

# Nociceptor Sensitization by Proinflammatory Cytokines And Chemokines

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**Abstract:** Cytokines are small proteins with a molecular mass lower than 30 kDa. They are produced and secreted on demand, have a short life span and only travel over short distances if not released into the blood circulation. In addition to the classical interleukins and the chemotactic chemokines, growth factors like VEGF or FGF and the colony stimulating factors are also considered cytokines since they have pleiotropic actions and regulatory function in the immune system. Despite the redundancy and pleiotropy of the cytokine network, specific actions of individual cytokines and endogenous control mechanisms have been identified. Particular local profiles of the classical proinflammatory cytokines are associated with inflammatory hypersensitivity and suggest an early involvement of TNF $\alpha$ , IL-1 $\beta$  and IL-6. An increasing number of novel cytokines and the more recently discovered chemokines are being associated with pathological pain states. Besides acting as pro- or anti-inflammatory mediators increasing evidence indicates that cytokines act on nociceptors. Neurons within the nociceptive system express neuronal receptors and specifically bind cytokines or chemokines which regulate neuronal excitability, sensitivity to external stimuli and synaptic plasticity. A first step towards a more mechanistic and individual pain therapeutic strategy could be avoidance of hypersensitive pain processing by either neutralization strategies for the proalgesic cytokines or by shifting the balance in favour of antialgesic members of the cytokine-chemokine network.

**Keywords:** Hypersensitivity, inflammatory pain, unrepathic pain, neuroimmune interaction.

## HYPERSENSITIVITY AND NOCICEPTOR SENSITIZATION

Tissue injury and inflammation commonly cause hypersensitivity of the affected body region, so that normally painful stimuli become more painful (hyperalgesia), and those usually associated with nonnoxious sensations evoke pain (allodynia). The neural bases for these sensory phenomena have been explored most extensively using heat injury and experimental arthritis as models. Heat and/or mechanical hypersensitivity is observed after burns, inflammation, nerve lesion and malignant tumour growth. In models of peripheral inflammation hypersensitivity has been attributed to sensitization of myelinated (A $\delta$ ) and unmyelinated (C) primary sensory neurons [1] that normally respond to potentially tissue damaging (noxious) stimuli. Since the first report on primary afferent fibres that responded only to damaging stimulation of the skin and therefore were termed nociceptors [2] our knowledge on the function of these fibres and their association with pain has increased substantially. Detailed analyses of nociceptor function have been performed and strict criteria are available for phenotyping distinct classes of nociceptors in mice, rats and men [1, 3-6]. Nociceptors occupy a prominent functional position in fast information detection, transduction and transmission of potentially noxious stimuli. They can undergo plastic changes and nociceptor sensitivity is modulated by a plethora of

mediators occurring in the extracellular space. These mediators activate ion channels or metabotropic receptors in the nociceptor membrane resulting in excitatory discharge or a drop of physical activation threshold frequently accompanied by an augmented response of single nociceptors to mechanical, heat or cold stimuli. These alterations result in hypersensitivity and/or ongoing pain [7, 8].

