Allergology International xxx (2016) 1–5

Contents lists available at ScienceDirect

Allergology International

journal homepage: http://www.elsevier.com/locate/alit

Invited review article

Spinal dorsal horn astrocytes: New players in chronic itch

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ARTICLE INFO

Article history:
Received 8 July 2016
Accepted 14 July 2016
Available online xxx

Keywords:
Astrocytes
Spinal dorsal horn
STAT3
Lipocalin-2
Mouse

Abbreviations:
ATF3, activating transcription factor 3; CNS, central nervous system; CV, conventional; GRP, gastrin-releasing peptide; GRPR, GRP receptor; GFAP, glial fibrillary acidic protein; JAK, Janus kinase; SDH, spinal dorsal horn; SPF, specific-pathogen-free; STAT3, signal transducer and activator of transcription 3; TLR4, toll-like receptor 4; TRPV1, transient receptor potential cation channel subfamily V member 1

ABSTRACT

Chronic itch is a debilitating symptom of inflammatory skin conditions, such as atopic dermatitis, and systemic diseases, for which existing treatment is largely ineffective. Recent studies have revealed the selective neuronal pathways that are involved in itch sensations; however, the mechanisms by which itch turns into a pathological chronic state are poorly understood. Recent advances in our understanding of the mechanisms producing chronic itch have been made by defining causal roles for astrocytes in the spinal dorsal horn in mouse models of chronic itch including atopic dermatitis. Understanding the key roles of astrocytes may provide us with exciting insights into the mechanisms for itch chronicity and lead to a previously unrecognized target for treating chronic itch.
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Introduction

Itch (or pruritus) is an unpleasant sensation that elicits the desire or reflex to scratch and normally serves as a self-protection mechanism from harmful external agents such as parasites.[1,2] In general, scratching can transiently relieve such itching sensations. However, under pathological conditions such as skin diseases like atopic dermatitis, other systemic disorders, such as liver and kidney diseases or HIV/AIDS, as well as metabolic disorders, itch becomes more severe and chronic, which leads to excessive, repetitive scratching.[1,3–5] Such scratching causes skin lesions, which can worsen the itch sensation and lead to further scratching (the vicious itch–scratch cycle).[1,3] Chronic itch affects millions of individuals worldwide,[6] but identifying the optimal treatment for it is a major clinical challenge.

A rapidly growing body of literature has revealed the existence of itch-specific neuronal circuitry in the peripheral and central nervous system,[1,2,7,8] and it has been proposed that aberrant modification of itch signaling at the levels of primary afferents and the spinal dorsal horn (SDH) might be involved in pathological chronic itch. Despite such progress in our understanding of the neuronal basis for itch sensation, the mechanisms by which itch turns into a pathological chronic state are poorly understood. In this review, recent advances in our understanding of the mechanisms that underlie chronic itch are highlighted, with a specific focus on the role of astrocytes, a type of glial cell in the central nervous system (CNS).

Glial cells in the CNS

Rudolph Virchow, a German anatomist, first found non-neuronal cells in the CNS and called them “glia”, the Greek word

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Peer review under responsibility of Japanese Society of Allergology.

http://dx.doi.org/10.1016/j.alit.2016.07.007
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for "glue", in the mid-nineteenth century.[9] However, it has now become apparent that glia are much more than "glue", but rather are active players having crucial roles in controlling neuronal functions.[10,11] Glial cells express a number of neurotransmitter receptors for receiving inputs from neighboring cells including neurons and also a number of biologically active substances for giving signals to the cells. Glial cells are mainly classified into three groups: astrocytes, oligodendrocytes and microglia. The former two cell-types are derived from neural stem cells,[12] whereas microglia originate from precursor cells in the yolk sac during early embryonic development.[13,14] In the CNS, astrocytes are the most abundant population. Based on their morphology and location, rodent astrocytes have been classified into two groups, protoplasmic astrocytes in the grey matter and fibrous astrocytes in the white matter.[15] Protoplasmic astrocytes ensheath synapses and are in contact with blood vessels, and fibrous astrocytes are in contact with the nodes of Ranvier.[15] However, recent studies have indicated a diverse cell population, with distinct properties in different brain regions.[16,17] Astrocytes occupy nonoverlapping spatial territories, and their processes are intimately associated with synapses.[15,18] The processes of one astrocyte are known to contact tens of thousands of synapses at a structure termed the tripartite synapse to recognize the structural and functional relationship between the astrocyte and the pre- and postsynaptic terminals.[18,19] Astrocytes do not produce action potentials, but they do increase intracellular Ca^{2+} levels in response to neurotransmitters (glutamate, dopamine, noradrenalin, serotonin, ATP, etc.) via activation of their cognate receptors.[20,21] By responding to such extracellular stimuli, astrocytes evoke various cellular responses including production and release of gliotransmitters (ATP, D-serine, and glutamate) and trophic factors, which act on neurons and modulate synapse formation, maturation, efficacy, and plasticity.[15,20,22] A growing body of evidence using astrocyte-specific molecular and genetic manipulations has revealed that astrocytes play a pivotal role in neuronal function under physiological and pathological conditions.[15,17,20,22–25]

