interaction. Finally, in this study, we illustrate how miRNA regulation by probiotics may lead to enhanced IL-10 production and partly explain the anti-inflammatory effects observed in clinical trials with specific probiotic strains.

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A histamine-independent itch pathway is required for allergic ocular itch

To the Editor:

Itch is the cardinal symptom of allergic conjunctivitis and afflicts 15% to 20% of the population worldwide. Histamine produced by conjunctival mast cells has been implicated as the principal itch mediator that activates histamine receptors on primary sensory fibers to induce allergic ocular itch.[1] However, antihistamines cannot completely relieve ocular itch in many cases, suggesting the involvement of a histamine-independent itch pathway. Herein, we sought to identify the histamine-independent neural pathway involved in allergic conjunctivitis and to develop new therapeutic strategies for allergic ocular itch.

Allergic ocular itch typically originates from the conjunctiva, a mucosal membrane that is anatomically distinct from the skin and covers the ocular surface over the sclera and the inner surface of the eyelid. However, our knowledge about the neural regulations of allergic ocular itch and its difference from skin itch is limited. Mast cells have been shown to secrete many bioactive compounds in addition to histamine.[2,3] Yet, the contribution of histamine-independent mediators to allergic ocular itch in comparison to histamine, and the neural pathway mediating the histamine-independent components in ocular itch remain unclear. TRPA1 is a cation channel that is often colocalized with TRPV1 in a subpopulation of primary sensory neurons in the trigeminal ganglion (TG) and the dorsal root ganglion. TRPV1 is known to be the downstream transduction channel of histamine H1 receptor in sensory neurons.[4] TRPA1 was recently found to be the downstream transduction channel of histamine-independent itch in the skin.[5] However, it is yet unknown whether TRPA1 is required for mast cell–mediated allergic itch. In this study, we characterized the role of TRPA1 as a histamine-independent modulator in ocular itch associated with allergic conjunctivitis.

To delineate the respective role of TRPA1 and TRPV1 in ocular itch, we first examined the acute behavioral responses of wild-type (WT), TRPA1 knockout (KO), and TRPV1 KO mice to pruritogens applied directly to their lower conjunctival sacs. We were able to differentiate ocular itch from pain using our behavioral models, in which itch-inducing compounds provoked mice to scratch the treated area using their hindpaw, whereas pain-inducing capsaicin elicited wiping behavior using the forelimb (Fig 1, *A*). We found that histamine challenge (46 μg in 2.5 μL PBS) induced 15 ± 1.8 scratching bouts in WT and 14.7 ± 3 scratching bouts in TRPA1 KO mice (Fig 1, *B*), but significantly less itch responses in TRPV1 KO mice (5 ± 1.3 bouts), suggesting that TRPV1—rather than TRPA1—is responsible for histamine-dependent ocular itch signaling. We then tested 2 histamine-independent pruritogens, chloroquine and serotonin, on evoking ocular itch. Chloroquine-induced (12.4 μg) ocular scratching was significantly decreased in TRPA1 KO mice (6.3 ± 1.1 bouts), compared with WT (16.6 ± 2 bouts) and TRPV1 KO mice (14.3 ± 2.3 bouts) (Fig 1, *C*), indicating that TRPA1 is required for histamine-independent itch induced by chloroquine. In contrast, there was no difference in ocular itch responses induced by serotonin (0.2 μg) among WT (11.1 ± 1.1 bouts), TRPA1 KO (12.3 ± 1.0 bouts) and TRPV1 KO mice (11.6 ± 1 bouts) (Fig 1, *D*). Vehicle control (PBS) induced only minimal scratching responses (Fig 1, *E*). These behavioral results indicate a pivotal role of TRPA1 in certain types of nonhistaminergic ocular itch.

