic approaches. **RESULTS:** Re-exposure to OVA increased pain signaling in the colon and small intestine only in mice exposed to OVA during WAS, in the absence of systemic allergy. OVA-induced increases in pain responses depended on mast cells, IgE, and signal transducer and activator of transcription 6 signaling. Notably, incubation of sensory neurons with ileum and colon supernatants from WAS/OVA+OVA mice lowered their threshold of excitability. Finally, treatment with histamine receptor H₁ antagonist pyrilamine blocked the increased

sensory neuron excitability, and reduced ileal afferent nerve firing to distension in WAS/OVA+OVA mice. **CONCLUSIONS:** Psychological stress induces a type 2 immune response to food antigens, with IgE-mediated mast cell activation and increased pain signaling in the small intestine and colon in response to food. These findings may explain the potential role of psychological stress in food-induced symptoms in IBS.

Keywords: Irritable Bowel Syndrome; Psychological Stress; Abdominal Pain; IgE; Mast Cells.

*Authors share co-first authorship; §Authors share co-senior authorship.

Abbreviations used in this paper: ANOVA, analysis of variance; CRH, corticotropin-releasing hormone; DRG, dorsal root ganglia; GI, gastrointestinal; H₁R, histamine receptor H₁; HPA, hypothalamic-pituitary-adrenal; IBS, irritable bowel syndrome; IgE, immunoglobulin E; IL, interleukin; OVA, ovalbumin; STAT6, signal transducer and activator of transcription 6; Tc, T cytotoxic; Th, T helper; Treg, T regulatory; VHS, visceral hypersensitivity; VMR, visceromotor response; WAS, water avoidance stress.

Most current article

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Irritable bowel syndrome (IBS) is a common gastrointestinal disorder^{1,2} characterized by abdominal pain and altered bowel habits and leads to a significant impairment in quality of life.³ Of these symptoms, patients report that abdominal pain has the greatest impact.⁴ Although the pathogenesis of this disorder remains poorly understood, alterations in gut-brain axis signaling, dysbiosis, and abnormal visceral pain signaling are among the most commonly proposed mechanisms. There is compelling evidence that an aberrant intestinal immune activation causing activation of mast cells is involved.^{5–8} Mast cell mediators can activate and/or sensitize intestinal afferent nerves leading to visceral hypersensitivity (VHS) in preclinical models of visceral pain.^{7–9} Furthermore, treatment of patients with IBS with the histamine receptor H1 (H1R)-antagonist ebastine increased symptom relief and reduced abdominal pain scores in these patients.^{9,10} What leads to this immune activation is unclear but common triggers of IBS symptoms, namely, diet and psychological factors, may play a role.

Diet is one of the most common triggers reported by patients with IBS.^{11,12} Although systemic food allergy—defined by the presence of circulating food-specific immunoglobulin E (IgE)—is poorly associated with food-related abdominal pain,^{13,14} several other neuroimmune mechanisms have been suggested to explain how food ingestion evokes symptoms. For example, bioactive compounds and food chemicals (such as food additives and metals) can activate the immune system leading to an increased pain sensitivity.¹⁵ Furthermore, fermentable oligo-disaccharides, and polyols, poorly digested carbohydrates that are fermented by the gut microbiota, can trigger abdominal pain potentially through a microbiota-immune signaling pathway.^{16,17} We have recently shown in a preclinical model that a self-limiting bacterial infection in the colon can lead to loss of local tolerance to a food antigen.⁸ Re-exposure to this food antigen resulted in VHS via IgE-mediated activation of colonic mast cells, in the absence of systemic allergy. Moreover, patients with IBS had an exaggerated mucosal edematous response to food antigen injection into the rectal mucosa,⁸ suggesting that loss of oral tolerance is a major mechanism of food-induced symptoms in patients with IBS. Whether other triggers lead to loss of oral tolerance and may involve mast cells more diffusely in the gastrointestinal (GI) tract, as is observed in IBS, remains unknown.

Importantly, many studies have identified psychological stress as a key risk factor for IBS.^{18–21} In both humans and rodents, activation of corticotropin-releasing hormone (CRH) signaling pathways underlies various biological components of the stress response, including the development of visceral pain.^{22–24} However, a randomized double-blind, placebo-controlled clinical trial found that a selective CRH receptor 1 antagonist had no significant effect on abdominal pain in patients with IBS,²⁵ suggesting that other pathways may be more relevant in explaining the role of psychological stress in chronic

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Although the link between stress and irritable bowel syndrome is well-recognized, the mechanisms by which stress triggers food-induced abdominal pain in these patients remain uncertain.

NEW FINDINGS

Prolonged psychological stress can disrupt the development of oral tolerance to dietary antigens and lead to IgE-mediated and mast cell-induced visceral hypersensitivity on subsequent antigen ingestion.

LIMITATIONS

This is a preclinical animal study investigating the effect of psychological stress on food-induced visceral pain and our observations need to be validated in clinical settings and/or using patient-derived tissue.

CLINICAL RESEARCH RELEVANCE

The findings in this study suggest that the loss of oral tolerance to dietary antigens may be responsible for abdominal pain after meal intake in a broader spectrum of patients beyond just those with postinfectious irritable bowel syndrome.

BASIC RESEARCH RELEVANCE

Our study provides new evidence that not only an enteric infection but also psychological stress can trigger a type 2 immune response directed against dietary antigens leading to increased pain signaling in the small intestine and colon in response to food ingestion.

abdominal pain in these patients. Importantly, the relationship between stress and GI symptoms triggered by food intake remains to be elucidated. Our recent post-infectious food antigen IBS model demonstrated that an infection confined to the colon can lead to production of local IgE antibodies without affecting the small intestine.⁸ In contrast, psychological stress can disrupt homeostasis along the entire GI tract,²⁶ consistent with the common finding that mast cells are activated within the small intestine, in addition to the colon.²⁷ Given that post-infectious IBS only represents a relatively small subgroup of patients with IBS,²⁸ we hypothesized that psychological stress may also break oral tolerance and cause a mast cell-dependent increase in pain signaling within both the small intestine and the colon.

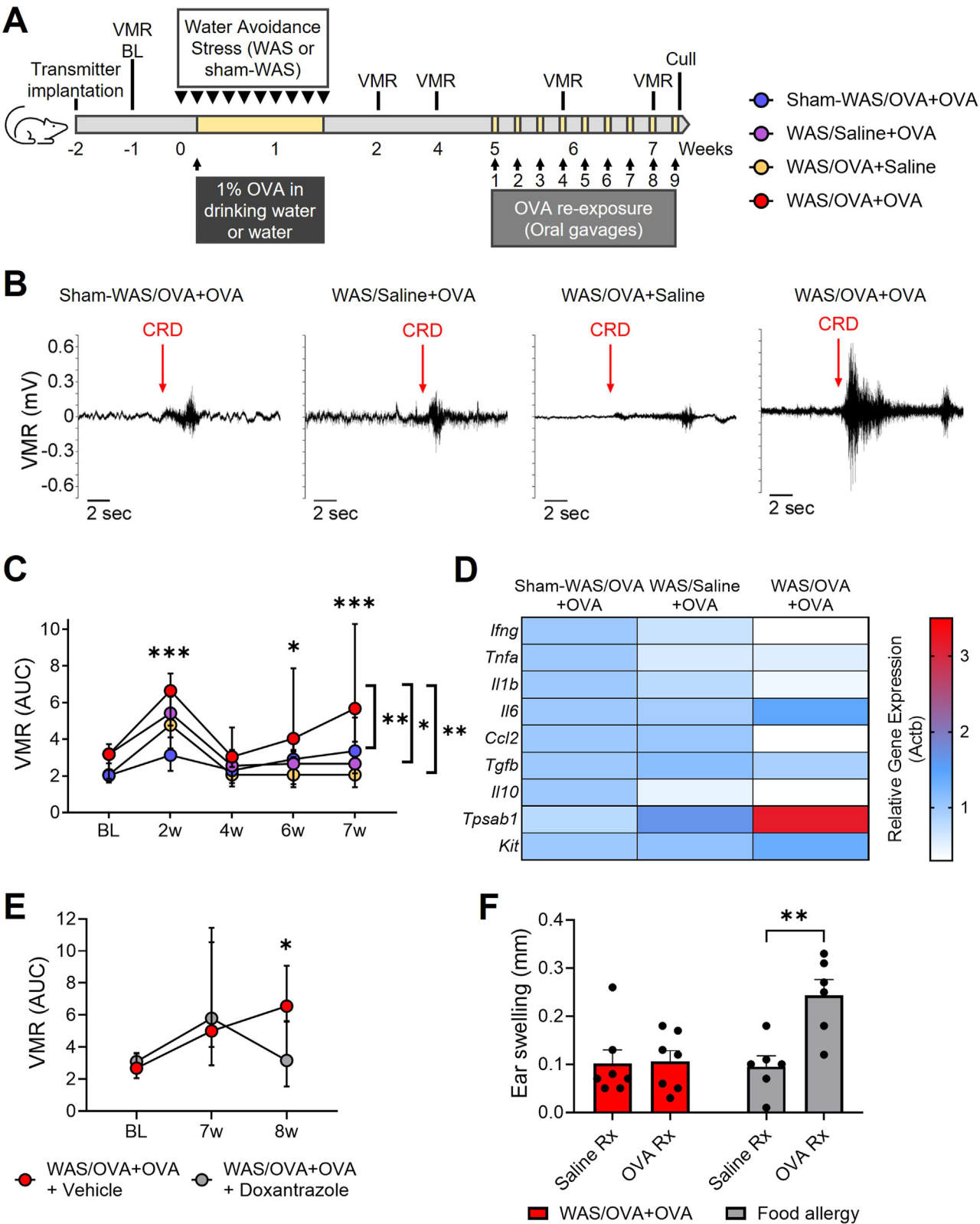
Methods

Animals

All studies were approved by the Queen's University Animal Care Committee (approval number 2019-1966) in accordance with guidelines from the Canadian Council of Animal Care, and the Animal Care and Animal Experiments Committee of the Medical Faculty of the KU Leuven in accordance with guidelines from the European Community Council of Animal Care. BALB/c

mice (males and females, 6- to 10-weeks old) were obtained from Charles River, The Jackson Laboratories (Queen's University) or Janvier Laboratories (KU Leuven). Experiments concerning visceromotor responses (VMR) were performed

only in males. While males were predominantly used throughout the study, females were used for some ex vivo afferent nerve recording experiments using wild-type mice as indicated in the figure legends.



