Neuroimmune modulation of pain and regenerative pain medicine

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Regenerative pain medicine, which seeks to harness the body's own reparative capacity, is rapidly emerging as a field within pain medicine and orthopedics. It is increasingly appreciated that common analgesic mechanisms for these treatments depend on neuroimmune modulation. In this Review, we discuss recent progress in mechanistic understanding of nociceptive sensitization in chronic pain with a focus on neuroimmune modulation. We also examine the spectrum of regenerative outcomes, including preclinical and clinical outcomes. We further distinguish the analgesic mechanisms of regenerative therapies from those of cellular replacement, creating a conceptual and mechanistic framework to evaluate future research on regenerative medicine.

While acute pain brings attention to injuries, chronic pain has no biological benefits. Chronic pain often arises from disease (e.g., arthritis, cancer) and trauma (e.g., nerve injury, spinal cord injury); it affects up to 30% of adults worldwide and costs the US economy more than $600 billion per year (1, 2). Arthritis alone affects more than 53 million individuals in the US (3), and current nonsurgical therapies have limitations due to low efficacy and side effects, such as steroid injections (4), hyaluronic acid (HA) viscosupplementation (5), and opioid therapy (6). Novel approaches to treating arthritis and other common painful conditions (e.g., back and neck injury, neuropathic pain) are critically needed. One of these advances, the field of regenerative pain therapies, seeks to harness the body's own reparative capacity, and is built on our improved understanding of the neurobiologic mechanisms that mediate and modulate pain perception and sensitization, coupled with our understanding of how inflammatory processes impact dynamic "pain circuits." Here, we discuss recent advances in research areas that are mechanistically related to regenerative pain medicine.

Primary afferent pathways that contribute to pain perception and sensitization

Specialized sensory neurons called nociceptors sense pain by detecting noxious stimuli (7–9). Nociceptor sensitization (or peripheral sensitization) may be the most direct cause of pathological or persistent pain (10) and the most appropriate target for peripheral regenerative therapies. Nociceptors are characterized by wide molecular and functional diversity, comprising both unmyelinated C-fibers and myelinated Aδ-fibers as the largest population of primary sensory neurons in the dorsal root ganglion (DRG), trigeminal ganglion, and glossopharyngeal ganglion. In addition to noxious thermal, mechanical, and chemical stimuli, light can also induce or suppress pain when nociceptors express light-sensing ion channels (11). Using transcriptional profiling analysis at the whole-population and single-cell levels, Chiu et al. revealed molecular diversity within six distinct groups of mouse DRG neurons (12). Usoskin et al. used unbiased single-cell RNA sequencing (RNA-Seq), revealing 11 types of mouse DRG neurons (3574 ± 2010 genes per cell), including three low-threshold mechanoreceptive, two proprioceptive, and six principal types of nociceptive neurons (13). Using high-coverage single-cell RNA-Seq (10,950 ± 1218 genes per neuron) with functional characterization, Li et al. identified 10 types and 14 subtypes of mouse DRG neurons (14). Deep sequencing of eight DRG neuron subtypes using individual mouse genetic lines revealed differentially expressed and functionally distinct genes, including the voltage-gated potassium channels Kv1–Kv4 (15).

Recent work has generated human nociceptors by reprograming fibroblasts; this technique recapitulated some aspects of human disease phenotypes in vitro to model "pain in a dish" (16). Humans and mice display some key differences in gene expression and function of DRG neurons (17, 18): human DRGs have higher expression of Nav1.7 (19), a sodium channel subtype critical for normal and abnormal pain sensation in humans (20, 21). Nav1.7 expression and function are upregulated in rodent and human DRG neurons by paclitaxel, a chemotherapy drug that induces neuropathy in rodents and humans (19, 22), as well as in DRG neurons of patients with neuropathic pain (22).

Touch hypersensitivity or tactile allodynia is a common feature of acute and chronic pain (23). Low-threshold Aβ-fiber neurons express *Tlr5* (13, 15), and recent evidence suggests that

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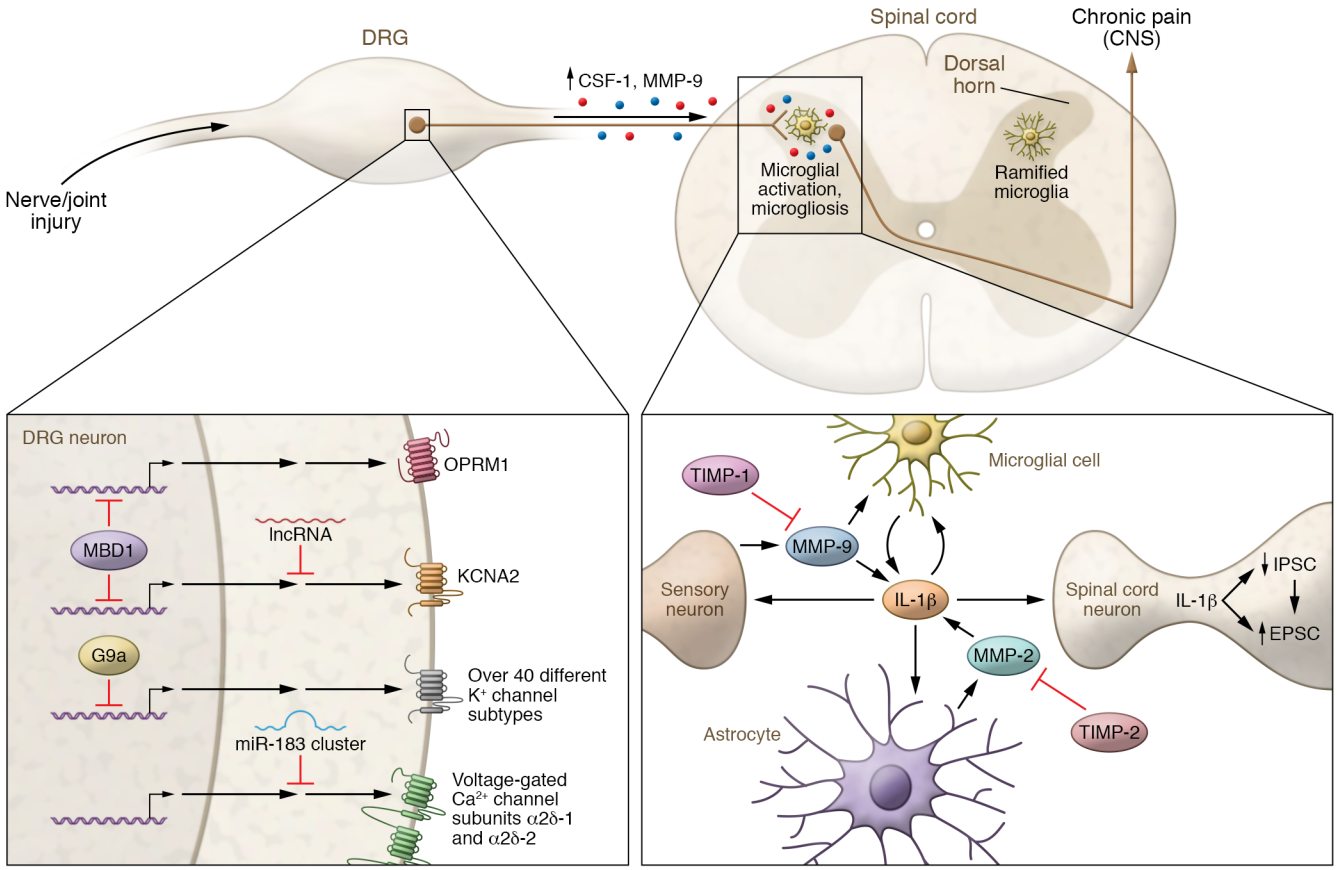

