

Cytokines in Cerebrospinal Fluid and Chronic Pain in Humans: Past, Present, and Future

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Keywords

Cerebrospinal fluid · Chronic pain · Cytokines · Neuroinflammation

Abstract

Background: That neuroimmune interaction occurs in chronic pain conditions has been established for over a century, since the discovery of neurogenic inflammation in the periphery. However, the central aspects of neuroimmune interactions have not been fully appreciated until the late 1900s, when a growing interest in how cytokines in the cerebrospinal fluid (CSF) might be relevant in chronic pain conditions emerged. Since then, the field has evolved, and nowadays neuroinflammation is considered to be involved in the pathophysiology of chronic pain. Whether or not pain conditions can be called “neuroinflammatory” is a matter of debate. This review summarizes the results from studies investigating cytokines in the CSF in various pain conditions, and critically discusses neuroimmune aspects of pain conditions using previously proposed hallmarks of neuroinflammation as a framework. **Summary:** Fifty-two papers were summarized and their results evaluated according to (a) the level of the measured cytokines in patients compared to controls, and (b) the correlation between cytokine level and pain intensity. A subdivision based on pain

type was also conducted for each of the 52 studies. A total of 49 proteins have been studied in at least 5 studies, 21 of which were upregulated in a majority of studies. IL-8 was specifically upregulated in a majority of studies of nociceptive pain conditions. Regarding correlation to pain intensity, there is a scarcity of data but 31 proteins were upregulated and correlated with pain in at least one study. Of these, 24 proteins were negatively correlated with pain, and 7 were positively correlated. None of the most studied cytokines, such as TNF, IL-1b, IL-6, IL-8, CCL2/MCP1, BDNF, or bNGF, were consistently correlated to pain. **Key Messages:** There is sufficient evidence to say that chronic pain conditions come with an upregulation of several cytokines. However, the majority of correlations to symptomatology seem to be negative, indicating that the cytokines might play a protective role that has not been broadly considered. Calling chronic pain conditions neuroinflammatory seems wrong; instead, a more suitable term for depicting the findings would, perhaps, be to talk about neuroimmune activation.

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Plain Language Summary

In this review, we have summarized the current evidence on signs of dysregulated cytokines, a family of proteins important for the function of both immune cells and nerve cells, in spinal fluid of chronic pain populations. Chronic pain is a complex entity, and alterations in cytokine activity has been proposed as a potential way in which some disease mechanisms might be mediated or maintained. In this review, we found 21 cytokines to be upregulated in chronic pain populations compared to controls. However, their association with pain intensity remains unclear. The few studies that have assessed cytokine levels in relation to pain intensity have found that a majority of cytokines that show some form of association to pain are negatively correlated to pain intensity, indicating possible pain-relieving roles of cytokine activity in the CSF.

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Introduction

The history of the relationship between neurons and inflammation is a rich and interesting one. For much of the 20th century the “immune-privileged status” of the brain parenchyma, based on the absence of the typical local immune responses following administration of antigen and the presence of a so-called blood-brain barrier or border (BBB), seems to often have been translated to “immune-deprived” [1, 2]. However, even before it became clear in the last quarter of the 20th century that the brain parenchyma can mount immune responses; it had already been shown in the early 20th century that the peripheral nervous system plays an active part in bringing about peripheral tissue inflammation and increased pain sensitivity [3, 4].

Indeed, primary sensory neural afferent fibers allow for neuropeptide (e.g., calcitonin gene-related peptide) release at their peripheral site of stimulation, which, in turn, promotes local vasodilation and hyperalgesia [5]. Beyond the role of these neurons in local inflammation, pro-inflammatory transcriptional changes in systemic neutrophils seem to play a role in the transition from acute to chronic pain [6]. In addition, activation of glial cells in the dorsal root ganglia, spinal cord, and brain, as well as production of cytokines and chemokines in the PNS and CNS can also contribute to peripheral and central sensitization [3]. Finally, nociception-associated inflammation in the periphery, but interestingly, also in the central nervous system, can increase BBB permeability, possibly through the actions of glial cells [7–9].

These considerations fit well with what has been coined neuroinflammation (NI), a term originally employed to describe the cerebral inflammatory processes observed in animal models of multiple sclerosis and HIV encephalitis [10, 11]. Although no systematic comparison has been undertaken to determine to what extent nervous tissue alterations during NI correspond to changes in cerebrospinal fluid (CSF) composition, immune cells and cytokines can be detected in CSF under various conditions, including those for which the label “neuroinflammation” has been classically used [12–14]. Subsequently, NI has been proposed to be characterized by four hallmarks: increased nervous tissue cytokine expression, activation of microglia, immune-cell recruitment and neurodegenerative tissue damage [15]. Over time, however, the term has been employed more loosely, e.g., after observations of only microglial activation or increased cytokine expression in the brain in response to peripheral and psychological stressors [15]. Unfortunately, as our realization of the complexity of neuro-immune interactions has grown, the precise meaning of the notion of NI seems to have fallen into darkness.

Today, the stripped-down version of NI is considered a key pathophysiological mechanism driving chronic pain [16, 17]. In animal models, glial cell activation has been linked to both central sensitization and pain behavior [18]. NI is often considered a driver of chronic pain, mediated by cytokines/chemokines released by both neurons and glia cells, and evidence of this is readily found in animal studies on the subject [19]. These interactions, characterized by a bidirectional communication between immune cells, glial cells, and neurons on different levels in the body, have collectively been coined the neuroimmune interface (Fig. 1) [16]. Despite the evident relation between neuroimmune activity and pain in animals, much less is known about the central aspects of the neuroimmune interface in humans, and virtually nothing about how it affects pain.

In humans, NI has been investigated by utilizing noninvasive neuroimaging methods such as positron emission tomography (PET) or magnetic resonance tomography in chronic pain populations [20, 21]. For ethical reasons, more invasive undertakings are limited to postmortem studies, which present with their own limitations, such as scarcity of subjects, unavailability of subjective measurements, etc. Therefore, the most common methodology to study central neuro-inflammatory processes has been to analyze CSF [22]. The CSF is an ultrafiltrate of blood, and its protein composition is to 80% derived from blood proteins and to about 20% from brain parenchymal or intrathecally