Water Avoidance Stress and Ovalbumin Exposure

BALB/c mice were randomly assigned to experimental groups and exposed to water avoidance stress (WAS) or sham stress (sham-WAS) for 1 hour daily for 10 days, as previously described.²⁹ During WAS, mice were placed on a dry 5-cm diameter platform in the center of a water bucket for 1 hour. Sham-WAS mice were placed under similar conditions but in the absence of water. On day 2, mice were administered 50 mg ovalbumin (OVA; grade II, Sigma-Aldrich) dissolved in 200 μ L 0.9% NaCl (saline) by oral gavage. Mice gavaged with ovalbumin also received 1% OVA (grade II) in the drinking water, replaced daily, during days 2–10. Five weeks after the first day of WAS or sham-WAS, mice were re-exposed to either OVA (50 mg) or saline by gavage every other day for 2 weeks (from day 35–49), yielding 4 study groups: WAS/OVA+OVA, WAS/OVA+saline, WAS/saline+OVA, and sham-WAS/OVA+OVA (Figure 1A).

VMR to Colorectal Distension

Mice of at least 20 g were implanted with Physiotel ETA-F10 telemetric transmitters (Data Sciences International). Transmitters were inserted in the abdominal cavity of anaesthetized mice (20 mg/kg ketamine [Nimatek, Eyurovet Animal Health B.V.]; and 1 mg/kg xylazine [Rompun 2%, Bayer]), and electrodes were tunneled through the abdominal wall and sutured into the left external abdominal oblique muscle. No wiring remained externalized, thus allowing for group housing during the entire WAS/OVA protocol. Colorectal distensions (volumes from 20–80 μ L) were performed using a distension catheter (Fogarty catheter for arterial embolectomy, 4F; Edwards Lifesciences) to evoke abdominal cramping as a read-out of visceral pain. Colorectal distensions were performed in freely moving animals. Data were presented as area under the curve (median $\pm$ interquartile range) of the VMR.³⁰ Colonic compliance was evaluated in mice submitted to VMR recordings to discard inflammation-mediated changes in wall stiffness that could influence nociception. No differences in colonic compliance were observed in mice submitted to different treatments (Supplementary Figure 1).

Ex Vivo Ileal Afferent Nerve Recordings

Segments of distal ileum (2–3 cm) along with attached mesentery were rapidly excised and placed into a recording

chamber continuously superfused (10 mL/min; 34°C) with gassed (5% CO₂ and 95% O₂) Krebs solution (composition, in mmol/L: NaCl, 118.4; NaHCO₃, 24.9; MgSO₄, 1.2; KH₂PO₄, 1.2; glucose, 11.7; CaCl₂, 1.9) supplemented with the L-type calcium channel blocker nifedipine (3 μ mol/L) and the muscarinic acetylcholine receptor antagonist atropine (5 μ mol/L) to suppress smooth muscle activity, as well as the cyclooxygenase inhibitor indomethacin (3 μ mol/L) to suppress potential inhibitory actions of endogenous prostaglandins.³¹

Preparations were cannulated with one end connected to an infusion pump to allow luminal perfusion of Krebs solution (0.2 mL/min) while the other end was connected to a pressure transducer (NL108; Digtimer, UK). Nerves were dissected from the perivascular mesenteric bundles and multiunit activity was recorded using borosilicate glass suction electrodes prefilled with Krebs buffer and attached to a head-stage (NL104; Digtimer, UK). Afferent nerve signals were amplified (NL104), filtered (NL125 band pass filter), and sampled at a rate of 10 kHz via a Micro 1401 interface and Spike2 software (Version 7, Cambridge Electronic Design, UK).

Following a 30-minute equilibration period, segments were distended up to 60 mm Hg by occluding luminal perfusion outflow of the cannulated tissue. The drain was then opened, returning the pressure to baseline. The afferent nerve response to ramp distension was assessed by plotting the firing frequency during distension using a 10-second bin width followed by using a custom-made script in Spike2 that obtained the frequency for each 1 mm Hg increase in pressure. Analysis was performed offline using the Spike sorting function of Spike2 software.

Perforated Patch Clamp Recordings

Dorsal root ganglia (DRG) of naïve BALB/c mice (8–12 weeks) from T9–T13 were dissected and collected on Hank's balanced salt solution. DRGs were enzymatically (60 U papain, 10 mg collagenase type 2, Worthington; 12 mg dispase II, Roche) and mechanically dissociated in F-12-supplemented media (10% fetal bovine serum, penicillin/streptomycin [100 U/100 μ g/mL]).³² Then, cell suspension was plated on Poly-D lysine/laminin (20 μ g/mL/20 μ g/mL)-coated coverslips and placed at 37°C (5% CO₂) for 2 hours before being incubated overnight with F-12-supplemented media and ileum or colon supernatants. The H₁R antagonist pyrilamine (Sigma-Aldrich) was incubated 1 hour before addition of supernatant where indicated.

Figure 1. Mast cell activation mediates increased colon pain signaling induced by OVA re-exposure in WAS/OVA+OVA mice. (A) Schematic of protocol indicating when VMR recordings were conducted during protocol timeline. BALB/c mice were exposed to WAS or sham-WAS for 10 days. *Inverted triangles* indicate WAS or sham-WAS. Mice were given 1% OVA in the drinking water or water from days 2–10. *Arrows* indicate OVA or saline exposure by oral gavage. During re-exposure phase OVA was given every other day. (B) Representative recordings of VMR to colorectal distension in the different groups. *Red arrows* indicate the beginning of the colorectal distension (CRD). (C) OVA re-exposure increased VMR to CRD (measured as area under the curve [AUC]) only in WAS/OVA+OVA mice (sham-WAS/OVA+OVA $n = 9$; WAS/saline+OVA $n = 9$; WAS/OVA+saline $n = 8$; WAS/OVA+OVA $n = 13$ mice; $P = .0013$, 2-way repeated ANOVA with Sidak post hoc test). (D) Relative gene expression of cytokines measured using reverse transcription-quantitative polymerase chain reaction in colon samples (sham-WAS/OVA+OVA $n = 8$; WAS/OVA+saline $n = 8$; WAS/OVA+OVA $n = 6$ mice). Expression of *Tpsab1* was higher in WAS/OVA+OVA mice ($P = .0167$; 1e-way ANOVA with Dunnett post hoc test). (E) Mast cell stabilizer doxantrazole decreased the VMR to CRD in WAS/OVA+OVA mice ($n = 6$ mice/group; adjusted $P = .0494$, 2-way repeated ANOVA with Sidak post hoc test). (F) Intradermal injections of saline (right ear) or OVA (1 μ g, left ear) in WAS/OVA+OVA mice did not induce ear swelling ($n = 7$ mice, $P = .9914$, 2-way repeated ANOVA with Sidak post hoc test) whereas OVA injection significantly increased ear swelling compared with saline injection in food allergy mice ($n = 6$ mice, $P = .0021$, 2-way repeated ANOVA with Sidak post hoc test).

All experiments were performed the following day at room temperature.³³ Neuronal excitability was assessed using perforated patch-clamp with amphotericin B (0.24 mg/mL) by recording the rheobase (minimum amount of current required to elicit an action potential) on cells with a membrane capacitance ≤ 30 pF and a resting membrane potential more negative than -40 mV.^{34,35} Borosilicate glass capillaries (Warner Instruments) were pulled (PC-100 Puller, Narishige) and fire-polished to obtain pipettes for patch-clamp with a resistance of 3–5 M Ω in bath solution. Signals were amplified using Multiclamp 700B or Axopatch 200B amplifiers, digitized by Digidata 1440A or Digidata 1550B AD converters, and recorded using pClamp 11.1 software (Molecular Devices).

Statistical Analysis

Graph Pad Prism 10.1.1 was used for statistical analyses. Results are expressed as mean $\pm$ standard error of the mean or median $\pm$ interquartile range. Data was analyzed using unpaired *t* test or 1-way, 2-way, or 2-way repeated analysis of variance (ANOVA) with Dunnett or Sidak post hoc test as indicated. *P* < .05 was considered significant.

