

MINISYMPOSIUM

Immune regulation of central nervous system functions: from sickness responses to pathological pain

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Classically, the central nervous system (CNS) and the immune system are thought to operate independently of each other. This simplistic view has been corrected in recent years, first with the recognition that the brain dynamically modulates the immune system, and later with the reverse; that is, that the immune system modulates the CNS as well. The evidence that the immune system regulates CNS functions is first reviewed. This immune-to-brain communication pathway triggers the production of a constellation of CNS-mediated phenomena, collectively referred to as 'sickness responses'. These sickness responses are created by

immune-to-brain signals activating CNS glia to release glial proinflammatory cytokines. The most recently recognized member of this constellation of changes is enhanced pain responsivity. The hypothesis is then developed that pathological, chronic pain may result from 'tapping into' this ancient survival-oriented circuitry, including the activation of immune and glial cells and the release of immune/glial proinflammatory cytokines. This can occur at the level of peripheral nerves, dorsal root ganglia, spinal cord, and likely at higher brain areas. The implications of this model for human chronic pain syndromes and clinical resolution of these chronic pain states are then discussed.

Keywords: allodynia, hyperalgesia, interleukin-1, -10, -6, proinflammatory cytokines, tumour necrosis factor, vagus.

Introduction

This review begins nearly two decades ago, with the discovery that peripheral immune challenges lead to the activation of discrete circuitries within the central nervous system (CNS) [1–3]. Such immune-to-CNS communication occurs using both blood-borne and neural pathways [4]. Activation of these pathways creates a constellation of changes known as sickness responses [1–3]. Following a discussion of immune-to-CNS communication in general, the review then narrows its focus to one specific sickness response: sickness-induced pain

facilitation. This focus was chosen because studies of this enhanced pain state ultimately led to the discovery that clinically relevant pathological pain states may be created by 'tapping into' the ancient CNS circuitries that evolved to ensure host survival [5]. The implications of this viewpoint for the treatment of human pain syndromes are then discussed.

Immune-to-CNS communication: overview

Since the late 1970s, there has been growing recognition that the CNS can dynamically regulate

the function of the immune system [6]. Neural and hormonal systems activated by stress, for example, can potentially alter the basal and evoked responses of immune cells. Considerable effort has been directed at understanding how, and under what circumstances, the brain alters normal immune function [2, 6].

It was only later appreciated that the immune system likewise communicates to, and controls the function of, the CNS. The argument was made that such an immune-to-CNS pathway must exist, based on changes in the behaviour and physiology of sick animals [1]. Such animals exhibit a broad array of so-called 'sickness responses'. Physiologically, sick animals exhibit fever, increased sleep, and alterations in the chemical and cellular composition of their blood. Hormonally, there is activation of both the hypothalamic-pituitary axis and sympathetic nervous system. Behaviourally, sick animals show diverse changes, including decreased food and water intake, decreased activity and exploration, and decreased social and sexual behaviour. As most, if not all, of these changes require alterations in brain function, information about infection/inflammation-induced peripheral immune activation must somehow be relayed to the CNS in order for sickness responses to occur [2, 3].

The release of proinflammatory cytokines by peripheral immune cells provides the critical signal for communicating with the CNS. Proinflammatory cytokines are a family of proteins, including tumour necrosis factor (TNF), interleukin-1 (IL-1), and IL-6. These powerful and synergistic cytokines are classically known for orchestrating the early immune response to challenge, by attracting and activating immune cells [7]. In addition, proinflammatory cytokines have come to be recognized as key mediators of immune-to-brain communication, based on the fact that blocking their actions by administration of specific antagonists can prevent the generation of sickness responses normally elicited by peripheral immune challenges. Furthermore, proinflammatory cytokines administered peripherally in the absence of peripheral immune challenge are sufficient to induce sickness responses [2, 3].

Proinflammatory cytokine actions within the CNS are also instrumental in creating sickness responses. Intriguingly, sickness responses do not, by-and-large, reflect central actions of proinflammatory cytokines that were released in the periphery by

immune cells. Rather, this action of central cytokines reflects *de novo* synthesis of proinflammatory cytokines by cells within the CNS in response to immune-to-CNS proinflammatory cytokine signalling. Glia (microglia, astrocytes) are the predominant sources of these cytokines [8, 9], although neurones may contribute as well [10]. Similar to the pivotal importance of peripheral proinflammatory cytokines reviewed above, proinflammatory cytokines released within the CNS also appear to be critical to the generation of sickness responses (Fig. 1). The importance of central cytokines is indicated by findings that (i) blockade of their actions in the CNS prevents peripherally stimulated sickness responses, and (ii) centrally administered proinflammatory cytokines induce the expression of sickness responses [2, 11]. Hence, proinflammatory cytokines have two key sites of action: at the site of peripheral immune challenge and within CNS sites that create the sickness sequelae.

Immune-to-brain communication: role of the sensory vagus

Whilst peripheral proinflammatory cytokines were recognized as being pivotal in immune-to-brain communication by the late 1980s to early 1990s [12, 13], the pathway(s) that they used to signal the CNS remained elusive. Ensuing investigations identified multiple immune-to-CNS pathways [4, 14], likely a testament to the importance of ensuring that sickness responses are generated in order to enhance host survival. The earliest pathways identified all involved blood-borne immune signals. Evidence accrued for direct signalling to the brain via entry at circumventricular structures where the blood-brain barrier is weak or absent, for active transport of immune products across the blood-brain barrier into brain, and for binding of immune products to brain endothelial cells, leading to the generation of endothelial cell-derived signals on the brain side of the blood-brain barrier [15, 16].