## ION CHANNELS FOR THERMAL AND MECHANICAL NOCICEPTIVE TRANSDUCTION

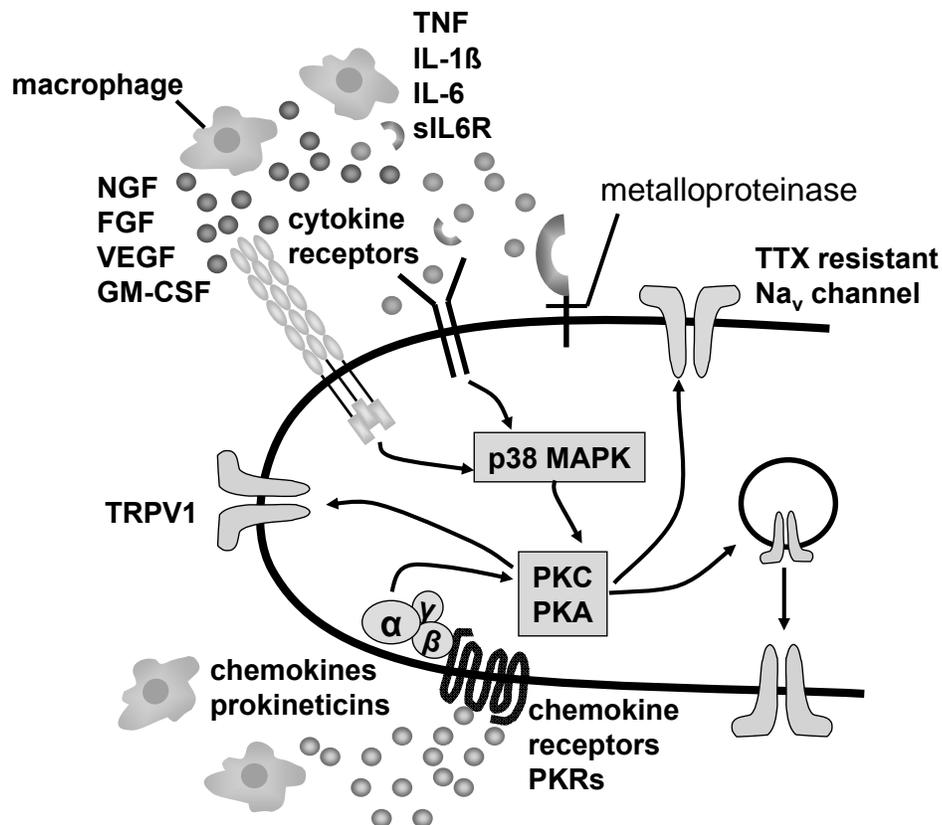
In normal tissue, the sensation of heat pain occurs at a temperature of  $\sim 44^{\circ}\text{C}$ . This correlates well with the activation threshold temperature of polymodal nociceptors and of the nociceptor-specific heat transducer transient receptor potential vanilloid receptor 1 (TRPV1 [9, 10]), a member of the thermoTRP family of ion channels [11, 12]. Nonetheless, nociceptors lacking TRPV1 have normal heat responses [13] and TRPV channel block with ruthenium red did not affect heat sensitivity of mechano-heat sensitive (polymodal) nociceptors, the most common nociceptor type, *in vitro* [14]. Other heat-sensitive ion channels must therefore be responsible for physiological transduction of heat stimuli. Thermosensitive TRPA1 so far has been found sensitive to cold stimuli (McKemy *et al.*, 2002; Story *et al.*, 2003), but possible candidates may be other members of the TRP superfamily, e.g. members of the TRPC subfamily which are also expressed in nociceptors [15-17]. The TRPC1 and TRPC6 ion channels co-operate with TRPV4 and may thus mediate mechanical hyperalgesia and nociceptor sensitization. However, it is well established that the capsaicin receptor TRPV1 is essential for the development of inflammatory hypersensitivity to heat stimuli in mice [18, 19]. The sensitivity of TRPV1 to heat and capsaicin depends

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on the phosphorylation status of the channel at intracellular serine/threonine or tyrosine sites [20-22] and this is regulated by a variety of inflammatory mediators including cytokines and chemokines (for review see [23, 24]). Intracellular signalling cascades are diverse but frequently converge on the activation of protein kinases (Fig. 1). Specific phosphorylation sites within TRPV1 intracellular sequence domains have been identified for PKC isoforms which are essential regulators of ion channel function and trafficking ([25-28] and see also the chapter of Camprubi-Robles and co-authors in this issue). Protein kinases of the PKA or CaMK type may share these phosphorylation sites and regulate TRPV1 [29-33] and even crosstalk between different arms of the signalling pathways may be relevant, however, are to date not fully understood. Nonetheless, drugs targeting TRPV1 are considered promising novel analgesics [23, 34].