Further detailed methods are included in the [Supplementary Methods](#).

Results

Psychological Stress Triggers Food Antigen–Dependent Mast Cell Activation and VHS

We previously reported that a self-limiting bacterial infectious colitis can break tolerance to food antigens to evoke increased pain signaling in the colon.⁸ First, to investigate whether psychological stress could also trigger an aberrant immune response to food antigens leading to increased visceral pain within the colon, we exposed mice to the food antigen OVA (OVA) for 9 days, starting from the second day of a 10-day period of repeated water avoidance stress (WAS/OVA). In a second phase (weeks 5–7), mice were re-exposed to OVA via oral gavage in the absence of WAS ([Figure 1A](#)). Although all animals exposed to WAS, but not sham-WAS, displayed VHS on colorectal distention independent of OVA exposure, this increased VMR response returned to baseline levels by 4 weeks. Notably, re-exposure to OVA evoked VHS (weeks 6–7) only in mice previously exposed (weeks 1–2) to WAS/OVA (ie, WAS/OVA+OVA mice), but not in any other group ([Figure 1B and C](#)). Gene expression in colonic samples revealed up-regulation of mast cell–restricted tryptase α/β -1 gene (*Tpsab1*) in WAS/OVA+OVA mice ([Figure 1D](#)), suggesting increased mast cell activation. To further examine the role of mast cells, in vivo administration of the mast cell stabilizer doxantrazole (16.5 mg/kg) normalized VHS to colorectal distention in WAS/OVA+OVA mice, compared with vehicle treatment ([Figure 1E](#)). Importantly, no differences were observed in ear swelling in WAS/OVA+OVA mice after intradermal ear injection of OVA or saline ([Figure 1F](#)), excluding the development of systemic allergy to OVA in WAS/OVA+OVA mice. Taken together, these results suggest that psychological stress can provoke local mast cell activation in response to a food antigen and lead to increased visceral pain perception.

Food Antigen Re-Exposure Increases Pain Signaling in the Small Intestine

In our previous study, we demonstrated that a bacterial infection with *Citrobacter rodentium*, known to mainly affect the large intestine, resulted in the generation of OVA-specific IgE antibodies restricted to the infected region.⁸ However, as psychological stress leads to systemic immunometabolic responses,^{36,37} we reasoned that psychological stress would affect the entire GI tract and could potentially explain increased pain responses demonstrated in the small intestine of IBS patients.^{38–40} Thus, we next investigated whether OVA re-exposure could also increase pain signaling in the small intestine in WAS/OVA+OVA mice ([Figure 2A](#)). To this end, we performed ex vivo extracellular recordings to measure the activity of afferent nerves innervating the ileum in response to ileal distension. Ileal nerves in WAS/OVA+OVA showed increased firing in response to distension compared with mice exposed to sham stress, or mice exposed to OVA only during the first phase or during the second phase ([Figure 2B and C](#)). When analyzing the afferent nerve subtypes, most fibers were low threshold and wide dynamic range fibers ([Supplementary Figure 2](#)), with the latter likely contributing to pain signaling in the GI tract.^{41–43} These results indicate that psychological stress indeed affects both the small and large intestine resulting in development of food-induced VHS.

Increased Pain Signaling in WAS/OVA+OVA Mice is Not Related to Changes in Immune Cell Populations

The development of OVA-induced VHS in WAS/OVA+OVA mice and absence of allergic-like responses ([Figure 1F](#)) suggested an immune response restricted within the gut. To investigate possible changes in intestinal immune cell populations, immune cells isolated from ileum and colon from the 4 groups of mice were stained with fluorophore-conjugated antibodies ([Supplementary Table 1](#)) and gated to identify the levels of neutrophils, eosinophils, monocytes, macrophages, mast cells, as well as B lymphocytes and cytotoxic (Tc) and helper T (Th) lymphocytes ([Figure 3A](#)). Flow cytometry analyses of these cell populations showed no differences between the groups ([Figure 3B and C](#)), indicating that the increased pain signaling in the small intestine and colon in WAS/OVA+OVA mice was not due to a change in global immune cell populations in these regions.

Increased Pain Signaling in WAS/OVA+OVA Mice is Mediated by IgE

Although we found no significant changes in intestinal immune cell populations, inhibition of mast cells during OVA re-exposure normalized the increased visceral pain perception in WAS/OVA+OVA mice ([Figure 1E](#)). Mast cells can be activated by antigen-induced crosslinking of IgE bound to the fragment crystallizable Epsilon receptor I expressed on the cell membrane.⁴⁴ To assess the role of IgE in the increased pain signaling observed in the ileum and

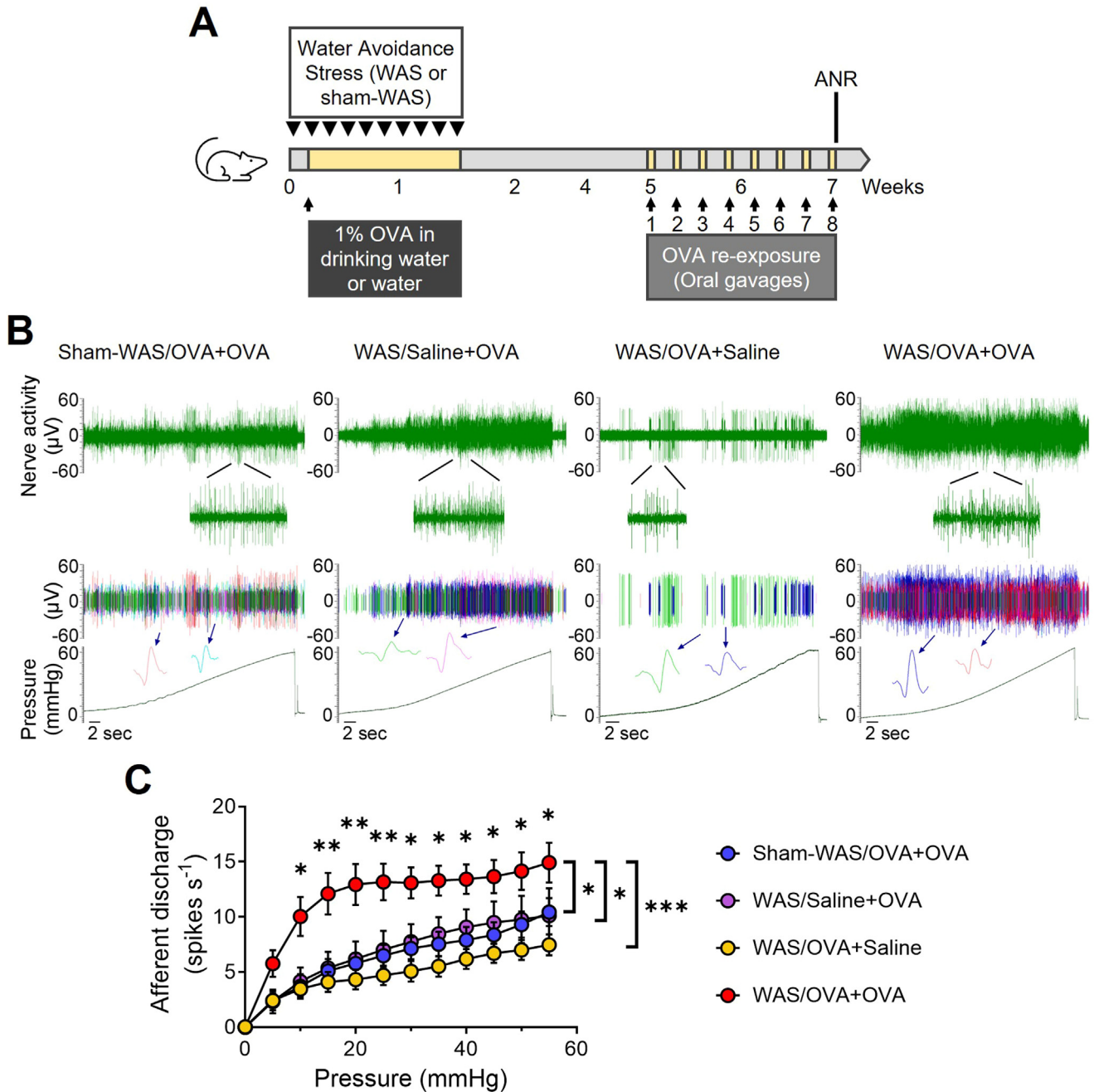
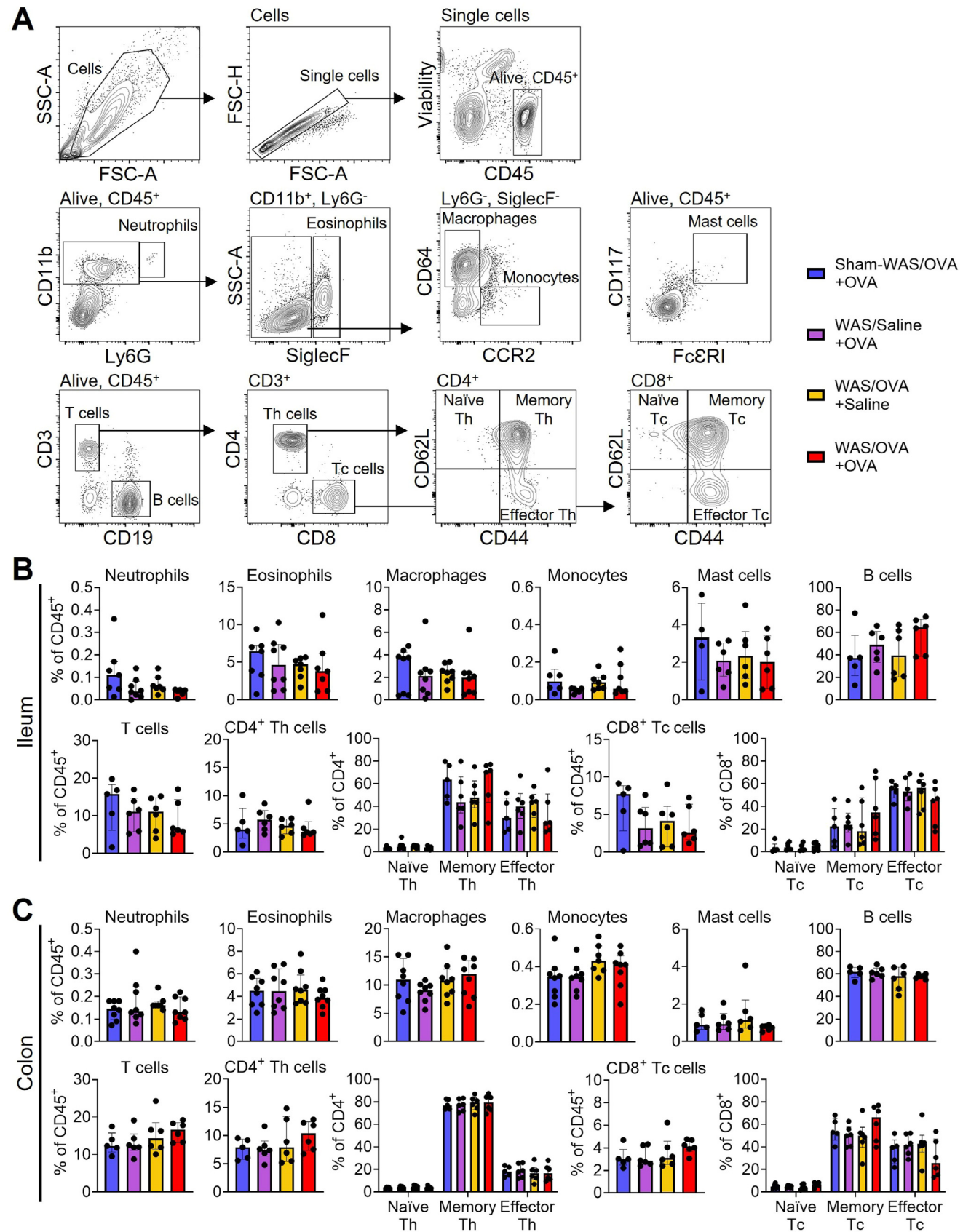