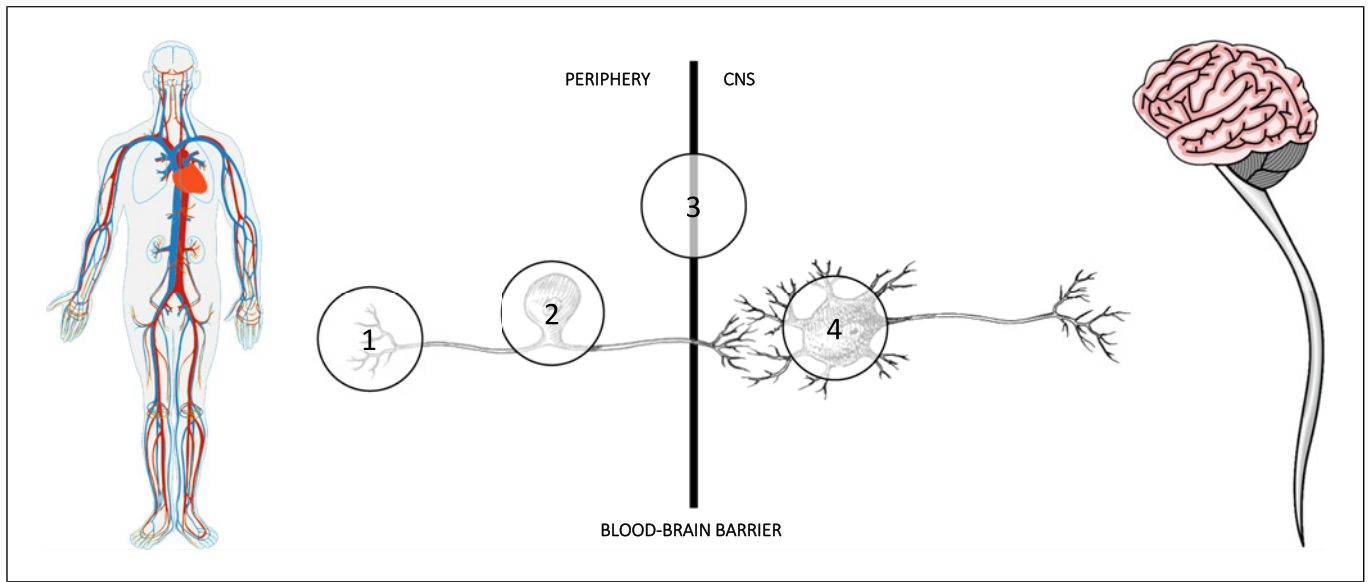


Fig. 1. A schematic overview of the neuroimmune interface. It is, in its essence, where the nervous system meets the immune system and how these two interact. Neuroimmune interaction occurs in (1) peripheral tissues, (2) in the dorsal root ganglia (DRG), (3) along the BBB, which function is to keep peripheral and central compartments separate from each other, and (4) within the central

nervous system (CNS). The cells, both immune and nervous system cells, are different in each of these four anatomical locations and alteration of neuroimmune interaction serves different purposes on each of these levels. Images are from the free-to-use image database pixabay.com and the illustration was made by Alexander Rosenström.

produced proteins [23]. Adult humans produce 450–600 mL of CSF per day, through the actions of ependymal cells in the choroid plexus of the ventricular system, but also through an ever-ongoing exchange of water and solutes through aquaporins that are lined along the membranes between CSF and interstitial fluid [24]. With increasing age and in neurodegenerative diseases, CSF turnover is reduced [25]. At any one time, an average adult has about 150 mL CSF [26], the composition of which is finely regulated by the autonomous nervous system. Indeed, the stability of CSF composition is the basis of its use in diagnosing different neurological diseases [25].

The interpretation of findings obtained in lumbar CSF depends on our understanding of (1) how this restricted fluid compartment relates to the rest of the CSF and (2) how the CSF compartment relates to other biological compartments. Although it has long been thought that the CSF was produced in one site, the choroid plexus, and reabsorbed in another site, the subarachnoid villi, this theory has recently been revised in favor of one in which CSF production and absorption are more widespread across the CNS [24, 27]. This, in turn, has also stimulated a more nuanced view on the CSF compartment being sealed off from the blood circulation by the so-called

blood-CSF barrier, with recent research indicating it being a much less regulated interface than the BBB, providing an alternative entry-point for peripheral cells and proteins [23, 28, 29]. The theory regarding drainage of CSF has also recently been revised to include cervical lymph nodes and the so-called glymphatic system, the latter mainly believed to depend on aquaporin activity [24]. This means that the interpretation of the level of a certain protein in a lumbar CSF puncture requires an understanding of its origin (blood/brain), a relation to the permeability of the blood-CSF-barrier (BCB), and an understanding of the inverse relationship between distance from brain lesion, if applicable, and concentration of the measured biomarker [23].

In the case of pain, there are currently three official pain categories according to the International Association for the Study of Pain (IASP): neuropathic, nociceptive, and nociplastic pain. Neuropathic pain is currently defined as secondary to a disease of lesion of the nervous system, and, therefore, likely to be associated with changes in the neuroimmune milieu, including changes in CSF composition [22, 30–32]. Neuropathic pain is classically encountered in conditions such as postherpetic neuralgia or diabetic neuropathy, although there are several conditions presenting with both neuropathic and

nociceptive pain, such as disk herniations that generally comes with nociceptive back pain on the one hand and neuropathic radiculopathy on the other hand. The pain in neuropathic conditions stands in contrast to nociceptive pain, where pain occurs with a normally functioning somatosensory nervous system due to actual or threatened tissue damage causing the activation of peripheral nociceptors (i.e., high-threshold sensory receptors of the peripheral somatosensory nervous system). Pain conditions that are classically considered to be nociceptive are, e.g., rheumatoid arthritis and other forms of inflammatory pain, osteoarthritis, and degenerative disk disease. In 2016 a new term, nociplastic pain was proposed to designate the large groups of patients with conditions characterized by altered nociceptive function not explained by neuropathic or nociceptive pain mechanisms [33]. Nociplastic pain has since been adopted by IASP as a third mechanistic descriptor of pain, next to nociceptive and neuropathic pain, defined as “pain that arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease or lesion of the somatosensory system causing the pain” [34]. Conditions typically considered as nociplastic include fibromyalgia, irritable bowel syndrome, complex regional pain syndrome type II [33]. Interestingly, a recent review, largely based on animal studies, suggested that microglial polarization toward a neuroinflammatory phenotype may be relevant for nociplastic pain [35]. However, this interpretation does not seem to be supported by PET or CSF studies of patients suffering from nociplastic pain, especially not when compared to patients with nociceptive pain [20, 36, 37]. It is, therefore, timely to address to what extent changes in CSF composition indicative of inflammation occur in nociplastic, as compared to nociceptive and neuropathic, pain.

The purpose of this historical overview was first and foremost to summarize findings from studies analyzing CSF regarding the concentrations of cytokines and the relation between cytokines in the CSF and pain, strictly in human populations. The presented articles are published between 1989 and 2024. More specifically, we aimed to: (a) identify cytokines with altered concentrations in CSF in chronic pain patients compared to controls, (b) analyze if chronic pain conditions characterized by nociceptive, neuropathic or nociplastic pain types present with specific cytokine profiles, (c) review the associations between cytokine levels in CSF and pain intensity, and (d) critically discuss the potential pathophysiological role of neuroinflammatory processes in chronic pain, viewed from a historical perspective.

Methods

Search Regime

We searched the PubMed database for published, original studies on human pain populations that investigated levels of any CSF cytokines. We used the search terms “csf cytokines pain,” filtered on human subjects, and “(cerebrospinal fluid) AND (cytokines) AND (pain),” also filtered on human subjects, and “CSF proteins pain” at several points in time, with the last search being conducted on February 7, 2024.

All original papers that studied cytokines in human CSF in any pain condition were included, provided that baseline data were sufficient, and are listed in Table 1. Some papers [38–53] did not report pain durations in their inclusion criteria or in the demographics of the studied populations but were deemed relevant for this publication after consideration since the included patients were reported to suffer from pain >3 months given the diagnosis (FM) or as they were undergoing treatments reserved for chronic pain patients. The only exceptions were the papers by Bø et al. [44], Cowan et al. [51], and Ludwig et al. [43], respectively, where the duration of the pain conditions could not be ascertained. In all included papers, only baseline data were used. In the case of longitudinal study designs, follow-up results are not covered in this paper, although the number of articles with such data is very few [54, 55].

The above-defined searches yielded 623, 138, and 347 results, respectively, out of which 30, 7, and 2 studies meeting our criteria were identified, respectively. In addition, 13 articles found in reference lists from other papers that did not show up in the search were included (Fig. 2).