The enigma of “the” nociceptor specific mechanosensitive ion channel so far has not unequivocally been resolved although a number of mechanosensitive ion channels has been identified (for review see [35]). Regarding the molecular correlates of mechano-nociceptor sensitivity several ion channels including members of the degenerin/ASIC family or the TRP family have been discussed as possible mechanosensors (for review [36-38]). While some of the ASIC

channels have been excluded as relevant channels for mechanical nociceptive transduction [39], some of the TRP channels expressed in nociceptive primary afferents are currently analysed by several groups. Recent data suggest a role in the detection of noxious mechanical stimuli for TRPA1 [40, 41] which is also a potential target for modulation by chemokines and cytokines [42]. The TRPV4 channel may also contribute to the development of mechanical hyperalgesia of diverse etiologies, presumably as part of a mechanoreceptor signalling complex [43, 44]. It interacts with cytoskeletal components and members of the TRPC subfamily and is inhibited by the stretch-activated channel (SACs) inhibitor GsMTx-4. Intradermal injection of GsMTx-4 into the rat hind paw reversed mechanical hypersensitivity induced by intradermal injection of inflammatory mediators. In addition, single fibre recordings showed that GsMTx-4 reversed inflammatory mediator-induced decrease in mechanical threshold in half of sensitized C-fibres. Furthermore, GsMTx-4 reduced hypersensitivity to both mechanical stimuli in models of inflammatory and neuropathic pain, but did not affect baseline mechanical nociceptive thresholds [43-45]. Therefore, TRPV4 may be relevant for mechanical hypersensitivity. Lastly, TRPC1, and TRPC6 are expressed in DRG neurons [15, 45, 46] and antisense RNAi to TRPC6, but not to TRPC1, reversed the mechanical hyperalgesia



**Fig. (1).** Cytokines bind specific membrane receptors to regulate nociceptive ion channels in primary afferent nociceptors. Macrophages release cytokines, chemokines or growth factors which bind to membrane receptors or soluble receptors. Intracellular signalling cascades involving protein kinases phosphorylate ion channels and increase neuron excitability or sensitivity for natural stimuli. NGF: Nerve growth factor, FGF: fibroblast growth factor, VEGF: vascular endothelial derived growth factor, GM-CSF: granulocyte macrophage colony-stimulating factor, TNF $\alpha$ : tumour necrosis factor  $\alpha$ , IL-1 $\beta$ : interleukin-1 $\beta$ , IL-6: interleukin-6, sIL-6R: soluble IL-6 receptor, TTX: tetrodotoxin, TRPV1: transient receptor potential vanilloid 1 receptor channel, PKR: prokineticin receptor, p38 MAPK: p38 mitogen-activated protein kinase, PKA: protein kinase A, PKC: protein kinase C.

induced by a thermal injury. TRPC1 and TRPC6 channels may cooperate with TRPV channels to mediate mechanical nociceptor sensitization [45].

### HYPERALGESIAS ASSOCIATED WITH PAIN STATES WITH INFLAMMATORY COMPONENT

While acute pain serves as an essential alarm system to protect our body's integrity, tissue injury including inflammation, nerve lesion and cancer generate pathological pain states characterised by mechanical and/or thermal (heat, cold) hyperalgesia and/or allodynia in humans. A number of animal models have been developed to study the associated changes within the nociceptive system [47] and investigate peripheral mechanisms of thermal and mechanical hypersensitivity [48]. Proinflammatory mediators which signal inflammation have been found to sensitize or excite nociceptors and cause hypersensitivity in animal models [7, 49-53]. Many of the findings obtained on nociceptor pathophysiology from animal models are translated into human pain models (for review see [54]). Regarding neuroimmune interactions, cytokines have emerged as the most important link between the immune system and nociception. Since the first report on the interferons [55, 56] the cytokine family has gained a considerable number of new members. Among the cytokine gene products associated with proinflammatory and proalgesic effects are several families including TNF and members of its superfamily, IL-1alpha, IL-1beta, IL-6, IL-8, IL-15, IL-18, IL-33 and the more recently discovered chemotactic cytokines (chemokines).

