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SKELETAL BIOLOGY AND REGULATION (MR FORWOOD AND A ROBLING, SECTION EDITORS)

When the Nervous System Turns Skeletal Muscles into Bones: How to Solve the Conundrum of Neurogenic Heterotopic Ossification

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Abstract

Purpose of Review Neurogenic heterotopic ossification (NHO) is the abnormal formation of extra-skeletal bones in periarticular muscles after damage to the central nervous system (CNS) such as spinal cord injury (SCI), traumatic brain injury (TBI), stroke, or cerebral anoxia. The purpose of this review is to summarize recent developments in the understanding of NHO pathophysiology and pathogenesis. Recent animal models of NHO and recent findings investigating the communication between CNS injury, tissue inflammation, and upcoming NHO therapeutics are discussed.

Recent Findings Animal models of NHO following TBI or SCI have shown that NHO requires the combined effects of a severe CNS injury and soft tissue damage, in particular muscular inflammation and the infiltration of macrophages into damaged muscles plays a key role. In the context of a CNS injury, the inflammatory response to soft tissue damage is exaggerated and persistent with excessive signaling via substance P-, oncostatin M-, and TGF-β1-mediated pathways.

Summary This review provides an overview of the known animal models and mechanisms of NHO and current therapeutic interventions for NHO patients. While some of the inflammatory mechanisms leading to NHO are common with other forms of traumatic and genetic heterotopic ossifications (HO), NHOs uniquely involve systemic changes in response to CNS injury. Future research into these CNS-mediated mechanisms is likely to reveal new targetable pathways to prevent NHO development in patients.

Keywords Neurogenic heterotopic ossification · Inflammation · Cytokines · Macrophages · Central nervous system

Introduction

Neurogenic heterotopic ossifications (NHOs) are abnormal extra-skeletal bone formations mostly in periarticular muscles [1] after severe damage to the central nervous system (CNS) such as spinal cord injury (SCI), traumatic brain injury (TBI), stroke, or cerebral anoxia [2] and hence their name "neurogenic." NHOs were first identified in spine-injured soldiers during World War I with the first use of radiography in battlefield injuries [3–5] and are still very prevalent in defense personnel with battlefield injuries, with up to 60% of blast and gunshot victims developing NHO when there is concomitant spinal damage [6–8]. NHO also occurs in up to 25% of civilians with severe SCI and 5–20% with TBI [9–12]. NHOs develop within a few months after CNS injury in periarticular muscles, with decreasing frequencies in the hip, elbow, knee, and shoulder (Fig. 1). NHO can be very incapacitating, mainly due to their large size (up to 2 kg), often causing significant pain and gradual reduction in the range of motion of affected limbs which often progresses to complete joint ankylosis. This exacerbates functional disabilities by increasing difficulty in sitting, eating, and dressing [13]. NHO growth can also cause nerve and blood vessel compression, further increasing patient morbidity [14, 15].

