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Neuroimmune Mechanisms Underlying Post-acute Sequelae of SARS-CoV-2 (PASC) Pain, Predictions from a Ligand-Receptor Interactome

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Abstract

Purpose of Review Individuals with post-acute sequelae of SARS-CoV-2 (PASC) complain of persistent musculoskeletal pain. Determining how COVID-19 infection produces persistent pain would be valuable for the development of therapeutics aimed at alleviating these symptoms.

Recent Findings To generate hypotheses regarding neuroimmune interactions in PASC, we used a ligand-receptor interactome to make predictions about how ligands from PBMCs in individuals with COVID-19 communicate with dorsal root ganglia (DRG) neurons to induce persistent pain. In a structured literature review of -omics COVID-19 studies, we identified ligands capable of binding to receptors on DRG neurons, which stimulate signaling pathways including immune cell activation and chemotaxis, the complement system, and type I interferon signaling. The most consistent finding across immune cell types was an upregulation of genes encoding the alarmins S100A8/9 and MHC-I.

Summary This ligand-receptor interactome, from our hypothesis-generating literature review, can be used to guide future research surrounding mechanisms of PASC-induced pain.

Keywords Musculoskeletal pain · COVID-19 · PASC · PBMCs · Immune · Long COVID

Introduction

Approximately 30% of individuals infected with COVID-19 will develop post-acute sequelae of SARS-CoV-2 infection (PASC) [1–3], which impacts a variety of systems and processes including cardiovascular [4], musculoskeletal [5], gastrointestinal [6], and metabolic [7]. To better understand PASC symptomology, large electronic health record databases and survey studies have identified clusters of individuals based on common PASC symptoms. One cluster that has been identified repeatedly consists of those who have a phenotype involving prolonged musculoskeletal pain, and this group comprises 10–30% of individuals with PASC [2, 3, 8, 9]. This musculoskeletal pain is typically accompanied by fatigue and post-exertional malaise that hinders function and engagement in work and social activities. Individuals with PASC report moderate levels of pain severity and individuals with both fibromyalgia and PASC have a higher level of pain severity than individuals with fibromyalgia alone, suggesting PASC can exacerbate pre-existing musculoskeletal pain [8]. However, it is unknown how an acute infection of COVID-19 could produce the musculoskeletal pain associated with PASC. A better understanding of this underlying mechanism could lead to the development of pharmaceuticals or other treatments aimed at relieving PASC-induced musculoskeletal pain.

The peripheral immune system has been heavily implicated in the production of chronic pain. For example, preclinical models of muscle pain demonstrate macrophages are necessary for the generation of muscle hyperalgesia [10–13], while both monocytes and T cells are involved in the production of neuropathic pain [14]. Alterations in circulating immune cells and increases in pro-inflammatory cytokines have been found in conditions associated with chronic musculoskeletal pain including rheumatoid arthritis [15, 16], osteoarthritis [17, 18], low back pain [19], and fibromyalgia [20–23]. These peripheral immune cells can produce

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prolonged hyperalgesia through neuroimmune interactions by increasing pro-inflammatory cytokines and other ligands that can act directly on nociceptors to drive changes in excitability [24]. Given how crucial immune signaling can be in the development of pain states, it is plausible that alterations in the peripheral immune system induced by COVID-19 infection are driving changes in the peripheral somatosensory system to lead to PASC-induced musculoskeletal pain. To identify potential neuroimmune mechanisms linking COVID-19 to the generation of PASC-induced musculoskeletal pain, we ran publicly available single-cell RNA sequencing data sets from peripheral blood mononuclear cells (PBMCs) from individuals with COVID-19 through a ligand-receptor interactome network that we developed previously and optimized for this study [25]. This comprehensive interaction network was built to identify potential connections between different cell types and human DRG (hDRG) sensory neurons to aid the discovery of drug targets for alleviating pain [25]. In this study, we aimed to use our interactome platform to generate predictions on mechanisms through which specific immune cells could produce PASC-induced musculoskeletal pain following COVID-19 infection.