In addition to blood-borne signalling, neural pathways via the sensory vagus and glossopharyngeal nerves have been identified as well [4, 17]. Specifically, it was found that sensory fibres of such peripheral nerves relay information to the brain about inflammation/infection. Vagal and glossopharyngeal afferents predominantly terminate within the nucleus tractus solitarius, a nucleus whose

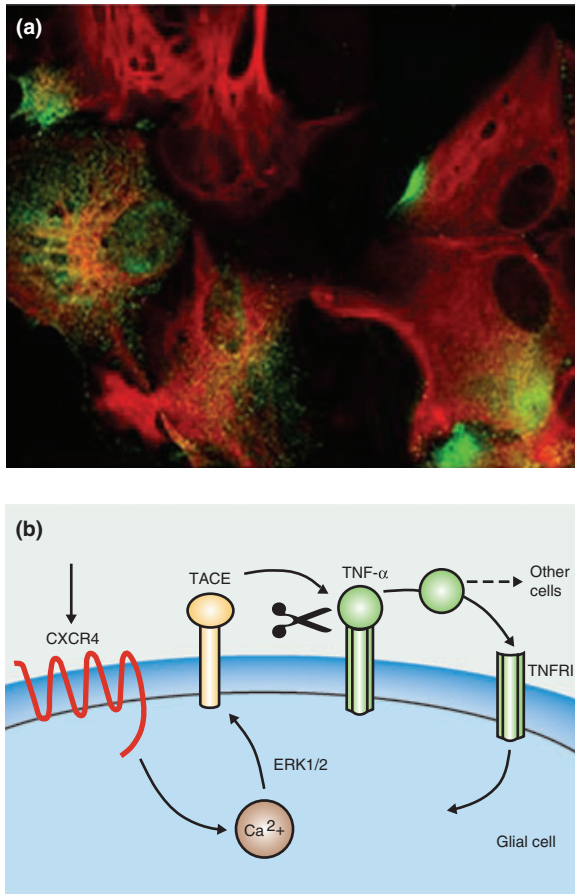


Fig. 1 Proinflammatory cytokines are constitutively expressed by glial cells within the CNS, allowing rapid release. Interleukin-1 (IL-1) is constitutively expressed intracellularly, as an inactive precursor molecule, allowing rapid release upon cleavage by IL-1-converting enzyme. As shown in this figure, tumor necrosis factor- α (TNF- α) is also constitutively expressed but on the extracellular surface of glia. This inactive pro-form is activated upon release from the cell surface by TNF- α -converting enzyme (TACE). (a) Expression of TNF on the extracellular surface of astrocytes. Red fluorescence reflects expression of the astrocyte marker, glial fibrillary acidic protein (GFAP). Green fluorescence reflects extracellular expression of the TNF precursor on approximately half of the astrocytes. (b) Illustration of the molecular events that couple receptor activation of astrocytes (binding to the chemokine receptor, CXCR4 is shown in this example). Intracellular signalling leads to the activation of extracellular signal-regulated kinase (ERK1/2), which activates TACE. TACE then cleaves the extracellular domain of membrane-bound (inactive) TNF to release the active form of TNF via ectodomain shedding. The shed (active) TNF molecule stimulates both the cell that produced it as well as nearby cells, including glia and neurones. Adapted with permission from Watkins & Maier, 2003 [105].

axons project to many brain regions implicated in the generation of sickness responses [18]. The glossopharyngeal nerve has received relatively little

study, but cutting the glossopharyngeal nerve has been reported to block fever induced by injection of IL-1 or bacterial cell walls into the oral cavity [17]. The effect of subdiaphragmatic transection of the vagus nerve has been more broadly investigated. Transecting this nerve is capable of disrupting or attenuating a wide array of sickness responses, including fever, activation of the hypothalamic-pituitary-adrenal axis, suppression of food and water intake, increases in sleep, and increases in pain [2]. This vagus-to-brain pathway has been argued to be especially important in localized immune responses, prior to the generation of blood-borne signals to brain [19, 20].

The vagus nerve is likely to be both directly and indirectly activated by proinflammatory cytokines. Direct activation follows from *in situ* hybridization evidence indicating that cell bodies of sensory vagal fibres express mRNA for IL-1 receptors [21]. Indirect activation is supported by the discovery of specialized sensory structures called paraganglia [22]. Paraganglia are scattered throughout the thorax and abdomen, positioned to sense immune products released in lymph nodes, visceral organs, and the intraperitoneal space. They are composed of glomus cells structurally similar to taste receptors. Discovery that these paraganglia express binding sites for at least IL-1 and that they form synapses onto sensory vagal fibres strongly suggests that these paraganglia serve as an interface between peripheral proinflammatory cytokine release and activation of sensory vagal signalling to the brain [22].

Pain facilitation: a recently recognized component of the sickness response

As reviewed above, the sickness response was first characterized in the mid-1980s [1]. In these original studies pain was simply never an endpoint measured. However, if pain were enhanced in response to peripheral immune challenges, it would fit the concept that, during sickness, behavioural changes are aimed at restricting behaviour, and thereby saving energy for use by host defence [1].

It was not until the early 1990s that it came to be recognized that pain facilitation is a potential sickness response [13, 23, 24] and is a natural consequence of exposure to stimuli that induce sickness responses. This production of pain facilitation has been most thoroughly examined by using

intraperitoneal Gram-negative bacteria cell walls (lipopolysaccharide, endotoxin) or proinflammatory cytokines as the sickness-inducing agents. Such manipulations create pain facilitation which: (i) is mediated by a vagus-to-nucleus tractus solitarius-to-ventromedial medulla-to-dorsolateral funiculus spinal cord pathway [25, 26], (ii) does not involve visceral-to-spinal cord afferents [26], (iii) is prevented by blocking the actions of proinflammatory cytokines [13, 27, 28], (iv) is correlated with glial (microglial and astrocytic) activation [29] and proinflammatory cytokine production [30] within the pain-responsive spinal cord dorsal horn and (v) is mediated at spinal levels by release of substance P, excitatory amino acids, cholecystokinin and nitric oxide [31, 32]. Thus, sickness-induced pain facilitation is created by a well-defined immune-to-brain-to-spinal cord pathway, in which the ventromedial medulla-to-spinal cord 'limb' of the pathway leads to the release of neurotransmitters/neuromodulators that activate spinal cord glia and enhance pain.

Pain facilitation as a consequence of immune activation: physiology versus pathology

As reviewed above, activation of the sensory vagus can induce exaggerated pain, along with other more classically studied sickness responses. These are normal, physiological sequelae to immune challenge. However, pain can also be amplified as a result of damage to peripheral nerve bundles as they course towards the spinal cord. Such damage can: (i) cause warm, cool and touch to be misinterpreted as pain, (ii) amplify the intensity of frankly painful stimuli, and (iii) induce spontaneous pain in the absence of an identifiable stimulus. Such abnormal pain responses, arising from inflammation and/or trauma to peripheral nerves, are referred to as 'neuropathic' pain [33]. Neuropathic pain is a major clinical problem as it is poorly managed by currently available therapeutics [34]. The question arises whether neuropathic pain, like sickness responses, may result from peripheral immune activation in/around peripheral nerves followed by central glial activation and proinflammatory cytokine release. That is, could pathological pain be created by tapping into, and dysregulating, the ancient circuitry that originally evolved to enhance host survival? (Fig. 2).