Articles meeting the criteria for inclusion in this work were assessed regarding pain type, using the main three categories for pain as defined by IASP: (a) nociceptive pain, defined as “pain that arises from actual or threatened damage to non-neural tissue and is due to the activation of nociceptors,” (b) neuropathic pain, defined as “pain caused by a lesion or disease of the somatosensory nervous system” and (c) nociplastic pain, defined as “pain that arises from altered nociception” not fully explained by nociceptive or neuropathic pain mechanisms, respectively [34]. In addition, studies on headache were separated into a group of their own for two reasons: (a) they are difficult to discretize into pain group and (b) they are usually investigated on their own. Finally, two studies were cumbersome to classify strictly by pain type and were categorized as “other.” These studies included data on patients with undefined chronic pain and failed back surgery syndrome patients, respectively (Table 1).

In total, 52 papers were classified by pain type: nociceptive ($N = 14$), neuropathic ($N = 20$), nociplastic ($N = 11$), headache ($N = 5$), or mixed/undefined ($N = 2$), respectively (Fig. 2). All reference papers, as well as their investigated biomarkers, can be found in online supplementary File 1 (for all online suppl. material, see <https://doi.org/10.1159/000540324>).

Classification of Results

The results from all 52 papers were discretized in two separate ways (symbol used in parentheses) (online suppl. File 2):

1. Whether the cytokine in question was (a) upregulated compared to controls (+), (b) downregulated compared to controls (–), (c) not up- nor downregulated compared to controls (0), or (d) either not detectable or not having any controls (?).

Table 1. A summary of all papers included in the review, along with their investigated diagnoses and classification of pain type

Classification of studies by pain type				
year	author	main diagnosis	pain type 1	pain type 2
1989	Vaeroy et al. [38]	Fibromyalgia	Nociplastic	n/a
1990	Hyyppä et al. [39]	Low back pain with rhizopathy	Neuropathic	n/a
1994	Skouen et al. [40]	Lumbar disc herniation, headache (tension HA/migraine w/o aura)	Neuropathic	Headache
1999	Brisby et al. [56]	Lumbar disc herniation	Neuropathic	n/a
1999	Giovengo et al. [41]	Fibromyalgia	Nociplastic	n/a
1999	Lindh et al. [57]	Orthopedic conditions	Nociceptive	n/a
1999	Yeager et al. [42]	Osteoarthritis	Nociceptive	n/a
2001	Sarchielli et al. [58]	Chronic daily headache, CDH	Headache	n/a
2002	Brisby et al. [59]	Lumbar disc herniation	Neuropathic	n/a
2002	Sarchielli et al. [60]	Chronic daily headache, CDH	Headache	n/a
2004	Baraniuk et al. [61]	Fibromyalgia, low back pain	Nociplastic	Nociceptive
2004	Kotani et al. [62]	Postherpetic neuralgia	Neuropathic	n/a
2005	Alexander et al. [63]	CRPS (83% CRPS I, 17% CRPS II)	Nociplastic	n/a
2006	Liu et al. [64]	Lumbar disc herniation	Neuropathic	n/a
2006	Sarchielli et al. [65]	Fibromyalgia, chronic migraine	Nociplastic	Headache
2007	Alexander et al. [66]	CRPS (83% CRPS I, 17% CRPS II)	Nociplastic	n/a
2007	Rozen et al. [67]	Migraine, daily persistent headache	Headache	n/a
2007	Sarchielli et al. [68]	Fibromyalgia, chronic migraine	Nociplastic	Headache
2008	Backonja et al. [69]	Painful diabetic neuropathy or post-traumatic neuralgia	Neuropathic	n/a
2008	Ludwig et al. [43]	Painful and non-painful polyneuropathy	Neuropathic	n/a
2008	Munts et al. [70]	CRPS	Nociplastic	n/a
2009	Bø et al. [44]	Migraine	Headache	n/a
2010	Lundborg et al. [71]	Osteoarthritis	Nociceptive	n/a
2010	Zin et al. [54]	Long-term pain, IT morphine	Other	n/a
2011	Ohtori et al. [72]	Lumbar spinal stenosis	Neuropathic	n/a
2012	Kadetoff et al. [45]	Fibromyalgia	Nociplastic	n/a
2013	McCarthy et al. [46]	Failed back surgery syndrome	Other	n/a
2015	Kosek et al. [37]	Fibromyalgia, rheumatoid arthritis	Nociplastic	Nociceptive
2016	Qin et al. [73]	Trigeminal neuralgia	Neuropathic	n/a
2017	Bäckryd et al. [74]	Peripheral neuropathic pain	Neuropathic	n/a
2017	Bäckryd et al. [75]	Fibromyalgia	Nociplastic	n/a
2017	Lim et al. [76]	Degenerative disc disease	Nociceptive	n/a
2017	Zhao et al. [77]	Postherpetic neuralgia	Neuropathic	n/a
2018	Andrade et al. [78]	Thoracic disc herniation	Neuropathic	n/a
2018	Azim et al. [47]	Osteoarthritis	Nociceptive	n/a
2018	Das et al. [79]	Radicular pain	Neuropathic	n/a
2018	Giron et al. [80]	Postlaminectomy syndrome	Nociceptive	n/a
2018	Kosek et al. [48]	Osteoarthritis	Nociceptive	n/a
2019	Ericson et al. [49]	Trigeminal neuralgia	Neuropathic	n/a
2019	Krock et al. [81]	Low back pain	Nociceptive	n/a
2019	Palada et al. [82]	Lumbar disc herniation, degenerative disc disease	Nociceptive	Neuropathic
2020	Bjurström et al. [83]	Osteoarthritis	Nociceptive	n/a
2020	Palada et al. [50]	Osteoarthritis	Nociceptive	n/a
2021	Cowan et al. [51]	Migraine	Headache	n/a
2021	Jönsson et al. [84]	Peripheral neuropathic pain	Neuropathic	n/a
2022	Liu et al. [85]	Osteoarthritis	Nociceptive	n/a
2023	Baroni et al. [86]	Trigeminal neuralgia	Neuropathic	n/a
2023	Chen et al. [52]	Postherpetic neuralgia	Neuropathic	n/a
2023	García-Fernández et al. [87]	Polyneuropathy (different kinds)	Neuropathic	n/a
2023	Kato et al. [88]	Osteoarthritis	Nociceptive	n/a
2023	Lafta et al. [53]	Trigeminal neuralgia	Neuropathic	n/a
2024	Rosenström et al. [89]	Lumbar disc herniation, degenerative disc disease	Nociceptive	Neuropathic