Figure 2. OVA re-exposure increased pain signaling in ileum of WAS/OVA+OVA mice. (A) Schematic of protocol. BALB/c mice were exposed to WAS or sham-WAS for 10 days. *Inverted triangles* indicate WAS or sham-WAS. Mice were given 1% OVA in the drinking water from days 2–10. *Arrows* indicate OVA or saline exposure by oral gavage. During re-exposure phase OVA was given every other day. (B) Representative recordings showing ileal afferent nerve response to distension in the different groups. (C) Ileal afferent nerve mechanosensitivity was significantly increased in WAS/OVA+OVA mice (sham-WAS/OVA+OVA $n = 5$, females; WAS/saline+OVA $n = 5$, females; WAS/OVA+saline $n = 7$, males; WAS/OVA+OVA $n = 7$, females; $P = .0012$, 2-way ANOVA with Dunnett post hoc test).

colon, WAS/OVA+OVA mice were treated with an anti-mouse IgE antibody (Figure 4A), which effectively blocks the interaction between IgE and fragment crystallizable Epsilon receptor I and prevents mast cell degranulation.⁴⁵ Interestingly, mice that received this treatment had decreased ileal afferent nerve discharge during distension

compared with mice treated with the isotype control antibody (Figure 4B and C, Supplementary Figure 3A). Similarly, WAS/OVA+OVA mice treated with the anti-IgE antibody showed a decreased VMR to colorectal distension compared with those treated with isotype control antibody (Figure 4D). Additionally, WAS/OVA+OVA mice with *Igh7*^{-/-}



genotype (IgE-deficient mice) also showed a decreased VMR to colorectal distension compared with wild-type WAS/OVA+OVA mice (Figure 4E), confirming the role of IgE-mediated mast cell activation in the increased visceral pain perception. Importantly, anti-IgE antibody treatment had no effect on the VMR of WAS/OVA+saline mice (Supplementary Figure 3B). The involvement of IgE signaling suggested a role for a type 2 immune response in the increased pain perception in WAS/OVA+OVA mice. To investigate this further, we used mice deficient for the transcription factor signal transducer and activator of transcription 6 (STAT6) (*Stat6*^{-/-}), the master regulator of type 2 immune responses required for IgE class-switching.⁴⁶ We observed that WAS/OVA+OVA *Stat6*-deficient mice showed decreased ileal afferent nerve response to distension compared with wild-type WAS/OVA+OVA littermates (Figure 4F, Supplementary Figure 4). Given the reduced nociceptive responses in mice treated with anti-IgE antibody and in *Stat6*-deficient mice, we assessed whether stress during the initial exposure to the food antigen sensitized the gut immune system toward a type 2 immune response on food antigen re-exposure. To this end, mesenteric lymph nodes were isolated from WAS/OVA+OVA mice after OVA re-exposure. Immune cells were cultured and incubated with OVA (200 μ g/mL) or vehicle and the type 2 cytokines interleukin (IL)4 and IL13 were measured. IL13 release was significantly increased in WAS/OVA+OVA mice compared with sham-WAS/OVA+OVA mice, whereas IL4 levels were increased albeit not statistically significant (Figure 4G). Together, these findings indicate that the increased pain signaling after OVA re-exposure involves a type 2 immune response that leads to mast cell activation via IgE signaling.

H₁R Antagonist Inhibits Increased Neuronal Excitability Induced by WAS/OVA+OVA Intestinal Tissue Supernatants

IBS tissue biopsy supernatants contain mast cell mediators that increase DRG neuron excitability.⁴⁷ Therefore, we investigated whether tissue supernatants from WAS/OVA+OVA mice would also excite DRG neurons. Overnight incubation of DRG neurons from naïve mice incubated with ileum supernatants from WAS/OVA+OVA mice decreased the rheobase (ie, increased the excitability) of these neurons compared with neurons incubated with supernatants from sham-WAS/OVA+OVA, WAS/saline+OVA or WAS/OVA+saline mice (Figure 5A and B). Similarly, colon supernatants from WAS/OVA+OVA mice also significantly

decreased the rheobase of DRG neurons compared with the other groups (Figure 5C). Notably, ileum (Figure 5D) or colon (Figure 5E) supernatants from WAS/OVA+OVA mice treated with anti-IgE antibody in vivo led to lower DRG neuron excitability than supernatants from WAS/OVA+OVA mice treated with isotype control antibody. Likewise, naïve DRG neurons incubated with ileum and colon supernatants from WAS/OVA+OVA *Stat6*-deficient mice were also less excitable compared with neurons incubated with supernatants from wild-type WAS/OVA+OVA littermates (Figure 5F and G). Taken together, these results indicate that, after OVA re-exposure in WAS/OVA+OVA mice, neuroactive mediators are released in both the small intestine and colon leading to an increase in neuronal excitability. Furthermore, inhibition of IgE and type 2 immune signaling (STAT6 signaling pathway) can prevent the release of these mediators.

Inhibition of the H₁R has been associated with decreased pain signaling in patients with IBS.⁹ To specifically assess whether blocking the H₁R was involved in the increased DRG neuronal excitability induced by ileal and colonic tissue supernatants, DRG neurons were incubated with tissue supernatants in the presence of pyrilamine, a H₁R antagonist. Although pyrilamine (1 μ mol/L) alone had no effect on the rheobase of control DRG neurons (Supplementary Figure 5A), neurons incubated with ileum or colon supernatants from WAS/OVA+OVA mice in the presence of pyrilamine had a larger rheobase (ie, were less excitable) compared with neurons incubated with supernatant alone (Figure 5H and I). Because pyrilamine inhibited tissue supernatants from increasing the excitability of DRG neurons, we next investigated whether inhibition of H₁R in vivo decreased nerve excitability. WAS/OVA+OVA mice treated with pyrilamine (5 mg/kg) (Figure 5J, Supplementary Figure 5B) had decreased ileal afferent nerve firing to distension compared with WAS/OVA+OVA mice treated with vehicle. Taken together, these findings suggest that activation of H₁R is involved in the increased neuronal excitability in the ileum and colon.

