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SYSTEMATIC REVIEW OPEN ACCESS

Buprenorphine's Effect on the Human Immune System and Inflammation

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ABSTRACT

Opioid use disorder is a persistent epidemic despite several FDA-approved medications for its treatment. While the pathogenesis of opioid use disorder has been classically attributed to dopamine pathways in the brain, there is emerging evidence and interest surrounding the role of inflammation and inflammatory signaling in its development and treatment. Buprenorphine has become the most prescribed medication for opioid use disorder, largely due to its ease of access and tolerability. This review aimed to better characterize contemporary knowledge of how buprenorphine modulates the human immune system and inflammatory functions in this population. A comprehensive review was conducted using 11 key databases, including Embase, MEDLINE, Cochrane Central Register of Controlled Trials, and ClinicalTrials.gov. This review captured 8177 records, and 14 studies were ultimately selected for inclusion and discussion in this review. Notably, all 14 clinical studies evaluated buprenorphine's effect on the peripheral immune system, and the majority of the studies supported the notion that initiation and maintenance of buprenorphine restore immune suppression caused by opioid use disorder. In addition, we discuss how recent and ongoing work utilizing advanced imaging and cellular technologies is advancing the understanding of how buprenorphine affects the immune and inflammatory signaling in the brain.

1 | Introduction

Opioid use disorder (OUD) is a persistent epidemic that has claimed over a half a million lives in the United States between 1999 and 2020 [1]. Currently, the gold standard for OUD treatment includes three FDA-approved medications for OUD (MOUD)—methadone, naltrexone, and buprenorphine [2]. In the recent decade, buprenorphine became the most commonly prescribed MOUD, with more than 16 million prescriptions written in 2022, largely due to ease of access compared to methadone [3] and a better tolerability profile compared to naltrexone [2]. Despite available MOUD, fatal overdoses and the economic burden have continued to rise. In 2021, fatal overdoses involving an opioid were responsible for more than 70,000 deaths, more than a sixfold increase since 1999 [4]. The total estimated economic burden of OUD rose from $78 billion in 2013 [5] to more than $1 trillion in 2017 [6]. Moving forward, an improved understanding of the pathogenesis of OUD and mechanisms of available treatments remain imperative.

The pathogenic mechanism of OUD in the brain is not fully understood, but a complex combination of dopamine-dependent

and -independent neuronal mechanisms has been described [7–10]. Emerging evidence also highlights that opioid abuse results in a pro-inflammatory state in both the periphery and the central nervous system (CNS) in humans [11–14]. Importantly, the inflammatory changes in the CNS resulting from opioid abuse have also been hypothesized to directly and indirectly modulate dopamine signaling in the brain, leading to OUD's development or further exacerbation [15, 16]. Therefore, further elucidation of immune and inflammatory mechanisms in OUD may lead to the development of novel therapies for OUD.

In light of inflammation as an emerging pathogenic pathway of OUD, there has also been an increased interest in immune or inflammatory modulation by MOUD. Recently, immunomodulatory profiles of various opioids have been reviewed by Franchi et al. [17] with a particular focus on methadone. In their review, methadone appeared to be immunosuppressive in preclinical studies, but clinical studies suggested that methadone may restore heroin- or other opioid-induced immune dysfunction [17]. However, this contrasts with another recent scoping review of methadone's effect on the hematological system, which suggests that prolonged methadone therapy may result in immune hyperactivation and derangements in circulating, with authors calling for a better substitute for OUD treatment [18]. The immune modulatory role of naltrexone has also gained increasing attention, with particular interest in its role in modulating immune function in cancer patients—these studies have been recently reviewed [19, 20].

The role of buprenorphine's impact on immune or inflammatory modulation is also of major interest, considering its wide and growing use in OUD. In preclinical studies, buprenorphine has been shown to have neutral or anti-inflammatory effects on the peripheral immune system function [21–25]. However, this is in contrast with preclinical findings within the CNS—which have shown that buprenorphine treatment resulted in increased microglial activation in a rat mode, suggesting a pro-inflammatory role [26]. In addition, sex-specific immune modulatory effects of buprenorphine have also been characterized in mice [27], highlighting the complexity of buprenorphine's effect within the CNS. To date, a comprehensive understanding of buprenorphine's immunomodulatory effect on the human immune system is lacking. In light of the increasing focus by the addiction research community on the role of inflammation in the pathogenesis of OUD, as well as a growing understanding of buprenorphine's role in modulating immune and inflammatory functions in recent preclinical studies, this review aimed to better characterize how buprenorphine modulates the human immune system and inflammatory functions.

2 | Methods

2.1 | Information Sources

We conducted a comprehensive search to find relevant literature on the effect of buprenorphine on the human immune system and inflammation. A medical librarian (M.M.) developed and deployed the search strategy in consultation with the research team (T.O., S.D.S., and R.B.). The search was built in Embase and reviewed by an independent medical librarian. Studies were identified via searches of the databases Embase, MEDLINE, Cochrane Central Register of Controlled Trials (CENTRAL), and the Cochrane Database of Systematic Reviews (CDSR)—all via the Wolters Kluwer Ovid interface; Science Citation Index Expanded (SCI Expanded), Emerging Sources Citation Index (ESCI), and Preprint Citation Index (PCI)—all via the Clarivate Analytics Web of Science interface and SCOPUS; the search engine Google Scholar (https://scholar.google.com/); and clinical trial registers ClinicalTrials.gov (https://clinicaltrials.gov/) and the International Clinical Trials Registry Platform (https://trialsearch.who.int/). English language database limits were applied as available or built into the search strategy when possible. Animal studies were restricted, when possible, via the search strategy. No published search filters were applied. The Embase search strategy was manually translated across all resources using syntax, controlled vocabulary, and search fields. MeSH thesaurus terms from MEDLINE, EMTREE thesaurus terms from Embase, and text words were used for search concepts related to buprenorphine, inflammation, and the immune system. All database, search engine, and trial register searches were performed on 11 October, 2023. Full search strategies are available in Supporting Information and https://osf.io/f25jg/. All records were downloaded or manually added to EndNote 20 Desktop version and deduplicated using a method by Bramer et al. [28]. To supplement database, search engine, and register searches, backward (cited) citation searches of the bibliographies of all 14 included studies were performed in April 2024 resulting in no additional records to screen. Forward (citing) citation searches of all 14 included studies were performed using SCOPUS on April 9, 2024, resulting in 439 records to screen after 35 duplicates were removed by Covidence's deduplication feature (28 records) and manually (7 records).