Figure 1. Pathogenesis of neuropathic pain and arthritic pain via gene regulation in DRG neurons and neuroinflammation in the spinal cord. Lower left: Epigenetic regulation in DRG neurons in peripheral sensitization after nerve injury. In primary sensory neurons, MBD1 epigenetically suppresses expression of μ-opiod receptor and potassium channel subtype Kv1.2 (encoded by *Kcna2*). *Kcna2* expression is also silenced by long noncoding RNA (lncRNA). Activity of G9a in DRG neurons increases following nerve injury, resulting in epigenetic silencing of more than 40 potassium channel subtypes. Voltage-gated calcium channel subunits α2δ-1 and α2δ-2, the molecular targets of gabapentin, are regulated by the miR-183 cluster. Right: Spinal cord microglia activation in chronic pain. Nerve injury and joint injury induce upregulation of MMP-9 and CSF-1 in DRG neurons. MMP-9 and CSF-1 undergo axonal transport to the spinal cord dorsal horn. Upon release, MMP-9 and CSF-1 induce microglia activation (e.g., p38 phosphorylation) and microgliosis (proliferation and morphological changes) in the ipsilateral spinal cord, leading to the development of chronic pain. Lower right: Spinal cord neuroinflammation in central sensitization and chronic pain. Upon activation, microglia produce and release IL-1β, which induces central sensitization and chronic pain via both presynaptic and postsynaptic regulations, leading to increased EPSCs and decreased IPSCs. IL-1β also modulates the activation of microglia and astrocytes in the spinal cord. Delayed but persistent MMP-2 production in astrocytes contributes to late-phase neuropathic pain. Both MMP-9 and MMP-2 are involved in regulating the cleavage and activation of IL-1β. Inhibition of MMP-9 and MMP-2 by TIMP-1 and TIMP-2 blocks neuropathic pain.

pharmacological inhibition of these neurons blocks mechanical allodynia (24). Interestingly, in both mice and humans, application of the TLR5 ligand flagellin onto primary afferent neurons results in increased membrane permeability to QX-314, a membrane-impermeable lidocaine derivative, and subsequent silencing of TLR5-expressing A-fibers, without affecting the function of C-fibers (24). Moreover, a combination of flagellin and QX-314 blocked Aβ-fiber–evoked compound potentials in the sciatic nerve (24), Aβ-fiber–evoked synaptic transmission in spinal cord neurons (25), and mechanical allodynia in mice after nerve injury and chemotherapy (24). In contrast, C-fiber blockade with capsaicin/QX-314 (26) inhibited heat hyperalgesia after nerve injury without affecting mechanical allodynia after chemotherapy (24).

Notably, single-cell RNA-Seq may miss certain low-expression but important genes in sensory neurons. For example, the autism-associated gene *Shank3* is not detected by single-cell analysis (13), but is indeed present in DRG neurons. SHANK3 loss in sensory neurons results in decreased heat sensitivity but increased mechanical sensitivity (27, 28). Thus, SHANK3 expression in sensory neurons contributes to pain and touch dysregulation in autism patients (27, 28). Partial knockdown of *SHANK3* with siRNA is sufficient to block the capsaicin response and TRPV1 function in human DRG neurons (27). Programmed death protein-1 (PD-1, encoded by *Pdcd1*) is typically expressed by immune cells and serves as a target of immune therapy for cancer (29). Electrophysiological studies revealed that functional PD-1 is present in both mouse and human DRG neurons, and its activation by the ligand PD-L1 silences nociceptive neurons (30). Behavioral studies further demonstrate that PD-L1/PD-1 signaling inhibits physiological and pathological pain in mice (30). In situ hybridization and immunohistochemistry showed *Pdcd1/Pd1* mRNA expression and PD-1 protein expression in mouse DRG neurons (30), although