Methods

To comprehensively explore existing data sets using single-cell RNA sequencing on PBMCs from individuals with COVID-19, the following search strategy was used on January 31, 2023, through PubMed: (Single cell [tiab]) AND ((Sequencing [tiab]) OR (seq [tiab])) AND ((COVID [tiab]) OR (SARS-CoV-2 [tiab])) AND (Peripheral blood mononuclear cell). This search strategy returned 161 articles which were subsequently screened for inclusion. We identified 5 publicly available data sets [26–30] from single-cell RNA sequencing data of PBMCs that included differential gene expression (DEG) analysis between individuals with COVID-19 and healthy controls (Table 1). The DEGs from each article were compiled and genes that had contrasting differential expression between articles, within a given immune cell subset, were removed. Upregulated DEGs from individuals with COVID-19 were then run through our ligand-receptor interactome platform which allows for the identification of interactions between specific cell types and receptors on nociceptors of hDRG [25]. The interactome database contains over 3000 ligand-receptor interactions, including lipid and small molecule ligands like prostaglandins and endocannabinoids [25]. Use of this interactome platform allows for the discovery of novel pain mechanisms, which can lead to testable hypotheses for the development of new therapeutic targets for treating chronic pain. For example, using this interactive platform, we previously identified a novel pro-nociceptive action of heparin-binding epidermal growth factor (HBEGF) [25].

Interactions were identified by intersecting the ligand gene list from each immune cell subtype with corresponding hDRG neuron receptors identified previously [31]. Following identification of all interactions between specific immune cells and different hDRG sensory neuron populations, we filtered the list to exclude those interactions in which the receptor was expressed only in non-nociceptive populations or in nociceptors that do not produce symptoms of musculoskeletal pain consistent with PASC, such as TRPM8 positive nociceptors which are believed to be responsible for detecting cold [32]. Thus, our final list of interactions focused on ligands produced from PBMCs that could activate receptors on 4 subsets of nociceptors (putative low threshold mechanosensitive C fibers, silent nociceptors, pro-enkephalin expressing,

Table 1 Details of study participants from data sets included in ligand-receptor interactome analysis. Samples sizes, sex, age, and immune cells analyzed for each data set included in the ligand-receptor interactome

Article	Participants	Sex	Age (Avg ± SD)	Cell types
Arunachalam[26]	COVID-19: 7	M: 2; F: 7	59.57 ± 11.63	Monocytes (classic, non-classic), CD4, CD8, B cells, NK
	HC: 5	M: 2; F: 3	70.0 ± 20.15	
Kramer[27]	COVID-19: 17	NR	NR	NK
	HC: 13	NR	NR	
Qi[28]	COVID-19: 21	M: 16; F: 5	51.13 ± 12.72*	Monocytes (classic, non-classic, intermediate), CD4, CD8 (effector, memory), B cells (naïve, memory), NK
	HC: 11	M: 6; F: 5	46.82 ± 10.82	
Silvin[29]	COVID-19: 3	M: 1; F: 2	38.66 ± 26.38	Monocytes
	HC: 3	M: 0; F: 3	46.33 ± 16.86	
Xu[30]	COVID-19: 13	M: 9; F: 4	53.31 ± 13.98	Monocytes, CD8 (effector)
	HC: 3	M: 1; F: 2	43.33 ± 18.93	

*Denotes average age was calculated with available information, but not for all samples. *HC* healthy control, *M* male, *F* female, NR not reported, *NK* natural killer cells

and transient receptor potential cation channel subfamily A member 1 (TRPA1) positive). Data is presented by grouping all the interactions for a given immune cell population (i.e., All Monocytes) and the interactions of specific immune cell subsets when data was available (i.e., classic, non-classic, and intermediate monocytes). For each immune cell group, each interaction was ranked by an aggregate score accounting for the adjusted p-value for the ligand's differential expression in COVID-19, the percentage of cells in the group that expressed the ligand in individuals with COVID-19, and the expression levels (in normalized counts) of the respective receptor in hDRG nociceptors. We then performed Gene Ontology (GO) term analysis for the ligand and receptor genes using Enrichr [33, 34] and classified the genes by the PANTHER classification of their protein products [35]. GO term analysis examines all the biological process or molecular function ontology annotations associated with each of the input genes and determines which terms are most enriched for a given set of DEGs, shedding insight into which signaling pathways and biological systems could be activated by the genes of interest. GO terms for biological processes and molecular function were identified and ranked based on adjusted *p*-value.

Results

Monocytes

Monocytes have been implicated in the etiology of musculoskeletal pain conditions including rheumatoid arthritis [36–39], osteoarthritis [40–42], fibromyalgia [20, 43], and low back pain [44]. Monocytes could play a role in the production of musculoskeletal pain associated with PASC as increased numbers of circulating classic monocytes is one of the hallmark differences found in PBMCs from individuals with COVID-19 [29, 45–47]. In moderate cases of COVID-19, there is also a decrease in non-classic monocytes [29, 45]; however, some report increases in non-classic monocytes in severe and fatal cases [48].