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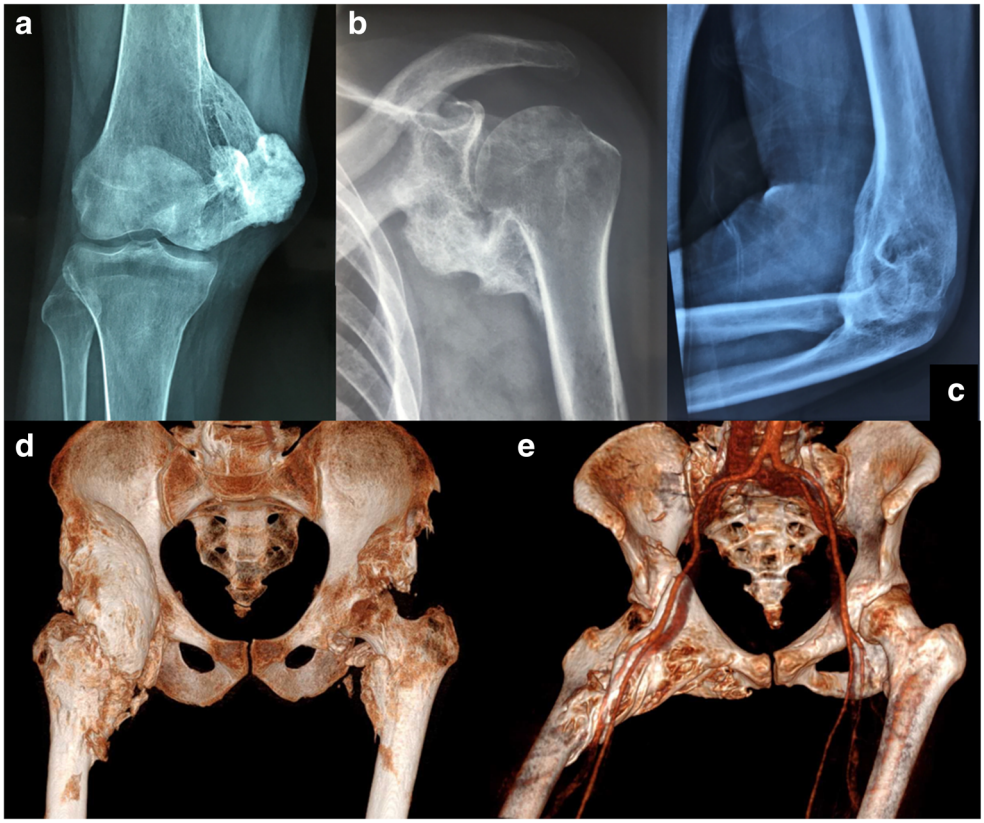

Fig. 1 NHOs are periarticular in CNS injured patients. X-ray radiographies showing NHO of the knee (**a**), shoulder (**b**), and elbow (**c**). Three dimensions CT scan without (**d**) and with (**e**) vessels injection showing NHO of the right hip

Treatment is still currently limited to surgical resection. Once NHOs have become troublesome, the orthopedic surgeon identifies a cleavage plan on the computed tomography (CT) scan, and patient's comorbidities are under control [16]. Surgical resection remains challenging, particularly when ossifications entrap the whole joint and adjacent large blood vessels and nerves. Following surgical resection, NHO can re-occur in around 6% of patients [2]. Additional treatment modalities have been investigated, but most provided limited efficacy. Radiation therapy (RT) has been explored as a treatment for multiple forms of heterotopic ossification (HO) [17, 18] and shown to be beneficial in patients following total hip arthroplasty [19]. However, similar treatment regimens did not effectively prevent the recurrence of NHO [20], and there are concerns that RT could delay repair of skeletal fracture associated with the initial CNS injury. While some studies suggested RT limited the progression of NHO around the hip after SCI [21] and reduced pain and increased mobility in patients with established NHO [22], other studies have shown less favorable outcomes [23]. A recent retrospective study suggests that RT was associated with an increased risk of postoperative sepsis [24]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are also used to treat NHO [25, 26], and a recent retrospective study on 108 SCI patients has shown significant reduction of NHO diagnosis following prophylactic indomethacin or celecoxib for 2 weeks after CNS injury [27•]. The mechanism is believed to be through the systemic inhibition of prostaglandins, with subsequent changes in mesenchymal cell differentiation into osteoblasts [25, 26]. While studies have shown that NSAIDs can be beneficial for NHO after SCI [25], with a reduction in NHO diagnosis and volume as well as reduced inflammatory symptoms, there has been concerns of long-term NSAID use on gastrointestinal complications [28], and delayed fracture healing was also reported in other studies [29, 30]. Bisphosphonates have been investigated as preventative treatment of NHO in SCI and TBI patients [31–34] with varying results, and some studies suggested bisphosphonates potentially increase the risk of developing heterotopic ossification (HO). However, an additional risk of delayed fracture healing comes with the additional musculoskeletal injuries that often accompany SCI and TBI patients [35–37]. Overall the development of improved treatments for NHO has been slow, and trials of pharmacological interventions have continued to show limited effectiveness [10] reflecting the current limited knowledge on the etiology and pathophysiology of NHO. This is in part due to previous studies in SCI/TBI patients being retrospective with little insight into early initiating cellular and molecular events which drive NHO pathogenesis. Therefore, it is essential to establish animal models of NHO to delineate the mechanisms of NHO pathogenesis in order to identify new biomarkers to predict NHO development after SCI/TBI, and new therapeutic treatments that would prevent or reduce NHO development.