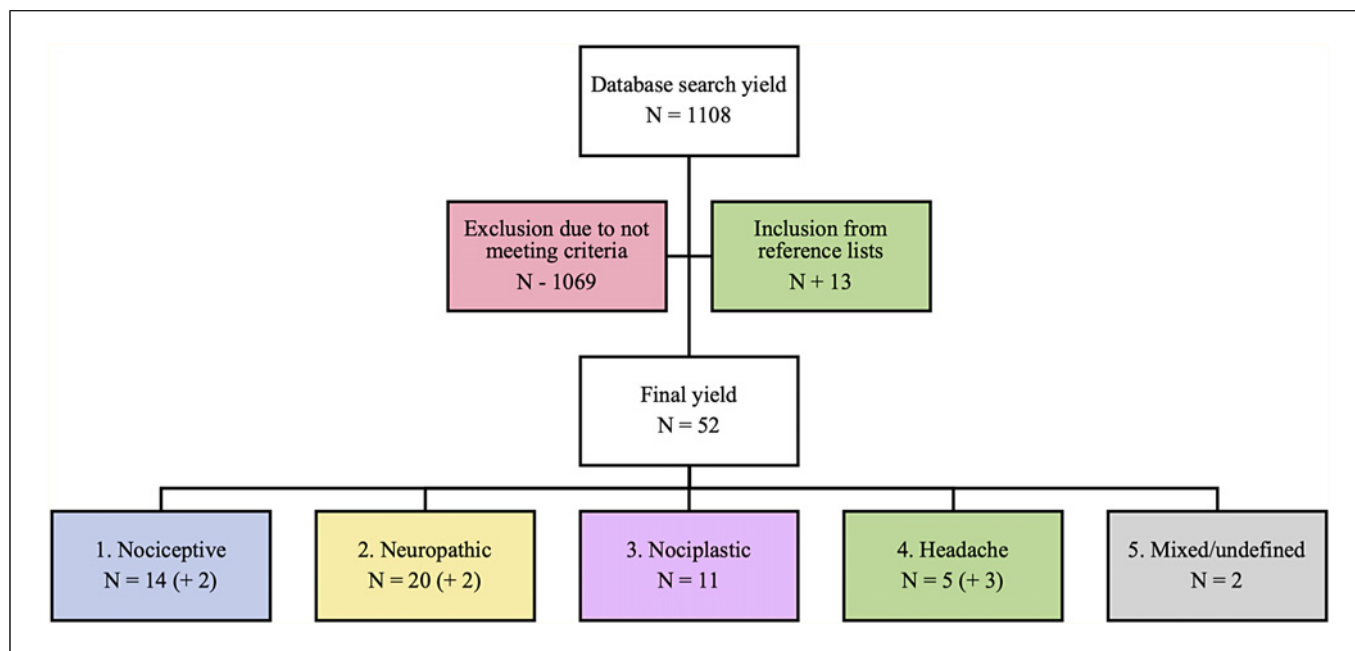


Fig. 2. Literature search and yields; N = number of articles in each stage. Numbers in parentheses indicate the number of papers covering several pain types that were classified as having a different main category. E.g., there were 22 papers on neuropathic pain, 20 of which were classified as investigating mainly neuropathic pain and an additional two being classified as investigating mainly another pain type (in this case, nociceptive pain, as can be seen from Table 1).

2. Whether the cytokine in question was (a) positively correlated to pain intensity (+), (b) negatively correlated to pain intensity (–), (c) not correlated to pain intensity (0), or (d) if pain intensity wasn't assessed/analyzed (?).

Unclear findings (categories “d” above), i.e., comprising studies that either did not have a control group, where the cytokine in question was undetectable, or where pain intensity was not assessed, have not been considered in our calculations since they constitute methodological issues rather than scientific findings. Hence, only the “net” number of studies, i.e., studies comprising groups a-c above, were regarded in the analysis of cytokine expression (Table 2; online suppl. File 3) and correlation to pain, respectively (Table 3; online suppl. File 4). We considered cytokines that were expressed in a similar fashion in more than 50% of the net number of studies to be cytokines of interest.

Results

The results from 52 studies analyzing a total of 212 proteins, mainly cytokines and neurotrophins, in human CSF have been reviewed. All studies, together with their pain type classification, are listed in Table 1. One-hundred cytokines have only been assessed in 1 study, 12 in 2 studies, 5 in 3 studies, and 46 in 4 studies. The remaining 49 cytokines, studied in 5 papers or more, are

summarized regarding expression in comparison to controls and expression in relation to pain intensity, in Tables 3 and 4, respectively. The complete dataset, containing all 212 proteins, their full names and their respective classifications, can be found in online supplementary File 2.

CSF Cytokine Levels in Chronic Pain Patients Compared to Controls across all Pain Types

Twenty-one cytokines assessed in at least 5 studies were upregulated while none were downregulated in more than 50% of the studies, and seven cytokines did not differ between patients and controls in the majority of studies (Table 2).

Cytokine Levels in CSF in Patients Suffering from Nociceptive, Neuropathic, or Nociplastic Pain

Table 3 depicts how the cytokines differed from controls in patients with nociceptive, neuropathic, nociplastic pain conditions as well as headache and mixed pain. Only four cytokines have been analyzed in five or more papers in a specific pain type. Regarding nociceptive pain, 6/9 (67%) of papers reported higher IL-8 levels in the patient's CSF. No significant differences between

Table 2. List of cytokines that are (1) studied in at least 5 papers and (2) expressed similarly in more than 50% of studies

Gene name	Expression compared to controls
4E-BP1	Upregulated
BDNF	Upregulated
bNGF	Upregulated
CCL11	Upregulated
CCL19/MIP-3 β	Upregulated
CCL2/MCP1	No difference in expression
CCL23/MIP-3	Upregulated
CCL3/MIP-1 α	Upregulated
CCL4/MIP-1 β	No difference in expression
CCL8/MCP-2	Upregulated
CD5	Upregulated
CX3CL1	Upregulated
CXCL1	No difference in expression
CXCL10	Upregulated
CXCL11/I-TAC	Upregulated
CXCL5/ENA-78	Upregulated
CXCL6	Upregulated
Flt3L	Upregulated
IL18	Upregulated
IL1b	Upregulated
IL6	No difference in expression
IL8	No difference in expression
LAP-TGF-b	Upregulated
LIF-R	Upregulated
OPG/TNFRSF11b	Upregulated
TNF-a/TNF	No difference in expression
TRAIL/TNFSF10	Upregulated
VEGF-A	No difference in expression

patients and controls were reported for IL-8 in neuropathic pain (67%, $n = 4/6$), IL-6 in nociceptive pain (89%, $n = 8/9$) and CCL2/MCP1 in nociceptive pain (83%, $n = 5/6$).

The Associations between Cytokine Levels in CSF and Pain Intensity

Unfortunately, only very few studies of cytokines in CSF of pain patients also included an analysis of their association with pain intensity. The results for the cytokines where associations to pain intensity were assessed in more than five papers are summarized in Table 4.

When including all pain types in the analysis, no cytokine correlated positively or negatively with pain intensity in chronic pain patients in more than 50% of the papers. No correlations with pain intensity was reported for BDNF (83%, $n = 5/6$), CCL2/MCP1 (100%, $n = 8/8$), IL-1b (83%, $n = 5/6$), IL-6 (86%, $n = 12/14$), IL-8 (92%, $n = 11/12$), bNGF (86%, $n = 6/7$), and

TNF (89%, $n = 8/9$) (Table 4). Refining the analysis according to pain type, no correlations were found between nociceptive pain and CSF levels of CCL2/MCP1 (100%, $n = 5/5$), IL-6 (86%, $n = 6/7$) and IL-8 (86%, $n = 6/7$), respectively, nor between neuropathic pain intensity and IL-6 (83%, $n = 5/6$).

Cytokines in CSF and Their Relation to Pain, a General Overview

The whole dataset of studied cytokines ($n = 212$) was mapped regarding their reported associations with pain intensity (online suppl. File 2) in at least one study. In Figure 3, all cytokines that were positively (+) or negatively (-) correlated with rated pain intensity in at least one study are visualized.