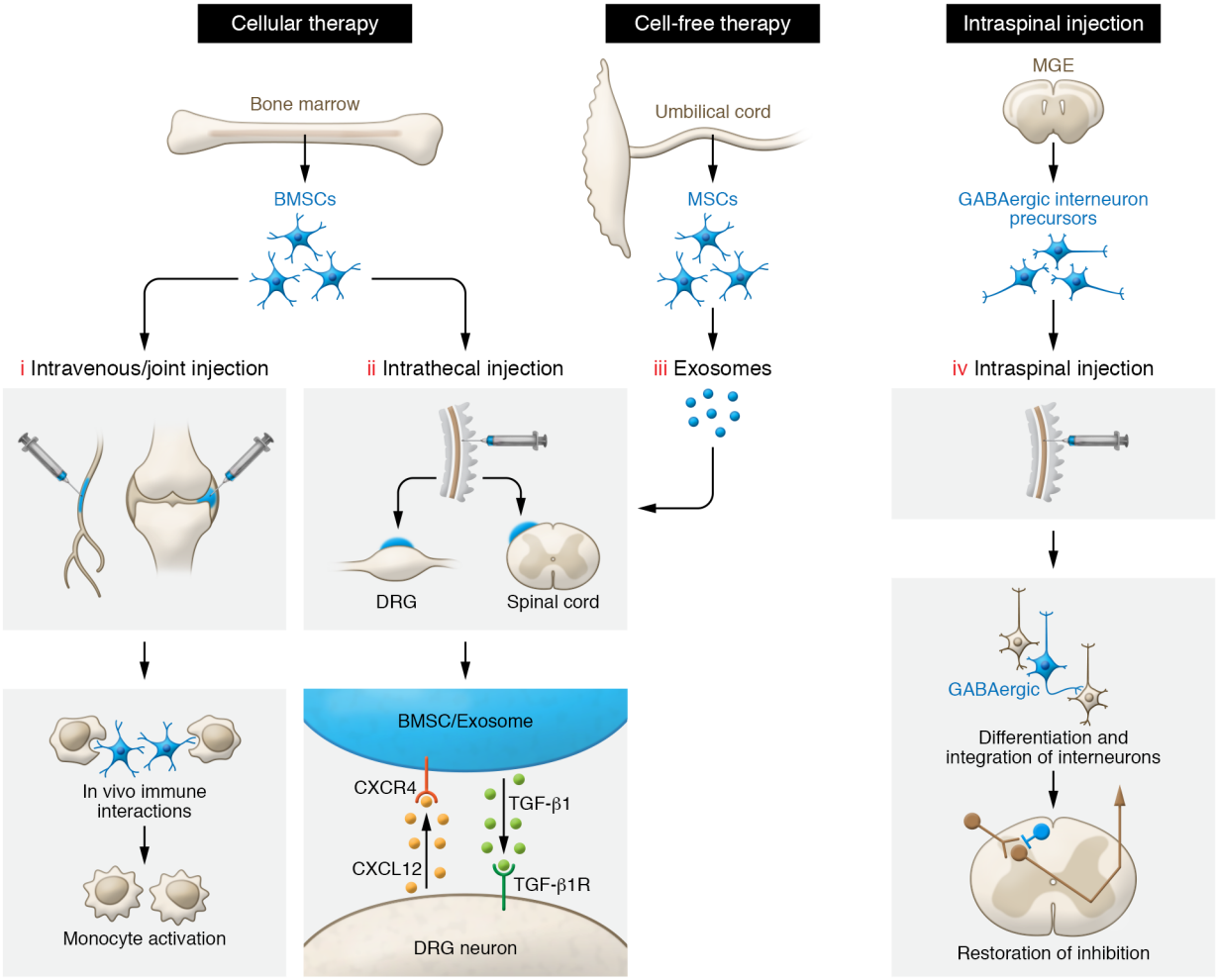

Figure 2. Preclinical models of cellular and cell-free exosome therapies for chronic pain. (i) Single systemic or local injection of BMSCs can reverse mechanical allodynia by in vivo immune interactions and activation of monocytes. (ii) Intrathecally injected BMSCs migrate to meninges of injured DRG neurons and spinal cord dorsal horn via a CXCL12/CXCR4 homing mechanism. TGF-β1 secretion by BMSCs confers potent long-term pain relief by activation of the neuronal TGF-β receptor (TGF-βR). (iii) Intrathecal injection of exosomes derived from human umbilical cord mesenchymal cells can serve as cell-free therapy for neuropathic pain. (iv) Transplantation of embryonic cortical GABAergic interneuron precursors from the medial ganglionic eminence (MGE) into the spinal cord leads to the development of inhibitory neurons. Furthermore, these GABAergic neurons integrate into spinal nociceptive circuits, mediating pain relief by release of GABA that acts on host-transplant inhibitory synaptic circuits.

single-cell RNA-Seq failed to detect *Pdcd1* mRNA expression (13). Together, these findings suggest that PD-L1/PD-1 may act as an endogenous inhibitory system for pain (30).

Accumulating evidence suggests an important role for epigenetic regulation of gene expression in primary sensory neurons for the pathogenesis of pain (Figure 1). Methyl-CpG–binding domain protein 1 (MBD1), an epigenetic repressor, regulates neuropathic pain by suppressing μ-opioid receptor (*Oprm1*) and potassium channel Kv1.2 (encoded by *Kcna2*) expression in primary sensory neurons (31). A long noncoding RNA that induces neuropathic pain by silencing *Kcna2* was also identified in primary afferent neurons (32). In DRG neurons, nerve injury increased the activity of euchromatic histone-lysine *N*-methyltransferase-2 (G9a), which drives neuropathic pain via epigenetic silencing of potassium channels (33). Furthermore, mechanical allodynia in neuropathic pain is controlled by sensory neuron expression of the microRNA-183 (miR-183) cluster, which regulates the expression of voltage-gated calcium channel subunits α2δ-1 and α2δ-2; these subunits are the molecular targets of gabapentin, a common treatment for neuropathic pain (34). Sensory neurons also express histone deacetylase 6 (HDAC6) after chemotherapy, and HDAC6 activation results in mechanical allodynia and a loss of intraepidermal nerve fibers (35).

Neuro-immune interactions and neuroinflammation in chronic pain

The past decade has also seen substantial progress in revealing non-neuronal mechanisms of pain (36, 37). Bidirectional signaling between the immune and nervous systems contributes to the development and maintenance of chronic pain (38–40). Microarray studies show that after nerve injury, immune-related genes are among the most differentially regulated genes in the spinal cord (41). Moreover, transcripts correlating with tactile hypersensitivity are immune cell–centric; depletion of macrophages or T cells reduced neuropathic tactile allodynia but not cold hypersensitivi-