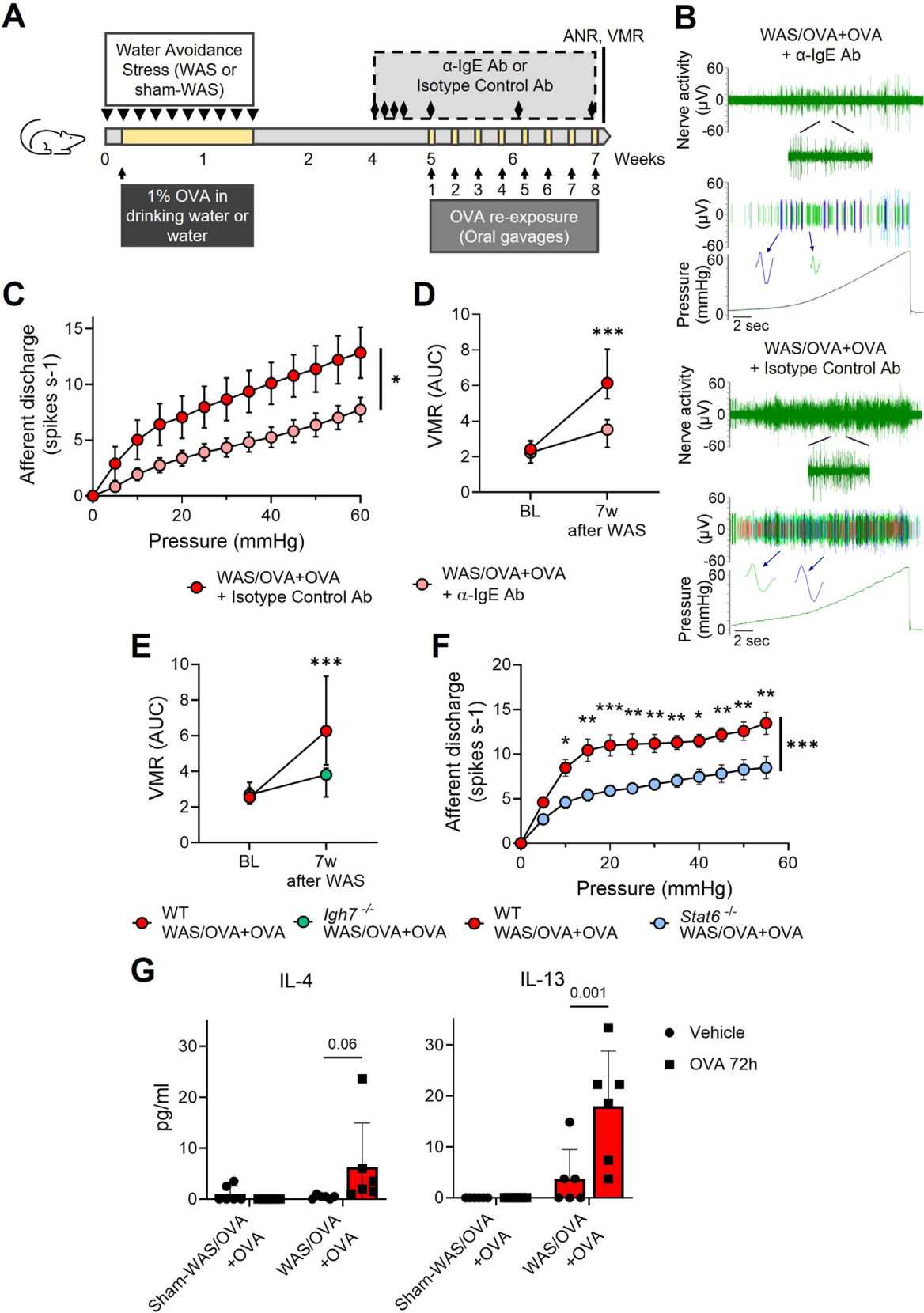
Discussion

Food is one of the most frequent triggers of abdominal pain in patients with IBS.¹¹ Although systemic food allergy has been ruled out as a cause of IBS,¹⁴ we recently found, in mice, that an enteric bacterial infection can break oral tolerance to food, leading to IgE-dependent mast cell activation and food-induced abdominal pain.⁸ In the current study, we expand these findings and provide evidence for the novel concept that not only an infection, but

Figure 3. Immune cell proportions in ileum and colon are unaltered in WAS/OVA+OVA mice. (A) Gating strategy used to identify innate and adaptive immune cell populations (Supplementary Table 3) in cells isolated from the lamina propria of ileum and colon of different mice groups. (B–C) Percentage of innate and adaptive immune cells in the ileum (B) and colon (C) of different mice groups. No differences were observed. Ileum granulocyte panel: sham-WAS/OVA+OVA n = 7; WAS/saline+OVA n = 8; WAS/OVA+saline n = 7; WAS/OVA+OVA n = 7. Ileum lymphocyte panel: sham-WAS/OVA+OVA n = 5; WAS/saline+OVA n = 6; WAS/OVA+saline n = 6; WAS/OVA+OVA n = 6. Colon granulocyte panel: n = 8 mice/group. Colon lymphocyte panel: sham-WAS/OVA+OVA n = 5; WAS/saline+OVA n = 6; WAS/OVA+saline n = 6; WAS/OVA+OVA n = 6. One-way ANOVA with Dunnet post hoc test).

psychological stress can trigger an immune response to food antigens leading to local neuroimmune signaling and subsequent VHS. Although psychological stress is a well-known

risk factor for IBS, insights into the mechanisms underlying its relationship with intestinal symptoms in IBS, especially food-induced abdominal pain, are limited. Our findings may



thus provide an explanation for the role of psychological stress in food-induced symptoms in these patients. Because postinfectious IBS only represents a relatively small subgroup of patients with IBS,²⁸ our findings suggest that loss of oral tolerance to dietary antigens might underlie abdominal pain in a much larger population. Moreover, we show that the local neuroimmune signaling occurs in both the colon and small intestine. Hence, although the applicability of our findings in humans requires confirmation, this study provides mechanistic insight into the increased mast cell activation and release of mast cell mediators in both of these regions of the GI tract, which may be relevant in patients with IBS and related disorders of gut-brain interaction.^{39,48}

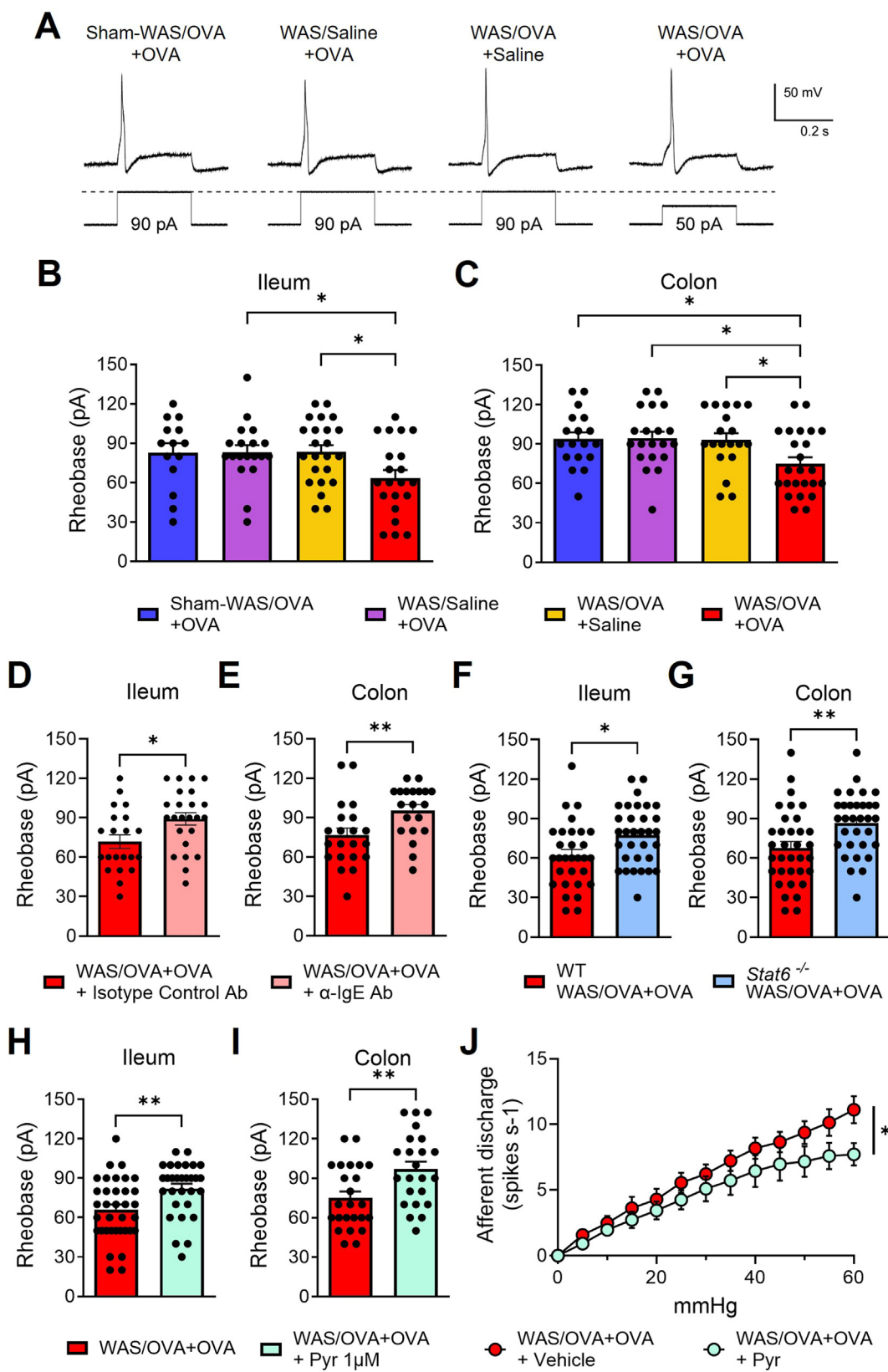
Psychological stress is a key factor for development of GI symptoms in many patients with IBS.^{20,21,23} Indeed, there is evidence of dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis in these patients.⁴⁹ Water avoidance stress is known to increase visceral pain in rodents⁵⁰ and this was observed in the current study with increased VMR in mice exposed to WAS, regardless of concurrent exposure to OVA, compared with mice exposed to sham-WAS. However, this increased VMR was not sustained with the responses returning to baseline 2 weeks after conclusion of WAS. During the re-exposure phase, only mice exposed to OVA during WAS had increased pain signaling on re-exposure to OVA. This suggests that psychological stress also promoted aberrant immune processing of OVA in the gut of these mice.