Reactive astrocytes in the SDH in models of chronic itch

Advances in the understanding of the mechanisms of acute and chronic itch have been achieved by the development of animal models. For chronic itch, several models have been established, most of which focus on cutaneous skin diseases, including atopic dermatitis, contact dermatitis and xerosis (dry skin).[8,26,27] Using the model of atopic dermatitis NC/Nga mice, an inbred strain of Japanese fancy mice that show spontaneous scratching behaviors when maintained under conventional (CV), but not specific-pathogen-free (SPF) conditions,[28] it was found that CV-NC/Nga mice have astrocytes with upregulated glial fibrillary acidic protein (GFAP), an astrocytic marker, in the SDH (Fig. 1).[29] Morphologically, the SDH astrocytes showed enlarged cell bodies and extensively arborized processes. These alterations are well-known histological criteria of reactive astrocytes.[30] Furthermore, reactive astrocytes in the SDH have also been reported in models of contact dermatitis[29] and dry skin.[31] Thus, reactive astrogliosis in the SDH occurs under chronic itch conditions.

Interestingly, astrocytic activation seems not to be induced in all SDH segments. Indeed, reactive astrocytes in the SDH are observed mainly in cervical and trigeminal segments that correspond to the nape of neck, rostral back, ear and face where the itchy mice have extensively scratched and skin lesions have developed.[29] In contrast, in the lumbar segments that do not corresponded with lesioned skin, GFAP expression is unchanged. Thus, reactive astrocytes may be restricted to the segments corresponding to the

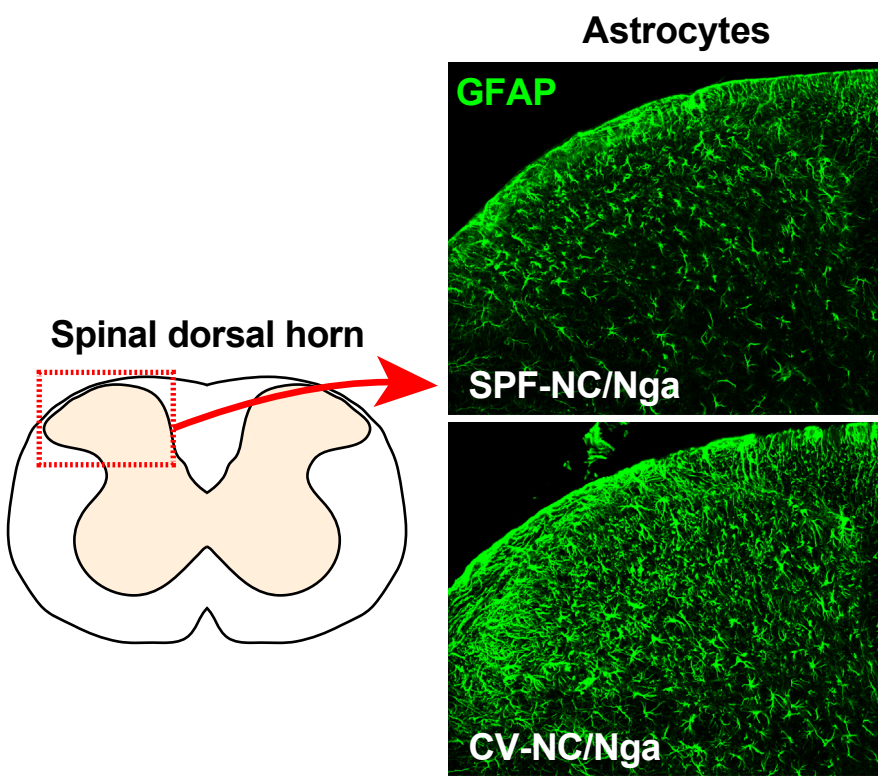

Fig. 1. Reactive astrocytes in the spinal dorsal horn in a model of atopic dermatitis. Immunofluorescence of the marker of astrocytes in the cervical spinal dorsal horn of SPF- and CV-NC/Nga mice. CV-NC/Nga mice have astrocytes with upregulated GFAP in the SDH. Morphologically, the SDH astrocytes showed enlarged cell bodies and extensively arborized processes.

