Loss of S1PR3 attenuates scratching behaviors in mice in the imiquimod model of psoriasis, but not in the MC903 model of atopic dermatitis

Rose Z. Hill, PhD\textsuperscript{a}, Ziad Rifi, BA\textsuperscript{a}, Cliff Vuong, BS\textsuperscript{a}, Diana M. Bautista, PhD\textsuperscript{a,\textast}\textsuperscript{a}

Abstract
Here we examine the role of sphingosine 1-phosphate receptor 3 (S1PR3) in chronic itch. We used 2 mouse models—the MC903 model of atopic dermatitis and the imiquimod model of psoriasis—to examine the contribution of S1PR3 to chronic itch. We measured scratching behaviors in these mouse models in S1PR3\textsuperscript{−/−}, +/+\textsuperscript{−}, and +/+\textsuperscript{+} animals. Whereas we observed no effect of loss of S1PR3 on itch behaviors in the MC903 model, imiquimod-evoked itch behaviors were reduced in S1PR3\textsuperscript{−/−} animals. Overall, the data support a role for S1PR3 signaling in the development of psoriatic but not atopic itch.

Keywords: Sphingosine 1-phosphate, Sensory neuron, Itch, Psoriasis, Atopic dermatitis, Sphingosine 1-phosphate receptor 3

The bioactive signaling lipid sphingosine 1-phosphate (SIP) and its receptor SIP receptor 3 (S1PR3) were recently shown to mediate acute itch and pain behaviors in mice via activation of TRPA1 and TRPV1 in somatosensory neurons\textsuperscript{[1]}. S1P is a proinflammatory lipid that has been implicated in a number of skin and systemic diseases, including psoriasis, atopic dermatitis (AD), chronic and neuropathic pain, multiple sclerosis, and inflammatory bowel disease\textsuperscript{[2–4]}. Despite the physiological relevance of elevated SIP levels to these disorders, the mechanisms by which SIP contributes to inflammatory disease remains unclear. For example, the inflammatory chronic itch disorders psoriasis and AD present distinct etiologies and underlying mechanisms. In particular, TRPA1 and TRPV1, thought to be the primary downstream targets of SIP/S1PR3 signaling in prurceptors and nociceptors that drive itch and pain behaviors, contribute little, and may even suppress, psoriatic itch in the commonly used imiquimod mouse model\textsuperscript{[5]}. By contrast, TRPA1 is required for itch in the MC903 mouse model of AD\textsuperscript{[6]}. And while SIP levels are elevated in skin and/or serum of humans in both psoriasis and AD\textsuperscript{[2,3,4]}, studies have presented conflicting findings of whether SIP signaling is protective or pathogenic in chronic itch\textsuperscript{[5–11]}. Many of these studies relied on pan-S1PR or S1PR1-targeted pharmacology, as S1PR1 is the main receptor expressed in the immune system\textsuperscript{[12,13]}. However, drugs targeting S1PR1 cause lymphopenia, the intended mechanism of action for treating autoimmune conditions, but which can also lead to potentially dangerous complications. Studies employing S1PR1-specific antagonists at selective doses show partial attenuation of psoriasis, whereas studies employing higher doses that target S1PRs 1 and 3 show more complete attenuation\textsuperscript{[14–16]}, suggesting additional sites of action. For example, both human and mouse sensory neurons express S1PR3. Thus, we set out to examine the specific role of S1PR3 in chronic itch so as to understand the role of somatosensory SIP signaling in AD and psoriasis.

Methods

Ethics
All experiments were performed under the policies and recommendations of the International Association for the Study of Pain and approved by the University of California Berkeley Animal Care and Use Committee.

Animals
All mice were housed in standard conditions (12 h light-dark cycle, 21°C). Wild-type C57BL6 mice were obtained from Jackson Laboratories. S1PR3\textsuperscript{−/−} mice\textsuperscript{[17]} were bred to wild-type animals and then crossed to generate S1PR3\textsuperscript{−/−} and +/+ mice. Previous work showed no effect of heterozygosity on itch behaviors, thus data from S1PR3\textsuperscript{+/-} and +/+ animals were pooled\textsuperscript{[18]}. Where appropriate, genotypes were assessed using standard polymerase chain reaction.

MC903 mouse model of AD
The MC903 model was performed on the cheek as previously described\textsuperscript{[8]} using daily treatment with 20 µL 0.2 mM calcipotriol/MC903 (Tocris) dissolved in 100% ethanol for 7 consecutive days. Animals were euthanized after behavioral recording on day 8.