Previous studies in rodents and humans have shown that stress-induced activation of the HPA axis can increase gut permeability,^{51,52} an effect that can be mimicked by peripheral CRH administration.^{52,53} This may lead to an increased passage of foreign luminal antigens to the lamina propria,^{54,55} which might facilitate the interaction of food compounds with the intestinal immune system. Indeed, a previous study showed that psychological stress can enhance immune response to oral antigens in a rat model of food allergy, leading to a shift in cytokine profiles toward a type 2 immune response and increasing the expression of IgE.⁵⁴ In our model, however, we did not observe signs of systemic allergy in WAS/OVA+OVA mice or any difference in global immune cell populations after oral re-exposure to

OVA compared with control animals. Notably, WAS/OVA+OVA mice showed an increased colonic expression of the gene encoding for the mast cell-mediator tryptase α/β -1 and the mast cell-stabilizer doxantrazole normalized increased visceral pain perception. These results highlight the important contribution of mast cell in food-evoked intestinal pain signaling on stress-induced break of oral tolerance.

Because the number of mast cells were not increased, the observed alterations in pain signaling are most likely explained by an increased activation of these cells. Mast cell activation can be considered as the signature of a type 2 immune response and proven to play a major role in diseases associated with type 2 immunity, including atopy and asthma.^{56,57} To further explore the role of type 2 immunity in the observed stress-induced dietary antigen-specific changes, we made use of *Stat6*-deficient mice. We observed decreased pain signaling in WAS/OVA+OVA *Stat6*-deficient mice on OVA re-exposure, in line with the increase in IL13 and the trend toward an increase in IL4 (which was not statistically significant) in intestinal draining lymph node in wild-type WAS/OVA+OVA mice. Although no systemic response to OVA was observed in these mice, a similar increase of intestinal type 2-related cytokines has previously been observed in models of food allergy after intragastric administration of an antigen during stress.^{54,58} Notably, stress-induced break of tolerance to OVA inhalation has been shown to be prevented by a glucocorticoid receptor antagonist⁵⁹ and B cells cultured with glucocorticoids and IL4 show increased IgE synthesis.^{60,61} This suggests that stress hormones released on activation of the HPA axis may promote isotype class switching. Evidence also indicates that psychological stress alters T regulatory cell (Treg) function in the gut⁶² and the lung^{59,63} in mice leading to an exacerbation of proinflammatory helper T-cell responses. In keeping with this, psychological stress in humans has been shown to reduce the levels of circulating Treg cells.⁶⁴ Given that an alteration of Treg function abolishes oral tolerance,⁶⁵ and considering the importance of local Treg subsets in controlling intestinal immunity,^{66,67} including local type 2 immune responses, it is tempting to hypothesize that a decrease in intestinal

Figure 4. Increased pain signaling induced by OVA re-exposure in WAS/OVA+OVA mice is dependent on IgE and STAT6. (A) Timeline used for anti-IgE (α -IgE) or isotype control antibody intraperitoneal injections in WAS/OVA+OVA mice. *Inverted triangles* indicate WAS or sham-WAS. *Arrows* indicate OVA exposure by oral gavage. *Diamonds* indicate antibody administration. (B) Representative recordings showing ileal afferent nerve response to distension in WAS/OVA+OVA mice treated with isotype control antibody or anti-IgE antibody. (C) Ileal afferent nerve mechanosensitivity in WAS/OVA+OVA mice was significantly decreased by anti-IgE antibody treatment ($n = 10$) compared with isotype control antibody treatment ($n = 8$) ($P = .0336$, 2-way ANOVA with Sidak post hoc test). (D) Anti-IgE antibody treatment ($n = 10$) decreased VMR to CRD compared with isotype control antibody treatment ($n = 9$) (adjusted $P = .0001$, 2-way repeated ANOVA with Sidak post hoc test). (E) IgE-deficient mice (*Igh7*^{-/-}; $n = 10$) had lower VMR to CRD compared with wild-type (WT; $n = 8$) WAS/OVA+OVA mice (adjusted $P = .0006$, 2-way repeated ANOVA with Sidak post hoc test). (F) *Stat6*-deficient mice (*Stat6*^{-/-}; $n = 3$ females + 5 males) had reduced ileal afferent nerve mechanosensitivity compared with WT ($n = 5$ females + 3 males) WAS/OVA+OVA littermates ($P = .0066$, 2-way ANOVA with Sidak post hoc test). (G) Type 2 core cytokines IL4 and IL13 were measured in supernatants of cultured cells from intestinal draining lymph nodes incubated 72 hours with OVA or vehicle isolated from WAS/OVA+OVA or sham-WAS/OVA+OVA mice. IL13 was elevated only in WAS/OVA+OVA supernatants ($n = 6$ mice/group, $P = .001$, 2-way ANOVA with Sidak post hoc test).



Treg cells or an alteration of their phenotype induced by stress could break oral tolerance against luminal antigens in WAS/OVA mice. Future studies are needed to characterize different intestinal subpopulations of immune cells induced by stress and elucidate their role in this process. Furthermore, the role of stress-induced increase permeability to food antigens, as well as how stress hormones modulate immune recognition of dietary peptides and subsequently impair function of tolerance-promoting intestinal immune cells in WAS/OVA mice remains to be characterized.

The increased pain signaling after OVA re-exposure observed in both the small intestine and colon of WAS/OVA+OVA mice involved release of excitatory mediators within the tissues. Blockade of H₁R inhibited the excitatory effect of both ileal and colonic supernatants and reduced ileal afferent firing to distension in WAS/OVA+OVA mice, suggesting histamine and derived metabolites, likely derived from mast cells, may be involved in this excitatory effect. In the postinfectious food antigen IBS model,⁸ the increased OVA-specific IgE was only observed in the colon, suggesting that the local inflammatory response to the infection may be responsible.⁸ In contrast, psychological stress may simultaneously prime the immune system in both organs, which then leads to mast cell activation on re-exposure to the food antigen. To prioritize potential targets to mitigate the development of these immune mechanisms, future studies are needed to elucidate which of these possible mechanisms are at play and the specific metabolites involved.

Psychological stress has also been shown to influence both the composition of the gut microbiota and its metabolites.⁶⁸ We recently showed that certain bacteria (ie, *Klebsiella aerogenes*) present in the GI tract of patients with IBS can release high levels of histamine, which in turn can recruit colonic mast cells through H₄R signaling leading to VHS in mice.¹⁶ However, in a different study, we were unable to replicate the increase in *K aerogenes* levels using another cohort of patients,⁶⁹ suggesting that this H₄R-dependent mechanism is likely involved in only a subpopulation of individuals with IBS. In the present

study, we did not observe increased mast cell recruitment in either the small intestine or the colon of WAS/OVA+OVA mice, hence arguing against the involvement of H₄R-driven mast cell migration. Nevertheless, further studies are required to interrogate the relative contribution of the gut microbiota to the loss of oral tolerance induced by psychological stress or bacterial infection and subsequent development of food-induced abdominal pain.

Although the present work and our previous study⁸ provide novel insights into neuroimmune mechanisms driving food-evoked abdominal pain, many questions require further investigation. For example, it is unclear why some patients report inconsistent adverse responses to the same foods. Variables such as the dose of the food antigen and episodic immune-modulating changes in the gut permeability and/or microbiota⁷⁰ may be relevant in this context. Interestingly, the immunogenicity of some antigens can be altered during food processing,⁷¹ which could affect the capacity of certain antigens to bind specific IgE antibodies and generate immune responses. Thus, additional studies are required to further characterize the mechanisms underlying the impact of stress, enteric infections, interaction with drugs, and gut microbiota on the intestinal immune processes driving food-evoked abdominal pain.