Animal Models of Heterotopic Ossification

Genetic Models

Beside NHO in patients with severe CNS injuries, rare genetically driven HOs are also well known in humans and have been at the forefront of animal model development in the last decade. This is particularly the case of fibrodysplasia ossificans progressiva (FOP, Stoneman syndrome). FOP is a very rare genetic disease (1 case in 2,000,000 humans) caused by dominant activating missense point mutations in the coding sequence of the *ACVR1* gene. *ACVR1* encodes the activin A receptor type I (ACVR1), also called activin receptor-like kinase-2 (ALK2), a bone morphogenetic protein (BMP) type I receptor [38–41]. Children with FOP develop a progressive ossification of an extensive portion of their body which is ultimately fatal. FOP animal models include the introduction (by means of mutant allele knock-in the *ACVR1* gene or expression of a mutant transgene) of causal missense point mutations in *ACVR1* such as $ACVR1^{R206H}$ which changes an arginine residue to a histidine residue [40, 42, 43]. In mice carrying the $ACVR1^{R206H}$ mutation, HOs develop locally in tissues following an injury or inflammation in a similar presentation to FOP patients with HO "flare-ups." Additional mouse models were developed based on alternative *ACVR1* mutations such as the constitutively active ALK2 model (caALK2) or $ACVR1^{Q207D}$ [44]. $ACVR1^{R206H}$, which is the most frequent causal *ACVR1* mutation in FOP patients [45], alters the signaling pathways that the ACVR1 receptor elicits upon binding of its physiological ligands. Specifically while wild-type ACVR1 inhibits bone morphogenetic protein (BMP) signaling in response to BMP-2 and BMP-4 binding to the BMP receptor 1A and BMP receptor 1B, the $AVCR1^{R206H}$ mutant loses this inhibitory function and gains BMP signaling function following direct binding of activin A [42, 46]. Mouse models in which recombinant BMPs are locally injected or surgically implanted or an inducible *BMP4* transgene is expressed have also been used to induce HO formation [38, 47–50]. Together, these models have been invaluable to demonstrate the role of inflammation, mast cells and macrophages in triggering FOP "flare-ups" [51•], and the over-activation of SMAD1/5/8-mediated signaling downstream of causal *ACVR1* mutations [52]. These models have been utilized for the development and pre-clinical testing of treatments for FOP and to identify cell types responsible for HO in FOP [43, 44, 53–55]. However, the relevance of these genetic models of FOP to NHO is questionable as pathological NHOs occur in genetically normal patients of a broad range of ethnicities, hence the necessity to develop animal models specific to NHO.