Our interactome analysis revealed similarities and differences between the upregulated genes and pathways in monocyte subsets. Ligands from classic, non-classic, and intermediate monocytes demonstrate increased activation of biological processes with GO terms associated with the immune response, leukocyte chemotaxis, and activation of neutrophils (Fig. 1). Increased circulating neutrophils are also routinely found in individuals with COVID-19 [49]; however, their role in the generation of pain has been

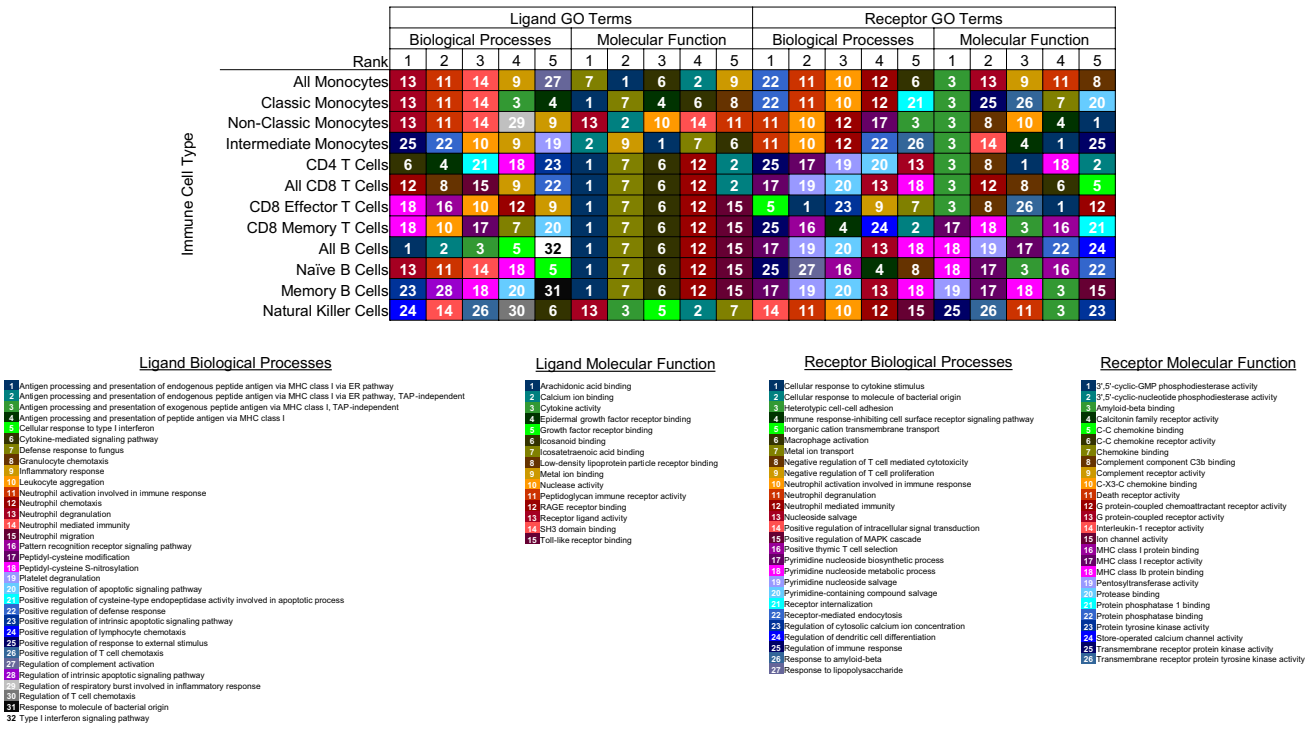

Fig. 1 Ligand and receptor GO terms from the interactome analysis of immune cells in individuals with COVID-19. Top 5 enriched biological processes and molecular function GO terms based on adjusted *p*-value for the differentially expressed ligand encoding genes for each PBMC immune cell type. The results are grouped by the ligand and receptor GO terms and then further divided into biological and molecular function and ranked from left to right