Immune activation can alter the function of somatic sensory nerves

Immune activation-induced alteration in peripheral nerve function has primarily been studied in sea slugs (*Aplysia*) and rats. From work with *Aplysia*, it is evident that sensory nerve damage induces enhanced pain responses. This change is associated with the recruitment of large numbers of immunocytes (macrophage-like immune cells of *Aplysia*) to the site of injury and the release of TNF-like and IL-1-like substances [35, 36]. The release of *Aplysia* proinflammatory cytokines is thought to participate in pain enhancement, as TNF and IL-1 alter ion channels in *Aplysia* neurones, causing hyperexcitability. In support of this conclusion, injury-induced hyperexcitability of *Aplysia* neurones is enhanced in the presence of activated immunocytes and direct application of proinflammatory cytokines to injured *Aplysia* nerves further increases hyperexcitability to painful stimuli. Indeed, simply attracting activated immunocytes to healthy *Aplysia* nerves is sufficient to induce hyperexcitability, suggesting that proinflammatory cytokines can exert pain-enhancing effects even in the absence of injury [35].

A strikingly analogous pattern of findings has developed from the study of rat neuropathic pain models. These models study the effects of partial nerve injuries, as human neuropathic pain occurs far less often after complete nerve transection than following subtotal injury [33, 37]. It was known by the mid-1980s that partial nerve injury causes exaggerated pain responses and nerve hyperexcitability, as well as Wallerian degeneration. Each of these phenomena has been linked to the activity of macrophages at the injury site [38]. Indeed, neuropathic pain and Wallerian degeneration can each be delayed simply by delaying the recruitment of macrophages to the area [39]. Conversely, neuropathic pain is enhanced by attracting activated macrophages to the injured nerve [40]. Available evidence implicates proinflammatory cytokines (TNF, IL-1 and IL-6) as key immune-derived substances in these pathological changes [41, 42]. Each cytokine is produced at nerve injury sites, being released by a variety of immune and immune-like cells that are either normally found in nerve and/or recruited to the site after injury. These cells include Schwann cells, endothelial cells, fibroblasts, and resident and recruited macrophages [37]. Blockade

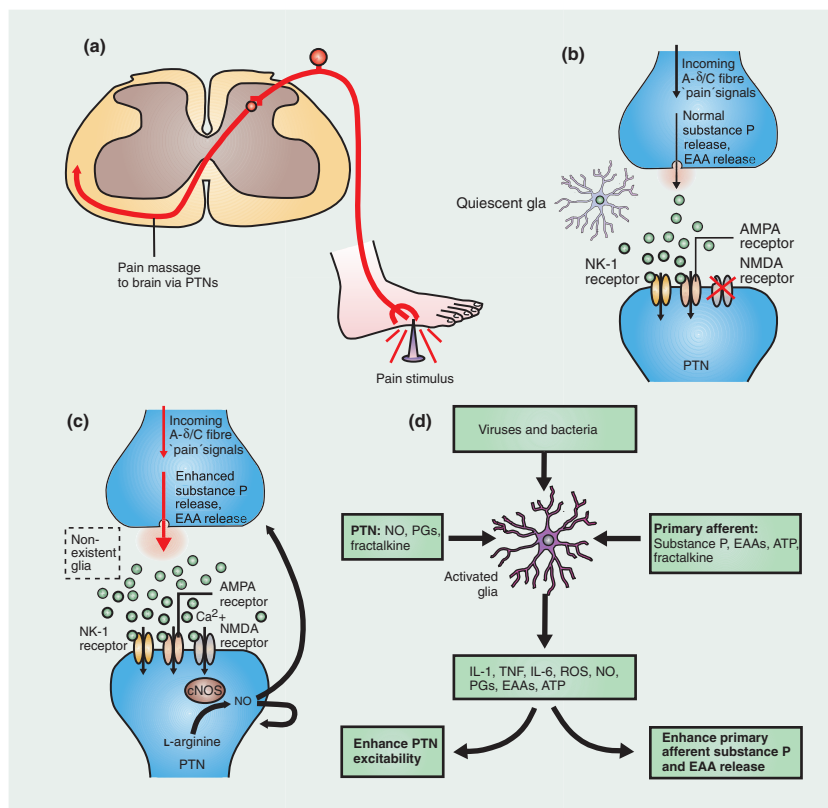


Fig. 2 Classical and nonclassical views of pain transmission and pain modulation. (a) Classical pain transmission pathway. When a noxious (painful) stimulus is encountered, such as stepping on a nail as shown), peripheral 'pain'-responsive A-delta and C nerve fibres are excited. These axons relay action potentials to the spinal cord dorsal horn. Here, neurotransmitters are released by the sensory neurone and these chemicals bind to and activate postsynaptic receptors on pain transmission neurones (PTNs) whose cell bodies reside in the dorsal horn. Axons of the PTNs then ascend to the brain, carrying information about the noxious event to higher centres. The synapse interconnecting the peripheral sensory neurone and the dorsal horn PTN is shown in detail in (b) and (c). (b) Normal pain. Under basal conditions, pain is not modulated by glia. Under these circumstances, glia are quiescent, and thus not releasing pain modulatory levels of neuroexcitatory substances. Information about noxious stimuli arrives from the periphery along A-delta and C fibres, causing the release of substance P and excitatory amino acids (EAAs) in amounts appropriate to the intensity and duration of the initiating noxious stimulus. Activation of neurokinin-1 (NK-1) receptors by substance P and activation of AMPA receptors by EAAs cause transient depolarization of the PTNs, thereby generating action potentials that are relayed to the brain. NMDA-linked channels are silent as they are chronically 'plugged' by magnesium ions. (c) Pathological pain: classical view. In response to intense and/or prolonged barrages of incoming 'pain' signals, the PTNs become sensitized and over-respond to subsequent incoming signals. The intense and/or prolonged barrage depolarizes the PTNs such that the magnesium ions exit the NMDA-linked channel. The resultant influx of calcium ion activates constitutively expressed nitric oxide synthase (cNOS), causing conversion of L-arginine to nitric oxide (NO). Because it is a gas, NO rapidly diffuses out of the PTNs. This NO acts presynaptically to cause exaggerated release of substance P and EAAs. Postsynaptically, NO causes the PTNs to become hyperexcitable. Glia have not been considered to have a role in creating pain facilitation in this neuronally driven model. (d) Pathological pain: new view. Here, glial activation is conceptualized as a driving force for creating and maintaining pathological pain states. The role of glia is superimposed on the NMDA-NO-driven neuronal changes detailed in (c), so only the aspects added by including glia in the model are described here. Glia are activated [shown as hypertrophied relative to (b), as this reflects the remarkable anatomical changes that these cells undergo on activation] by three sources: bacteria and viruses which bind specific activation receptors expressed by microglia and astrocytes; substance P, EAAs, fractalkine, and ATP released by A-delta and/or C fibre presynaptic terminals (shown here) or by brain-to-spinal cord pain enhancement pathways (not shown); and NO, prostaglandins (PGs) and fractalkine released from PTNs. Following activation, microglia and astrocytes cause PTN hyperexcitability and the exaggerated release of substance P and EAAs from presynaptic terminals. These changes are created by the glial release of NO, EAAs, reactive oxygen species (ROS), PGs, proinflammatory cytokines (for example, IL-1, IL-6 or TNF), and nerve growth factor. Modified with permission, from Watkins *et al.*, 2001 [118].