SCI-Induced NHO Models

To fill this knowledge gap, we have developed the first clinically relevant animal model of NHO in genetically non-modified mice, without artificial implantation of osteogenic proteins such as BMPs [56•]. In our model, we perform a transection of the spinal cord between T11 and T13 rendering mice paraplegic. Without any additional intervention, NHO never develops in mice with SCI. The prevalence of NHO is significantly associated with (1) the severity of the CNS injury [57], (2) the presence of additional injuries or inflammation [12], and (3) is particularly high in combat-related casualties which are mostly multi-traumatic [6, 7]. For this reason, we hypothesized that an associated muscle injury was required for NHO to develop. To test this, we modeled muscle damage via an intramuscular injection of cardiotoxin (CDTX) purified from *Naja* snake venom, a well-accepted model of muscle damage and repair [58]. Likewise, CDTX-mediated muscle injury alone never caused HO with CDTX-injured muscles repairing within 1–3 weeks [56, 58]. However when combined with a SCI, NHOs develop within the CDTX-injured muscle over 1–3 weeks and recapitulate most clinical features of NHO in SCI patients [56•]. We also established that SCI with an intramuscular CDTX injection lead to NHO development in multiple mouse strains such as C57BL/6, C3H/He, and BALB/c (Fig. 2), confirming that this phenomenon is not restricted to the genetic make-up of any particular inbred strain. Therefore the development of NHO requires a dual insult of both the CNS and soft tissue [56•] (Fig. 3). Unlike genetically altered or BMP-implant models of FOP, our new model provides a powerful tool for determining the pathogenesis of NHO and assessing preventative therapies which is discussed further in sections below.

TBI-Induced NHO Models

More recently, small animal models of TBI-NHO have also been developed. A rat model using a polytrauma approach of TBI, femoral fracture, and muscle crush injury combined [59] has illustrated ectopic bone development in injured limbs. Other groups have utilized a combination of TBI with an Achilles tendon rupture (tenotomy) to establish NHO and illustrated changes in matrix metalloproteinase expression levels during TBI-NHO development [60]. Others used a combination of TBI and fracture, which increased serum calcitonin-related peptide release [61]. All these models have the potential to further identify mechanisms of TBI-NHO.

Trauma-Induced HO Models

As the development of HO is common in polytrauma patients [2, 62, 63], numerous models of trauma-associated HO have been developed, some of which do not have an accompanying CNS injury [64]. It is well established that HO develops after severe burns and other forms of soft tissue trauma [65]. A small animal model of burn-induced HO has been developed with a combination of burn and tenotomy with HO developing

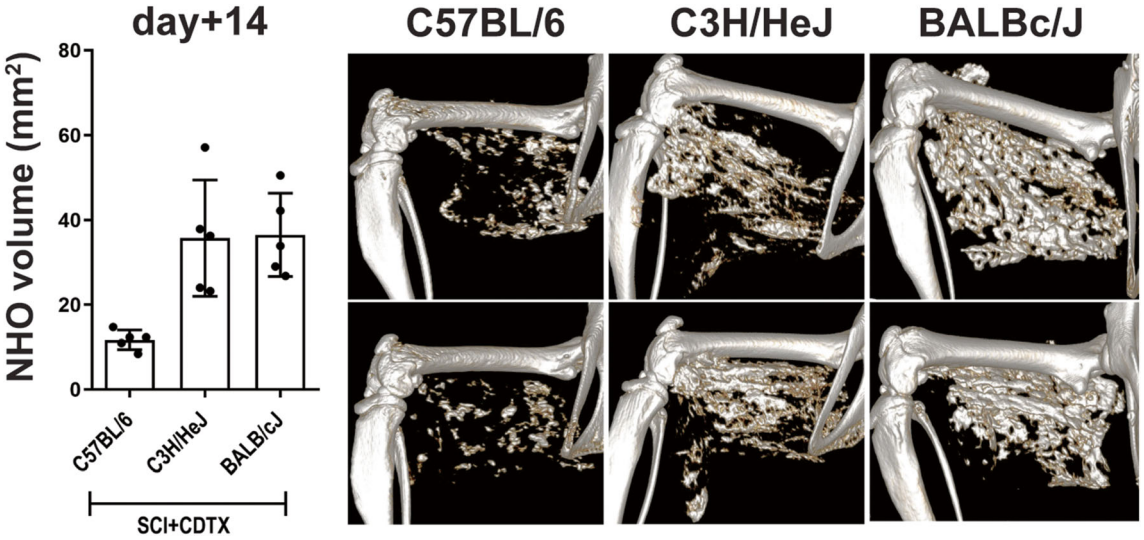

Fig. 2 NHOs develop in multiple inbred mouse strains after combined SCI and muscle injury. C57BL/6, C3H/He, and BALB/c mice ($n = 5$/group) all underwent SCI between T12 and T13 together with an intramuscular injection of CDTX. NHO volume was quantified at 14 days post-surgery by micro-CT. Results show that NHOs develop in all mouse strains confirming that NHO development is not restricted to the genetic make-up of mouse strains. Data are presented as mean ± SD