debated as some show pro-nociceptive [50, 51] and other anti-nociceptive effects [52]. Classic and intermediate monocytes also show a specific upregulation of the genes *S100A8/9* that encode for a class of alarmins that bind to toll-like receptor 4 (TLR4) and drive activation of the innate immune response by increasing cytokine production and release [53] (Fig. 2). S100 proteins are intracellular calcium sensors which are released passively following cellular apoptosis or are actively secreted and signal to initiate pro-inflammatory cascades [54]. S100A8/9 are implicated in the production of pain in animal models of osteoarthritis, rheumatoid arthritis, and intervertebral disc degeneration [55–57]. S100A8 administered to cultured mouse DRGs increases production of MCP-1 that promotes a local influx of monocytes in the DRG which could induce sensitization of the DRG neurons and subsequent hypersensitivity [58]. However, recent preclinical work demonstrates that S100A8/9 can reduce pain duration in an animal model of inflammation induced pain, suggesting a potential role in pain resolution [52]. Ligands from classic monocytes also demonstrate activation of antigen processing and presentation through major histocompatibility class I (MHC-I) and an upregulation of the gene *PKM* both of which suggest increases in phagocytic activity and cell death [59, 60] (Fig. 2). Increases in cell death can cause a release of intracellular contents, notably ATP, which binds to purinergic receptors on nociceptors in the DRGs, which in turn can lead to sensitization and hypersensitivity [61].

Non-classic monocytes exhibit upregulation of the gene *NAMPT* which is involved in producing an inflammatory response through interferon gamma (IFNγ) signaling [62, 63] (Fig. 2). IFNγ is implicated in the production of pain as increased IFNγ-stimulated genes are found in the DRGs of individuals with neuropathic pain [64]. Increased plasma levels of *NAMPT* are also found in individuals with rheumatoid arthritis [62, 65]. Non-classic monocytes also show an increase in the ligand gene *GRN* which encodes for the protein progranulin (Fig. 1). This demonstrates the attempted healing action of non-classic monocytes as this gene is involved in wound repair and anti-inflammation [66, 67]. GRN knockout mice demonstrate an increased pain response to nerve injury and progranulin administration shows therapeutic effects in animal models of inflammatory arthritis [68, 69]. In sum, ligands from monocytes show activation of several pathways responsible for leukocyte chemotaxis and pro-inflammatory pathways which could be responsible for the production of musculoskeletal pain. Interestingly, individuals with PASC-induced post-exertional fatigue have increased numbers of monocytes in skeletal muscle, which further supports the notion that alterations in the presence of this cell type, and its signals, could lead to PASC-induced musculoskeletal pain [70].

CD4 T Cells

Following COVID-19 infection, there is an overall decrease in the proportion of CD4 T cells in the blood and further decreases are reported with increased severity of symptoms suggesting dysregulation in immune response in severe cases [47–49]. While the total number of CD4 T cells may decrease, there is a reported shift in the phenotype of CD4 T cells with increases in Th1 acutely following infection in those with mild cases [30, 71]. An increase in the proportion of Th2 T cells is reported in severe cases and is a predictor of subsequent hospitalization and death due to COVID-19 [72–74]. Animal models of neuropathic pain have extensively studied the role of CD4 T cells in the production of prolonged hypersensitivity and suggest these subsets of CD4 T cells play opposing roles in pain development. Pro-inflammatory Th1 cells seem to promote pain, while anti-inflammatory Th2 cells appear to protect against it. Following nerve injury, there is a subsequent infiltration of CD4 T cells into the DRG [75–77] and mice lacking CD4 T cells are protected against the development of hypersensitivity [78]. When rats lacking T cells were reconstituted with Th1 T cells, the pain behaviors were restored but not when reconstitution was performed with Th2 T cells suggesting pro-inflammatory Th1 T cells play a role in the production of pain [79]. Similarly, studies of chronic pain conditions have implicated a causative role for pro-inflammatory CD4 T cells that infiltrate into the painful joints of individuals with rheumatoid arthritis [80–82] and osteoarthritis [83–85]. Thus, an overall increase in the proportion of Th1 T cells in individuals with COVID-19 could be a factor in the production of PASC-induced musculoskeletal pain.