of TNF, IL-1 or IL-6 activity after peripheral nerve injury or inflammation reduces exaggerated pain responses [43–46]. In fact, the magnitude of neur-

opathic pain has been found to directly correlate with the number of proinflammatory cytokine-generating macrophages that are present at the site

[47, 48]. Recently, such investigations have been extended to human nerve biopsy samples, in which a direct correlation was found between nerve cytokine content and degree of axonal degeneration, endoneurial macrophages and epineurial T cells. The patients with higher cytokine content in their nerve biopsies were the ones with neuropathic pain [49].

Similar to results obtained in *Aplysia* immune activation in the vicinity of healthy (uninjured) nerves is sufficient to create exaggerated pain responses. Pain enhancement is observed following exposure of healthy peripheral nerves to gut suture (which activates macrophages), killed bacteria, algae protein (carrageenan), yeast cell walls (zymosan), or HIV-1 envelope protein gp120 [50–52]. Furthermore, these effects are mimicked by direct application of proinflammatory cytokines. For example, TNF applied to nerve induces ectopic activity in pain-responsive nerve fibres and TNF injected into sciatic nerve causes pain facilitation, as well as nerve inflammation, demyelination and degeneration [53–55].

Whilst proinflammatory cytokines have been the immune products that have been the focus of most studies [42], they are not the only immune products that can alter pain by actions at mid-axonal sites. Perisciatic administration of inflammatory mediators such as phospholipase A2 [56] and HMG-1 (high mobility group-I, a recently recognized cytokine) [52] can each induce pain facilitation. In addition, nerve damage is associated with rapid increases in cyclo-oxygenase-2 (COX-2) expression by Schwann cells, and a delayed but prolonged upregulation of COX-2 in macrophages and other infiltrating inflammatory cells [57, 58]. These changes are associated with increases in prostaglandin E2 levels in the nerve and ipsilateral dorsal root ganglia (DRG), but not spinal cord [59]. The elevations in COX-2 expression may be a mediator in neuropathic pain as pain facilitation induced by nerve injury is attenuated by COX-2 inhibitors [59]. In addition to COX-2, neuropathic pain can be blocked or attenuated by scavengers of reactive oxygen species and peroxynitrite [45, 60], inhibitors of membrane attack complexes (a cell-destructive product of complement activation) [45], and inhibitors of histamine and mast cell degranulation [61], as well as by depletion of neutrophils or macrophages [47, 62]. Thus, multiple immune cells and

immune cell products are involved in the production of neuropathic pain.

In the discussion of vagal sensory fibre activation by proinflammatory cytokines above, it was noted that vagal activation was either direct via proinflammatory cytokine receptors expressed on the sensory vagal fibres themselves and/or indirect via expression of these receptors on sensory paranglia. The situation is clearly different in the present context of neuropathic pain. Now, proinflammatory cytokines are inducing neural activity via effects exerted mid-axonally along large nerve bundles. How this activation can occur is controversial. Similar to vagal sensory neurones, somatic sensory neurones can express receptors for proinflammatory cytokines. The existence of this receptor expression is supported by *in situ* hybridization analysis of mRNA in sensory afferent cell bodies in the DRG [63]. However, it is unknown whether receptors for proinflammatory cytokines are inserted along the length of the axon, or whether they are only functional at nerve terminals within innervation sites such as skin. Given that evidence has recently accrued for mid-axonal expression of receptors for ATP (released by damage and activated immune cells) [64, 65], excitatory amino acids [66], and capsaicin [67], it appears likely that the receptor repertoire of sensory nerve axons will prove far broader than previously thought. Immune-derived substances may also affect peripheral axons indirectly. For example, ATP has been reported to first activate Schwann cells, followed by increased excitability of adjacent axons, suggestive of an ATP-induced release of neuroexcitatory substance(s) from Schwann cells [68].

An alternative mechanism has been proposed for mid-axonal excitation of sensory neurones, based on studies of lipid membranes. Using this model system, it has been demonstrated that TNF can dimerize and insert into cell membranes, forming voltage-dependent sodium channels [69]. Insertion and pore formation are facilitated under low pH conditions that are typical of inflammation sites [69]. Indeed, direct application of TNF to sciatic nerve rapidly alters neural activity, suggestive of a direct effect at mid-axonal sites [53]. Finally, TNF, IL-1 and IL-6 have each been documented to produce long-lasting increases in the conductance of endogenously expressed voltage-sensitive sodium and calcium channels, leading to increased neuronal excitability

[70, 71]. Thus, immune activation in the vicinity of nerve bundles can increase neural excitability and pain by mechanisms unique from those previously documented for the vagus nerve.