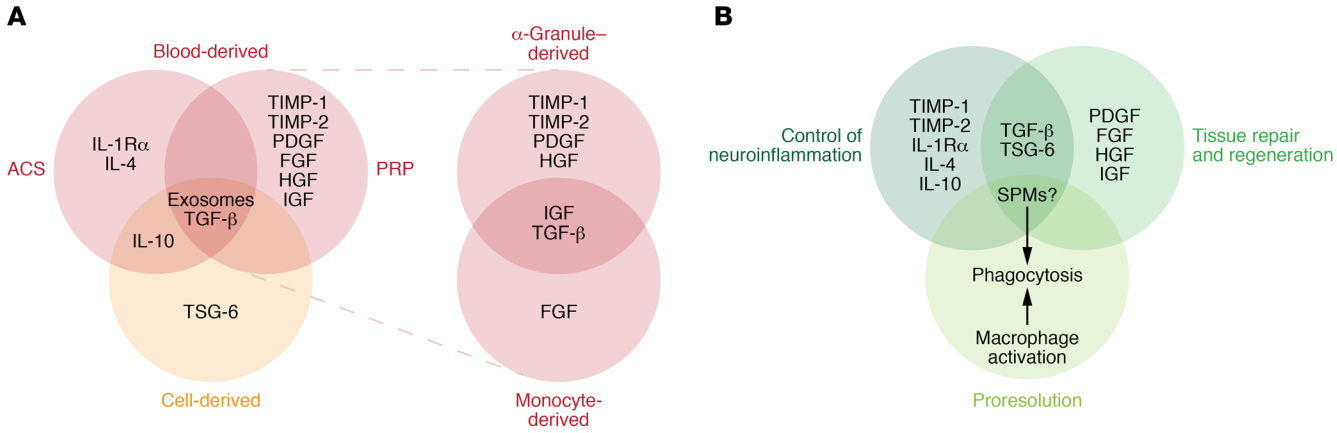

Figure 3. Clinically used blood-derived and cell-derived pain therapies and their mechanisms of action via production of therapeutic mediators. (**A**) PRP contains (a) α-granule–derived growth factors such as PDGF, TGF-β, and HGF, as well as TIMP-1 and TIMP-2 and (b) monocyte-derived factors including TGF-β, FGF, and IGF. ACS provides factors including IL-1 receptor antagonist (IL-1Rα), IL-4, IL-10, and TGF-β. MSCs have been found in clinical treatments to alter macrophage phenotypes, leading to direct and indirect production of IL-10 and TGF-β. MSCs also produce TSG-6 to inhibit inflammation and promote wound healing. Blood- and cell-derived therapies could also contain exosomes.(**B**) Common therapeutic mediators and mechanisms of action include (a) control of neuroinflammation, (b) tissue repair, and (c) pro-resolution processes. Notably, PRP, ACS, and MSCs may also contain or produce SPMs that produce multiple beneficial effects. ACS, autologous conditioned serum; MSC, mesenchymal stromal cells; PRP, platelet-rich plasma; SPM, specialized pro-resolving mediators.

ty (42). Neuroinflammation is local, resulting from glia activation in the PNS (e.g., Schwann cells and satellite glial cells) and the CNS (e.g., microglia and astrocytes), as well as from the activation and infiltration of immune cells (e.g., macrophages and T cells) (43–45). While neuroinflammation in the PNS drives peripheral sensitization, neuroinflammation is also implicated in central sensitization, widespread chronic pain, and comorbidities, such as fibromyalgia and temporomandibular disorders (46). Central sensitization, which is mediated by the enhancement of pain processing within the spinal cord and brain, manifests as an increase in excitatory neurotransmission and/or disinhibition, i.e., reduction or loss of inhibitory synaptic transmission in CNS pain circuits (47). Central sensitization opens the spinal cord "gate," rendering low-threshold Aβ-fiber stimulation such as light touch sufficient to induce mechanical allodynia (48).

Matrix metalloproteinases (MMPs) are extracellular matrix proteins with major roles in neuroinflammation and pathological pain (49) (Figure 1). The gelatinases MMP-2 and MMP-9 are among the most studied MMP-s and contribute to the development and maintenance of neuropathic pain in mice (50). Nerve axonal injury induces a rapid but transient increase in MMP-9 expression and activity in DRG neurons. Secreted MMP-9 activates microglia, leading to early-phase neuropathic pain. Intrathecal injection of MMP-9 is sufficient to evoke persistent mechanical allodynia and p38 phosphorylation in spinal microglia, a critical event of microglial signaling in pathological pain (51–53). MMP-9 has also been observed to be upregulated in the synovial fluid of patients with joint fracture (54). Nerve injury also causes delayed but persistent MMP-2 production in glial cells, leading to late-phase neuropathic pain mediated by ERK phosphorylation in astrocytes (51), a critical event for astrocyte activation during the maintenance of neuropathic pain (55). Tissue inhibitor of MMPs (TIMP) proteins, endogenous MMP inhibitors with relative selectivity of TIMP-1 for MMP-9 and TIMP-2 for MMP-2, suppress neuropathic pain in different phases: TIMP-1 alleviates early-phase neuropathic pain, and TIMP-2 attenuates late-phase neuropathic pain (51). Mice lacking TIMP-1 exhibited rapid onset of thermal and mechanical hypersensitivity at the site of inflammation (56). Genetic pathway analysis revealed a major role for extracellular matrix organization in inflammatory and neuropathic pain (57).

MMP-9 and MMP-2 are involved in IL-1β activation and signaling in neuropathic pain (51). The proinflammatory cytokine IL-1β induces pain hypersensitivity in rodents by increasing nociceptor excitability and modulating spinal synaptic transmission through neuronal receptors (58, 59). In particular, IL-1β powerfully modulates both excitatory and inhibitory synaptic transmission in the spinal dorsal horn (Figure 1). At the presynaptic level, IL-1β increases NMDA receptor activity and inhibits the frequency of spontaneous inhibitory postsynaptic currents (IPSCs) in the spinal pain circuit (59, 60). At postsynaptic and extrasynaptic sites, IL-1β reduces the IPSC amplitude and GABA- and glycine-induced currents (59, 61). In contrast, IL-1 receptor antagonist (IL-1Ra), which opposes the actions of IL-1β, is downregulated in chronic pain conditions (62). IL-1β release results from NLRP3 inflammasome activation and is associated with enhanced neuropathic pain after chronic opioid exposure (63).

TGF-β1, an antiinflammatory cytokine, is downregulated in chronic pain conditions in both animals (64) and patients (65). TGF-β1 is a potent inhibitor of neuropathic pain, and has been shown to suppress spinal cord glial activation and neuroinflammation after nerve injury (66, 67). In addition to canonical signaling through gene transcription, TGF-β1 plays an unconventional role in neuromodulation in the DRG: it can rapidly activate TGF-β1 receptors on neurons, normalizing nerve injury–induced DRG neuronal hyperexcitability and spinal cord synaptic plasticity within minutes (66). Interestingly, TGF-β1 is a target of miR-30c-5p, which is increased in the spinal cord, DRG, cerebrospinal

Table 1. Preclinical and clinical evidence supporting the use of PRP in the treatment of osteoarthritis in knee and hip, tendinopathy, and spine disease