The genes and GO terms from our interactome analysis demonstrate that ligands from CD4 T cells activate cytokine-mediated signaling, the complement system, and antigen processing and presentation through MHC-I, all of which are implicated in the production of pain (Fig. 1). Pro-inflammatory cytokines such as IL-6, IL-1β, and TNFα can bind to their receptors on nociceptors to cause excitation and sensitization [86, 87]. The complement system is part of the innate immune system, its activation can lead to immune cell recruitment and inflammation, and it is linked to the production of persistent pain through its ability to sensitize nociceptors [88, 89]. Antigen processing and presentation involves immune system communication through either MHC class I or class II signaling [90]. MHC-I is responsible for alerting the immune system that a cell is virally infected so that it can be tagged for destruction [91]. *B2M* and *HLA-C*, both of which are genes encoding components of the MHC-I protein, were the ligands for two of the top predicted interactions between CD4 T cells and DRGs (Fig. 2). Genetic variants in *B2M* are associated with neuropathic pain [92], while variants in *HLA-C* are linked

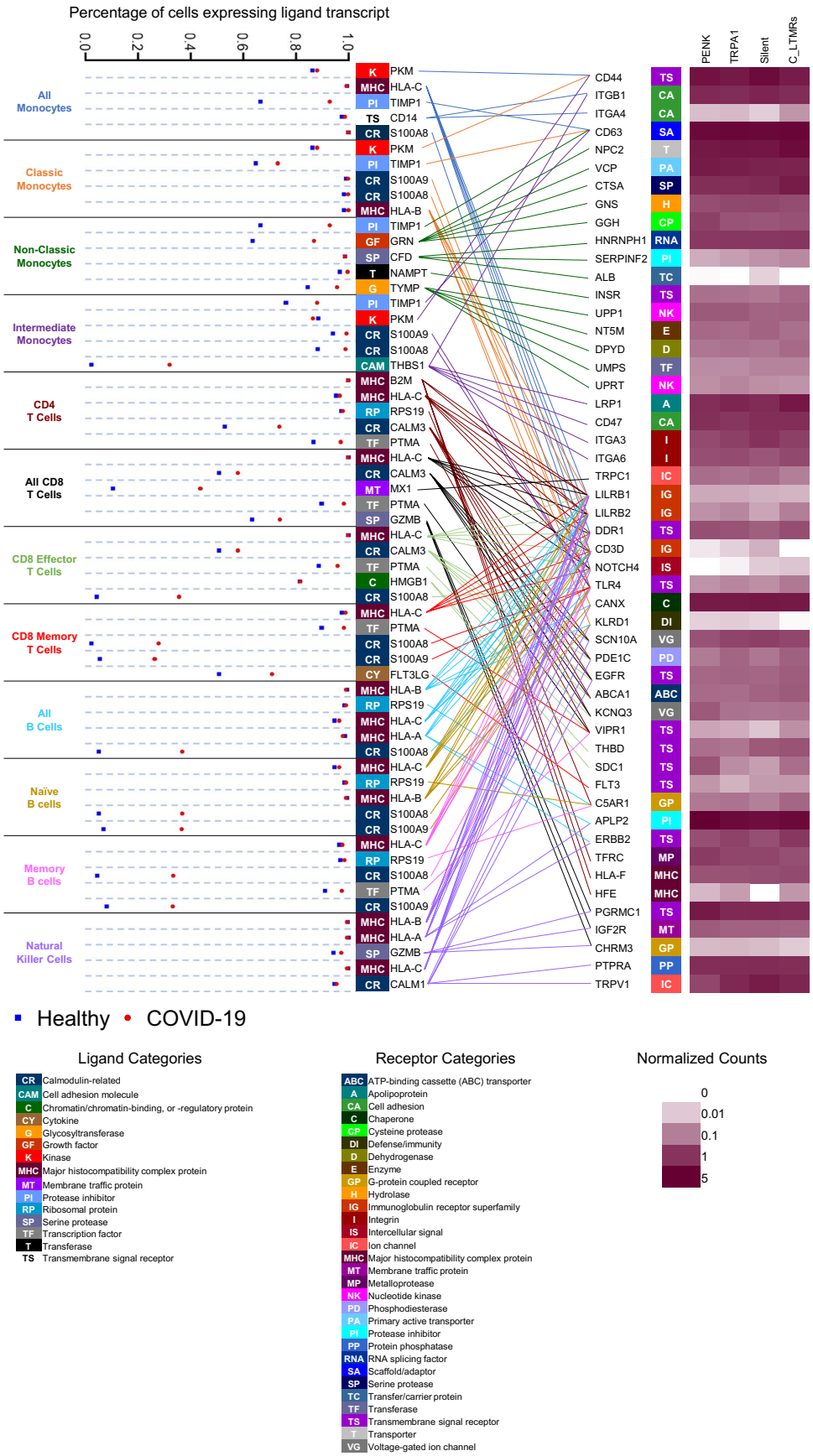

Fig. 2 Differential expression and interactome analysis of ligands from immune cells in individuals with COVID-19. Differential expression of ligand encoding genes from PBMC immune cells with COVID-19 compared with healthy individuals grouped by immune cell type. The top 5 predicted ligand-receptor interactions are listed for each PBMC immune cell type. Left panel illustrates the percentage of each PBMC immune cell expressing the transcript of the given gene from individuals with COVID-19 (red) and healthy controls (blue). The middle panel illustrates the given differentially expressed ligand gene with its category and the corresponding top receptors for that ligand on human DRGs with its subsequent category. The right panel illustrates the normalized counts of each receptor on different classes of nociceptors (PENK = pro-enkephalin expressing, TRPA1 = transient receptor potential cation channel subfamily A member 1 expressing, silent nociceptors, C_LTMR = putative low threshold mechanoreceptor C fibers) from human DRGs [31]

with painful conditions including rheumatoid arthritis [93], psoriatic arthritis [94], and endometriosis [95]. Although not extensively studied in the context of pain, increased signaling of MHC-I is implicated in an animal model of cancer-induced bone pain through increased neuronal apoptosis, and siRNA knockdown of *B2M* attenuates this hyperalgesia [96]. Again, cellular apoptosis can lead to DRG sensitization due to release of intracellular components, such as ATP and S100 proteins, and could be responsible for the production of prolonged PASC-induced musculoskeletal pain.

CD8 T Cells

As seen with CD4 T cells, there is a decrease in the number of circulating CD8 T cells following COVID-19 infection, with further decreases reported as severity of symptoms increase [47–49]. CD8 T cells are part of the adaptive immune system and can be classified into a cytotoxic or regulatory phenotype [97]. CD8 T cells recognize antigens displayed by MHC-I and cytotoxic CD8 T cells are programmed to destroy virally infected cells [90]. In the context of chronic pain, changes in circulating levels of CD8 T cells are not routinely found; however, a