Immune activation can alter the function of DRG and spinal nerves

Peripheral nerve is not the only site where immune activation may affect sensory function so as to enhance pain. Immune activation may also impact neuronal function at the level of the cell bodies in the DRG and their adjoining nerve processes. In addition to neurones, the DRG contains a variety of immune and immune-like cells, including endothelial cells, resident macrophages, dendritic cells, and glially derived satellite cells, so-named as groups of these cells closely appose and encircle each DRG neuronal cell body [37]. Retrograde signals transported to the DRG from sites of peripheral nerve damage activate these DRG non-neuronal cell types, potentially via the release of ATP or nitric oxide from the damaged neurones [72, 73]. Activation induces the release of proinflammatory cytokines and growth factors from the DRG non-neuronal cells [74–76]. Peripheral nerve damage also greatly increases gap junctional connections between satellite cells that encircled *different* neurones, a putative mechanism for spreading electrical currents and/or second messengers from glia surrounding injured neurones to distant glia apposing healthy, neighbouring neurones [68, 77]. Such gap junctional spread of excitation may alter the responsivity of uninjured neurones within the DRG [68, 77]. In addition, peripheral nerve injury leads to the recruitment of activated immune cells into the DRG from the circulation [78]. Peripheral nerve injury is not the only stimulus for such immune cell migration as it has recently been reported that a long duration influx of immune cells into DRGs occur in response of spinal cord trauma, as well [79].

Taken together, these resident and recruited non-neuronal cells are well positioned to influence DRG neuronal function. For example, DRG neurones upregulate their expression of TNF receptors [80–82] and increase their sensitivity to TNF in response to damage of their peripheral nerves [83], in tandem with upregulation of TNF production by their surrounding glial satellite cells [81]. Furthermore, satellite cell-derived neurotrophins have been linked

to pain enhancing noradrenergic sprouting into the DRG following peripheral nerve injury [75, 76, 84]. Finally, proinflammatory cytokines stimulate DRG neurones to release substance P [85, 86] and calcitonin gene-related peptide [87], two neurotransmitters that relay information about pain to the spinal cord.

The herniation of discs (extruded nucleus pulposus tissue) is another means, in addition to peripheral nerve injury, by which immune activation can influence DRG neuronal function and create pain facilitation. Herniated discs produce a variety of pain-enhancing substances including TNF, IL-1 and IL-6 [88–90] as well as other inflammatory mediators, including phospholipase A2, prostaglandin E2, and nitric oxide synthase I [88, 91]. Components of herniated discs can also be responded to as 'foreign' to the host and thus elicit an inflammatory response independent from substances released by the disc itself. That autoimmune responses may contribute to disc-associated pain enhancement is supported by the finding that depletion of peripheral immune cells can prevent the development of nucleus pulposus-induced pain responses [92]. Interestingly, associations between human IL-1 gene locus polymorphisms and both intervertebral disc degeneration and low back pain have recently been reported [93, 94], again suggesting that alterations in proinflammatory cytokine production may be a contributing factor. Studies of herniated discs in animal models reveal that application of nucleus pulposus to nerve roots induces oedema and spontaneous electrical activity in sensory afferents and frank pain behaviours [95–98]. Importantly, all of these effects of nucleus pulposus are prevented by blocking the actions of proinflammatory cytokines, most notably TNF [89, 98, 99]. Indeed, direct application of TNF to DRG and nerve roots elicits abnormal spontaneous electrical activity [100, 101], presumably via binding to TNF receptors known to be expressed by sensory neurones [63]. In addition, exposure of dorsal roots to such disc material increases production of proinflammatory cytokines within the DRG [91]. Beyond proinflammatory cytokines, inhibitors of other inflammatory mediators (phospholipase A2, nitric oxide synthase I) can also attenuate the pain-enhancing effects of herniated discs [102]. Thus, multiple mechanisms exist for immune activation to alter sensory signals relayed from the DRG to spinal cord.

Immune-derived alterations in spinal pain transmission via glial activation and proinflammatory cytokine release

Recall that sickness-induced proinflammatory cytokines in the periphery are recapitulated by the *de novo* production of proinflammatory cytokines within the CNS (above). A parallel recapitulation also occurs upon peripheral nerve injury. That is, proinflammatory cytokines released at sites of peripheral nerve inflammation/injury lead, in turn, to the release of proinflammatory cytokines by glial cells within the spinal cord [103, 104].

Glial activation and proinflammatory cytokine release appears to be critical for the production of pathological pain states, just as they are critical for the production of sickness responses induced by immune-to-CNS communication. That is, glial activation and proinflammatory cytokine release are not simply correlated with pathological pain. They appear to be causal [105]. Given this parallel between pathological pain and sickness responses, it is tempting to speculate that pathological pain may arise by tapping into the spinal 'limb' of the neural circuitry mediating sickness responses more generally (see above).

The convergence of two disparate literatures drew glia to the attention of pain researchers in the early 1990s. One was the literature on sickness responses, which had already documented the key involvement of glial activation and proinflammatory cytokine release within the brain [2, 9, 11]. This, in turn, predicted that exaggerated pain, newly recognized as part of the constellation of sickness responses, would be mediated by glial activation and proinflammatory cytokine release as well. Indeed, as reviewed above, this turned out to be so. A natural extension of this line of investigation was to explore whether glial and proinflammatory cytokine involvement may extend to situations in which pain facilitation is induced by pathological, rather than physiological, conditions. This again turned out to be the case. As one example, neuropathic pain resulting from release of proinflammatory cytokines at the level of the sciatic nerve [45] is, in turn, mediated by the activation of glia and the release of these same proinflammatory cytokines by glial cells at the level of the spinal cord [106–108].

The second, unrelated literature documented that peripheral nerve damage somehow led to glial activation within CNS sites affected by this damage [109]. That is, beginning in the 1970s, activated