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lesioned itchy skin. However, it appears unlikely that astrocytic activation might be due to direct damage to a peripheral nerve by repetitive scratching because primary afferent sensory neurons do not express activating transcription factor 3 (ATF3),[29] a neuronal injury marker.[32]

Temporal correlation between reactive astrocytes and itch–scratch cycle

A clinically important element of chronic itch is the vicious cycle of itching and scratching. In CV-NC/Nga mice, scratching behavior is induced at the age of 4 weeks, but skin lesions due to scratching are only observed from approximately 8 weeks of age.[29] It is thus conceivable that the itch–scratch cycle might also be established around this time in CV-NC/Nga mice. Interestingly, GFAP upregulation in the SDH is observed only between the ages of 8 and 15 weeks, much later than 4 weeks.[29] Thus it appears that the time course of the appearance of reactive astrocytes matches that of the initiation of the itch–scratch cycle. Furthermore, preventing scratch-induced skin damage by trimming the toenails of CV-NC/Nga mice leads to a recovery from their skin lesions and attenuates GFAP upregulation, as well as reductions in scratching. Similar results were obtained in a model of contact dermatitis. Furthermore, protecting mice from scratching by using an Elizabethan collar prevents GFAP upregulation in the SDH.[31] Thus, it seems likely that SDH astrogliosis under chronic itch conditions might be associated with physical and/or chemical stimulation to the skin by scratching or inflammatory factors.

The mechanism underlying the induction of reactive astrocytes remains to be determined, but the segment in the SDH receiving primary afferent neurons connected with the lesion itchy skin matches that having reactive astrocytes, therefore, astrocytic activation might require a signal from the skin. Tominaga *et al.* have demonstrated that hyperinnervation of primary afferent C-fibers expressing the transient receptor potential cation channel subfamily V member 1 (TRPV1) to the epidermis occurs in lesioned itchy skin of CV-NC/Nga mice.[33] In patients with atopic dermatitis, the density of epidermal nerve fibers is higher in the skin,[34,35] suggesting that hyperinnervation might be responsible for chronic itch. It was found that ablation of TRPV1-positive primary afferent C-fibers by systemically administering the ultrapotent TRPV1 agonist resiniferatoxin (RTX)[36] suppresses GFAP upregulation.[29] Thus, reactive astrocytes under chronic itch conditions might be maintained by the lesioned skin in a manner that requires TRPV1^{+} C-fibers. What biologically active substances are released from primary afferent terminals in the SDH and activates astrocytes under chronic itch condition is a major issue for future investigations.

SDH astrocytes are necessary for chronic itch

Recent studies have implicated SDH reactive astrocytes in chronic itch by demonstrating that repetitive scratching behaviors in models of chronic itch requires the transcription factor signal transducer and activator of transcription 3 (STAT3)[29] and the receptor toll-like receptor 4 (TLR4),[31] both of which in the SDH are selectively activated and expressed in reactive astrocytes.

STAT3 is a member of the STAT family of transcription factors, which has been implicated in the astrocytic activation[30] that appears required for chronic itch. Evidence implicating STAT3 came from convergent results of pharmacological and molecular investigations. It was found that the SDH cells activating STAT3 are astrocytes and because acute pharmacological inhibition of spinal cord STAT3 by intrathecal administration of AG490, an inhibitor of the STAT3 activator Janus kinase (JAK), to the cervical spinal segments suppresses GFAP upregulation and scratching behaviors in models of chronic itch.[29] It appears that ongoing STAT3 activation is crucial for maintaining reactive astrocytes and chronic itch. Moreover, a similar reduction was observed in *Gfap-cre*:*Stat3*$^{flox/flox}$ mice, in which astrocytic STAT3 is inactivated by an astrocyte-selective Cre recombination. In contrast, these mice have normal, acute itch responses to histamine and chloroquine (non-histaminergic itch). Thus, STAT3-dependent reactive state of astrocytes in the SDH is necessary for the progression and maintenance of chronic itch.[29]