2.2 | Selection Criteria and Study Categorization

Study inclusion criteria included in vivo, ex vivo, or in vitro studies investigating buprenorphine's role in modulating inflammation in humans. Studies were categorized as in vivo if buprenorphine was administered directly to human subjects and samples were analyzed at collection without further manipulation. Studies were categorized as ex vivo if buprenorphine was administered directly to human subjects and samples underwent secondary treatment in cell culture before analysis. Studies were categorized as in vitro if buprenorphine was administered to human samples in cell cultures. Exclusion criteria included animal models, human studies with comorbid disease states (such as HCV, HIV, cancer, chronic pain, or autoimmune conditions), confounding pharmacological interventions in addition to buprenorphine, incomplete studies, review articles, and studies without clear experimental outcomes such as case reports. Studies that met the inclusion and exclusion criteria were reviewed and categorized based on study design (i.e., prospective vs. cross-sectional, randomized vs. case–control, in vivo vs. ex vivo or in vitro), sample type, sample size, and immune or inflammatory parameters measured by the study.

3 | Results

This review captured 8177 records, and 14 studies were ultimately selected for inclusion and discussion in this review (Figure 1). The study design, sample type and size, and key

immune and inflammatory markers investigated in the 14 studies are detailed in Table 1. Three studies were completed in the United States, while the remaining studies were completed in Asia and Europe. Seven of the 14 studies were categorized as in vivo and/or ex vivo human studies based on their study design (Table 1). The other seven studies were categorized as in vitro mechanism studies, utilizing primary human cells for at least a portion of their work in order to further elucidate the mechanism of buprenorphine's effect on the human immune system (Table 1). Only one study was designed as a prospective randomized control trial [29], while the other in vivo/ex vivo studies were designed as prospective or cross-sectional case–control studies. With regard to sample types, three studies included active heroin or other opioid users [30–32] and four studies included ex-heroin users in their design [29–31, 33]. Nine studies were completed with healthy patients or samples from healthy patients (treated with buprenorphine or other opioids in vivo or in vitro) without active- or ex-heroin/opioid group (Table 1).

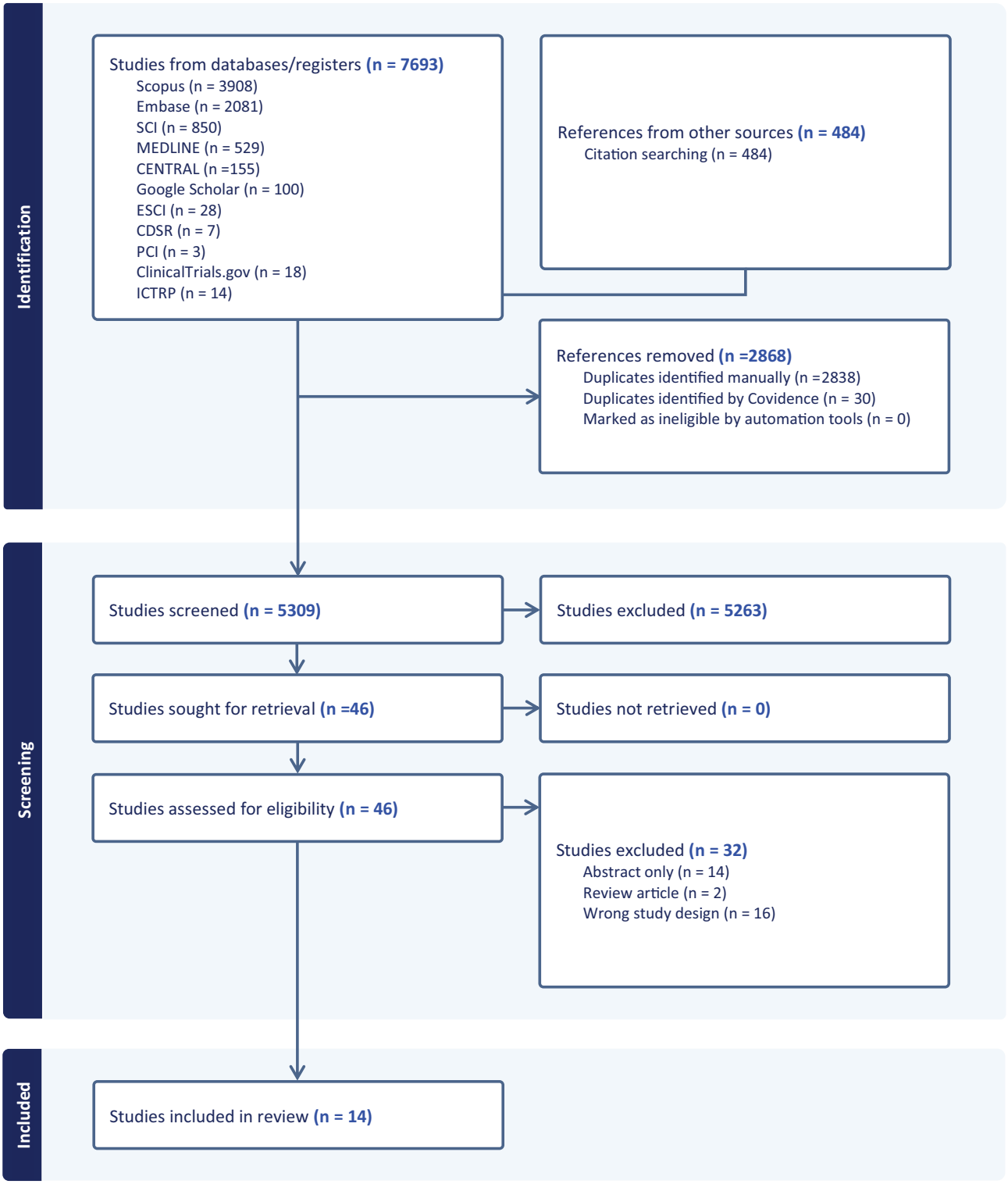

FIGURE 1 | Flow of information through literature search and review.