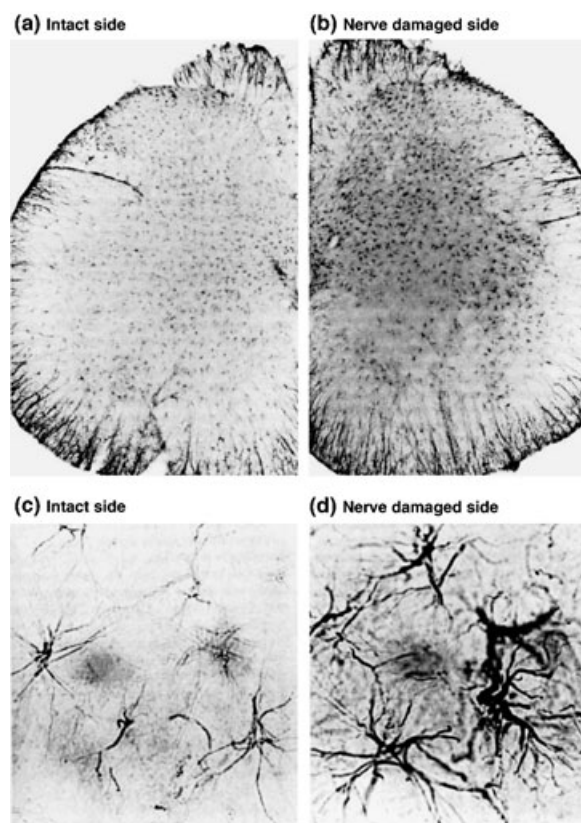


Fig. 3 A historical look at glial involvement in pain. The first anatomical evidence that glia may be involved in pain modulation came from the work of Garrison *et al.* who showed that astrocytes in spinal cord were activated (as reflected by immunohistochemistry for the astrocyte-specific activation marker, glial fibrillary acidic protein; GFAP) in response to sciatic nerve damage. They examined the effect of chronic constriction injury (CCI), as it is one of the best-validated animal models of partial nerve injury leading to chronic pain. Although previous studies had identified CNS glial activation as a rapid response to peripheral nerve injury, the work of Garrison *et al.* was the first to link such glial activation to a functional outcome; namely, enhanced nociception. In the upper panels (a, b), the spinal cord ipsilateral to sciatic damage is compared with the spinal cord on the healthy sciatic side. Clearly, these two sides look different. To more clearly see what this difference is due to, the lower panels (c, d) provide a high-power image of dorsal horn astrocytes. Compared with astrocytes on the healthy spinal cord side (c), astrocytes on the nerve-damaged side (d; same magnification as c) are hypertrophied and more darkly stained, indicating astrocyte activation. Modified with permission, from Garrison *et al.*, 1991 [111].

glia were observed around the axotomized, degenerating motor neurones subsequent to injury of peripheral motor nerves. Similarly, subsequent to injury of peripheral sensory nerves, activated glia were observed in the terminal field regions of damaged sensory afferents (Fig. 3). Whilst the focus

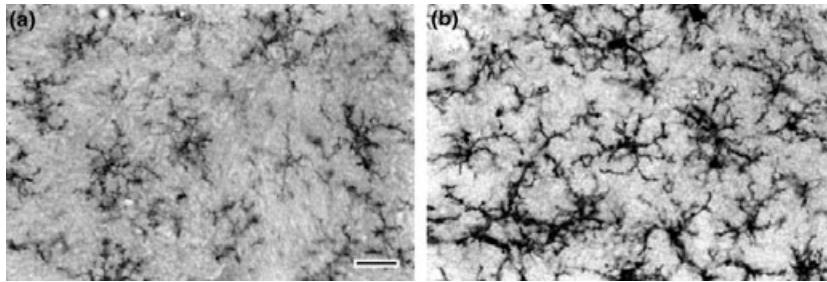


Fig. 4 Microglia, as well as astrocytes, are activated by procedures that create enhanced pain responses in animal models. Although the earliest studies focused on the activation of astrocytes, microglia are also activated. These photomicrographs provide one example of microglial activation in response to a manipulation (intrathecal HIV-1 glycoprotein 120) that produces enhanced pain responses. Microglia are stained for expression of complement-type 3 receptor, which is upregulated when microglia are activated. (a) Normal microglia in the dorsal horn of the spinal cord after peri-spinal injection of vehicle (control). (b) Same magnification view of dorsal horn microglia after peri-spinal injection of a viral protein (HIV-1 glycoprotein 120), which induces exaggerated pain responses. Modified with permission, from Watkins *et al.*, 2001 [118].

of these studies had no link to pain, it did document that robust spinal cord glial activation was produced by nerve damage that would be expected to produce neuropathic pain.

Once such linkages were recognized, numerous studies followed, documenting that every animal model of exaggerated pain tested is associated with the activation of glia (microglia and astrocytes) within the pain-responsive regions of the spinal cord [107, 110–113] (Fig. 4). Subsequent experimentation substantiated that such exaggerated pain states are: (i) mediated by glial activation as they are blocked by drugs (fluorocitrate, minocycline) that block glial activation [106, 107, 114–117], and (ii) mediated by the release of proinflammatory cytokines, based on the effects of selective proinflammatory cytokine antagonists [105, 118]. Thus, parallels again arise between physiological induction of pain facilitation via sickness response circuitry versus pathological induction of pain facilitation via peripheral nerve damage. Both involve activation of glia in pain-modulatory regions of spinal cord, and both are mediated by spinal release of proinflammatory cytokines, most likely of glial origin.

The diverse ways in which glia may facilitate pain within the spinal cord is a topic of ongoing investigation. Neurones express receptors for proinflammatory cytokines and proinflammatory cytokines increase the excitability and 'windup' of dorsal horn neurones that respond to painful stimuli [119, 120]. In addition, proinflammatory cytokines and as-yet unidentified 70 kDa products of activated microglia can exert indirect effects on

nociceptive neuronal function, such as potentiating the efficacy of *N*-methyl-D-aspartate (NMDA) channel openings [121, 122]. Astrocytes can also potentiate NMDA receptor activation via glutamate-induced release of glutamate [123] and homocysteic acid, an endogenous, potent NMDA agonist [124]. Clearly, given the central role of NMDA activation in pain, such modulation of NMDA function would be predicted to enhance pain. NMDA function would also be predicted to be enhanced by downregulation of glial glutamate transporters, such as GLAST (glutamate-aspartate transporter) and GLT-1 (glutamate transporter-1). Intriguingly, these glial-specific glutamate transporters are indeed downregulated in response to peripheral nerve injury [125].

One major issue that is just beginning to be investigated is what triggers spinal cord glial activation in response to peripheral nerve injury. Here, the circuitry must be at variance with that previously defined for sickness-induced hyperalgesia. As reviewed above, sickness-induced hyperalgesia in response to intraperitoneal immune challenge involves a vagus-to-nucleus tractus solitarius-to-ventromedial medulla-to-spinal cord circuit, wherein spinal cord glia are thought to be activated as a result of the release of medullospinal neurotransmitters such as substance P, cholecystikinin (CCK), and/or glutamate [26, 31, 32]. A direct pathway from the periphery to spinal cord does not account for this phenomenon [26]. In contrast, after injury to a peripheral nerve in a hindlimb, something(s) are most likely released by sensory afferents that project to the lumbosacral spinal cord in order for glia to

become activated. At least four potential classes of mediators are currently being considered.