### NEUROIMMUNE COMMUNICATION USING CYTOKINES AND CHEMOKINES

In general, cytokines are small proteins with a molecular mass lower than 30 kDa. They are produced and secreted on demand, have a short life span and only travel over short distances if not released into the blood circulation. *In vivo* concentrations are in the range of a few pg to ng per ml. They bind specifically to receptor molecules on the cell surface with binding constants between  $10^{-12}$  and  $10^{-10}$  M. Cytokines are a chemically diverse group of proteins but share common functions as regulators in the immune system. In addition to the classical interleukins and the chemotactic chemokines, growth factors like VEGF or FGF and the colony stimulating factors are also considered cytokines since they have pleiotropic actions and regulatory function in the immune system. Some cytokines and cytokine receptors are shedded by metalloproteinases (see below) which are also briefly reviewed in this article since they regulate cytokines and cytokine receptors in inflammation and cancer [57-59]. Despite the redundancy and pleiotropy of the cytokine network, specific actions of individual cytokines and endogenous control mechanisms have been identified. Particular local profiles of the classical proinflammatory cytokines are associated with inflammatory hypersensitivity and suggest an early involvement of TNF $\alpha$ , IL-1 $\beta$  and IL-6 [60-62]. Moreover, an increasing number of further proinflammatory cytokines and the more recently discovered chemokines are associated with pathological pain [63-66]. Besides acting as inflammatory mediators increasing evidence indicates that cytokines act on nociceptors [65, 67] where they specifically interact with neuronal receptors and

ion channels regulating neuronal excitability, sensitivity to external stimuli and synaptic plasticity [67].

### Tumour Necrosis Factor TNF $\alpha$

TNF $\alpha$  initiates the activation cascade of cytokines, chemokines and growth factors in the inflammatory response and therefore is generally accepted as the prototypic proinflammatory cytokine. Converging evidence points to a strong correlation between the number of macrophages, the level of TNF $\alpha$  production and the development of heat-hyperalgesia in inflammatory and neuropathic animal models [68, 69]. Moreover, histology of experimental tumours shows a pronounced infiltration of the neoplastic tissue with macrophages and immune cells producing TNF $\alpha$  [70-72]. The TNF $\alpha$  antagonist etanercept<sup>®</sup> attenuates nociceptor sensitization and heat and mechanical hypersensitivity in rodent cancer model [70, 73]. TNF $\alpha$  is accordingly synthesized and released in tumour tissue and induces heat hypersensitivity and pain by directly affecting nociceptors innervating the tumour area. Therefore, it is not surprising that, anti-TNF $\alpha$  treatment of refractory pain in selected pain patients significantly improved pain scores [74, 75]. Lastly, there is a strong link for TNF to the generation and maintenance of neuropathic pain [69, 76-79]. In animal models, injection of TNF induces mechanical and thermal hypersensitivity [70, 80, 81]. TNF seems to affect nociceptors directly, since sensitization of cutaneous nociceptors to heat also occurs *in vitro* at physiological pH which largely excludes secondary effects [70, 82, 83]. TNF $\alpha$  elicits neuronal discharges in dorsal root ganglion (DRG) neurons, and injured as well as neighboring uninjured afferent neurons exhibit an increased sensitivity to TNF [84]. TNF $\alpha$  binds to TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2) in the cell membrane. Both receptor subtypes are expressed by primary afferent nociceptors [70, 85] and upregulated following experimental nerve lesion or inflammation [68, 83, 85]. While neuropathic pain largely seems to depend on TNF $\alpha$  TNFR1 [85-90] TNFR2 seems to be more relevant for the development of cancer-induced heat pain and hyperalgesia since upregulation of TRPV1 and heat hypersensitivity is found in wild type but not in TNFR2-/- mice with experimental cancer [70].

Downstream of its receptors TNF $\alpha$  activates protein kinases including mitogen activated kinase **p38/MAPK** and **PKC** [91]. TNF-induced hypersensitivity to heat is mediated via p38 MAPK [92-94] and TNF $\alpha$  induces a fast sensitization of responses to both heat and the specific TRPV1 agonist capsaicin which requires activation of p38/MAP kinase and PKC [70]. Although PKC phosphorylates TRPV1 at specific sites and regulates channel function [95-97] some of the phosphorylation sites of the TRPV1 channel protein do not show preference for PKC, PKA or CaMKII and could be possible targets for phosphorylation by p38/MAPK [29, 30, 98-100]. Besides regulating TRPV1 channel function at the cell membrane, TNF $\alpha$  also induces up-regulation of TRPV1 expression [101]. Both mechanisms cooperate to sensitize nociceptors to heat. To provide nociceptors with even greater capacity to generate facilitated responses, cytokines also regulate nociceptor excitability (for review see [102]). TNF increases nociceptor excitability in a dual mode of action. First, via p38 activation it enhances TTX-resistant sodium currents which are a critical site of modulation underlying