TABLE 1 | Main data contained in 14 selected articles.

References	Country	Study type	Study goal	Study design	Sample type/size	Immune cells studied	Immune/ inflammatory markers	Key results
Girotra et al. 1990 [34]	India	In vivo, exploratory pilot study	Investigate dermal buprenorphine's clinical safety profile regarding histamine release	Healthy patients underwent dermal injection of various dilutions of buprenorphine ranging from 1:10000 to 1:10, with positive histamine control	Healthy patients: $N=11$	Skin mast cell (by proxy)	Histamine release measured by skin reaction (wheal and flare)	No cutaneous reaction observed with buprenorphine
Stellato et al. 1992 [37]	Italy	In vitro	Investigate opioid-mediated histamine release and changes in inflammatory markers	Human peripheral blood basophils, skin mast cells, and lung mast cells were treated with buprenorphine, morphine, or fentanyl in vitro	Basophils: $N=11$, skin mast cell: $N=15$, lung mast cells: $N=13$	Peripheral basophils Skin mast cells (breast CA or cosmetic resection) Lung mast cells (lung CA resection)	Histamine release, tryptase release, and de novo PGD2 and LTC4 syntheses in response to buprenorphine	Peripheral basophils and skin mast cells did not release histamine in response to buprenorphine. Concentration-dependent release of histamine and tryptase release seen in lung mast cells but not skin mast cells. Buprenorphine induced PGD2 and LTC4 only in lung mast cells
Blunk et al. 2004 [35]	Germany	In vivo, randomized double-blind study	Investigate mast cell response in healthy subjects who received intradermal buprenorphine	Healthy subjects received intradermal buprenorphine, codeine, meperidine, fentanyl, alfentanil, sufentanil, remifentanil, or naloxone	Total $N=60$ (31 women and 29 men)	Skin mast cells (by proxy)	Localized dose-dependent intradermal histamine and tryptase release, measured by intradermal microdialysis	Buprenorphine did not induce histamine or tryptase release

(Continues)

TABLE 1 | (Continued)

References	Country	Study type	Study goal	Study design	Sample type/size	Immune cells studied	Immune/ inflammatory markers	Key results
Neri et al. 2005 [29]	Italy	In vivo, prospective randomized controlled trial	Investigate the effects of MOUD on the immune system	Ex-heroin users were randomized to 12 months of MOUD tx (methadone or buprenorphine); samples were collected before and after tx and compared to healthy control	Methadone: $N=31$, sublingual buprenorphine: $N=31$, healthy (unmatched healthy paramedical staff): $N=35$	Monocytes	CD14+ monocyte population and plasma concentration of cytokines (TNF-α, IL-1β, and IL-2)	Prior to tx, heroin users had lower CD14+ monocyte count than healthy controls. After 12 months of buprenorphine, CD14+ count was restored to levels higher than healthy controls. Cytokine levels (TNF-α, IL-1β, and IL-2) were also noted to be lower in heroin users, which were also restored to levels higher than healthy controls after MOUD tx
Mikawa et al. 2006 [49]	Japan	In vitro	Investigate the effects of buprenorphine on human neutrophil function	Neutrophils isolated from healthy volunteers were treated with buprenorphine, pentazocine, or butorphanol in vitro	Healthy: $N=12$	Neutrophils	Neutrophil function measured by chemotaxis and production of superoxide, hydrogen peroxide, and hydroxyl radicals	Buprenorphine inhibited chemotaxis of neutrophils in a dose-dependent manner. At clinically relevant concentrations (1 and 10 ng/mL), buprenorphine impaired superoxide production independent of intracellular calcium concentration

(Continues)

TABLE 1 | (Continued)

References	Country	Study type	Study goal	Study design	Sample type/size	Immune cells studied	Immune/ inflammatory markers	Key results
Sacerdote et al. 2008 [30]	Italy	Ex vivo, cross-sectional case–control	Investigate the difference in human PBMC in active heroin users compared to patients receiving MOUD and healthy controls	PBMCs isolated from active heroin users, patients on MOUD (methadone or buprenorphine), and sex- and age-matched controls; samples were treated in ex vivo cultures	Active heroin users: $N=9$, methadone: $N=12$, buprenorphine: $N=12$, healthy: $N=15$	PBMC: lymphocytes (T and B), NK cells, monocytes, dendritic cells	Primary human PBMC lymphoproliferation and cytokines (TNF-α, IFN-γ, IL-2, and IL-4)	PMBCs from active heroin users displayed decreased lymphoproliferation and decreased secretion of TNF-α, IFN-γ, and IL-4 in response to PHA. No change in IL-2 secretion. Lymphoproliferation and cytokine secretion in the buprenorphine group was equivalent to healthy controls
Riss et al. 2012 [31]	Germany	In vivo and ex vivo, cross-sectional case–control	Investigate the effects of various opioid maintenance therapies on T cell function	Samples isolated from active heroin users, ex-heroin users on MOUD (methadone/ levomethadone, buprenorphine/ buprenorphine–naloxone), and healthy controls; direct assessment of samples (in vivo) plus additional experiments in ex vivo cell cultures	Active heroin: $N=27$, MOUD analyzed as a single group: $N=27$ (N for each medication: methadone/ levomethadone: $N=18$, buprenorphine: $N=7$, buprenorphine + naloxone: $N=2$), healthy: $N=25$	T cells (CD4+) T-regs (CD4 + CD25 [high])	In vivo levels of CD4 + CD25high T-regs by flow cytometry. Ex vivo experiments: 1. Anti-CD3/ anti-CD28–induced proliferation of CD4+ in ex vivo culture. 2. Proliferation of CD4+ cells with depletion of CD25(high) T-regs. 3. Cytokine secretion (TNF-α, IFN-γ, and IL-10)	Increased percent of CD4+/CD25(high) T-regs was noted in active heroin users compared to controls and MOUD. CD4+ cells from heroin users displayed decreased proliferative activity and depletion of CD4+/ CD25high T-regs restored the proliferative capability of remaining CD4+ cells of heroin users (ex vivo)

(Continues)

TABLE 1 | (Continued)