In summary, our results show that psychological stress triggers a type 2 immune response to a food antigen leading to mast cell sensitization. Re-exposure to this food antigen results in mast cell activation and increased pain signaling in both the small intestine and colon. Importantly, there was no systemic response to the food antigen in keeping with the gut-specific symptoms induced by food experienced by many patients with IBS. These results provide novel insight into how stress may contribute to food-induced abdominal pain, which could be of great relevance in the pathophysiology of IBS and related disorders. Moreover, our findings broaden our understanding of the factors contributing to the loss of oral tolerance and food-induced abdominal pain, alongside enteric infections.⁸

Figure 5. Nociceptor sensitization in VHS mice involves mast cells mediators and is dependent of IgE, H₁R, and STAT6. (A) Representative recordings of the rheobase of nociceptive DRG neurons measured using perforated patch clamp after overnight incubation with ileum supernatants from: sham-WAS/OVA+OVA, WAS/saline+OVA, WAS/OVA+saline, and WAS/OVA+OVA mice. (B-C) Ileum (B) and colon supernatants (C) from WAS/OVA+OVA mice increased the excitability of DRG neurons (ie, decreased rheobase) from naïve mice (ileum supernatants collected from sham-WAS/OVA+OVA n = 4 females; WAS/saline+OVA n = 4 females; WAS/OVA+saline n = 5 males; WAS/OVA+OVA n = 4 female, *P* = .0341; colon supernatants collected from 4 mice/group, *P* = .0121; 1-way ANOVA with Dunnett post hoc test). (D-E) Ileum (D) and colon supernatants (E) from WAS/OVA+OVA mice treated with anti-IgE (α -IgE) antibody decreased excitability of DRG neurons (ie, increased rheobase) from naïve mice compared with supernatants from WAS/OVA+OVA mice treated with isotype control antibody (ileum and colon supernatants collected from WAS/OVA+OVA + isotype control Ab n = 3; WAS/OVA+OVA + α -IgE Ab n = 4). (D) *P* = .0175, (E) *P* = .0104, unpaired *t* test). (F-G) Ileum (F) and colon supernatants (G) from WAS/OVA+OVA *Stat6*^{-/-} mice decreased the excitability of DRG neurons from naïve mice compared with supernatants from WT WAS/OVA+OVA littermates (ileum supernatants collected from *Stat6*^{-/-} n = 3 female + 4 male; WT n = 4 female + 2 male, *P* = .0128; colon supernatants collected from 5 male/group, *P* = .0042, unpaired *t* test). (H-I) Pyrilamine prevented the increased excitability of DRG neurons induced by ileum (H) and colon supernatants (I) from WAS/OVA+OVA mice (ileum supernatants collected from 8 mice, *P* = .0048; colon supernatants collected from 4 mice, *P* = 0.0053; unpaired *t* test). (J) Ileal afferent nerve mechanosensitivity in WAS/OVA+OVA mice was significantly decreased by in vivo pyrilamine treatment (n = 7) compared with vehicle treatment (n = 7) (*P* = 0.0354, 2-way ANOVA with Sidak post hoc test).

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at <https://doi.org/10.1053/j.gastro.2025.01.246>.

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Conflicts of interest

The authors disclose no conflicts.

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Data Availability

The data supporting the findings of this study are available on reasonable request to the senior authors.

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Supplementary Materials and Methods

Animals

Stat6^{-/-} mice (RRID: IMSR_JAX:002828) males and females with BALB/c background and their wild-type littermates were from The Jackson Laboratories and bred at Queen's University. *Igh*⁷^{-/-} mice (lacking IgE) male with BALB/c background^{e1} were provided by Prof. J. Strid (Imperial College, London, UK) and experiments using these mice were performed at the KU Leuven. Mice were maintained under controlled temperature (22 ± 1°C) and light (12-hour light/dark cycle) with free access to food (Ssniff V1535-000 at KU Leuven and LabDiet 5015 at Queen's University) and water. Mice were not fasted before any procedure/analysis and all interventions were performed during the light period. Bedding material was spruce wood granulate (SAFE BK 8-15, SAFE, Augy, France) at KU Leuven and corn cob bedding blend of 1/8" and 1/4" (Anderson's lab bedding, Maumee, Ohio) at Queen's University. Mice were housed in individually ventilated cages at both institutions.

Food Allergy Model

BALB/c mice were intraperitoneally (ip) injected with 100 µg OVA (grade V) mixed with 1 mg of alum (Alhydrogel, InvivoGen) on days 0 and 7. Mice were challenged with 50 mg of OVA (grade II) by oral gavage on days 20, 22, 24, 26, 28, and 30.

Ear Allergy Test

Mice were anesthetized with isoflurane and subjected to intradermal injections of 10 µL OVA (1 µg, grade V, Sigma-Aldrich) dissolved in sterile saline in the left ear and 10 µL saline in the right ear. Ear swelling was measured with a caliper 30 minutes after injection.

Anti-IgE Treatment

Four weeks after the first day of WAS, mice were injected with either anti-IgE antibody (1 mg/kg, rat anti-mouse IgE, clone R1E4, Kerafast, or clone R35-92, BD Biosciences) or isotype control antibody (1 mg/kg, Ultra-LEAF Purified Rat IgG1, κ Isotype control Antibody, BioLegend) ip during days 28–31, and during OVA re-exposure on days 35, 42, and 48. Both antibodies were diluted in sterile saline. On day 28, antibodies were administered at increasing doubling or tripling doses every 90 minutes starting at 50 ng to complete 1 mg/kg dose. The other days mice were injected with a single 1 mg/kg dose.^{e2}

Mast Cell Stabilizer Treatment

After undergoing the "Water avoidance stress and ovalbumin exposure" protocol, WAS/OVA+OVA mice were treated with doxantrazole (16.5 mg/kg ip, Sigma-Aldrich) daily 1 hour before OVA re-exposure for 7 consecutive days.^{e3} NaCHO₃ (0.5% wt/vol) was used as vehicle treatment. VMR was measured 1 hour after OVA gavage, and thus 2 hours after the last doxantrazole treatment.

H₁R Antagonist Treatment

Four weeks after the first day of WAS, WAS/OVA+OVA mice were injected with either pyrilamine (5 mg/kg, pyrilamine maleate salt, Sigma-Aldrich) or vehicle (NaCl 0.9%) ip during days 28, 30, 32, and 34 and 1 hour before OVA re-exposure on days 36, 38, 40, 42, 44, 46, 48, and 50.

Perforated Patch Clamp Recordings Solutions

The extracellular solution composition was as follows (in mmol/L): NaCl, 140; KCl, 5; MgCl₂, 1; CaCl₂, 2; HEPES, 10; D-glucose, 10; pH 7.4. The pipette solution composition was as follows (in mmol/L): K-gluconate, 110; KCl, 30; HEPES, 10; MgCl₂, 1; CaCl₂, 2; pH 7.25.

Supernatant Collection

Ileum and colon were harvested, and mesenteric fat was removed. Tissues were washed with 0.9% NaCl, cut into small pieces (~2 mm) and collected in cold Hank's balanced salt solution. Tissue pieces were transferred to RPMI supplemented media (10% fetal bovine serum [FBS], penicillin/streptomycin [100 U/100 µg/mL, Sigma-Aldrich], and gentamicin/amphotericin B [10 µg/0.25 µg/mL, Thermo Fisher]) as 1 mL per cm of tissue and incubated in presence of OVA (grade V, 100 µg/mL, Sigma-Aldrich) for 4 hours at 37°C (5% CO₂). Supernatants were aliquoted (200 µL) and stored at -80°C until use.^{e3} Supernatants in F-12 supplemented media (1:20) were filtered before DRG neuron incubation.

Gene Expression

RNA was isolated with RNeasy Mini Kit (Qiagen) and transcribed to complementary DNA (cDNA; Quanta Biosciences) following the manufacturer's instructions. Next, polymerase chain reactions were prepared with FastStart Sybr Green Master (Merk) following the manufacturer's instructions. In brief, 10 µL reaction mix per well containing cDNA (20 ng), oligonucleotides (200 nmol/L), and DNase/RNase free water was loaded onto 96-well plates. Gene quantification was performed using real-time quantitative reverse transcription polymerase chain reaction using a LightCycler 480 multi-well plate 96 (RocheGmbH). Gene expression was calculated using the Pfaffl method and normalized to control groups.^{e4,e5} At the end of thermocycling, a melt curve was performed to assess the specificity of primers, confirming single peaks for all primer pairings. To determine primer efficiency, we generated a standard curve primers set at cDNA concentrations of 50, 5, 0.5, 0.05, and 0.005 ng/µL, where we plotted Ct against log concentration. Efficiency was calculated following the equation $\text{Efficiency} = 10^{-(1/\text{slope})} - 1$.^{e6,e7} Efficiency of reaction values between 90% and 110% was considered acceptable. Relative gene expression was calculated as $2^{-\Delta\Delta\text{Ct}}$.^{e8} Primers are listed in [Supplementary Table 2](#); *Actb* (Actin) was used as reference gene.