hypersensitivity [103] and second, TNF suppresses sustained potassium currents which regulate membrane potential [104]. Both effects leave the nociceptive neuron in a hyperexcitable state and increase the probability of action potential firing following an increased generator potential by TNF $\alpha$ .

### Interleukin-1 $\beta$

Inflammation induces hypernociception that is mediated by an initial release of TNF- $\alpha$  which triggers the subsequent release of IL-1 $\beta$  [105, 106]. IL-1 $\beta$  is the most studied member of the expanding IL-1 family because of its role in mediating autoinflammatory diseases (reviewed in [107]). More than any other cytokine family, the interleukin-1 (IL-1) family is closely linked to the innate immunity response [108]. The IL-1 family consists of two major agonistic proteins, IL-1 $\alpha$  and IL-1 $\beta$ , which are pleiotropic and affect mainly inflammation, immunity, and hemopoiesis. In their secreted form, IL-1 $\alpha$  and IL-1 $\beta$  bind to the same receptors and induce the same biological function but differ in their compartmentalization within the producing cell or the microenvironment. Thus, IL-1 $\beta$  is solely active in its secreted form, whereas IL-1 $\alpha$  is mainly active in cell-associated forms (for review see [109]). Major cellular sources for IL-1 $\beta$  in the context of pain include macrophages, glial cells and both sympathetic and sensory neurons [63, 110, 111]. Inflammatory hyperalgesia can be prevented by experimental administration of endogenous IL-1 receptor antagonist (IL-1ra), and pain-associated behavior in mice with experimental neuropathy is reduced by neutralizing antibodies to IL-1 receptors [105, 112]. IL-1 $\beta$  levels are locally increased in mice with experimental tumours. Osteosarcoma-induced thermal and mechanical hyperalgesia is inhibited by high doses of systemic anakinra, a neutralizing anti-IL1 $\beta$  antibody but not when anakinra is given intrathecally. This suggests that some hyperalgesic symptoms observed in the mouse model of bone cancer pain are mediated by peripheral IL-1 $\beta$  induced nociceptor sensitization and may be inhibited by antagonists of IL-1 receptors type I [113]. However, the reduced mechanical hypersensitivity following treatment with exogenously given IL-1ra, the naturally occurring soluble IL-1 decoy receptor, is mainly explained by regulation of spinal nociceptive processing by IL-1 $\beta$  [114-116]. Local injection of IL-1 $\beta$  induces pain reflexes which might occur due to a secondary increase in prostaglandins [117]. The peripheral pronociceptive IL-1 action is likely mediated by a complex intracellular signalling cascade and secondary production of nitric oxide, bradykinin or prostaglandins by which sensitization or excitation of nociceptors may be explained [118-122]. Expression of IL-1 receptor type I (IL-1RI) mRNA in sensory neurons suggests a possible direct influence of IL-1 $\beta$  on sensory processing [110, 123]. IL-1 $\beta$  facilitates heat-evoked release of calcitonin gene-related peptide [82] and sensitizes heat-activated inward currents ( $I_{\text{heat}}$ ) in sensory neurons via PKC and tyrosine kinases [123]. In addition, IL-1 $\beta$  acts in a p38 MAP kinase-dependent manner, to increase the excitability of nociceptors by regulating TTX-resistant voltage-gated sodium channels [124]. IL-1 $\beta$  induced pain hypersensitivity is largely reduced in mice carrying a null mutation for the voltage-gated sodium channel Nav1.9 [125]. In addition, IL-1 $\beta$  induced activation of c-Src kinase regulates preprotachykinin gene expression in rat sensory

ganglia and substance P (SP) secretion [126]. Both, IL-1 $\alpha$  and IL-1 $\beta$  increase the neuronal content of SP. Interestingly, IL-1 $\alpha$  was significantly more efficient than IL-1 $\beta$  in inducing SP expression [127]. Taken together, all data suggest that IL-1 $\beta$  has a significant role in peripheral nociceptor sensitization which similar to TNF $\alpha$  converges on TRPV1 regulation whereas mechanical hypersensitivity mainly depends on indirect signalling pathways.