References	Country	Study type	Study goal	Study design	Sample type/size	Immune cells studied	Immune/ inflammatory markers	Key results
Boland et al. 2014 [50]	United Kingdom	In vitro	Investigate the effects of various opioids on innate and adaptive immune cell functions	Isolated primary human PBMCs from healthy subjects were treated with buprenorphine, morphine, tramadol, fentanyl, methadone, oxycodone, diamorphine, or codeine	Healthy volunteers (ages 25–33), $N = 3$–5 samples per drug/ concentration studied	Neutrophils, monocytes, NK cells, T cells	1. Neutrophil and monocyte phagocytosis and oxidative burst. 2. CD6CD56 NK cell function (ability to kill K562 cells). 3. T cell activation and cytokine secretion	Buprenorphine-treated neutrophils and monocytes showed a trend toward (not statistically significant) a decrease in phagocytic response. Mixed results between neutrophil and monocyte oxidative burst. No change in NK cell function or T cell activation with buprenorphine tx
Carvallo et al. 2015 [41]	USA	In vitro	Investigate the effect of buprenorphine on human monocyte function	Human primary monocytes from healthy subjects were treated with buprenorphine in vitro	Human primary monocytes from healthy subjects (Leukopaks from blood bank). N of human donors = unknown, N of independent replicated experiments = 4–8	Monocytes	1. CCL-2–mediated migratory phenotype of primary isolated monocytes (measured by % cells with membrane projections), monocyte chemotaxis, and migration. 2. CCL-2 receptor (CCR2) recycling in monocytes	Buprenorphine decreased CCL2-induced monocyte migration by decreasing surface cytoskeletal protein rearrangements, intracellular signaling, and CCL-2 receptor recycling
Sun et al. 2017 [36]	China	In vitro	Investigate the effects of buprenorphine on M1- and M2-polarized macrophages	Primary macrophages were isolated from human umbilical cord blood of healthy subjects, differentiated into M1 or M2 macrophages in vitro, and treated with buprenorphine in vitro	Healthy subjects: $N = 8$	Macrophages	Key inflammatory cytokines and transcription factors that represent M1 versus M2 macrophage activity.	In M1 macrophages, buprenorphine decreased mRNA of pro-inflammatory cytokines TNF- α, IL-6, and IL-12. In M2 macrophages, buprenorphine increased mRNA of Ym1 and Fizz1 (markers of M2 activation, which is anti-inflammatory)

(Continues)

TABLE 1 | (Continued)

References	Country	Study type	Study goal	Study design	Sample type/size	Immune cells studied	Immune/ inflammatory markers	Key results
Jaureguiberry-Bravo et al. 2018 [45]	USA	In vitro	Investigate the effects of buprenorphine on chemotaxis of mature monocytes	Isolated human monocytes from healthy subjects (Leukopaks from blood bank) were treated with buprenorphine in vitro	Human primary monocytes from healthy subjects (Leukopaks from blood bank), *N* of human donors = unknown, *N* of independent replicated experiments = 6–15	Monocytes	1. Mature monocyte binding to BMVEC, ICAM-1 versus VCAM-1 binding, chemotaxis. 2. THP-1 cell-line study for buprenorphine's effect on CCL-2–mediated association of FROUNT with CCR2 (time-course study)	Buprenorphine decreased CCL-2–mediated adhesion of mature monocytes to BMVEC by decreasing binding of monocytes to ICAM-1 but not VCAM-1. Buprenorphine also decreased CCL-2–mediated chemotaxis of mature monocytes. In a separate in vitro cell-line experiment (THP-1), buprenorphine decreased CCL-2–mediated association of FROUNT with CCR2
Maher et al. 2019 [51]	USA	In vitro	Investigate the effects of buprenorphine or 8 other opioid medications on human NK cell function	NK cells isolated from healthy volunteers treated with buprenorphine (or 8 other medications) in vitro	Healthy male volunteers (ages 25–39), *N* = 5 of separate human donors for the buprenorphine portion of the study	NK cells	NK cell function (cytotoxicity) measured by % apoptosis of K562 leukemia cells by NK cells after tx with various opioids	Buprenorphine decreased NK cell cytotoxicity at low doses (1 and 5 ng/mL) but higher dose (10 ng/mL) showed no significant difference in cytotoxicity compared to control

(Continues)

TABLE 1 | (Continued)

References	Country	Study type	Study goal	Study design	Sample type/size	Immune cells studied	Immune/ inflammatory markers	Key results
Arezoomandan et al. 2022 [33]	Iran	In vivo, cross-sectional case–control	Investigate the effects of buprenorphine on various peripheral inflammatory markers	Peripheral inflammatory markers were compared between ex-heroin/opium users after at least 12 months of MOUD (methadone or buprenorphine) vs. healthy matched controls	Methadone: $N=30$, buprenorphine: $N=30$, healthy: $N=30$	Other peripheral inflammatory markers	Ferritin Malondialdehyde Total antioxidant capacity High-sensitivity CRP	Ferritin: healthy > methadone > buprenorphine. Malondialdehyde: buprenorphine > methadone > healthy. Total antioxidant capacity: methadone > healthy > buprenorphine. High-sensitivity CRP: methadone > buprenorphine > healthy
Mahintamani et al. 2023 [32]	India	In vivo, prospective case–control	Investigate the effects of buprenorphine on various immune cell counts and plasma TLR4 levels	Buprenorphine naive opioid-dependent patients underwent 2–6 weeks of sublingual buprenorphine-naloxone tx; samples collected were before and after, and compared to healthy age- and sex-matched controls	Buprenorphine–naloxone: $N=30$, healthy: $N=30$	Neutrophils, lymphocytes (total), T cells (CD4 and CD8)	Total counts of neutrophils, lymphocytes, CD-4, and CD-8 T cells. Plasma TLR-4 level (as a proxy for brain TLR-4)	Plasma TLR-4 levels were equivalent in opioid-dependent and healthy control group at baseline; TLR4 levels increased in opioid-dependent group after tx. Neutrophil–lymphocytes ratio was increased in opioid-dependent group at baseline, and this normalized after tx. No changes in T cells noted before and after tx

Abbreviations: BMVEC, brain microvascular endothelia cells; CA, cancer; CCL-2, chemokine ligand 2; CRP, C-reactive protein; ICAM-1, intercellular adhesion molecule 1; LTC4, peptide-leukotriene C4; MOUD, medications for opioid use disorder; NK cells, natural-killer cells; PBMC, peripheral blood mononuclear cell; PGD2, prostaglandin D2; PHA, polyclonal mitogen phytohemagglutinin; TLR4, toll-like receptor 4; T-reg, regulatory T cells; Tx, treatment; VCAM-1, vascular cell adhesion molecule 1.