To further explore the underlying mechanisms, we examined whether TRPA1 is functionally required for the responsiveness of conjunctival sensory fibers to pruritogens. We found that the conjunctival mucosa is abundantly innervated by $TRPA1^+$ and $TRPV1^+$ neurons, but not cold-sensing $TRPM8^+$ neurons, in TG (see Fig E1 in this article's Online Repository at www.jacionline.org). GCaMP3-assisted calcium imaging of conjunctival sensory fibers showed that on stimulation by pruritogens, subpopulations of conjunctival nerve fibers displayed moderate to high calcium mobilization (Fig 1, *F-K*). Interestingly, TRPA1 deficiency does not affect conjunctival nerve response to histamine stimulation, but significantly reduces the nerve response to chloroquine stimulation. In contrast, TRPV1 is required for nerve fiber response to histamine but not to chloroquine in the conjunctiva. Finally, deficiency in TRPA1 or TRPV1 did not affect serotonin-induced nerve activity (Fig 1, *L*). These data reveal a segregation of TRPA1-dependent and TRPV1-dependent pathways in conjunctiva-innervating sensory neurons.

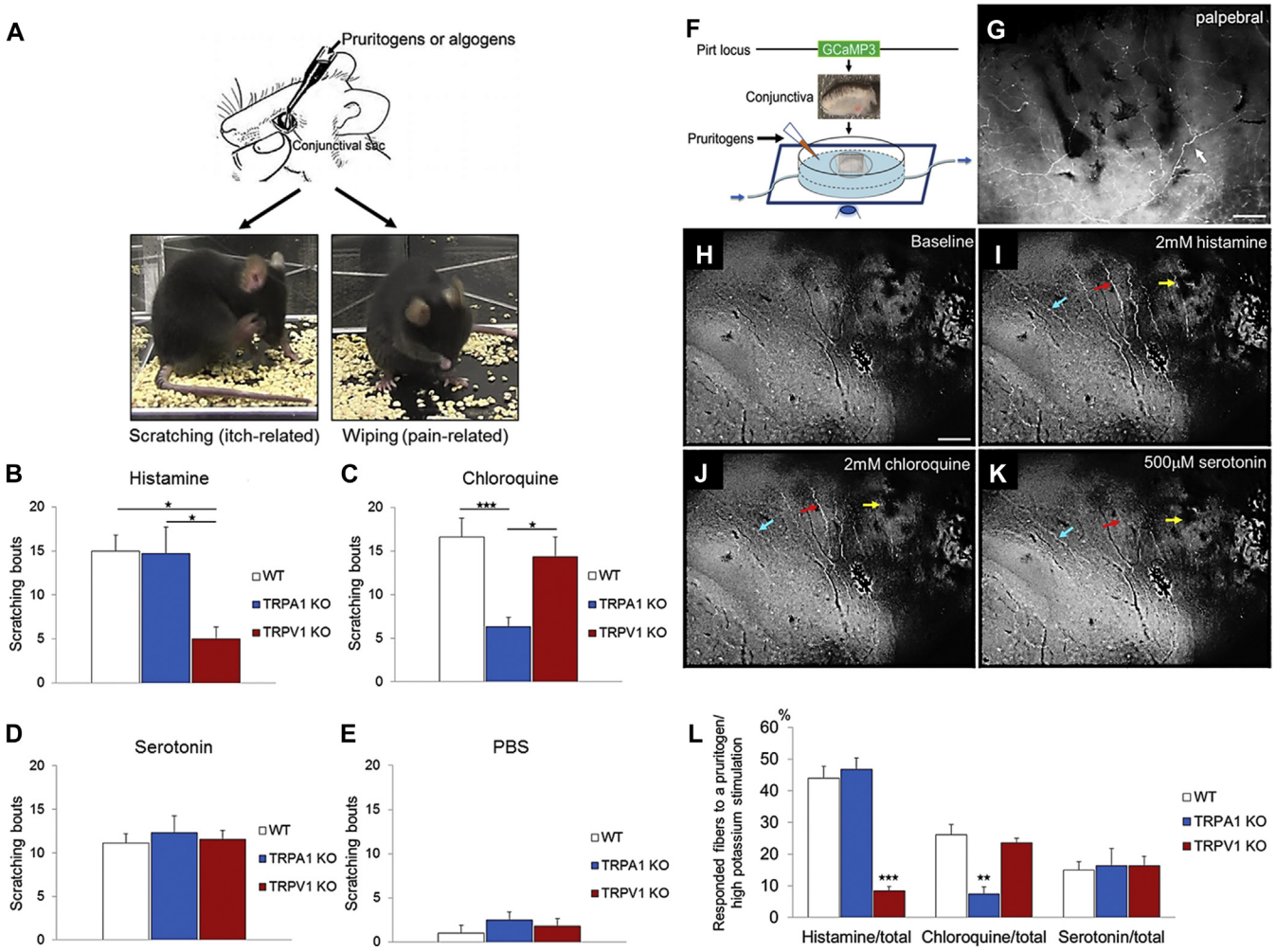

FIG 1. Behavioral and cellular mechanisms of pruritogen-induced ocular itch. **A,** Behavioral distinction between ocular itch and pain in mice. **B,** Histamine-evoked scratching responses were reduced in TRPV1 KO mice (n = 5-9 per group). **C,** Chloroquine-induced scratching behavior was attenuated in TRPA1 KO mice (n = 6-13 per group). **D,** Serotonin evoked comparable number of scratching bouts among 3 genotypes (n = 7-14 per group). **E,** PBS elicited minimal and significantly less scratching responses than any group receiving pruritogen challenge (n = 5-9 per group). **F,** Imaging conjunctival nerve fiber activities using Pirt-GCaMP3 mouse. **G,** Whole-mount immunofluorescence of GCaMP3 signal in a $Pirt^{GCaMP3/+}$ conjunctiva. *White arrow*, $GCaMP3^{+}$ sensory fiber. **H-K,** Responses of sensory fibers to pruritogens. *Arrows* in different colors indicate conjunctival fibers with differential receptivity to pruritogens. **L,** Percentage of $GCaMP3^{+}$ conjunctival fibers activated by pruritogens among $Pirt^{GCaMP3/+}$, TRPA1 KO; $Pirt^{GCaMP3/+}$, and TRPV1 KO; $Pirt^{GCaMP3/+}$ mice (n ≥ 5 per group). *Scale bar* represents 100 μm. $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$.