higher CD4/CD8 ratio has been demonstrated in those with chronic headache [98], another common pain complaint in PASC patients [9]. In animal models of pain, CD8 T cells have exhibited both pain-promoting and pain-resolving effects. In a model of chemotherapy-induced peripheral neuropathy, intrathecal administration of CD8 T cells increases pain hypersensitivity in mice [99]. However, in mice lacking T cells, the resolution of hypersensitivity produced by chemotherapy-induced neuropathies is delayed, and reconstitution of these animals with CD8 T cells restores the alleviation of pain, suggesting CD8 T cells have pain-resolving effects [100]. In fact, CD8 T cells were shown to promote the resolution of cisplatin-induced neuropathies by stimulating the release of IL-10 from macrophages [101]. Similarly in an animal model of arthritic pain, CD8 T cell depletion enhances pain hypersensitivity [102], and CD8 T cells play a pivotal role in both endogenous and exogenous opioid induced-analgesia [102, 103]. This data suggests the loss of circulating CD8 T cells could play a role in the development of musculoskeletal pain in individuals with PASC due to a loss of pain-resolving and analgesic mechanisms.

Our interactome analysis revealed that ligands from CD8 T cells could bind to receptors on DRGs to activate pathways causing inflammation and the recruitment of immune cells, including neutrophils (Fig. 1). Activation of inflammatory pathways will cause an increase in the number of immune cells, cytokines, and chemokines that if left unresolved can cause sensitization of nociceptors and DRGs leading to hypersensitivity and prolonged pain [86, 104]. Our interactome also displayed similar biological processes and molecular functions for pathways activated by CD8 effector and memory T cells, and CD8 T cells showed upregulation of ligand genes *HLA-C* and *S100A8/9* similar to other immune cells in our analysis (Fig. 2). Of note, one of the top hits for CD8 effector T cells was the ligand gene *HMGB1* (Fig. 2). The protein HMGB1 is an alarmin that can be released by immune cells; plays a role in mediating immune cell migration, proliferation, and differentiation; and is implicated in the production of chronic pain through neuroinflammation [54]. In rodents, intrathecal, sciatic nerve, and intraplanar administration of HMGB1 produces mechanical hypersensitivity [105–107]. Furthermore, increases in HMGB1 are found in nociceptors, DRG, and spinal cord following induction of neuropathic pain [108–110], and pharmacological blockade of HMGB1 alleviates neuropathic pain [110–113]. In individuals with rheumatoid arthritis, increases in HMGB1 are found in the synovial fluid of joints, suggesting a role in the pathophysiology of this painful disease [114–116]. A top hit for CD8 memory T cells was the ligand gene *FLT3LG* that encodes for the cytokine FMS-like tyrosine kinase 3 ligand (FLT3) which has recently been implicated in the production of pain (Fig. 2). In mice, intrathecal injection of FLT3 alone produces paw hypersensitivity, genetic knockdown and pharmacological inhibition of FLT3 protects against and alleviates neuropathic and post-incisional pain, and FLT3 produces sensitization of cultured DRG neurons [117, 118]. Thus, CD8 T cells could also produce PASC-induced musculoskeletal pain through induction of prolonged neuroinflammation through mechanisms involving HMGB1 and FLT3LG.