TABLE 2 | Summary of buprenorphine's effect on various immune cell functions and inflammatory markers.

Immune cell/marker	Effect of buprenorphine
Mast cells/histaminergic response	No significant histaminergic response, no reports of anaphylaxis [34, 35, 37]
Monocyte count	Restores CD14+ monocyte counts and plasma cytokine levels in heroin users to levels higher than healthy controls, suggesting hyperactivation of the immune system [29]
Monocyte chemotaxis	Dampens CCL-2–mediated monocyte chemotaxis and migration in vitro, potentially contradicting in vivo findings of immune restoration [41]
Monocyte adhesion	Decreases monocyte adhesion to brain microvascular endothelial cells, specifically inhibiting monocyte–ICAM-1 binding [45]
Macrophage phenotype	Decreases pro-inflammatory cytokine mRNA in M1 macrophages and increases markers of M2 macrophage activation, indicating an anti-inflammatory effect [36]
Lymphoproliferative capacity	Restores the lymphoproliferative capacity of PBMCs in heroin users to levels comparable with healthy controls [30]
Neutrophil function	Inhibits neutrophil chemotaxis and superoxide production, suggesting a dampening effect on neutrophil function [32, 49, 50]
NK cell function	No significant effect on cytotoxic function [50, 51]
T cell function	No change in T cell activation markers [50] Restores the T-reg phenotype in opioid users, normalizing CD4+/CD25(high) T-reg counts to levels seen in healthy controls [31]
Inflammatory markers	Buprenorphine and methadone differentially affect inflammatory markers, with buprenorphine increasing malondialdehyde levels and methadone increasing high-sensitivity C-reactive protein levels [33] Buprenorphine increased plasma TLR4 [32]

Each study in this review analyzed various aspects of the human immune and inflammatory changes related to buprenorphine. It is important to note that all studies characterized peripheral immune and inflammatory changes. Two studies analyzed direct skin reactions to characterize histamine release and mast cell function [34, 35], while isolated immune cells were analyzed in the remaining 11 studies. One study focused on buprenorphine's effect on other inflammatory markers, including acute phase reactants and oxidative activity [33]. One study assessed cytokines directly from the plasma of ex-heroin users randomized into MOUD groups, including a buprenorphine group [29]. Two studies assessed cytokine secretion in ex vivo culture mediums of peripheral blood mononuclear cell (PBMC) samples from human subjects [30, 31], and one study measured cytokines released into the in vitro culture medium of primary human macrophages [36]. Five studies measured cytokines as outlined in Table 1; cytokines measured in these studies include TNF-α, IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-10, and IL-12. All five studies measured protein levels of cytokines utilizing ELISA or Luminex; one of the five studies also measured mRNA changes via qPCR in addition to ELISA [36]. Lastly, one study measured immune modulators prostaglandin D2 and peptide leukotriene C4 [37].

4 | Discussion

4.1 | Early Clinical Safety Studies of Buprenorphine

The initial studies of buprenorphine's effect on the human immune system were conducted around the time of the official US FDA approval of buprenorphine. These studies focused on the clinical safety profile of a new medication, with particular interest from anesthesiologists regarding histamine release and anaphylaxis in response to buprenorphine (Table 1). The three studies related to the topic reported no significant safety concerns, and the lack of significant histaminergic or anaphylactic response with buprenorphine treatments in these studies contributed to the approval and ultimate widespread use of buprenorphine [34, 35, 37]. One study utilized mast cells isolated from skin and lung samples for mechanistic insight regarding prostaglandin D2 and peptide-leukotriene C4 release in response to buprenorphine, although the tissue samples were collected from breast cancer and lung cancer patients (resected skin and lung parenchymal tissue during surgery) [37].

4.2 | Buprenorphine's Effect on Monocytes

Buprenorphine's effect on peripheral monocyte function is most studied to date. Neri et al.'s study in 2005 was the first and only randomized and prospective controlled trial included in this review that studied buprenorphine's effect on the human immune system, with a particular focus on monocyte function [29]. In this work, the authors investigated changes in inflammatory markers before and after long-term buprenorphine treatment in ex-heroin users and compared these changes to healthy subjects. CD14+ monocyte count (isolated from PBMCs) and plasma cytokine levels were measured as key markers of inflammation in the human peripheral immune system. Prior to buprenorphine treatment, heroin users had a lower CD14+ monocyte count compared to the healthy controls, and there were no pretreatment differences between the two groups of heroin users who were

subsequently randomized into methadone or buprenorphine for the 12 months of MOUD treatment. The authors also reported lower plasma levels of TNF-α, IL-1β, and IL-2 in heroin users compared to healthy controls. These baseline characteristic findings of heroin users versus healthy controls are consistent with previous work in OUD, which demonstrated that active heroin users have an overall decreased immune response [38]. For example, patients who abuse opioids have been shown to have a poor response to vaccines [39], and the results from Neri et al.'s work are mechanistically consistent with the crucial role of monocytes in modulating proper immune response to vaccines [40]. In Neri et al.'s work, CD14+ monocyte counts were shown to be restored in both MOUD groups following 12 months of MOUD treatment with buprenorphine or methadone [29]. Interestingly, the CD14+ monocyte count in both groups surpassed that of the healthy control group, and plasma levels of TNF-α, IL-1β, and IL-2 also surpassed healthy controls. These results formed the basis of the theory that MOUD treatment leads to "hyperactivation" of the immune system in chronic heroin users. Overall, Neri et al.'s work characterized a potential key inflammatory marker of buprenorphine treatment and provided evidence for a potential pragmatic solution to a previously described clinical problem (i.e., revaccinating previous opioid users after consistent treatment with buprenorphine) [39].