	OA: knee	OA: hip	Tendinopathy	Spine
PRP	Proposed mechanisms: Therapeutic effects are believed to be due to concentrated antiinflammatory cytokines, growth factors such as TGF-β, monocyte activity, and reduction of proinflammatory cytokines such as IL-1β.			
Evidence for clinical effectiveness	Several supportive RCTs (120, 121) and meta-analyses demonstrate superiority over placebo and active controls such as HA (90, 123, 124).	RCTs demonstrate superiority (159) or equality (160) of PRP to HA at 12 months. A meta-analysis of limited data supports improvement of symptoms and function up to 12 months (161).	Several supportive RCTs (162, 163) and 1 supportive meta-analysis (164) for PRP effectiveness in LE. Limited evidence for benefit in rotator cuff (165) and Achilles tendinopathy (166).	RCT demonstrates significant improvement following intradiscal PRP vs. control (167). Case series additionally support improved symptoms following intradiscal PRP (168).
Laboratory evidence for tissue regeneration	PRP induces in vitro chondrocyte proliferation and collagen production (113). Restoration of cartilage demonstrated in animal models of knee arthritis (169).	Majority of animal studies for PRP effect on cartilage restoration have been performed in knee OA models.	Animal models demonstrate improved tendon morphology and strength with PRP injection (170).	PRP use in experimental degenerative disc disease model improves disc morphology (171). Nerve regeneration is noted following experimental spinal cord injury model (172).
Clinical evidence for tissue regeneration	No significant cartilage restoration noted by MRI, including in patients who experienced pain reduction following PRP (115).	Case reports demonstrate radiographic improvement of osteonecrosis after PRP injection (173). No RCTs or larger observational trials available.	Ultrasound study demonstrates improved LE tendon morphology after PRP treatment (174). Post-ACL MRI demonstrates faster remodeling with PRP (175).	Observational data demonstrate no improvements in MRI assessment of disc degeneration, including in patients who noted pain reduction following PRP (176).
Comments	PRP appears to demonstrate greater benefit in younger patients with earlier-stage disease (120, 177). Leukocyte-poor preparations appear superior to leukocyte-rich preparations in treatment of knee OA (117).	PRP appears to demonstrate better response noted in earlier-stage hip OA (178).	PRP has demonstrated greater results for LE than for other tendinopathies. Clinical response for other indications (rotator cuff, Achilles, etc.) may be limited by initial inflammatory response (179).	Significant percentage of spine-based PRP studies have been performed in treatment of intervertebral disc pathology. Safety for intrathecal or epidural use not established.

Major mechanisms include production of antiinflammatory mediators and growth factors and activation of monocytes/macrophages. ACL, anterior cruciate ligament; HA, hyaluronic acid; LE, lateral epicondylopathy; OA, osteoarthritis; RCT, randomized controlled trial; PRP, platelet-rich plasma.

fluid, and plasma after sciatic nerve injury in rats (68). TGF-β1 is downregulated by miR-30c-5p and upregulated by miR-30c-5p inhibitor after nerve injury.

IL-10 is probably the best-studied antiinflammatory cytokine in pain research. In early life, neuropathic pain after nerve injury is constitutively suppressed by IL-10–mediated antiinflammatory neuroimmune regulation in the mouse spinal cord (69). Gene therapy via enhancement of endogenous production of IL-10 produces long-term relief of neuropathic pain in rats (70). Endogenous IL-10 is also implicated in pain relief by exercise (71), acupuncture (72), and $CD8^+$ T cell transplantation (73) in rodent models of neuropathic and muscle pain. Mechanistically, IL-10 suppresses abnormal paclitaxel-induced spontaneous discharges in DRG neurons in vitro (73). Infant nerve injury also triggers upregulation of another antiinflammatory cytokine, IL-4, which is correlated with lack of neuropathic pain in early life in mice (69). By contrast, nerve injury decreases spinal IL-4 levels in adult mice, and IL-4 mediates the analgesia produced by low-intensity exercise in neuropathic pain (74).

Resolution of inflammation requires the production of specialized pro-resolving mediators (SPMs) (e.g., resolvins, maresins, and protectins), which are derived from omega-3 unsaturated fatty acids such as docosahexaenoic acid and eicosapentaenoic acid (75), during the resolution phase of inflammation. Notably, resolvin D1 is induced after sham surgery, a resolution condition that is associated with acute inflammation and acute pain in mice (76). SPMs not only possess potent antiinflammation and pro-resolution actions but also produce powerful antinociception via both immunomodulation and neuromodulation (75, 77). For example, resolvin E1 inhibits inflammatory pain in mice in part by blocking TRPV1 signaling in nociceptors via its G protein–coupled receptor ChemR23 (77).

Tremendous progress has been made in elucidating the neurocircuits of pain, including those that mediate mechanical allodynia in the spinal cord (78–81). Nerve injury–induced allodynia in mice is mediated by direct cortical-spinal projections (82), and molecular and inflammatory mediators within these circuits represent important potential therapeutic targets for regenerative pain medicine (46).

Recent progress has demonstrated sex dimorphism in neuroinflammation regulation in pathological pain. For example, spinal microglia regulate inflammatory and neuropathic pain in male animals (83–85), whereas T cell signaling appears more critical in female animals (83). However, sex dimorphism in astrocytes in inflammatory and neuropathic pain is less evident (86).

Sex differences in peripheral immune regulation of pain have also been revealed. Adoptive transfer of paclitaxel-activated macrophages can elicit mechanical allodynia in both sexes. However, macrophage-derived TLR9 regulates chemotherapy-induced peripheral neuropathy in male mice (87). It is of great interest to investigate sex

Table 2. Preclinical and clinical evidence supporting the use of ACS for osteoarthritis in knee and hip, tendinopathy, and spine disease

	OA: knee	OA: hip	Tendinopathy	Spine
ACS	Proposed mechanisms: Therapeutic effects are believed to be due to enriched concentrations of antiinflammatory cytokines such as IL-1Ra, IL-4, and IL-10, growth factors such as TGF-β, and exosomes.			
Evidence for clinical effectiveness	A large RCT demonstrates superiority over HA (129). A second RCT shows significant improvement in KOOS vs. sham injection, but primary outcome not met (180).	Positive observational data support use of ACS in hip OA (131).	RCT demonstrates ACS is superior to steroid injection for rotator cuff pathology (133), and observational data support that ACS is superior to physical therapy for Achilles tendinopathy (181).	RCT demonstrates improvements in radicular pain following epidural injection and superiority to lower-dose epidural steroid (182). Other supportive evidence is observational.
Laboratory evidence for tissue regeneration	Equine model of OA demonstrates improved synovial hyperplasia without significant cartilage regrowth (128).	No direct evidence for cartilage regeneration in other OA models.	Evidence for histologic and mechanical collagen repair in Achilles injury model (183) and tendon healing in equine model (184).	No published studies investigating the histologic/regenerative impact of ACS on disc or spine pathology.
Clinical evidence for tissue regeneration	Clinical studies have not employed MRI or imaging outcomes.	Clinical studies have not employed MRI or imaging outcomes.	MRI evidence of Achilles tendon healing with ACS injection (185).	No published studies investigating radiographic restoration of disc or spine pathology.
Comments	Longer-term positive outcome data for ACS when compared with PRP in the treatment of knee OA are required.	No RCTs for use in hip OA.	Animal models with ACS demonstrate thickening of tendons, increases in type I collagen, and decreases in synovial membrane hyperplasia.	ACS data are more limited in spine applications.

Major mechanisms include production of antiinflammatory mediators, growth factors, and exosomes and activation of monocytes/macrophages. HA, hyaluronic acid; KOOS, Knee Injury and Osteoarthritis Outcome Score; OA, osteoarthritis; RCT, randomized controlled trial; ACS, autologous conditioned serum.

differences in macrophage signaling and the underlying mechanisms of sex-dependent macrophage-nociceptor interactions.