TLR4 has been also reported to be required for chronic itch.[31] TLR4 is a member of the TLR family and mediates innate and adaptive immunity by recognition of exogenous ligands, pathogen-associated molecular patterns after viral and bacterial infection, and also detection of endogenous ligands, danger-associated molecular patterns produced by tissue injury.[37] TLR4 and other TLRs have been reported to be expressed in glial cells in the CNS, and activation of these receptors produces a variety of proinflammatory mediators such as cytokines and chemokines.[38] Liu *et al.* have shown that TLR4-knockout mice display reduced spontaneous scratching and touch-evoked scratching (alloknesis) in chronic itch models associated with dry skin and contact dermatitis.[31] A similar reduction is observed in mice intrathecally treated with an antagonist for TLR4, suggesting an involvement of TLR4 in the spinal cord. Spinal TLR4 expression is upregulated in chronic itch models and restricted to astrocytes. Interestingly, TLR4-knockout mice showed a reduction in the GFAP upregulation observed in chronic itch models,[31] suggesting that TLR4 might play a role in astrocyte activation in the SDH. In addition, intrathecal treatment with an astroglial toxin suppressed spontaneous scratching and alloknesis.[31]

Thus, the above findings showing suppression of chronic itch by inhibiting two distinct types of signaling molecule, STAT3[29] and TLR4,[31] whose expression within the SDH is restricted to astrocytes makes a strong case that reactive astrocytes play a causal role in chronic itch.

Modulation of SDH itch signaling by reactive astrocytes

Given the evidence that the astrocytic molecules, STAT3 and TLR4, are necessary for chronic itch,[29,31] astrocytes must influence activity of SDH neurons involved in the itch processing. Indeed, pharmacological and genetic manipulations of STAT3 decreased SDH neurons positive for c-Fos,[29] a marker of neuronal activity. Furthermore, CV-NC/Nga mice show an increase in scratching behavior evoked by intrathecal administration of gastrin-releasing peptide (GRP),[29] which specifically induces itch by acting on GRP receptor (GRPR)-expressing SDH neurons,[39,40] which, interestingly, is abolished by intrathecal administration of AG490,[29] a regimen that is effective to suppress reactive astrocytes in the SDH. Thus, astrocytic STAT3 activation under chronic itch conditions contributes to enhanced GRP-evoked itch signaling in the SDH.

A major issue is then to determine the mechanism(s) by which astrocytes promote itch signaling in the SDH. One candidate for astrocytic factors that modulate itch signaling has been identified, specifically lipocalin-2 (LCN2).[29] Several studies have implicated LCN2 as a protein secreted from reactive astrocytes in response to neuronal inflammation[41–43] and in the modulation of neuronal excitability.[44] LCN2 mRNA and protein levels in models of chronic itch are upregulated and this upregulation is suppressed in *Gfap-cre*:*Stat3*$^{flox/flox}$ mice.[29] The localization of LCN2 in the SDH is specific to astrocytes. Furthermore, LCN2-knockout mice display a reduction in scratching, and knockdown of LCN2 by intrathecal administration of LCN2 siRNA reverses chronic itch. Moreover, the pivotal role of astrocytic LCN2 has been demonstrated by