In 2015, Carvallo et al. [41] (Berman laboratory) further investigated the mechanisms of buprenorphine's effect on monocyte function by specifically characterizing monocyte chemotaxis and migration. In this in vitro study, CD14+ monocytes were isolated from PBMCs of unspecified healthy human donors and treated with CCL-2, the most potent monocyte chemoattractant [42], to induce morphological change and migration. In this work, when monocytes were co-treated with buprenorphine and CCL-2, buprenorphine demonstrated a dampening effect on CCL-2–mediated phenotypic changes and cellular migration in primary human monocytes. Furthermore, a time-course experiment in this study showed that buprenorphine decreased the rate of CCR-2 (CCL-2 receptor) recycling back to the monocyte cellular membrane, thus possibly explaining how buprenorphine may counteract CCL-2–mediated monocyte chemotaxis and migration. In the context of previous work, these findings may appear potentially contradictory. Specifically, Neri et al. showed presumed (in vivo) improvement or restoration of immune function in heroin users (measured by absolute CD14+ count and cytokines) following extended buprenorphine treatment, while Carvallo et al.'s work appears to suggest a dampening effect in CD14+ monocyte function following buprenorphine treatment in vitro. One plausible explanation for this difference is in the experimental design (in vivo vs. in vitro), as immunologic response to various stimuli is almost never exclusively carried out by one single-cell type as is often observed in an in vitro setting. Carvallo et al. [41] reported several additional mechanistic findings, including changes in phosphorylation patterns of p38 MAPK and JAM-A junctional protein, as well as proteomic analysis of buprenorphine-treated and CCL-2–induced monocytes. One key finding from this proteomic analysis is buprenorphine's effect on decreasing CCL-2–induced phosphorylation of leukosialin/CD43. CD43 is expressed in all leukocytes and has been implicated in T cell migration in the periphery [43]. Interestingly, CD43 is also expressed in microglia in the CNS [44], and this finding suggests that buprenorphine may directly modulate microglial function in the CNS.

In 2018, the same laboratory (Berman laboratory) reported additional findings on buprenorphine's effect on human monocyte function. Jaureguiberry-Bravo et al. [45] demonstrated that buprenorphine decreased monocyte adhesion to brain microvascular endothelial cells (BMVECs), an in vitro assay that models the human blood brain barrier. Interestingly, buprenorphine decreased CCL-2–mediated monocyte binding to ICAM-1 but not VCAM-1, suggesting a specific buprenorphine-mediated inhibition of monocyte–ICAM-1. Similar to their previous work, Jaureguiberry-Bravo et al. utilized monocytes isolated from healthy donors to study buprenorphine's effect; therefore, buprenorphine's effect in changing monocyte function in vivo still remains to be further elucidated. A notable strength of their study is that the authors specifically considered an important physiological state of human monocytes in vivo, which is that most monocytes exist in an immature CD14+/CD16 state within the human body, until subsequently activated by a stimulus. Therefore, the authors conducted a series of studies to characterize and differentiate the immature CD14+/CD16 monocytes into mature CD14+/CD16+ monocytes prior to conducting any buprenorphine experiments—thereby adding clinical relevance to their work.

4.3 | Buprenorphine's Effect on Macrophages

Macrophages are a heterogeneous population of cells that share the same hematopoietic lineage as monocytes (myeloid lineage), and Sun et al. [36] investigated buprenorphine's effect on the macrophage phenotype in human umbilical cord blood. While newly recruited macrophage populations arise from circulating monocytes that infiltrate various tissues, some resident tissue-specific macrophages arise during early embryonic development [46]. Nevertheless, macrophages in the tissue play a key role in potentiating a pro- or anti-inflammatory process, orchestrated by M1 macrophages that secrete pro-inflammatory cytokines (i.e., TNF-α, IL-6) or M2 macrophages that secrete anti-inflammatory cytokines (i.e., IL-10) [47]. In their work, Sun et al. showed that buprenorphine decreased the mRNA of inflammatory cytokines TNF-α, IL-6, and IL-12 in M1 macrophages, effectively displaying an anti-inflammatory response. Further mechanistic work by Sun et al. showed that buprenorphine decreased the transcription of IRF5, a key modulator of IL-6. In addition, Sun et al. showed that buprenorphine increased the mRNA of Ym1 and Fizz [36], which are previously reported markers of anti-inflammatory M2 macrophage activation [48]. Overall, studies that examined the effects of buprenorphine in monocytes and macrophages suggest a net anti-inflammatory effect.

4.4 | Buprenorphine's Effect on General Lymphoproliferative Capacity

Sacerdote et al.'s [30] cross-sectional ex vivo study in 2008 examined buprenorphine's ability to modulate the general

lymphoproliferative capacity of PBMCs, which includes various peripheral immune cells such as monocytes, B cells, T cells, and NK cells. This study utilized PBMCs isolated from active heroin users that were compared to PBMCs isolated from patients who were on long-term MOUD treatment with either buprenorphine or methadone, as well as sex- and age-matched healthy controls. In this work, PBMCs from active heroin users demonstrated decreased ability to proliferate in an ex vivo culture setting (in response to polyclonal mitogen phytohemagglutinin [PHA]), while PBMCs from those who were receiving long-term MOUD with buprenorphine or methadone showed equivocal response when compared to healthy controls. PBMCs from heroin users also displayed decreased secretion of TNF-α, IFN-γ, and IL-4 in response to PHA-induction, while PBMCs from the buprenorphine or methadone group demonstrated equivalent cytokine secretions when compared to sex- and age-matched controls [30]. Together, these findings provided insights into buprenorphine's potential in restoring the chronically suppressed immune function in OUD. However, PBMCs in this study were not further separated into individual cell types; therefore, this remains a general finding.