### Interleukin-6

The classical proinflammatory IL-6 is an important neuronal survival and neurite elongation factor [128-132] and neurons including nociceptors express signal transducer components present at the cell membrane [133, 134]. IL-6 is produced and excreted by immune cells including macrophages, glia cells and even neurons (reviewed in [135]). Increased levels of IL-6 have been correlated with sickness behaviour in humans [136] and treatment associated symptoms like pain, fatigue and others [137]. Apart from controlling immune cell interactions, IL-6 may account for the pain and hypersensitivity associated with inflammation, neuropathy or cancer by directly regulating the gain of pain-sensing neurons. IL-6 $^{-/-}$  mice present with reduced thermal hyperalgesia after carrageenan inflammation or nerve constriction [138-140]. Antisera neutralizing endogenous IL-6 inhibit inflammatory hyperalgesia [141]. In neuropathic mice, nerve injury correlates well with upregulated IL-6 levels and development of thermal hyperalgesia and allodynia [138, 142, 143]. Some tumours produce interleukin-6 [144] and elevation of serum IL-6 levels is found in up to 60 % of lung cancer patients in advanced stages [145]. Mice with a selective deletion of the signal transducer protein gp130 in neurons develop significantly reduced levels of inflammatory and tumour-induced pain independent of the degree of inflammation or tumour growth [146]. In addition, IL-6 sensitises peripheral nociceptors to mechanical stimuli [147]. Both peripheral as well as central sites of action may also be relevant for the side effects of cancer chemotherapy which may increase plasma levels of IL-6 [148]. IL-6, although involved in the generation of neuropathic pain states [149], may on the other hand protect against chemotherapy induced neuropathies without impairing anti-tumoural activity of the anti-mitotic drugs [150] and therefore global neutralization of IL-6 signalling is controversially discussed. However, most experimental studies report pro-inflammatory and pro-nociceptive roles for IL-6 [142, 151, 152]. In most systems including sympathetic neurons, IL-6 effects depend on the presence of the soluble IL-6 receptor (sIL-6R) [129] which after ligand binding heteromerizes with the signal transducer molecule gp130 that is also utilized by other cytokines of the same family, e.g. LIF [153, 154]. IL-6/sIL-6R complex or Hyper-IL-6 (HIL-6), a fusion protein mimicking the effects of the IL-6/sIL-6R complex [128, 155], increase nociceptor responsiveness and induce thermal hypersensitivity [82, 134, 156]. A dual regulation of heat sensitivity by IL-6 and its soluble receptor sIL-6R has been reported [156]. The sensitization involves activation of the Janus tyrosine kinase (Jak), the adapter proteins Gab1 and Gab2 and finally PKC-delta which regulates the heat transducer ion channel TRPV1 [134, 146]. Currently, the launch of inhibitors IL-6 or gp130 as a novel class of anti-inflammatory drugs not only gives rise to great hopes for the

treatment of inflammation in rheumatoid arthritis [157-159] but may also alleviate its most quality of life worsening symptom, pain.

## OTHER PROINFLAMMATORY CYTOKINES

### Colony-Stimulating Factors

Granulocyte- and granulocyte-macrophage colony-stimulating factors (*G-CSF* and *GM-CSF*) were originally defined as haematopoietic-cell growth factors, but have also been shown to act directly on mature myeloid cells. Recent data from animal models indicate that the depletion of CSFs has therapeutic benefit in many inflammatory and/or autoimmune conditions. As a result, early-phase clinical trials targeting granulocyte/macrophage colony-stimulating factor and macrophage colony-stimulating factor have now commenced (reviewed in [160]). CSFs mediate tumour nerve interactions and bone cancer pain in a mouse model [161]. Local injection of GM-CSF causes hypersensitivity to mechanical and thermal stimuli and GM-CSF sensitizes nerves to mechanical stimuli and capsaicin *in vitro* and *in vivo* [161]. Specific membrane receptors are expressed and are functional on nociceptive afferents [161, 162] and inhibition of G-CSF and GM-CSF signalling *in vivo* reduces tumour growth and nerve remodeling, and abrogates bone cancer pain [161].