The relationship between the cytokine levels in CSF, i.e., if patients had higher, lower or no significant difference compared to controls and the association between protein levels and pain intensity within the patient groups are summarized in Table 5. Looking at all upregulated cytokines that have been found to be correlated with pain, 77% ($n = 24/31$) have been negatively correlated and 23% ($n = 7/31$) have been positively correlated. TGF- β 1 was significantly lower in patients suffering from nociceptive pain and negatively correlated to pain intensity, and VEGF-A is the only cytokine that has been found to be both positively and negatively associated with pain.

Discussion

Main Findings

In this article, we summarize the current knowledge regarding altered cytokine levels in the CSF of patients suffering from different chronic pain conditions, different pain types as well as the associations between cytokine levels and rated pain intensity. Our main finding is that cytokines tend to be upregulated in the CSF of chronic pain patients when compared to controls, with 21 cytokines studied in at least 5 papers being upregulated in the majority of studies, while the correlation to pain intensity remains largely elusive, mainly due to inconsistent and inconclusive data.

The Origin and Role of Cytokines in Pain Conditions

We have sufficient evidence to say that there are at least two of the hallmarks of NI present in at least some chronic pain conditions, namely upregulation of cytokines and signs of glial activation that is not seen in controls. However, the presence of peripheral immune

Table 3. A summary of the cytokines studied in five papers or more, in relation to levels in the studied control group(s)

Gene name	Upregulated, n					Downregulated, n					Not up- nor downregulated, n					"Net" studies, n					All studies, n								
4E-BP1	<u>4</u>	2	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	5	2	2	1	0	0	5	
BDNF	<u>4</u>	0	1	1	1	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	5	1	1	1	1	1	11	
bNGF	<u>2</u>	2	1	2	2	0	0	0	0	0	0	0	0	0	0	2	0	1	1	0	0	9	2	2	3	2	0	11	
CCL11	<u>5</u>	2	2	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	6	2	3	1	0	0	6	
CCL13 / MCP-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	5	
CCL19 / MIP-3β	<u>5</u>	2	2	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	6	2	3	1	0	0	6	
CCL2 / MCP-1	2	1	0	0	1	0	0	0	0	0	0	0	0	0	0	<u>2</u>	<u>5</u>	2	1	0	1	11	6	2	1	1	1	14	
CCL20 / MIP-3α	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	5	
CCL23 / MIP-3	<u>5</u>	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	2	2	1	0	0	5	
CCL25 / TECK	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	1	0	0	4	2	1	1	0	0	5	
CCL3 / MIP-1α	<u>3</u>	1	2	0	0	0	0	0	0	0	0	0	0	0	0	2	1	1	0	0	0	5	2	3	0	0	0	7	
CCL4 / MIP-1β	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	<u>4</u>	1	2	1	0	0	5	2	2	1	0	0	5	
CCL7 / MCP-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	5	
CCL8 / MCP-2	<u>4</u>	2	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	5	2	1	0	0	0	6	
CD5	<u>4</u>	2	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	5	2	2	1	0	0	5	
CGRP	2	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	4	0	3	0	1	0	5	
CSF1	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	1	0	0	4	2	1	1	0	0	5	
CX3CL1	<u>5</u>	2	2	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	7	2	3	1	0	0	9	
CXCL1	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	<u>4</u>	1	2	1	0	0	6	2	3	1	0	0	6	
CXCL10	<u>5</u>	2	2	1	0	0	0	0	0	0	0	0	0	0	0	3	1	1	1	0	0	8	3	3	2	0	0	9	
CXCL11 / I-TAC	<u>4</u>	2	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	5	2	2	1	0	0	6	
CXCL5 / ENA-78	<u>4</u>	1	2	1	0	0	0	0	0	0	0	0	0	0	0	2	1	1	0	0	0	6	2	3	1	0	0	6	
CXCL6	<u>5</u>	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	2	2	1	0	0	5	
Fib3L	<u>3</u>	2	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	1	0	0	5	2	2	1	0	0	5	
GDNF	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	1	0	0	0	3	1	1	1	0	0	7	
IFNγ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	1	0	11	
IL10	1	0	1	0	0	0	1	0	0	1	0	0	0	0	0	3	0	2	0	0	0	4	0	3	1	0	0	20	
IL13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
IL17A	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
IL17C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	5	
IL18	<u>3</u>	1	1	1	0	0	0	0	0	0	0	0	0	0	0	2	1	1	0	0	0	5	2	2	1	0	0	5	
IL1b	<u>4</u>	1	2	1	0	0	0	0	0	0	0	0	0	0	0	3	0	2	1	0	0	7	1	4	2	0	0	16	
IL1ra	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	3	1	2	0	0	0	4	1	2	0	1	0	7	
IL2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
IL33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	5	
IL4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	12	
IL5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	6	
IL6	5	1	1	3	0	0	0	0	0	0	0	0	0	0	0	<u>13</u>	<u>8</u>	3	1	1	0	18	9	4	4	1	0	28	
IL7	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	5	
IL8	9	<u>6</u>	2	1	0	0	0	0	0	0	0	0	0	0	0	<u>10</u>	3	<u>4</u>	2	1	0	19	9	6	3	1	0	22	
LAP-TGF-b	<u>3</u>	0	2	1	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	5	2	2	1	0	0	5	
LIF-R	<u>4</u>	2	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	5	2	2	1	0	0	5	
NT-3	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	5	
OPG / TNFRSF11b	<u>3</u>	2	1	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	1	0	0	5	3	1	1	0	0	5	
TNF-a / TNF	2	0	1	0	1	0	0	0	0	0	0	0	0	0	0	<u>2</u>	3	2	1	0	0	9	3	3	1	2	0	26	
TNF-b / LTA	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	1	0	0	4	2	1	1	0	0	5	
TRAIL / TNFSF10	<u>3</u>	2	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	1	0	0	5	2	2	1	0	0	5	
TSLP	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	5	
VEGF-A	3	2	1	0	0	0	0	0	0	0	0	0	0	0	0	<u>4</u>	1	1	1	0	1	7	3	2	1	0	1	9	

Column color depicts pain type; white = all studies, blue = nociceptive pain, yellow = neuropathic pain, purple = nociplastic pain, green = headache, and grey = other pain (mixed or undefined). Numbers written in underlined bold indicate that >50% of studies found the same expression of the cytokine compared to controls when the net number of studies exceeds 5, while numbers written in italics indicate that more than 50% of studies found the same expression compared to controls, with a net number of studies of less than 5. CGRP is a neurotrophin, while the rest are considered cytokines.

Table 4. A summary of cytokines studied in five or more papers, in relation to pain intensity