4.5 | Buprenorphine's Effect on Neutrophil Function

Mikawa et al.'s report in 2006 demonstrated that buprenorphine had a dampening effect on neutrophil function—decreased chemotaxis and superoxide production by neutrophils following in vitro buprenorphine treatment (Table 1) [49]. Specifically, a high dose of buprenorphine (100 ng/mL) inhibited neutrophil chemotaxis, while low doses (1 and 10 ng/mL) impaired superoxide production in neutrophils—together suggesting that neutrophils are particularly sensitive to buprenorphine. Two other studies (Boland et al. and Mahintamani et al.) reviewed in this work also studied the effects of buprenorphine on neutrophils with inconclusive findings [32, 50]. In Boland et al.'s work in 2014, in vitro treatment of isolated primary human neutrophils with buprenorphine did not result in statistically significant changes in phagocytosis or oxidative bursts with the exception of a mild decrease in oxidative burst in response to *E. coli* only with 0.8 and 20 ng/mL of buprenorphine but not with a 4 ng/mL dose (of note, no significant changes in oxidative bursts induced with fMLP or PHA) [50]. In Mahintamani et al.'s work in 2023, total neutrophil count was analyzed for neutrophil–lymphocyte ratio in opioid-dependent patients undergoing sublingual buprenorphine–naloxone treatment compared to healthy controls in a prospective case–control study [32]. In this work, total neutrophil counts decreased with buprenorphine–naloxone treatment but no mechanistic findings about buprenorphine's effect on neutrophil function were reported.

4.6 | Buprenorphine's Effect on NK-Cell Function

Briefly discussed above, Boland et al.'s 2014 work was an in vitro mechanistic study that investigated the effects of eight different opioids on the immunologic function and phenotype of several immune cells, including neutrophils, monocytes, NK cells, and T cells [50]. In this work, NK cell function, measured by cytotoxicity against K562 cells, did not show significant changes following buprenorphine treatment. Of note, this finding is consistent with a previous preclinical study that showed that buprenorphine does not affect NK cell cytotoxicity in mice [23]. Maher et al. in 2019 also investigated the effects of buprenorphine and eight other opioids on isolated human NK cell functions in vitro [51]. Interestingly, the authors reported a mixed response in NK cell function due to buprenorphine in which lower dose treatments (1 and 5 ng/mL) resulted in decreased NK cell cytotoxic functions, while the higher dose treatment (10 ng/mL) did not show any significant difference in cytotoxic functions of NK cells.

4.7 | Buprenorphine's Effect on T Cell Function

Boland et al.'s 2014 work also studied buprenorphine's effect on T cell activation, measured by CD69 and IL-6 expressions. However, their results did not show significant changes with in vitro buprenorphine treatments [50]. Riss et al.'s work in 2012 took a different approach to study the mechanisms that drive the chronic immune suppression in OUD by investigating the role of CD4+/CD25(high) regulatory T cells (T-regs) in active heroin users compared to those undergoing long-term MOUD treatment (multiple agents, including buprenorphine and buprenorphine–naloxone, grouped into one) or sex-matched healthy controls [31]. CD4+/CD25(high) T-regs are thymic-derived cells that maintain immune tolerance and are critical in regulating self-reactive T cells and therefore preventing autoimmune disease [52]. In Riss et al.'s work, PBMCs from active heroin users demonstrated a significant increase in the absolute number of CD4+/CD25(high) T-regs, compared to PBMCs from those undergoing long-term MOUD treatment or sex-matched healthy controls [31]. It is important to note that there was no difference in the absolute number of PBMCs or CD4+ T cells isolated among all three groups, providing in vivo evidence that the difference in regulatory T cells, rather than absolute T cell count (i.e., total CD4+ count), may be responsible for chronic immune suppression in OUD. Furthermore, the CD4+/CD25(high) T-reg count was equal between the MOUD group and healthy controls, suggesting that MOUD can restore the T-reg-immunologic phenotype of OUD.

The heroin group in Riss et al.'s study also demonstrated an increase in CD4+/CD25(high)/Foxp3+ T cells compared to both the control and MOUD groups [31]. This is an interesting finding in the context of the previously identified problem of poor vaccine response in OUD [39], because Foxp3+ T-regs have been shown to inhibit proper vaccine response, and subsequent depletion of Foxp3+ T-regs resulted in the restoration of the immune system's ability to mount a therapeutic immune response to vaccines [53]. Similarly, Riss et al. carried out an additional ex vivo study in which they depleted the CD4+/CD25(high) T-regs from the total CD4+ T cells of each group and studied their ability to proliferate in ex vivo cultures [31]. Prior to the depletion of the T-regs, CD4+ cells from heroin users demonstrated decreased ability to proliferate under anti-CD3/anti-CD28 induction, while subsequent depletion of T-regs from CD4+ cells of heroin

users restored their proliferative capability equivalent to that of healthy controls and the MOUD group. Altogether, this is an insightful study that provides a mechanistic understanding of how MOUD may restore another aspect of immune suppression of OUD. However, it is important to note that this study combined those receiving different MOUD (i.e., buprenorphine, methadone, etc.) into one single MOUD group as detailed in Table 1. Therefore, further work is needed to differentiate the pattern of immunologic restoration unique to each MOUD included in this study.

4.8 | Buprenorphine's Effects on Other Markers of Inflammation

In contrast to previous work that focused on specific immunologic markers as they related to a specific subset of the peripheral immune system, Arezoomandan et al.'s work in 2022 investigated general peripheral inflammatory and oxidative stress markers in vivo by analyzing serum levels of high-sensitivity C-reactive protein (hsCRP), ferritin, malondialdehyde (MDA), and total antioxidant capacity. HsCRP is a nonspecific peripheral marker of chronic inflammation previously known to be elevated in opium users [54, 55]. In that regard, it is interesting to note that hsCRP expression correlated with μ-opioid receptor agonism; hsCRP was highest in the methadone treatment group, followed by the buprenorphine-treated group and healthy controls [33]. However, MDA, a marker of lipid peroxidation, was highest in the buprenorphine group, followed by the methadone group and healthy controls [33]. The mechanistic difference between methadone and buprenorphine appears to play a direct role in the differential expression of hsCRP and MDA, suggesting that full versus partial agonism of the μ-opioid receptor activates different inflammatory mechanisms in OUD.