Importantly, these exciting advancements in our mechanistic understanding of pain may facilitate development of new therapeutic solutions. In Figures 2 and 3, we present where regenerative pain medicine, including blood-derived products (platelet-rich plasma and autologous conditioned serum) and cell-derived products (mesenchymal stromal cells and stem cells), can produce long-lasting pain relief via control of neuroinflammation in the pain-transmitting system.

Regenerative pain medicine

Regenerative medicine, defined as "the process of creating living, functional tissues to repair or replace tissue or organ function lost due to age, disease, damage, or congenital defects" (88), has garnered a great deal of enthusiasm over the past few years, not only because of its tissue-restoring potential, but also because of the emerging evidence of analgesic benefit in degenerative arthritis and neurologic conditions. Current regenerative pain therapies cover various treatments and technologies and may be split into two general categories: cellular products derived from bone marrow, lipid, umbilical cord, etc., and blood-derived products such as platelet-rich plasma (PRP) and autologous conditioned serum (ACS) (89–91). Stem and precursor cells are now available from a wide variety of sources (e.g., embryos, gestational and adult tissues, and reprogrammed differentiated cells).

Mesenchymal cells exist in the perivascular space of nearly all organs. These multipotent cells are capable of differentiating into other mesodermic tissues such as cartilage, fat, muscle, and bone with appropriate culturing techniques. These cells, sourced from a broad array of tissues such as bone marrow, adipose tissue, umbilical cord tissue, and peripheral blood, are the basis of what is broadly termed "mesenchymal stem cells" and what many now refer to as "mesenchymal stromal cells" or "medicinal signaling cells" (92). In this Review, we collectively refer to this heterogeneous cell population as "MSCs." These cellular constituents are extracted from the source tissues and may or may not be cultured/expanded. As such, these processes create a divergent spectrum of cell lines with various cell surface markers and characteristics.

Preclinical studies of MSCs and neuronal precursor cells. Bone marrow–derived MSCs (BMSCs) were first described in 1968 by Friedenstein et al. (93), and the culturing techniques used to form mesodermal phenotypes from these cells were later developed and described by Caplan (94). Preclinical studies have examined the efficacy of a variety of cellular therapies in different animal models of clinical neuropathic pain conditions. Special attention has been paid to BMSCs, as numerous early studies investigated the efficacy of BMSC treatment by intravenous (systemic) injection or localized injection directly into the site of injury (95). These studies demonstrated the analgesic effects of BMSC treatment in a wide range of rodent models of neuropathic pain after nerve injury, spinal cord injury, streptozotocin-induced diabetic neuropathy, and arthritic pain, using cells sourced from mouse, rat, and human bone marrow (95).

In 2011, Guo et al. showed that a single intravenous or local (lesion site) injection of rat BMSCs reversed mechanical allodynia in rats after tendon injury. The opioid receptor antagonist naloxone blocked this anti-allodynic effect, suggesting endogenous opioid involvement (96). The group's follow-up study further demonstrated that in vivo

Table 3. Preclinical and clinical evidence supporting the use of MSCs for osteoarthritis in knee and hip, tendinopathy, and spine disease

	OA: knee	OA: hip	Tendinopathy	Spine
MSCs	Proposed mechanisms: Therapeutic effects believed to be secondary to (a) paracrine activity and macrophage induction of cytokines such as IL-10 and TGF-β, and (b) potential direct cellular differentiation. MSC sources include bone marrow, adipose, umbilical cord, synovium, and peripheral blood.			
Evidence for clinical effectiveness	RCTs demonstrate effectiveness of MSCs as a stand-alone technique (139) and as part of a surgical procedure (140). Meta-analyses support the effectiveness of both approaches with noted variability of technique and cell sources (89, 143).	Observational and preliminary data support potential therapeutic effect of MSCs for hip arthritis (186, 187).	RCT of MSCs vs. PRP demonstrates short-term advantages of MSCs in Achilles tendinopathy (188). Observational trial of MSC for rotator cuff demonstrated improved symptoms and MRI findings after treatment (189).	Observational data and small randomized trials support the use of MSCs for discogenic pain (156, 190). The use of MSC for spine-related conditions is predominantly as a surgical adjuvant (191).
Laboratory evidence for tissue regeneration	Cartilage growth noted in models using MSCs with surgical scaffold (192, 193) as well as intra-articular injection of culture-expanded cells (194).	Positive evidence of MSC-mediated cartilage regeneration in various OA models (195).	Evidence of MSC differentiation into tenocytes with enhanced tendon strength in rabbit Achilles (196).	Radiographic and histologic evidence of intervertebral disc regeneration in a canine model (197).
Clinical evidence for tissue regeneration	4-year observational trial of BMAC in surgical scaffold demonstrates pain reduction and MRI improvements in cartilage defects (198). Observational trial of intra-articular cultured MSCs demonstrates improvement in pain and function, with MRI evidence of cartilage regeneration (199).	2.5-year observational trial of cultured bone marrow MSCs injected into hip, ankle, or knee demonstrated improved pain and function with MRI evidence of cartilage regrowth in the majority of patients with hip OA (200).	MRI and arthroscopic evidence for tendon regeneration after MSC injection for rotator cuff tears (189).	Observational trials demonstrate some patients have improvement in MRI-assessed disc disease after MSC injection (156, 201).
Comments	Evidence for tissue regeneration/ cellular replacement is stronger with the use of cultured MSCs and surgical scaffolds.	No RCTs for use of MSCs in hip OA.	Limited data for MSC use in tendinopathy.	Limited data for safety and efficacy with intrathecal administration.

Major mechanisms include paracrine activity, production of antiinflammatory mediators and growth factors, and activation of monocytes/macrophages. Tissue regeneration may be a mechanism, especially with the use of culture-expanded cells and surgical scaffolds. BMAC, bone marrow aspirate concentrate; MSCs, mesenchymal stromal cells; OA, osteoarthritis.

immune interactions and monocyte activation underlie the long-lasting pain-relieving effects of these cells (97) (Figure 2). Sustained analgesia by BSMCs requires activation of central brain stem μ-opioid receptors and CXCL1/CXCR2 chemokine signaling (97).

Chen et al. demonstrated that intrathecal administration is also an effective way to deliver BMSCs for long-term pain relief (66). A single injection of 250,000 murine BMSCs via lumbar puncture provided rapid-acting, potent, and long-lasting pain relief for more than 6 weeks in mouse models of neuropathic pain (66). After intrathecal injection, dye-labeled BMSCs migrated to the DRG and spinal cord meninges, where they survived for up to 3 months (66) (Figure 2). Interestingly, after nerve ligation, the injured DRG neurons upregulated CXCL12, a chemotactic signal that guides BMSCs to the damaged DRGs via a CXCR4/CXCL12 homing mechanism. Importantly, TGF-β1 secretion by BMSCs was the specific factor conferring potent pain relief (66). Notably, intrathecal administration of anti–TGF-β1 neutralizing antibody selectively reversed the analgesic effect conferred by intrathecal BMSC treatment (66). Intrathecal BMSCs also effectively reduce nerve injury–induced neuroinflammation in the spinal cord, including microglia and astrocyte activation and increased expression of IL-1β, IL-6, and TNF (66) (Figure 2).