Gene name	Positive correlation, n	Negative correlation, n	No correlation, n	"Net" studies, n	All studies, n
4E-BP1	0 0 0 0 0 0	1 1 0 0 0 0	1 1 0 0 0 0	2 2 0 0 0 0	5
BDNF	1 0 0 0 0 1	0 0 0 0 0 0	<u>5</u> 1 2 1 1 0	6 1 2 1 1 1	11
bNGF	0 0 0 0 0 0	1 1 0 0 0 0	<u>6</u> 1 2 1 2 0	7 2 2 1 2 0	11
CCL11	0 0 0 0 0 0	0 0 0 0 0 0	3 2 1 0 0 0	3 2 1 0 0 0	6
CCL13 / MCP-4	0 0 0 0 0 0	0 0 0 0 0 0	1 0 1 0 0 0	1 0 1 0 0 0	5
CCL19 / MIP-3β	0 0 0 0 0 0	1 1 0 0 0 0	2 1 1 0 0 0	3 2 1 0 0 0	6
CCL2 / MCP-1	0 0 0 0 0 0	0 0 0 0 0 0	<u>8</u> <u>5</u> 2 0 0 1	8 5 2 0 0 1	14
CCL20 / MIP-3α	0 0 0 0 0 0	0 0 0 0 0 0	1 0 1 0 0 0	1 0 1 0 0 0	5
CCL23 / MIP-3	0 0 0 0 0 0	0 0 0 0 0 0	2 2 0 0 0 0	2 2 0 0 0 0	5
CCL25 / TECK	0 0 0 0 0 0	0 0 0 0 0 0	2 2 0 0 0 0	2 2 0 0 0 0	5
CCL3 / MIP-1α	0 0 0 0 0 0	1 1 0 0 0 0	2 1 1 0 0 0	3 2 1 0 0 0	7
CCL4 / MIP-1β	0 0 0 0 0 0	0 0 0 0 0 0	3 2 1 0 0 0	3 2 1 0 0 0	5
CCL7 / MCP-3	0 0 0 0 0 0	0 0 0 0 0 0	1 0 1 0 0 0	1 0 1 0 0 0	5
CCL8 / MCP-2	0 0 0 0 0 0	0 0 0 0 0 0	3 2 1 0 0 0	3 2 1 0 0 0	6
CD5	0 0 0 0 0 0	0 0 0 0 0 0	2 2 0 0 0 0	2 2 0 0 0 0	5
CGRP	1 0 1 0 0 0	0 0 0 0 0 0	2 0 1 0 1 0	3 0 2 0 1 0	5
CSF1	0 0 0 0 0 0	1 1 0 0 0 0	2 2 0 0 0 0	3 3 0 0 0 0	5
CX3CL1	0 0 0 0 0 0	1 1 0 0 0 0	3 2 1 0 0 0	4 3 1 0 0 0	9
CXCL1	0 0 0 0 0 0	0 0 0 0 0 0	3 2 1 0 0 0	3 2 1 0 0 0	6
CXCL10	0 0 0 0 0 0	0 0 0 0 0 0	4 3 0 1 0 0	4 3 0 1 0 0	9
CXCL11 / I-TAC	0 0 0 0 0 0	0 0 0 0 0 0	2 2 0 0 0 0	2 2 0 0 0 0	6
CXCL5 / ENA-78	0 0 0 0 0 0	0 0 0 0 0 0	3 2 1 0 0 0	3 2 1 0 0 0	6
CXCL6	0 0 0 0 0 0	0 0 0 0 0 0	2 2 0 0 0 0	2 2 0 0 0 0	5
FR3L	0 0 0 0 0 0	0 0 0 0 0 0	3 2 1 0 0 0	3 2 1 0 0 0	5
GDNF	0 0 0 0 0 0	0 0 0 0 0 0	1 0 1 0 0 0	1 0 1 0 0 0	7
IFNγ	0 0 0 0 0 0	0 0 0 0 0 0	1 0 1 0 0 0	2 0 1 0 0 0	11
IL10	0 0 0 0 0 0	2 0 2 0 0 0	2 0 2 0 0 0	4 0 4 0 0 0	20
IL13	0 0 0 0 0 0	0 0 0 0 0 0	0 0 0 0 0 0	0 0 0 0 0 0	8
IL17A	0 0 0 0 0 0	0 0 0 0 0 0	0 0 0 0 0 0	0 0 0 0 0 0	6
IL17C	0 0 0 0 0 0	0 0 0 0 0 0	1 0 1 0 0 0	1 0 1 0 0 0	5
IL18	0 0 0 0 0 0	0 0 0 0 0 0	3 2 1 0 0 0	3 2 1 0 0 0	5
IL1b	1 0 1 0 0 0	0 0 0 0 0 0	<u>5</u> 0 3 2 0 0	6 0 4 2 0 0	16
IL1ra	1 0 1 0 0 0	0 0 0 0 0 0	2 1 1 0 0 0	3 1 2 0 0 0	7
IL2	0 0 0 0 0 0	0 0 0 0 0 0	0 0 0 0 0 0	0 0 0 0 0 0	8
IL33	0 0 0 0 0 0	0 0 0 0 0 0	1 0 1 0 0 0	1 0 1 0 0 0	5
IL4	0 0 0 0 0 0	0 0 0 0 0 0	1 0 1 0 0 0	1 0 1 0 0 0	12
IL5	0 0 0 0 0 0	0 0 0 0 0 0	1 0 1 0 0 0	1 0 1 0 0 0	6
IL6	2 1 1 0 0 0	0 0 0 0 0 0	<u>11</u> <u>6</u> <u>5</u> 1 0	14 7 6 1 0 0	28
IL7	0 0 0 0 0 0	0 0 0 0 0 0	3 2 1 0 0 0	3 2 1 0 0 0	5
IL8	1 1 0 0 0 0	0 0 0 0 0 0	<u>11</u> <u>6</u> 4 1 0 0	12 7 4 1 0 0	22
LAP-TGF-β	0 0 0 0 0 0	0 0 0 0 0 0	2 2 0 0 0 0	2 2 0 0 0 0	5
LIF-R	0 0 0 0 0 0	1 1 0 0 0 0	1 1 0 0 0 0	2 2 0 0 0 0	5
NT-3	0 0 0 0 0 0	0 0 0 0 0 0	1 0 1 0 0 0	1 0 1 0 0 0	5
OPG / TNFRSF11b	0 0 0 0 0 0	1 1 0 0 0 0	2 2 0 0 0 0	3 3 0 0 0 0	5
TNF-α / TNF	1 0 1 0 0 0	0 0 0 0 0 0	<u>8</u> 4 3 1 0 0	9 4 4 1 0 0	26
TNF-β / LTA	0 0 0 0 0 0	0 0 0 0 0 0	2 2 0 0 0 0	2 2 0 0 0 0	5
TRAIL / TNFSF10	0 0 0 0 0 0	0 0 0 0 0 0	3 2 1 0 0 0	3 2 1 0 0 0	5
TSLP	0 0 0 0 0 0	0 0 0 0 0 0	1 0 1 0 0 0	1 0 1 0 0 0	5
VEGF-A	1 0 1 0 0 0	2 1 0 0 0 0	1 2 1 1 0 0	5 2 2 0 0 1	9

Column color depicts pain type; white = all studies, blue = nociceptive pain, yellow = neuropathic pain, purple = nociplastic pain, green = headache, and grey = other pain (mixed or undefined). Numbers written in underlined bold indicate that >50% of studies found the same expression of the cytokine compared to controls when the net number of studies exceeds 5, while numbers written in italics indicate that more than 50% of studies found the same expression compared to controls, with a net number of studies of less than 5. CGRP is a neurotrophin, while the rest are considered cytokines.