Single-Cell Suspensions

Colon and small intestine were harvested and mesenteric fat removed. Tissues were longitudinally opened and cleaned with cold phosphate-buffered saline (PBS) and

collected in wash medium (Hank's balanced salt solution supplemented with 1% FBS and penicillin/streptomycin [100 U/100 μ g/mL]). Epithelial cells were removed by a 15-minute incubation in warm wash medium (37°C, 200 rpm shaking) supplemented with dithiothreitol (1 mmol/L, Sigma-Aldrich) and EDTA (1 mmol/L, Invitrogen). Tissues were washed, cut into 1- to 2-mm pieces, and digested using a 20-minute incubation in warm Minimum Essential Medium α (37°C, 200 rpm shaking) containing penicillin/streptomycin, β -mercaptoethanol (50 μ mol/L, Sigma-Aldrich), FBS (5%), DNase I (5 U/mL, Roche), Collagenase V (0.7 mg/mL, Sigma-Aldrich), Collagenase D (0.9 mg/mL, Roche), and Dispase II (1 mg/mL). For adaptive immune cells staining, epithelial cells were removed with wash medium supplemented with EDTA (1 mmol/L) and tissues were digested using a 45-minute incubation in warm Minimum Essential Medium α (37°C, 200 rpm shaking) containing penicillin/streptomycin, FBS (10%), DNase I (5 U/mL), and Collagenase D (1 mg/mL). After digestion, cells were filtered through 70- μ m and 40- μ m cell strainer and lamina propria cells were purified using a Percoll (Sigma-Aldrich) gradient (40%–80%). Purified cells were resuspended in PBS and counted before staining for flow cytometry.

Flow Cytometry

Single-cell suspensions were incubated with viability dye (Zombie-NIR, BioLegend) for 15 minutes in the dark to identify living cells. Then, cells were incubated with Fc-block (BioLegend) for 10 minutes at 4°C before incubation with fluorophore-conjugated antibodies (Supplementary Table 1) for 30 minutes at 4°C. Gating strategies were designed to identify innate immune cells (neutrophils, eosinophils, macrophages, monocytes, mast cells) and adaptive immune cells (naïve, memory, and effector helper T cells; naïve, memory, and effector cytotoxic T cells; and B cells) as shown in Figure 2A and Supplementary Table 3. Samples were acquired using Cytoflex S (Beckman) and analyzed with FlowJo software.

Fluorescence minus one was evaluated for every antibody to assess specificity of the staining. Moreover, each antibody was in-house titrated to ensure optimal separation between positive and negative populations.

Mesenteric Lymph Node Culture

Mesenteric lymph nodes (mLN) were harvested from WAS/OVA+OVA and sham-WAS/OVA+OVA mice, fat was removed, and mLN were mechanically dissociated through a 70- μ m cell strainer to obtain a single-cell suspension. Cells were cultured in 96-well plates with RPMI supplemented media (10% FBS, penicillin/streptomycin [100 U/100 μ g/mL, Sigma-Aldrich], and 50 μ mol/L β -mercaptoethanol) with or without OVA (grade V, 200 μ g/mL, Sigma-Aldrich) for 72 hours at 37°C (5% CO₂). Supernatants were collected and stored at –80°C until used.

Cytokine Enzyme-Linked Immunosorbent Assay

IL4 and IL13 cytokines in mLN supernatants were determined using sandwich enzyme-linked immunosorbent assay (ELISA) DuoSet kits (Mouse IL4 DuoSet ELISA: DY404;

Mouse IL13 DuoSet ELISA: DY413; R&D Systems) according to the manufacturer's instructions. Briefly, plates were coated overnight with capture antibody at room temperature. Next morning, plates were blocked with bovine serum albumin (1% in PBS) for 1 hour. Then, samples and the standards (50 μ L) were incubated for 2 hours at room temperature. Plates were incubated 2 hours at room temperature with the detection antibody followed by 20-minute incubation with streptavidin–horseradish peroxidase and 20-minute incubation with TMB substrate (Thermo Fisher). The reaction was stopped with stop solution (R&D Systems) and plates were read in an ELISA plate reader at 450 nm.

Statistical Analysis

Normal (Gaussian) distribution was determined for all datasets using the Shapiro–Wilk normality test and datasets where one (or more) group did not pass the normality test (assuming $\alpha = .05$) were analyzed using a nonparametric test. A *P* value < .05 was considered as significant.

For in vivo experiments, the number of mice was determined using power calculations based on previous analyses of the same or similar nature (procedure, pharmacologic treatment, or murine genetic background) carried out in our laboratory.^{e3} To this end, a priori statistical calculations were performed to compute the required sample size between 2 independent groups (power = .8; α error probability = .05), allocating a group ratio of N2/N1 = 1.

Supplementary References

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Supplementary Table 1.Antibodies for FACS Analysis

	Antibody	Fluorophore	Clone	Catalog no.
Panel 1, granulocytes	Anti-CD45	AlexaFluor700	30-F11	BioLegend 103128
	Anti-CD64	APC	X54-5/7.1	BioLegend 139305
	Anti-CCR2	PE-Cy7	SA203G11	BioLegend 150611
	Anti-Ly6G	AlexaFluor488	1A8	BioLegend 127625
	Anti-SiglecF	BV786	E50-2440	BD Biosciences 740956
	Anti-CD11b	BV510	M1/70	BioLegend 101245
Panel 2, lymphocytes + mast cells	Anti-CD45	AlexaFluor700	30-F11	BioLegend 103128
	Anti-CD19	BV421	6D5	BioLegend 115537
	Anti-CD3	PE-Cy7	17A2	BioLegend 100219
	Anti-CD4	APC	RM4-5	BioLegend 100515
	Anti-CD8a	BV785	53-6.7	BioLegend 100749
	Anti-CD44	PE	IM7	BioLegend 103007
	Anti-CD62L	BV605	MEL-14	BioLegend 104437
	Anti-FcεRI	AF488	MAR-1	BioLegend 134329
	Anti-CD117	BV510	2B8	BioLegend 105839

Supplementary Table 2.Primers for Gene Expression

Gene	Forward	Reverse	NCBI access no.
<i>Actb</i>	CATTGCTGACAGGATGCAGAA	GCTGATCCACATCTGCTGGAA	NM_007393.5
<i>Ifng</i>	GCCATCAGCAACAACATAAGCGTC	CCACTCGGATGAGCTCATTGAATG	NM_008337.4
<i>Tnf</i>	CCCCAAAGGGATGAGAAGTT	CACCTGGTGGTTTGCTACGA	NM_013693.3
<i>Il1b</i>	GGGCCTCAAAGGAAAGAATC	TACCAGTTGGGGAACCTCTGC	NM_008361.4
<i>Il6</i>	AAGTCGGAGGCTTAATTACACATGT	CCATTGCACAACCTCTTTCTCATTC	NM_031168.2
<i>Ccl2</i>	CCCCACTCACCTGCTGCTACT	GGCATCACAGTCCGAGTCACA	NM_011333.3
<i>Tgfb</i>	TGACGTCACCTGGAGTTGTACGG	GGTTCATGTCATGGATGGTGC	NM_011577.2
<i>Il10</i>	AGAAGCATGGCCCTGAAATCAAGG	CTTGTAGACACCTTGGTCTTGAG	NM_010548.2
<i>Tpsab1</i>	TTGCTGACCCCAACAAGGTC	GGACGATGTAGAAGTCGGGG	NM_031187.4
<i>Kit</i>	TGGGAGCTCTTCTCCTTAGGAA	TGCTCCGGGCTGACCAT	NM_001122733.1

Supplementary Table 3.Gating Strategies

Innate immune cells	Neutrophils	FSC/SSC, alive/CD45 positive, CD11b positive, Ly6G positive
	Eosinophils	FSC/SSC, alive/CD45 positive, CD11b positive, Ly6G negative, Siglec F positive
	Macrophages	FSC/SSC, alive/CD45 positive, CD11b positive, Ly6G negative, Siglec F negative, CD64 positive, CCR2 negative
	Monocytes	FSC/SSC, alive/CD45 positive, CD11b positive, Ly6G negative, Siglec F negative, CD64 negative, CCR2 positive
	Mast cells	FSC/SSC, alive/CD45 positive, CD117 positive, FcεRI positive
Adaptive immune cells	B cells	FSC/SSC, alive/CD45 positive, CD3 negative, CD19 positive
	T cells	FSC/SSC, alive/CD45 positive, CD3 positive, CD19 negative
	Helper T cells (Th)	FSC/SSC, alive/CD45 positive, CD3 positive, CD4 positive, CD8 negative
	Naïve Th cells	FSC/SSC, alive/CD45 positive, CD3 positive, CD4 positive, CD8 negative, CD62L positive, CD44 negative
	Memory Th cells	FSC/SSC, alive/CD45 positive, CD3 positive, CD4 positive, CD8 negative, CD62L positive, CD44 positive
	Effector Th cells	FSC/SSC, alive/CD45 positive, CD3 positive, CD4 positive, CD8 negative, CD62L negative, CD44 positive
	Cytotoxic T cells (Tc)	FSC/SSC, alive/CD45 positive, CD3 positive, CD4 negative, CD8 positive
	Naïve Tc cells	FSC/SSC, alive/CD45 positive, CD3 positive, CD4 negative, CD8 positive, CD62L positive, CD44 negative
	Memory Tc cells	FSC/SSC, alive/CD45 positive, CD3 positive, CD4 negative, CD8 positive, CD62L positive, CD44 positive
	Effector Tc cells	FSC/SSC, alive/CD45 positive, CD3 positive, CD4 negative, CD8 positive, CD62L negative, CD44 positive