The MSC treatment reversed microglia and astrocyte activation, suggesting that MSCs may regulate immune cells and neurons by paracrine activity of TGF and IL-10 (98, 99). With this further confirmation of the analgesic effects of intrathecally and intravenously administered BMSCs and adipose-derived MSCs in rat chronic constriction injury (CCI) nerve injury models (100), the preclinical studies of MSC populations and products demonstrate powerful potential in treating chronic neuropathic conditions. Hua et al. found new applications of stem cell therapy in the realm of pain medicine, particularly the therapy's ability to also prevent and reverse opioid tolerance (OT) and opioid-induced hyperalgesia (OIH) (98). MSC transplantation (intrathecal or intravenous) had significant therapeutic effects in both preventing the onset of OT and OIH when delivered prior to initiation of daily morphine injections, and reversing established OT and OIH in rats and mice.

It is noteworthy that exosomes derived from human umbilical cord MSCs also serve as a cell-free therapy for nerve injury–induced neuropathic pain in rats (101) (Figure 2). A single intrathecal injection of exosomes reversed mechanical and thermal hypersensitivities for 24 hours, and continuous infusion of exosomes into the intrathecal space prevented and reversed nerve ligation–induced pain for 2 weeks. The exosomes migrated specif-

ically to the spinal dorsal horn, DRG, and peripheral axons associated with the ipsilateral nerve injury, and suppressed glial activation. Exosomes depressed TNF and IL-1β levels and reciprocally enhanced levels of IL-10, brain-derived neurotrophic factor, and glial cell line–derived neurotrophic factor in DRGs with axonal injury (101). Future studies are warranted to compare the analgesic impact of MSC-derived exosomes versus MSCs per se.

Several antiinflammatory cytokines, including TGF-β, IL-10, IL-4, and TIMPs, are implicated in the analgesic actions of MSCs (Figures 2 and 3). MSCs mediate immunomodulatory actions via TGF-β (102, 103), and TGF-β is required for generating persistent analgesia following intrathecal administration of BMSCs (66). IL-10 release from BMSCs was relatively low, and IL-10 neutralization failed to reverse BMSC-induced inhibition of neuropathic pain in mice (66). However, a recent study showed that IL-1β–pretreated BMSCs enhanced analgesia of intrathecally injected BMSCs in rat neuropathic pain after spinal nerve ligation, and these analgesic effects were reversed by both TGF-β1 and IL-10 antibody neutralization (104). Recently, human umbilical cord plasma was found to be enriched with TIMP-2, an endogenous inhibitor of MMP-2 and neuroinflammation; this study revealed that systemic treatments with umbilical cord plasma and TIMP-2 increased synaptic plasticity and hippocampal-dependent cognition in aged mice (105). Intra-articular injection of MSCs produces TNF-stimulated gene 6 protein (TSG-6) that acts as an arthritis-associated hyaluronan binding protein, and displays antiinflammatory and cartilage-protective actions (106). BMSC-secreted TSG-6 also attenuates intervertebral disc degeneration by inhibiting the TLR signaling in rats (107).

In addition to MSCs, Braz et al. showed that embryonic cortical GABAergic interneuron precursors from the medial ganglionic eminence (MGE) could survive in mouse spinal cord after transplantation (108). MGE cell transplantation in the spinal cord either before or after nerve injury induced development of inhibitory neurons that integrated into nociceptive circuits, forming GABA-A–mediated inhibitory synapses in host mice, ultimately leading to either prevention or reduction of neuropathic pain (108, 109). Additional studies revealed that MGE cell transplantation mediates pain relief specifically via synaptic GABA release into the newly formed host-transplant inhibitory synaptic circuits (110) (Figure 2).

PRP in clinical applications. The use of PRP for the treatment of musculoskeletal conditions and arthritis has grown substantially over the past couple of decades, secondary to its rich supply of α-granule–based growth factors (111) (Table 1). Platelet-derived growth factors include TGF-β, PDGF, and hepatocyte growth factor (HGF). TGF-β appears to promote chondrocyte activity and cartilage growth (65). PRP-based factors have been shown to reduce inflammatory cytokine activation (112), promote collagen and proteoglycan production in vitro (113), and enhance endogenous hyaluronic acid (HA) secretion in arthritis patients (114). However, PRP does not appear to contribute substantially to radiographic restoration of cartilage in humans (115).

PRP contains monocytes and neutrophils in varying concentrations, depending on the method of preparation. While proinflammatory neutrophil activity was shown to be detrimental to synoviocytes (116) and associated with worse outcomes in the treatment of osteoarthritis (117), monocytes, especially macrophages with antiinflammatory M2 phenotype, secrete growth factors including TGF-β, FGF, and IGF (118) that promote endogenous hematopoietic stem cell and progenitor cell recruitment to the delivery site (119). Other important anabolic constituents of PRP include TIMPs, with previously discussed important roles in attenuating the early- and late-phase development of neuropathic pain (51).

The majority of randomized clinical trials using PRP explored the treatment of knee osteoarthritis (120, 121) and support its functional benefits. Not all studies demonstrate PRP's superiority over current treatments (e.g., HA) in osteoarthritis patients (122), although the method of platelet preparation in one of the negative trials was criticized for a potentially high leukocyte count and greater inflammatory signal (112). There are now multiple meta-analyses and systematic reviews of randomized clinical trials supporting the superiority of PRP versus HA at 6 months (123) and at 12 months or greater (90, 124).

ACS in clinical applications. Another blood-derived regenerative product, ACS, was developed with the knowledge that augmented levels of IL-1Ra, a cartilage-protective cytokine, reduces both pain and joint damage in arthritis (91) (Table 2). Laboratory studies furthermore revealed that whole blood, given proper incubation conditions, produced not only substantial amounts of IL-1Ra, but also anabolic growth factors such as TGF-β, antiinflammatory cytokines such as IL-4 and IL-10, and extracellular vesicles such as exosomes, collectively described as a whole blood clot secretome (91, 125, 126). The multifactorial analgesic mechanisms of ACS-induced analgesia are supported by the observation that intra-articular recombinant IL-1Ra alone does not produce clinically meaningful results in osteoarthritis patients (127). Processing techniques have been subsequently studied and standardized for clinical use, avoiding the variability seen in PRP production (91). In animal models, ACS treatment produces tendon thickening, greater concentrations of type I collagen, and decreases in synovial membrane hyperplasia (128).