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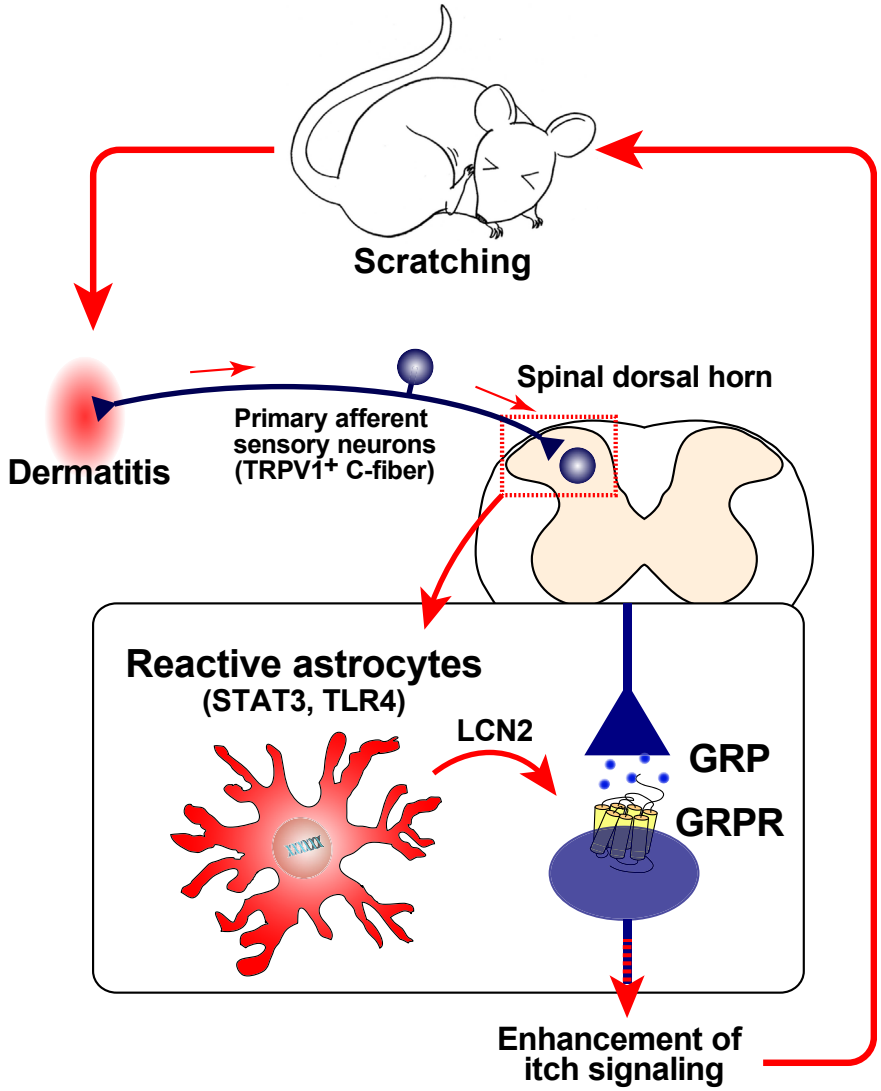

Fig. 2. Schematic illustration of astrocyte-mediated amplification of spinal itch signaling. Under chronic itch conditions, scratching causes skin lesions, which in turn lead to activation of STAT3 in SDH astrocytes in a manner that requires TRPV1$^+$ C-fibers. Activation of STAT3 and TLR4 causes astrocytes to be reactive. Induction of LCN2 production involves STAT3. LCN2 then sensitizes an itch processing neuronal network involving GRPR$^+$ SDH neurons. Enhanced itch signaling leads to excessive scratching, worsening skin lesions, which maintains enhanced astroglial reactivity, and thereby contributes to the vicious cycle of itching and scratching, and to chronic itch.

microinjecting adeno-associated virus (AAV)[45] containing a gene encoding an LCN2 shRNA into the parenchyma of the cervical SDH of CV-NC/Nga mice.[29] Intrathecal co-administration of LCN2 with GRP markedly enhances GRP-evoked scratching. These results together indicate that LCN2 is an astrocytic factor that plays a pivotal role in the progression and maintenance of chronic itch.

In addition, a recent study has revealed a neuronal pathway in the SDH for mechanical itch,[46] which is distinct from the pathway for chemical itch involving GRP-GRPR signaling. From the finding showing that inhibiting spinal TLR4 expressed in astrocytes suppresses the touch-induced itch alloknesis,[31] it is possible that reactive astrocytes in the SDH may also influence mechanical itch signaling through a GRP-GRPR-independent SDH neuronal circuit.

Conclusions

This article primarily focused on the role of astrocytes in chronic itch. A model of the mechanisms underlying astrocytic modulation of chronic itch in the SDH is presented in Figure 2. Of note, pharmacological, molecular, and genetic manipulations of the function or expression of astrocytic STAT3 and TLR4 substantially influences chronic itch behaviors and, importantly, have no effect on acute histaminergic and non-histaminergic itch behaviors under normal conditions.[29,31] Therefore, astrocytic molecules might be a promising target for treating chronic itch.[47] This hypothesis would be strengthened by providing clinical evidence for the presence of reactive astrocytes in the SDH of patients with chronic itch such as atopic dermatitis (which is an important issue in the future) because in patients with atopic dermatitis, intense itch sensations appear only in the lesioned skin, which is considered to be mediated by spinal sensitization.[48] It is expected that an increased understanding of the functions of SDH astrocytes will provide us with exciting insights into the mechanisms for itch chronicity and clues to develop novel therapeutic agents for treating chronic itch.

Acknowledgments

This work was supported by JSPS KAKENHI Grant Numbers 15K15203, and The Uehara Memorial Foundation.

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Conflict of interest

The author has no conflict of interest to declare.

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