Imiquimod mouse model of psoriasis
The imiquimod model was performed on the rostral back as previously described\textsuperscript{[19]} using daily treatment with 41.6 mg pharmacy-grade 5%...
imiquimod cream (Perrigo). Animals were euthanized after behavioral recording on day 7. A variety of imiquimod formulations have been used by published studies; however, the differing compositions of these formulations have been shown to affect experimental results[7,19-21]. The imiquimod vehicle itself contains inactive ingredients that stimulate inflammation and contribute to the effects of the model, including isostearic acid[21]. Published studies have used a variety of vehicle controls for comparison, including commercial lotions such as Vaseline Lanette cream, which do not perfectly mimic the inactive ingredients of the pharmaceutical-grade imiquimod formula[20]. Because of these potentially confounding factors, we chose to compare imiquimod-evoked scratching between S1PR3−/− and +/+ animals, rather than between imiquimod and vehicle treatment. Although our imiquimod experiments lacked a true vehicle control, we did find that imiquimod animals scratched significantly more than our ethanol vehicle controls from the MC903 model (*P = 0.0337, F(3,37) = 3.96; Holm-Sidak’s multiple comparisons: **P_{MC903} vs. EtOH = 0.0029, P_{MC903-KO} vs. MC903 = 0.4389). N = 12, 11, 9, 7 mice per group (left to right). B, Immune cell infiltration on day 8 of MC903 treatment in S1PR3−/− and +/+ animals for N = 4 mice per genotype. Two-tailed t tests were performed for each immune cell subtype and all P-values > 0.05.

**Results**

We first examined the effect of loss of S1PR3 on MC903-evoked itch behaviors at day 8 of the model, when itch-evoked scratching is most robust and skin displays the molecular, cellular and histologic hallmarks of mature AD lesions[8,23]. We observed no difference in itch behaviors between S1PR3−/− and control animals on day 8 (Fig. 1 A). In addition, infiltration of myeloid cells to the cheek skin of S1PR3−/− animals was consistent with previous studies (Fig. 1 B)[22], suggesting no major effects of loss of S1PR3 on the MC903-evoked immune response. Interestingly, there was a trend toward increased inflammatory monocytes and mast cells in MC903-treated S1PR3−/− skin, suggesting that the immune response may be even greater in S1PR3-deficient mice, consistent with their robust itch behaviors. By contrast, in the imiquimod model, we observed a significant reduction in itch-evoked scratching at all time points, with the greatest effect on day 7 (Fig. 2 A).

**Discussion**

S1PR3 signaling mediates imiquimod itch. We hypothesize that S1PR3 is signaling via sensory neurons to promote itch; however, it is possible that S1PR3 is acting indirectly via non-neuronal cells in the skin, such as keratinocytes that express multiple S1P receptors. Further study using tissue-specific genetic knockout of S1PR3 will be required to dissect its cell-specific contributions to psoriatic itch.

We speculate that the controversy surrounding S1P signaling in psoriasis is due to the diverse and possibly opposing effects of the various S1P receptors present in immune cells, skin cells, and sensory neurons. Here we have shown a role for S1PR3 specifically in psoriatic itch. However, a subpopulation of pruriceptors also express S1PR1, and may also co-express S1PR3[24]. Given the essential roles of S1PR1 in the immune system, use of neuron-specific S1PR1 knockout animals will be necessary to determine any potential contribution of neuronal S1PR1 to chronic itch.

**Measurement of itch-evoked scratching**

Itch-evoked scratching measurements were performed as previously described[8]. Mice were shaved and singly housed 1 week before itch behavioral measurements. Briefly, mice were placed in plastic 4-part behavioral chambers (IITC) with opaque dividers (TAP plastics) and recorded from below using a mirror. Scratching bouts were defined as the time between when the hind paw lifted to when the hind paw touched the floor. Bout length and time spent scratching were recorded. Behavior was scored while blinded to genotype and treatment. Male mice were used for all experiments and were randomly assigned to treatment groups.

**Flow cytometry**

Myeloid cells were measured in MC903-treated and ethanol-treated cheek skin using previously established methods[23].
While we were surprised to find no effect of S1PR3 deletion in the MC903 model, many other factors are thought to contribute to MC903 itch and inflammation, including: proteases, 5-HT, leukotrienes, prostaglandins, IL-4, IL-31, TSLP, CXCL10, mast cells, basophils, ILC2s, CD4 T cells, and neutrophils\(^{[8,22,23,25]}\). It is possible that these immune-derived signals override whatever S1P-S1PR3 signaling might be occurring in this model to drive itch even in the absence of S1PR3. Taken together, our findings reveal a new role for S1PR3 signaling in chronic itch. It will be essential to determine the mechanisms and cells underlying S1PR3-dependent psoriatic itch.

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**Authors’ contribution**

R.Z.H. and D.M.B. conceived the study and wrote the manuscript. R.Z.H. performed the experiments with experimental assistance from Z.R. C.V. also provided experimental assistance.

**Conflict of interest disclosures**

The authors declare that they have no financial conflict of interest with regard to the content of this report.

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