A randomized, blinded trial with 376 participants revealed superior outcomes of ACS over intra-articular HA or placebo. The ACS group's improvements were maintained for at least 2 years (129). The longer-term impact of ACS is also supported by a 2-year observational trial in 118 patients in combination with physical therapy (130). Patients noted 62% and 56% decreases in Visual Analog Scale (VAS) and WOMAC pain scale scores, respectively. Positive observational results also support the use of ACS for hip arthritis (131). A randomized trial of ACS injection in patients who had undergone anterior cruciate ligament reconstruction additionally demonstrated superior pain scale scores and reduced bone-tunnel widening in the treatment group at all time points (132). ACS furthermore demonstrates superiority to steroid injection for the treatment of rotator cuff tendon pathology (133).

MSCs in clinical applications. Extensive preclinical work demonstrated the potential therapeutic effects of MSCs through expanded and cultured cell lines (95). MSC treatment is known to suppress the release of inflammatory factors from chondrocytes and improve radiographic and histologic markers of osteoarthritis in experimental arthritis models (134, 135). It appears that cartilage regeneration is further facilitated when differentiated chondrocytes are used (136). Systemically infused MSCs have a short

life expectancy, but induce a phenotypic change in macrophages with subsequent production of anabolic and antiinflammatory cytokines such as IL-10 and TGF-β (137).

MSC therapies defy standard classification systems to an even greater extent than PRP does, given not only the multiple methodologies for preparation, but diverse cellular sources (Table 3). MSCs additionally go through several types of mechanical or biochemical extraction, and may be injected immediately or cultured for days to weeks prior to injection. At the time of administration, they have variable phenotypes with cell surface markers consistent with mesenchymal cells, hematologic cells, or a hybrid. In human clinical studies, important distinctions should be made between products that are cultured and expanded, demonstrating mesenchymal cell surface molecules such as CD73, CD90, or CD105, and products that are processed and injected in the same surgical/procedural setting. This multitude of processes for isolating and culturing MSCs prompted the International Society for Cellular Therapy to establish and identify criteria for MSCs (138). However, these criteria are rarely used in current publications.

A growing number of trials demonstrate favorable results for the use of MSCs in the treatment of osteoarthritis, including studies using cells derived from bone marrow (139, 140), adipose tissue (141), and peripheral blood (142) (Table 3). The positive therapeutic impact of MSCs for the treatment of arthritis is supported by both recent systematic reviews and meta-analyses (89, 143), although concerns about bias have been expressed (144).

Enthusiasm about the possibility of cartilage regrowth with MSC therapies was further inspired by demonstration that MSCs alleviated cartilage defects to a greater extent when added to surgical interventions such as microfracturing (145) or as an isolated nonsurgical procedure (146). As a result, MSC therapies are now commonly employed as a stand-alone procedure (143), or in conjunction with surgical repair, using bioengineered scaffolds to support cellular growth (140). A divergence between clinical effect and cartilage regrowth was observed in studies demonstrating improved function and pain despite persistent cartilage defects (147), implying that the analgesic benefits may result from nonregenerative mechanisms.

Concluding remarks and future directions

Neuroinflammation is associated with various chronic pain conditions and contributes to central and peripheral sensitization (46). Genetic and psychological factors such as chronic stress and comorbidities such as depression, anxiety, and cognitive decline are also associated with neuroinflammatory upregulation and chronic pain (46, 148). Furthermore, treatments for chronic pain, such as opioid usage, may paradoxically worsen neuroinflammation, leading to opioid-induced tolerance, hyperalgesia, and subsequent dose escalation, highlighted in the ongoing opioid crisis (149, 150). Peripheral drivers of osteoarthritis pain, including inflammatory cytokines such as IL-1β and TNF (151), are especially important in early disease (152). Conversely, diminished production of antiinflammatory cytokines such as IL-1Ra (62) and TGF-β (65) plays an important role in the progression of degenerative joint disease.

Based on the growing biochemical understanding of pain in osteoarthritis, the use of biologically based regenerative pain therapies has grown dramatically over the past decade. Common treatments include autologous blood products such as PRP and ACS, and cellular therapies such as MSCs. It has historically been believed that tissue regeneration with MSC-based interventions is critical to the pain and functional improvements seen after treatment; however, recent studies highlight that the analgesic effects of these therapies may be largely independent of cellular replacement (Figure 3) and more related to paracrine effects and immune modulation (153–155). Supporting this concept, Pettine et al. actually noted that positive clinical outcomes of MSC treatment for degenerative disc disease correlated with the concentration of CD34 cell surface markers (a marker for hematologic cells, not mesenchymal cells) detected on injected MSCs (156). Furthermore, it has been noted that the majority of BMSCs are trapped in the lung immediately after intravenous infusion (157) and their survival time in the host is inconsistent with their duration of pain relief (several months) (97). It has been hypothesized that the cartilage regeneration noted in some MSC-based clinical trials may be due to the cellular components acting as an anabolic substrate for endogenous cartilage growth; analgesia across the spectrum of regenerative therapies may be secondary to common immune mechanisms.

There remain several outstanding questions for future studies. First, it is increasingly clear that regenerative therapies need ongoing mechanistic studies and rigorous clinical trials to better define the optimal indications, safety, sources, and processing for this wealth of products. Second, SPMs play critical roles in the resolution of inflammation and pathological pain (75, 77), and human peritoneal stem cells release SPMs during antimicrobial activities (158). It remains unknown whether SPMs are also among the therapeutic mediators produced by PRP, ACS, and MSCs (Figure 3). Third, sex dimorphism has been revealed in immune regulation of pain in animals (83, 86, 87). It is of great interest to investigate whether pain relief by BMSCs is sex-specific. Last but not least, clinical guidelines based on preclinical scientific research and clinical evidence must be established to provide a framework for decision-making in the application of these blood and cellular products. The potential to ameliorate symptoms and modulate disease processes with regenerative therapies exists but will require a conscientious and scientifically driven approach to be fulfilled.

In summary, regenerative therapies play a growing role in the treatment of degenerative musculoskeletal and neurologic conditions with widespread use across the world. The effectiveness of these treatments is supported by a growing body of evidence that demonstrates improvements in function and pain after treatment, and some evidence for tissue regeneration. The observed divergence between the positive clinical outcomes and evidence for tissue regeneration further highlights the importance of immune modulation and neuro-immune interactions across the spectrum of regenerative therapies (Figure 3). Thus, this Review creates a conceptual and mechanistic framework to evaluate future research of regenerative medicine.

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