The latest study included in this review is by Mahintamani et al. in 2023, in which the authors investigated the effects of buprenorphine on various immune cells (neutrophils, total lymphocytes, CD4+, and CD8+ T cells), with a particular focus on Toll-like receptor 4 (TLR4) levels [32]. In this in vivo prospective case–control study, buprenorphine-naïve and opioid-dependent (i.e., morphine) patients were treated with 2–6 weeks of sublingual buprenorphine–naloxone, and samples from before and after treatments were compared to age- and sex-matched healthy controls. In regard to cell populations, the neutrophil–lymphocyte ratio was noted to be increased in the opioid-dependent group compared to healthy controls in the pretreatment phase. However, this ratio was subsequently normalized with buprenorphine treatment, which led the authors to make a positive correlation between increased pain and increased neutrophil count (pain scales were measured in the study). There were no significant differences in T cell populations between the two groups (as measured by CD3, CD4, CD8, as well as CD4:CD8 ratios). Mechanistically, Mahintamani et al. measured TLR4 in the plasma by ELISA as a proxy for brain TLR4. At baseline, plasma TLR4 levels in the opioid-dependent group were equivalent to those of the healthy controls. However, plasma TLR4 increased significantly following 2–6 weeks of sublingual buprenorphine–naloxone treatment, suggesting that buprenorphine may exert its effects by activating TLR4 and its downstream pathways. The authors also speculated that the equivocal TLR4 levels at baseline between opioid-dependent (i.e., morphine) and healthy controls may be due to subsequent silencing of TLR4 by morphine following chronic exposure, and that buprenorphine reactivated TLR4—thereby resulting in the phenotype observed following the treatment. No other correlations were found between TLR4 and other parameters measured by the authors.

Of note, TLR4 is a key immune receptor that belongs to the family of pattern recognition receptors, an important aspect of innate immunity that recognizes pathogen-associated molecular patterns (PAMPs, i.e., gram-negative lipopolysaccharide) and damage-associated molecular patterns (DAMPs) [56, 57]. Classically, activation of TLR4 by PAMPs or DAMPs results in a downstream pro-inflammatory response, but emerging evidence suggests that aberrant activation of TLR4 or its downstream pathways may also be involved in noninfectious sterile inflammation and various neuropathological processes as well [56–58]. As for opioids as ligands for TLR4, some opioids (such as morphine) have been shown to bind TLR4 directly [59, 60]. However, how various opioids affect the TLR system remains poorly characterized [61] and a recent in vivo animal study demonstrated that various opioids failed to bind or illicit TLR4 response at physiologic concentrations [62]. In regard to buprenorphine, there is currently limited information about buprenorphine's binding and modulation of TLR4—one preclinical study has shown that the buprenorphine treatment can activate TLR4 signaling in vitro [59], while another animal study reported improved pain relief by buprenorphine with TLR4 blockade in rats [63]. Additional work is needed to further characterize the role of direct TLR4 modulation by buprenorphine in humans.

4.9 | Challenges and Future Directions

Understanding how opioids and buprenorphine modulate the inflammatory mechanisms in the human CNS appears to be the next frontier in OUD research. A key challenge in studying buprenorphine's effect on human CNS inflammation is the invasive nature of CNS studies in humans (i.e., lumbar puncture for CSF studies) and logistical, ethical, and safety challenges in conducting an invasive study in a vulnerable opioid-dependent population. One potential solution to this challenge is the utilization of novel imaging techniques in OUD research. Advances in novel imaging techniques for neuroinflammation have been reviewed by several groups [64–66]. Our literature search did not reveal any completed neuroimaging study related to buprenorphine and neuroinflammation but revealed two meeting abstracts with incomplete study updates from Woodcock et al. in which the authors are currently investigating the effect of buprenorphine on neuroinflammation, utilizing positron emission tomography (PET) with 18 kDa translocator protein (TSPO) as a marker of neuroinflammation [67, 68]. To date, TSPO is a widely used in in vivo imaging marker of neuroinflammation in many neurological, neurodegenerative, and psychiatric disease research studies [69]. Preliminary work from Woodcock et al. shows that the induction of OUD treatment with buprenorphine

may initially result in increased neuroinflammatory signaling (increased TSPO levels), followed by time-dependent neuroimmune recovery with continued buprenorphine treatment [67, 68].

Another potential solution to the challenges of clinical neuroinflammation research in OUD is the utilization of induced pluripotent stem cells (iPSCs) and iPSC-derived organoids in OUD research. Unlike animal models or immortalized human cell lines, iPSCs are derived from primary human cells and lack immortalizing genetic alterations, thus resulting in greater potential for direct translation. In this context, our group has previously studied the effects of oxycodone and buprenorphine on iPSC-derived brain organoids from patients with OUD [70]. In that work, oxycodone and buprenorphine treatments resulted in distinct differential transcriptional responses in both neural and glial cell populations, highlighted by the induction of Type I interferon signaling and activation of STAT1 by oxycodone but not by buprenorphine [70]. Type I interferon signaling is a key inflammatory signaling pathway that activates Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway, including STAT1, and subsequently regulates the transcription of interferon-stimulated genes, including several key cytokines and chemokines [71, 72]. As STAT1 is closely related to the regulation of CCL-2 and CCL-2–mediated immune response [72–74], buprenorphine may exert its mechanistic effect on the immune system via modulation of STAT1. Therefore, our work with iPSC-derived brain organoids provides a novel mechanistic insight into the molecular mechanism of buprenorphine at single-cell resolution.

Emerging evidence from past decades has characterized neuroinflammation as not only a result of a peripheral disease process (i.e., by invasion of peripheral inflammatory cells into the CNS) [75] but also a potential initial inciting event in the CNS that drives early pathogenesis of several neurological and psychiatric conditions [76–79]. Consistent with the authors' understanding of the current literature, all 14 clinical studies included in this review focused on buprenorphine's effect on the peripheral immune system, and the majority of the studies seem to support the notion that the initiation and maintenance of buprenorphine restore immunosuppression caused by OUD, as summarized in Table 2. However, OUD is a brain disease and has been described as pro-inflammatory in the CNS. No current human studies describe the direct effects of buprenorphine on the human immune system within the CNS, which should be the next important direction in this research. Despite challenges, future work utilizing advanced imaging and cellular technologies will continue to uncover how buprenorphine affects the human immune system and inflammation, particularly in the CNS. These collective advances in the field may result in the development of novel therapies that work synergistically with or independently from currently available therapies.

Author Contributions

S.D.S., R.B., A.S., M.M., M.-F.H., V.M.K., and T.O. wrote the manuscript; S.D.S., R.B., M.M., and T.O. designed the research; S.D.S., R.B., A.S., and M.M. performed the research; and S.D.S., R.B., A.S., M.-F.H., and T.O. analyzed the data.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.