Although a number of other proinflammatory cytokines have recently been associated with hyperalgesia, we are just beginning to understand their role in regulating nociceptor sensitivity. Frequently, their mode of action is indirect via control of downstream immediators of inflammation which in turn may affect nociceptive primary afferent. Particularly for the development of mechanical inflammatory hypersensitivity a crucial role for leukocytes has been reported and more recently discovered leukocyte products like IL-15, IL-18, IL-33 and the chemokine CINC-1 have come into focus of pain researchers. These cytokines frequently trigger sequential release of interferon-gamma, endothelin and prostaglandins which in turn affect nociceptor phenotypes [163-166].

### Chemokines

When tissue is invaded by immune cells chemokines are released as constituents of the inflammatory soup [167]. Chemokines are small chemotactic cytokines of about 10 kD which are secreted in damaged tissue not only by leucocytes but also by activated glia cells or neurons. More than 45 chemokines have been identified and chemokine classification is based on the presence and position of cysteine residues. The CC group has two cysteines next to each other, in the CXC group the two cysteine residues are separated by one other amino acid, and the CX3C chemokine CX3CL (alias fractalkine) where the cysteines are separated by three other amino acid residues is the only member of its class [168]. Chemokine actions are mediated by seven-transmembrane domain receptors that couple to the inhibitory G-protein  $G_i$ . Currently, nineteen chemokine receptors have been identified and they are expressed on a variety of cells, including immune cells, endothelial cells and neurons (for review see [169-172]). Chemokines promote immune cell migration, induce astrocyte migration and proliferation of microglia regulating nociceptive transmission

in the spinal dorsal horn (for review see [167]). The chemokines CCL2 (and CXCL1) triggers calcitonin gene-related peptide release by exciting nociceptive neurons [173, 174], and induces mechanical hyperalgesia after intradermal injection [175]. In addition, it functions as a neuromodulator in neuropathic pain [175, 176]. The chemokine network is activated at multiple levels of the peripheral and central nervous system and has recently been identified as new target for pain relief [64]. Small molecule antagonists for particular chemokine receptors may therefore not only be promising for the treatment of acute and chronic inflammation [170, 177] but may also be of relevance in pain biology and therapy (for review see [64, 178]).

## GROWTH FACTORS

A number of growth factors have been associated with the development of nociceptor hypersensitivity; however, for many of them we are just beginning to understand their role in pathological pain.

*Nerve growth factor (NGF)* was originally identified as an essential neuronal survival factor in the developing nervous system. In adults, NGF has a crucial role in generating pain and hyperalgesia. The expression of NGF is high in inflamed tissue and anti-NGF treatment provides effective pain control in animal models of inflammatory pain (for review see [179]). NGF and NGF receptor expression are high in immune cells and certain types of cancer [180-182] and more recently, crosstalk between NGF and TNF $\alpha$  has been associated with painful diseases, however, is not yet fully understood (for review see [183]). In inflammatory pain animal models, NGF is involved in thermal hyperalgesia and nociceptor sensitization [184, 185] and neutralization of NGF improves bone cancer pain and reduces up-regulation of ATF3 and other biochemical markers of nociceptor activation [186, 187]. NGF sensitizes nociceptive neurons to heat and capsaicin by binding to specific neurotrophin receptors activating PI3 and p38/MAP kinase dependent pathways [188] and rapidly increases TRPV1 expression in the nociceptor membrane [26, 27, 92]. Several pharmaceutical companies have developed approaches to antagonize NGF including NGF capture blocking the binding to its receptors, and NGF antagonism is expected to provide effective treatment for chronic pain states [179, 189].