Involvement of TRPA1-mediated itch pathway in allergic ocular itch was next evaluated using an ocular allergy model. Mast cell–mediated allergic conjunctivitis was induced in mice using an ovalbumin (OVA) sensitization regime (see this article's Methods section in the Online Repository at www.jacionline.org). Topical OVA challenge (250 μg) into unilateral conjunctival sac provoked targeted scratching responses in WT mice (21.6 ± 2.1 bouts; Fig 2, *A*). This itch was caused by allergen (OVA)-specific immune reaction because OVA challenge before sensitization or vehicle treatment after sensitization did not elicit significant scratching responses (Fig 2, *A*). More importantly, our allergic conjunctivitis model recapitulates histologic changes associated with severe seasonal allergy or atopic keratoconjunctivitis in humans,[6] as evidenced by our serial OVA challenges leading to inflammatory cells infiltrations and loss of goblet cells in the conjunctiva (Fig 2, *B*). Interestingly, in this model, ocular itch was significantly attenuated in TRPA1 KO (10.8 ± 1.9 bouts) and TRPV1 KO (13.3 ± 2.9 bouts) mice, suggesting that both TRPA1 and TRPV1 are required for allergic ocular itch. The observed behavioral changes were not due to defects in mast cell activation of TRPA1 KO or TRPV1 KO mice because mast cell degranulation was comparable among WT, TRPA1 KO, and TRPV1 KO mice (see Fig E2 in this article's Online Repository at www.jacionline.org).

To further explore the potential of TRPA1 as a therapeutic target for allergic conjunctivitis, we examined the effect of pharmacologically blocking TRPA1 in ocular allergy. Compared with the vehicle-treated group (23.4 ± 1.7 bouts), pretreatment with TRPA1 antagonist HC-030031 significantly alleviated allergic ocular itch in mice (10.7 ± 1.3 scratching bouts). More