- (1) Neurotransmitters released by activated sensory afferents or brain-to-spinal cord pathways may bind to and activate glia. In support of this possibility, the spinal cord is one of the rare sites in the CNS where glia express receptors for substance P [126, 127]. In addition spinal cord glia express receptors for glutamate [128] and ATP [129], furthering the possibility that pain-related neurotransmitters may activate dorsal horn spinal cord glia as well as neurones. In support of this idea, ATP [130] activates glia and induces their release of proinflammatory cytokines [131–133]. P2X4 purinergic receptors selectively upregulate on microglia after peripheral nerve injury, and blockade of these microglia-specific P2X4 receptors can suppress neuropathic pain [129]. Blockade of all subtypes of P2 ATP receptors with suramin also inhibits spinal cord microglial activation and long-duration pain facilitation induced by subcutaneous formalin [134]. Indeed, if cultured microglia are
- activated with ATP and then these activated microglia are injected over spinal cord, this is sufficient to induce pain facilitation [129].
- (2) Neuromodulators released by activated neurones, such as nitric oxide or prostaglandins may activate glia. For example, nitric oxide is a potent stimulus for proinflammatory cytokine transcription, protein production and release, as recently documented in dorsal spinal cord [135].
- (3) Neurones may release glial excitatory chemokines, such as fractalkine [136, 137]. Fractalkine is a protein that may serve as a selective neurone-to-microglial signal as, in dorsal spinal cord, fractalkine is only expressed by sensory afferent fibres and dorsal horn neurones, whereas the fractalkine receptor is expressed by microglia, and not neurones or even astrocytes [136] (Fig. 5). Fractalkine is tethered to the extracellular surface of neurones in an inactive form, breaking free to form a soluble signalling molecule upon strong neuronal activation [138]. Spinal administration of fractalkine induces pain enhancement, and spinal

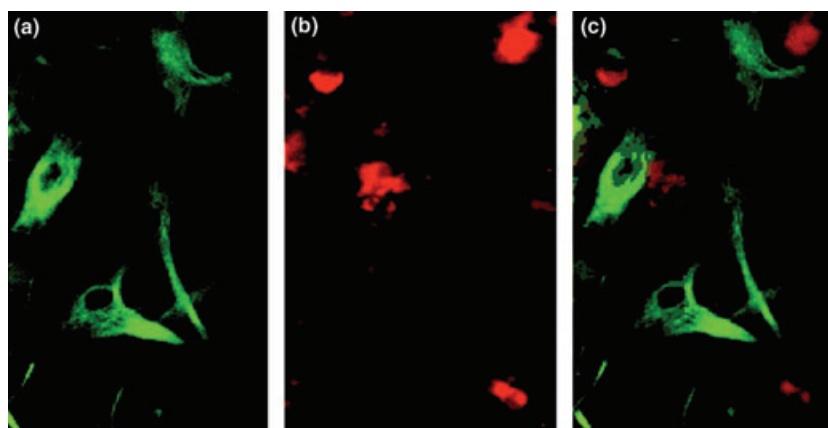


Fig. 5 Neurone-to-glia communication. When pain processing is enhanced by inflammation or damage to peripheral tissues or peripheral nerves, signals must somehow be relayed from sensory nerves to spinal cord glial cells to cause glial activation. There are several possible routes of neurone-to-glia communication that could lead to glial activation and consequent enhancement of pain. One way is that neurones could release a selective neurone-to-glia signal that binds to and activates glia. This avenue of neurone-to-glia signalling has only very recently begun to be productively explored. One candidate signal is fractalkine, a protein expressed on the extracellular surface of neurones that, on strong neuronal activation, can be released into the extracellular fluid. In spinal cord, only microglia express receptors for fractalkine, making it a putative neurone-to-glia signal. Fractalkine, either injected exogenously or released endogenously in response to peripheral nerve damage, enhances pain responses in animal models. The photomicrographs are of astrocyte and microglia mixed cultures. These photomicrographs demonstrate that microglia, but not astrocytes, express fractalkine-binding sites. Green fluorescence (a, c) reveals glial fibrillary acidic protein (GFAP), as astrocyte-specific marker. Red fluorescence (b, c) reveals binding of fluorescent fractalkine. The lack of yellow co-localization of green and red indicates that astrocytes do not express binding sites for fractalkine. By contrast, all microglia in the field bind this putative neurone-to-glia signal. (c) Shows the mixed glial culture with superimposed fluorescence images. Modified with permission, from Watkins & Maier, 2003 [105].

administration of a fractalkine receptor antagonist delays the initiation of neuropathic pain [137]. Even more intriguing is the fact that well established neuropathic pain can also be reversed by administering a fractalkine receptor antagonist, suggesting that peripheral nerve damage leads to prolonged spinal release of fractalkine [137].

- (4) Finally, glial-excitatory signals may be released from sensory afferent fibres within the spinal cord following damage of their peripheral nerve axons. Damaged and dying neurones can release a variety of substances that activate glia, such as ATP, prostaglandins, and heat shock proteins (HSP). HSPs are a family of proteins that normally act intracellularly to support survival from cellular stresses. However, when HSPs are released extracellularly, such as occurs with cell damage and death, they can bind to specific receptors expressed by glia, such as Toll-like receptor 4 (TLR4) [139]. This binding can lead to glial activation and the release neuroexcitatory substances, including nitric oxide and proinflammatory cytokines [140]. This process may be relevant to neuropathic pain as HSP27, for example, has been identified in degenerating sensory afferent fibres in the dorsal horn following peripheral nerve injury [141] and HSP27 synergizes with other glial activators to elicit proinflammatory cytokine release from rat dorsal spinal cord glia *in vitro* [142]. Lastly, the HSP receptor TLR4 upregulates in microglia following a spinal nerve transection procedure that induces neuropathic pain [143]. Taken together, recent studies are beginning to define a variety of ways in which neurones can signal glia to become activated to release neuroexcitatory substances.