*Prokineticins PK1* (vascular endothelia growth factor, *VEGF*) and *PK2* are tissue-specific angiogenic factors which share certain aspects of cytokines: PKs are highly expressed by neutrophils and other inflammatory cells and play a role in immune-inflammatory responses. PK-like hyperalgesic activity was demonstrated in extracts of rat inflammatory granulocytes and PKs seem to be new pronociceptive mediators in inflammatory tissues (for review [190]). VEGF/PK1 is significantly elevated in cancer patients [191] and it is secreted by islets and stellate cells in pancreatic cancer [192]. Nociceptors express prokineticin receptors PKR1 and PKR2 under the control of glia derived neurotrophic factor GDNF. The receptors for prokineticins are present in a fraction of peptidergic C-fibre neurons and in a fraction of myelinated A fiber neurons. PKR-expressing neurons also express TRPV1, and Bv8, an agonist of both PKR1 and PKR2, has recently been shown to sensitize

TRPV1 channels [193]. Intraplantar injection of recombinant PK2 results in a strong and localized hyperalgesia with reduced thresholds to nociceptive stimuli. PK2 mobilizes calcium in dissociated dorsal root ganglion (DRG) neurons and mice lacking the PK2 gene display strong reduction in thermal and chemical nociception. However, PK2 mutant mice showed no difference in inflammatory response to capsaicin [194]. Mice lacking the PKR1 gene exhibit impaired Bv8-induced hyperalgesia, develop deficient responses to noxious heat, capsaicin and protons and show reduced thermal and mechanical hypersensitivity to paw inflammation, indicating a requirement for PKR1 signalling associated with activation and sensitization of primary afferent fibres [195]. This may also be the case for other growth factors including fibroblast growth factor [196] which has pleiotropic effects and at the same time may regulate nociception and pain sensation [197].

### METALLOPROTEASES

Matrix Metalloproteases (MMP) are a family of enzymes which contribute to the degradation of the extracellular matrix and this is generally accepted to regulate leukocyte migration, inflammation, and wound healing [58, 198]. MMPs function as regulators of entire groups of cytokines and their downstream signalling pathways since they determine the degree of cytokine receptor activation by shedding of membrane bound receptor proteins or of target proteins relevant for nociceptor or immune cell function [199]. Studies propose the involvement of matrix metalloproteinases *MMP-1,-2,-3,-9,-13* and *ADAM-17(TACE)* and *ADAMTS5* as major *in vivo* mediators of extracellular matrix degradation [200, 201]. They represent promising therapeutic targets to treat osteoarthritic symptoms and more selective inhibitors are currently developed (for review see [202]). MMPs are also emerging as modulators of neuropathic pain [203-205]. Up-regulation of MMP-3 and following macrophage activation caused in the dorsal root ganglion found in animal models of neuropathic pain might be a significant event to trigger a series of reactions occurring along primary nociceptive afferents after nerve lesion [206]. Although little evidence is available for a direct role of MMPs at peripheral nociceptors, MMP inhibitors could be potentially interesting for pain therapy induced by inflammation and nerve lesion since they can control cytokine and chemokine substrates in health and disease. A significant role for MMPs is emerging for neuropathic pain [192, 207, 208] but cleavage of specific substrates and transsignalling by MMPs may be a more general mechanism for regulating nociceptor sensitivity by MMPs.

### CONCLUSION AND OUTLOOK

Severe pain persists in many patients even with high dose analgesic therapies. Individual variations in the severity of pain and in the responsiveness to treatment have been assumed to result from either sociodemographic characteristics (age, sex, race, marital status), clinical health status (performance status, comorbid conditions) or disease-related variables (stage of disease). Cytokines are strongly linked to inflammation, neuropathy and cancer and there is increasing evidence that the balance between proalgesic and antialgesic cytokines is relevant for the severity and persistence of the

accompanying pain [209, 210]. Understanding the molecular epidemiology of pathological pain offers the opportunity of identifying specific genes involved in the cytokine network that could be used for a more personalized treatment of pain. A first step towards a more mechanistic pain therapeutic strategy could be avoidance of generation of hypersensitive pain processing by either neutralization strategies in order to prevent triggering the proinflammatory cytokine avalanche or by shifting the cytokine balance in favour of anti-inflammatory cytokines.

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