B Cells

B cells are part of the adaptive immune system and produce antibodies to protect the host against specific pathogens [119]. Upon activation, naïve B cells can differentiate into memory B cells or antibody producing plasma cells which are increased in individuals with COVID-19 [49, 119]. Although not studied as in depth as monocytes and T cells, there is emerging evidence illustrating the potential role of B cells in the production of pain. Pharmacological depletion of B cells in individuals with rheumatoid arthritis results in improvement of rheumatological symptoms, which include pain [120]. In an animal model of complex regional pain syndrome, B cell-deficient mice and depletion of B cells results in prevention or alleviation of mechanical hypersensitivity [121]. However, reducing B cells in mice does not protect against neuropathic or postoperative incisional pain suggesting B cells may play a role in the etiology of only specific pain producing conditions [121, 122].

The interactome analysis demonstrates that as a whole, B cells activate pathways associated with MHC-I and type I interferon (IFN) signaling and show increases in the ligand

genes *S100A8/9* (Figs. 1 and 2), which were common across cell types. IFNs are a class of cytokines that play a role in interfering with virus replication and are classified as either type I, type II, or type III [123]. The major classes of type I IFNs are IFNα and IFNβ, which have recently been shown to act directly on nociceptors. In animals, virally induced pain is driven by a pathway mediated by type I IFN-stimulated increases in indoleamine-2,3-dioxygenase [124, 125], and intraplantar administration of type I IFNs alone produces mechanical hypersensitivity [126]. Type I IFNs bind and sensitize DRG neurons through the phosphorylation of eIF4E [126]. Our interactome analysis also revealed that ligands from different B cells activate unique biological pathways. Ligands from naïve B cells activate pathways involving neutrophil activation while memory B cells initiate pathways mediating cellular apoptosis (Fig. 1). One of the top interactions for all B cells involved the ligand gene *RPS19* which encodes a ribosomal protein that binds to the complement receptor C5AR1 and is responsible for immune cell activation and monocyte recruitment [127, 128] (Fig. 2). Activation of C5AR1 mediates the production of pain in several animal models including neuropathic, complex regional pain syndrome, and postsurgical pain models through its ability to sensitize DRG nociceptors [129–132]. Thus, B cells could produce prolonged PASC-induced musculoskeletal pain through activation of MHC-I, type I IFNs, and complement system signaling.

Natural Killer Cells

Natural killer (NK) cells are a member of the innate immune system that release cytokines and possess cytotoxic capabilities tasked with killing virally infected and tumor cells [133]. It has been reported that circulating NK cells are decreased in individuals with COVID-19 [49]. Little attention has been given to NK cells in terms of their role in producing persistent pain. Levels of circulating NK cells have been measured in individuals with fibromyalgia, neuropathic pain, complex regional pain syndrome, and low back pain without a clear consensus on alterations in NK cell levels, as some articles show slight decreases in the NK cell population and activity while others show no change [134–139]. However, a recent assessment in individuals with neuropathic pain demonstrated that decreases in the frequency of NK cells in the cerebrospinal fluid is correlated with increased mechanical pain sensitivity assessed via quantitative sensory testing, suggesting a protective role of NK cells in neuropathic pain [139]. Thus, decreased circulating levels of NK cells in individuals with COVID-19 could be involved with the generation of PASC-induced musculoskeletal pain. Our interactome analysis revealed that ligands from NK cells activate pathways involved in T cell and neutrophil chemotaxis and several of the top ligand genes from NK cells are involved in MHC-I signaling including *HLA-A,B,C* (Figs. 1 and 2). Thus, NK cells could be producing PASC-induced musculoskeletal pain through T cell activation and MHC-I-induced cellular apoptosis.