A related but as yet unexplored issue is whether the triggers for acute pain enhancement and perseverative pain enhancement differ. It can be argued that pain enhancement in response to acute tissue inflammation/injury is adaptive whereas pain enhancement as a result of prior nerve damage is not. Are glia involved in both of these sensory changes and are these situations mechanistically identical? Regarding the first question, glial activation is importantly involved in pain enhancement induced by acute inflammation [113–115, 144]

and, as reviewed above, also involved in pathological pain such as neuropathic pain. To date, the cascade of events is only known, even in part, for the consequences of peripheral nerve damage leading to neuropathic pain. Here, present evidence suggests that microglia are activated first, and their activation induces the initiation of exaggerated pain responses [116, 117]. This is in keeping with the 'reactive' nature of microglia, as sensors of pathological events [145]. Consequent to microglial activation, astrocytes become activated and become prominently involved in the perseverative stages of neuropathic pain [116, 117].

Whilst these data are clear, they do not make inherent sense if glial activation is the result of the release of neurotransmitters. This is because of the intimate proximity of neuronal synaptic junctions to astrocytes (which enwrap, receive signals from, and send signals to the majority of synapses) [146, 147] versus microglia (which are totally divorced from synapses). If neuronal release of neurotransmitters were the primary signal for glial reactivity, then astrocytes would seem to be far better positioned to react than microglia. Whilst speculative, it might be expected that astrocytes would be found to be the glial cell first triggered in response to acute injury/infection in which the predominant signal from neurones is enhanced synaptic activity, whereas microglia would be the first to be triggered under pathological conditions in which HSPs, fractalkine, and other nonsynaptically released substances would readily reach microglia, the 'sensors of pathology' [145].

Implications for human chronic pain control

The implications of the circuitries defined above for human pain control are great. [105]. Whilst it would not be desirable to disrupt the normal generation of fever or other primary sickness responses or immune responses central to host survival, disrupting pain facilitation could well be a valuable goal. This is especially true in the case of pathological pain in which pain syndromes such as neuropathic pain remain as yet unresolved dilemmas for drug development.

The problem of human pathological pain should not be underestimated. Currently available drugs for treating pain were developed under the assumption

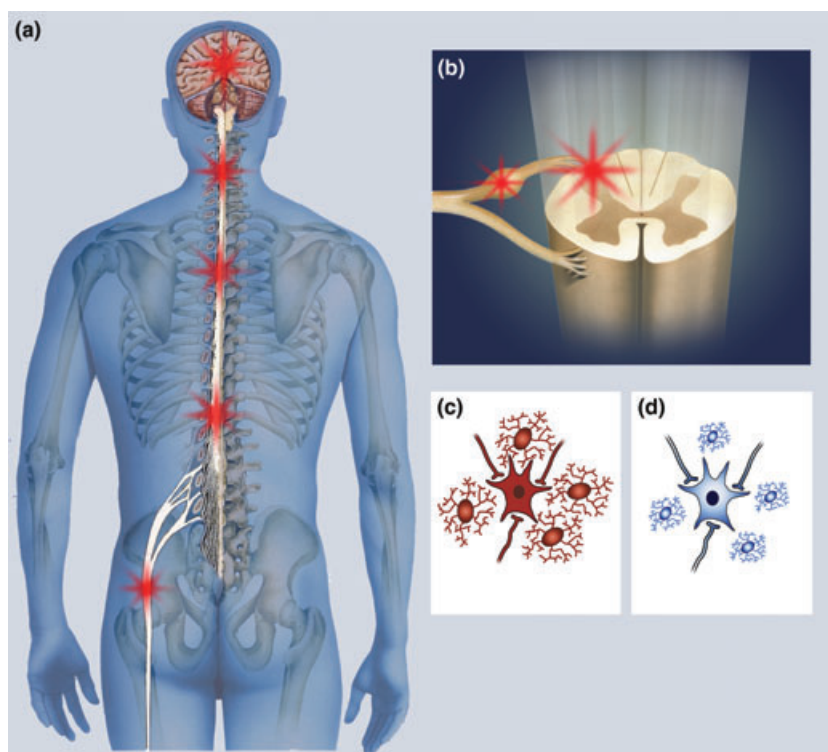


Fig. 6 Implications of glial regulation of pain for human pain control. The data reviewed from animal studies suggests that glial activation may potentially be powerfully modulating pain in human chronic pain states as well. (a) A schematic of human neuropathic pain where trauma, infection or inflammation of one sciatic nerve at hip level, for example, leads to amplification of pain signalling, first to the spinal cord and then up to the brain. (b) Illustrates that upon the pain signal reaching the spinal cord dorsal horn the pain message is greatly amplified, thus amplifying the transmission of pain to higher centres. This pain amplification can occur by neuronal as well as glial mechanisms. (c) Illustrates hypertrophied, activated glia enhancing the activation of neighbouring neurones and sensory afferent fibres. In situations where the glia assume this activated profile, they release neuroexcitatory substances which can enhance release of 'pain' neurotransmitters from incoming sensory fibres as well as enhance the excitability of pain transmission neurones that relay pain messages to the brain. (d) Illustrates the aim of newly developing therapies for human clinical pain that target glial activation. That is, data from animal studies of glial modulating drugs (such as propentofylline and methotrexate) and intrathecal anti-inflammatory cytokines (such as interleukin-10) suggest that pathological pain states can be effectively treated by 'calming' the hypertrophied, activated glia, causing them to revert to their quiescent, basal state. Under such conditions, glia would resume their normal everyday functions in the CNS and not release pain-modulatory neuroexcitatory substances.

that neurones were the only cell type involved in the dysregulation of pain. These drugs fail to control pathological pain [34]. Even a 'good' drug for controlling pathological pain is one that leaves three to four of every five pain patients with no pain relief [148, 149]. The remaining patients generally receive partial relief at best. This is an abysmal profile.

The hope raised by the research reviewed here, for finally gaining control over clinical pain, is that the premises defined above are wrong. That is, pain should now be viewed as being powerfully modulated by immune cells and glia, rather than only by neurones. The convincing conclusion across animal models, across laboratories, and across methodologies is that peripheral immune cells and spinal cord

glia are profoundly involved in the amplification of pain [105]. New therapies need to be developed which target these non-neuronal cells in humans to test their involvement in clinical pain states (Fig. 6). Whilst a number of drugs, as well as novel gene therapies, targeting immune/glial activation have proved successful in multiple animal models [105], this success has yet to move into clinical trials for pain. The hope, and the promise of this research, is that this will soon change.

Conflict of interest statement

Whilst we do not have conflicts of interest, we have received research support in terms of supplied

reagents or research funding from several of our industry collaborators, including Amgen, Avigen, GlaxoSmithKline, Johnson & Johnson and Astra-Zeneca.

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