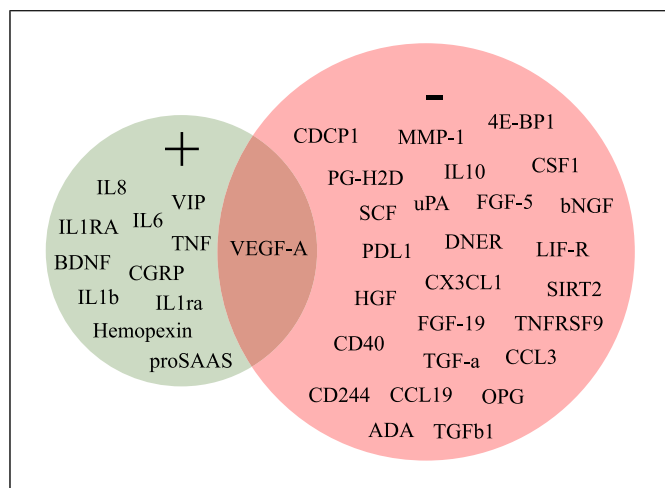


Fig. 3. Cytokines that have been found to be either positively or negatively correlated to pain in at least one study. To date, the only protein found in both categories is VEGF-A. Cytokines in CSF and chronic pain.

cells or overt neurodegenerative effects have not been reported in human pain populations as far as we know, and the evidence that exists does not suggest a fulminant neuroinflammatory state such as in, e.g., MS. Hence, unless the pain is caused by autoimmune diseases affecting CNS, such as MS, it does not seem accurate to call the reported observations “neuroinflammation.” Perhaps a more nuanced way to discuss these observations would be in terms of altered neuroimmune activation/signalling that covers a spectrum: from a changed neuroimmune milieu and glial activation in mild-moderate disease states, to increased permeability of the BBB/BCB and infiltration of peripheral immune cells in its most extreme forms. The question of neurodegeneration in NI is complicated, as we see pronounced neurodegenerative effects during fulminant neuroimmune activity (e.g., MS), but also neurodegenerative effects of the long-term neuroimmune activation of lower grades that has been investigated in, e.g., Alzheimer’s disease [90], and cannot be definitely addressed in the question of chronic pain with the current state of evidence. However, there is a growing concern that pain might be an important factor in the development of different forms of dementias; e.g., a recent observational study found “non-cancer chronic pain conditions” to be an independent risk factor for the development of Alzheimer’s disease and related dementias, and there are several experimental, cross-sectional, and longitudinal studies that have found pain to, at least in some aspects, be associated with cognitive decline [91].

A Case for Neuroimmune Activation in Pain?

If neuroimmune activation (without fulminant NI) can be defined as an upregulation of cytokines in the CSF, and if this is related to different kinds of chronic pain, we would expect neuroimmune activation to be a distinguishing feature in pain populations. Indeed, several cytokines were upregulated in the CSF of chronic pain patients compared to controls in $\geq 50\%$ of the reviewed studies, whereas none were downregulated. There were 21 cytokines upregulated across all pain types (section 3.1.), and when looking at separate pain types, IL-8 was upregulated specifically in nociceptive pain (67% of studies). No specific patterns were detected for the remaining cytokines regarding concentrations in CSF in association to different pain types.

On the other hand, if we believe that neuroimmune activity is a relevant algogenic mechanism in the context of chronic pain, we would expect to find a positive correlation between rated pain intensity and measures of CSF concentrations of cytokines. Our clinical review of cytokines in the CSF of human pain patients suggests a more complicated picture. In fact, across pain types, no correlations between pain intensity and CSF levels were found for many cytokines regarded as key players in NI and chronic pain, such as TNF, IL-1b, IL-6, IL-8, CCL2/MCP1, BDNF, and bNGF. In addition, in 86% of the studies, no correlation was found between nociceptive pain intensity and CSF levels of IL6 and IL8, respectively, nor between IL6 and neuropathic pain (83% of studies). These results do not support a general (= across pain types) association between neuroimmune activity and chronic pain intensity. However, it may be argued that these associations are only relevant to study in situations when the cytokine of interest is either up- or down-regulated in the patient group compared to controls.

If we accept these hypotheses, cytokines of special interest would be (a) upregulated in the CSF of chronic pain patients and (b) positively correlated to the rated pain intensity. Our overview of clinical studies would support the existence of many cytokines meeting the first condition, as we found 95 cytokines that were upregulated in the CSF of chronic pain patients, while only two were downregulated (in at least one study). However, a positive correlation with pain intensity was only reported regarding 10% of the 21 cytokines assessed in five or more studies and upregulated in $>50\%$ of these (Table 3), or regarding 7% ($n = 7/95$) of the cytokines upregulated in at least one study. Surprisingly, a negative correlation with pain intensity was seen regarding 33% of the 21 upregulated cytokines and regarding 25% ($n = 24/95$) of the cytokines upregulated in at least one study; however, it

Table 5. Cytokines found to be correlated either positively or negatively with pain in at least a single study

Category	Positive correlation to pain	Negative correlation to pain
Upregulated in CSF	BDNF, CGRP, Hemopexin, IL1b, IL8, TNF, VIP	4E-BP1, ADA, bNGF, CCL3, CCL19, CD244, CD40, CDCP1, CSF1, CX3CL1 (n = 2), DNER, FGF-5 (n = 2), FGF-19, HGF, IL10, LIF-R, OPG, PDL1, PGH2D, SCF, SIRT-2, TGFRSF9, uPA, VEGF-A
Downregulated in CSF		TGF-β1
Not up- or downregulated	IL-1RA, IL-6 (n = 2), ProSAAS	CDCP1, IL-10, MMP-1, TGF-α, VEGF-A
No controls	VEGF-A	

CGRP, calcitonin gene-related peptide. "n" indicates the number of studies the finding is replicated in (if >1).

must be pointed out that the latter observations are mainly based on one single study of patients suffering from painful knee osteoarthritis (KOA) [50]. In another study, complex, mainly u-shaped, associations were found between back pain intensity and CSF cytokine levels in patients with lumbar disc herniation, while no significant associations were seen in patients suffering from disc degenerative disease, suggesting disease-specific mechanisms [89]. However, in this review those results were considered not to be correlated with pain as the direction of correlation is difficult to determine in the study in question.

Given the paucity of data, no firm conclusions can be drawn regarding the relationship between pain intensity and cytokine expression in the CSF. The data at hand would support that neuroimmune activity can be observed in chronic pain conditions, but the association to pain is unclear and the data suggests a bidirectional role of the neuroimmune response that also entails analgesic and neuroprotective effects, as previously suggested [50, 89]. The latter is in accordance with Bjurström et al. [55], showing increased CSF levels of interferon gamma induced protein (IP-10) in patients with hip osteoarthritis compared to controls before surgery, with a further increase 18 months following total hip arthroplasty despite significant reductions in clinical pain as well as measures of central sensitization. Two key questions arise: where do the cytokines we measure in the CSF come from, and why does it matter?

The Neuroimmune Interface and the BBB

The answer to these questions might offer clues to the organization of peripheral-central communication, as well as how and where neuroinflammatory processes in general are created, maintained, and terminated. Cytokines and neurotrophins are used for para- and autocrine signalling throughout the body, produced by cells of both the immune and nervous systems, and their modulatory

mechanisms of action have been proposed to entail BBB/BCB disruption as well as alteration of glial cells. For example, in mice, Huber et al. [92] found a relationship between experimental inflammatory pain and BBB permeability. Separately, Tenorio et al. [93] concluded that glia cells alone, without alterations of BBB permeability, were not sufficient to uphold a chronic pain condition. Based on findings of microglia activation that mirror the activated brain regions in chronic pain (reviewed in [94]), and evidence of the need for nociceptive input for BBB alteration [7], DosSantos and colleagues [94] hypothesized that the BBB-alterations might be driven by “a central-mediated response conducted through the spinothalamic tract.” This hypothesis is supported by studies showing that NSAIDs seem to decrease CNS uptake of sucrose in inflammatory pain [7] and by studies showing that bupivacaine, a local anesthetic, reduces CNS uptake of sucrose as well as inhibits the change in expression of genes that are integral to the tight junctions and adherence junctions of the BBB in neuropathic pain [95]. In summary, there seems to be a link between neuroimmune activation and BBB disruption in certain animal pain models, with directionality remaining elusive.