Overview, Limitations, and Future Directions

Overall, results from our interactome analysis revealed the enrichment of pathways including immune cell activation and chemotaxis, response to cytokines, complement system activation, and type I IFN signaling that could be driving the production of musculoskeletal pain in individuals with PASC. The most consistent finding across all immune cell subtypes that were analyzed was increases in the genes encoding the alarmins *S100A8/9* and ligands involved in the activation of MHC-I; thus, they serve as great targets for future research in the mechanisms producing PASC-induced musculoskeletal pain. The interactome also revealed similarities across adaptive immune cells (T and B cells) in their ligand gene molecular function GO terms. First, adaptive immune cells showed activation of pathways involving arachidonic acid binding which is driven by the upregulation of the alarmins *S100A8/9* [140] (Fig. 1). Arachidonic acid is a fatty acid that is a precursor to the synthesis of many eicosanoids including prostaglandin, which is implicated in the production of pain [141]. Increases in prostaglandins are found in the DRGs of rodents following induction of neuropathic, low back, and post-incisional pain [142–144], and cultured DRGs produce prostaglandin upon stimulation with pro-inflammatory cytokines [145]. Prostaglandin signaling increases nociceptor excitability and expression of several ion channels and receptors including sodium channels, calcium channels, purinergic receptors, and TRPV1 receptors, to drive peripheral sensitization leading to the production of persistent pain [141]. Secondly, the adaptive immune cells showed activation of receptor for advanced glycation end products (RAGE), which is also driven by the upregulation of *S100A8/9* [54] (Fig. 1). RAGE can bind with several ligands including S100 proteins and HMGB1 [146]. Activation of RAGE by S100A8/9 and HMGB1 produces pro-inflammatory signaling and immune cell migration [147, 148] which could lead to a sensitization of DRG neurons to produce long-term musculoskeletal pain associated with PASC.

There are several limitations to this work. First, we utilized single-cell RNA sequencing data sets from individuals who were recently diagnosed with COVID-19. While our analysis can be useful about making predictions about PASC-induced musculoskeletal pain mechanisms, future research should focus on collecting tissues from individuals with PASC to further determine molecular mechanisms underlying symptomology. Secondly, as only 30% of individuals with COVID-19 go on to develop PASC,

the majority of individuals included in the data sets used in this analysis likely did not develop PASC. If possible, future work should follow up with the individuals whose PBMCs were collected acutely after COVID-19 diagnosis so that further comparisons can be made between individuals that did and did not develop PASC. Lastly, due to the heterogeneity of the patient characteristics used in each study, we were unable to draw any conclusions regarding potential impact of sex or disease severity on the ligand-receptor interactome. Future work aimed at underpinning the molecular mechanisms of PASC associated pain should power their studies to be able to explore for sex differences as the immune system's mechanistic role in producing pain has been demonstrated to be sex dimorphic [149–154].

Studies on individuals with PASC have consistently shown pain as a major feature of the disorder [8, 9]. Our work suggests interactions between circulating immune cells and nociceptors could drive the production of PASC-induced pain. We recommend targeted prospective studies on individuals with PASC with specific pain symptoms, such as musculoskeletal pain, to examine changes in transcriptomes of the immune cell types highlighted here to better understand how these cells are altered in this patient population. Integrating that data with the interactome platform described here can help to hone in targets that might have the greatest value for alleviation of pain in PASC patients. These types of prospective experiments could also potentially confirm targets described here in patients that are in a later stage of the disease. Such targets would likely have the greatest value for moving ahead into clinical trials for patients suffering from persistent pain post-COVID-19.

Conclusion

Our interactome analysis revealed that PBMC populations express several ligands that are upregulated in COVID-19 and could bind to receptors on DRG neurons to activate several pro-nociceptive pathways, including immune cell activation and migration. The ligands most consistently upregulated across monocyte and lymphocyte cell types involved the alarmins S100A8/9 and MHC-I signaling. We hope that this interactome analysis will provide data for the generation of future hypotheses and research regarding the mechanisms underlying PASC-induced musculoskeletal pain.

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Declarations

Conflict of Interest The authors declare no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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