In humans, migraine patients were found to have an increased serum-CSF albumin quotient compared to healthy controls [51], and the same was found in patients with lumbar disc herniation [40]. Meanwhile, García-Fernández et al. [87] found a positive correlation between neurodegeneration, inflammation and CSF/serum albumin quotient in polyneuropathies that was possibly modulated by intrathecal IL-8. In addition, our group found a positive correlation between BCB permeability, as measured with serum-CSF albumin quotient, and CSF levels, but not serum-CSF quotients of CCL11, CCL23, CCL25, CXCL9, and IL-12b, as well as a positive correlation between albumin quotient and both

IL-18-quotient and CSF-IL-18 levels in patients with either degenerative disc disease or lumbar disc herniation, suggesting a BBB/BCB effect on central levels of cytokines [89]. Thus, there seems to be a case to be made in favor of increased BBB/BCB permeability on the upregulation of certain cytokines in the central compartment.

Cells of the immune and nervous systems share many common receptors and pathways that facilitate their close communication both in the periphery and within the CNS. Due to the great mobility of immune cells, the exception being resident macrophages, the cytokines we measure in serum, CSF or any other biological fluid are not necessarily produced in cells that originate in said fluid. Instead, they can be transported there either through cytotoxicity followed by cytokine release, or through active/passive transport over the BBB/BCB. This would be supported by previous work, suggesting that proteins measurable in the CSF originate from both blood and brain structures in an 80/20 relationship [23]. In addition, positive correlations between serum and CSF levels of cytokines have been demonstrated with a fairly consistent pattern for a great number of cytokines in patients suffering from KOA [50], as well as lumbar disc herniation or disc degenerative disease [89]. Since CSF is an ultrafiltrate of blood, this makes sense. However, some cytokines in the CSF are derived more exclusively from the leptomeninges and brain parenchyma, such as Cystatin C, Prostaglandin-D synthase (PGDS), neuron-specific enolase (NSE), and glial fibrillary acidic protein (GFAP) [23]. These players have not been studied extensively in chronic pain conditions as far as we know; Cystatin C has been studied and been found upregulated compared to controls in two studies on nociceptive and neuropathic pain, respectively [64, 76], GFAP was upregulated in one study on nociplastic pain [66] and did not differ in expression from controls in a study on neuropathic pain [56], while NSE has not been found to be up- or downregulated in one study on neuropathic pain [56], and in one additional study on neuropathic pain NSE levels were not compared to controls [86].

All in all, we have sufficient evidence to claim that there is some form of neuroimmune alteration going on in chronic pain that at least in some cases may alter the BBB/BCB. What about glial cells in humans?

Glial Cells as Part of the Neuroimmune Axis

As should be evident from the above discussion, the role of NI in chronic pain conditions is a very complex question. This is further illustrated by the results from neuroimaging studies in chronic pain, which constitutes the preferred method for investigating central neuro-

immune alteration in humans. Cerebral glial cell activation in chronic pain patients has been indirectly studied by using PET ligands, such as the translocator protein (TSPO) for which increased binding is interpreted as a sign of glia activation. Increased TSPO binding has been reported in patients suffering from chronic low back pain (CLBP) [21, 96], as well as in widespread cortical areas in fibromyalgia patients compared to healthy controls [20, 97, 98]. However, the relation to pain was inconclusive as negative correlation (CLBP), no correlation (FM) as well as positive correlation (FM) has been reported [20, 21, 97]. In addition, patients with rheumatoid arthritis did not differ from HC in cerebral gray matter in terms of TSPO binding, nor did they present with a correlation between TSPO binding and pain intensity. Instead, a strong negative correlation between TSPO binding and disease activity was found in the patient group suggesting protective and disease modifying effects [36]. Finally, TSPO binding patterns in the primary somatosensory cortex were related to the widespreadness of pain in CLBP patients [99].

Furthermore, magnetic resonance spectroscopy (MRS) has been used to assess metabolites, such as myo-inositol, which is mainly localized in glia and considered a (potential) marker of NI [100]. In fibromyalgia patients, Lee et al. [101] reported a negative correlation between myo-inositol concentrations in anterior midcingulate cortex and thalamus, and pain intensity, while Fanton et al. [102] reported a negative correlation between scyllo-inositol (a stereoisomer of myo-inositol) in rostral anterior cinguli and severity of fibromyalgia symptoms, and lower levels of scyllo-inositol in the rostral anterior cinguli of fibromyalgia patients compared to controls. On the other hand, Weerasekera et al. [100] reported a positive correlation between knee pain intensity and myo-inositol concentrations in the thalamus of KOA patients. The KOA patients had higher levels of myo-inositol compared to controls before surgery and a further increase in thalamic myo-inositol levels was reported 4 weeks following total knee arthroplasty despite no significant change in pain intensity. Given that the postoperative increase in myo-inositol was accompanied by a proportional normalization of a neuro-metabolite regarded as a marker of neuronal integrity (NAA/Cr), the authors speculated that “surgery-induced neuroinflammation might have a beneficial role in promoting the restoration of neuronal metabolism and/or viability, possibly supporting a dual role of neuroinflammation: adaptive in the acute/subacute context, such as in response to surgery, but pathogenic and maladaptive when dysregulated or in the chronic context” [100]. However,

an alternative interpretation would be possible, namely, that certain types of glia activation reflect a neuro-protective mechanism.

Conclusions

In the current literature review of cytokines in the CSF of chronic pain patients, evidence of an altered neuro-immune environment in chronic pain was found as 21 cytokines were upregulated in the majority of studies they have successfully been measured in, while none were consistently downregulated. However, surprisingly, no consistent correlations between pain intensity and CSF levels were found for many cytokines traditionally regarded as key players in neuroinflammation and chronic pain, such as TNF, IL-1b, IL-6, IL-8, CCL2/MCP1, BDNF, and bNGF. In fact, when examining cytokines that were upregulated in the CSF in chronic pain patients in at least one study and had a correlation with pain, 77% were associated with *lower* pain intensities, and only 23% with higher intensity. The evidence from cytokine studies of the CSF and from neuroimaging studies on chronic pain conditions is still inconclusive. This is due to the natural elusiveness of pain as a phenomenon but also reflects the limitations that are commonplace in the field of pain research, such as the lack of longitudinal studies, making it difficult to assess causal relationships, the heterogeneity of pain conditions studied, which makes data interpretation cumbersome and prone to errors, and the so-called healthy control groups used, as they often undergo evaluation for some other form of symptoms. Despite these shortcomings, our analysis supports the

idea that chronic pain conditions are characterized by an altered neuroimmune environment that in at least some conditions present with glial cell activation and BBB/BCB disruption. Regarding the role of this neuroimmune alteration, it will be important to conduct studies where we also consider the potentially beneficial effects of neuro-immune interactions, which could enable the development of radically new treatment strategies for chronic pain. At the same time, it is important to remember that cytokines alone do not account for the whole experience of pain, and that neuroimmune activity reflects only part of the phenomenon that is pain. Finally, our findings suggest that the notion of “neuroinflammation” needs a revision, from regarding all of it strictly as pathological and pain-promoting, to viewing it as part of a spectrum of neuroimmune alteration, where analgesic and neuro-protective effects might also play a role.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Conceptualization, writing, and revising were done jointly by A.R., J.K., and E.K. A.R. collected and curated the data, analyzed it, and created the tables and figures.

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