in the damaged limb [66]. This model has recently unraveled numerous pathways involved in burn-induced HO [67–69] and more recently excluded a role for activin A in burn-induced HO [70•]. Interestingly, these trauma models share some similarities with our SCI-NHO model and illustrate the importance of multiple traumas in the development of HO, as the incidence of HO was significantly higher with burn plus tenotomy compared with each insult alone [66]. Rat models of polytrauma have been developed using a combination of blast-related limb injury, bone fracture, quadriceps crush, amputation, and infection with methicillin-resistant *Staphylococcus aureus* (MRSA), reflective of combat-associated HO [71, 72]. Other models of trauma HO include mouse and rat tenotomy models [73–75] and a rabbit HO model where HO develops after hip surgery [76].

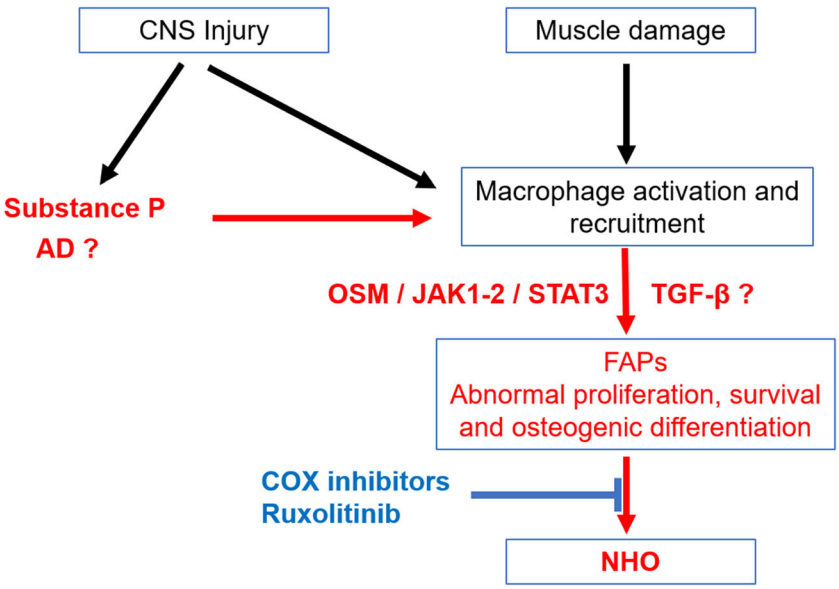

Fig. 3 Schematic representation of SCI-NHO pathogenesis deduced from mouse models and retrospective studies in patients. Damage to the central nervous system (CNS) causes release of substance P. High-level SCI can also cause autonomic dysreflexia (AD). Systemic factors released after CNS injury and possibly AD causes abnormally high and prolonged activation of macrophages in injured muscles with persistent release and accumulation of OSM and TGF-β1. This may induce uncontrolled proliferation of fibro-adipogenic progenitors (FAPs) within injured muscles, FAP osteogenic differentiation, and formation of NHOs. Cyclooxygenase (COX) inhibitors such as indomethacin and celecoxib reduce NHO incidence in CNS-injured patients whereas the selective JAK1/2 tyrosine kinase inhibitor ruxolitinib reduces NHO volumes in mice with SCI

Overall, there has been substantial development of multiple small animal models for both neurogenic and non-neurogenic HO in the last decade [64]. These animal models will be vital to further understand HO pathogenesis and for development and pre-clinical testing of new therapeutics. Interestingly, multiple models demonstrate a commonality of multi-level trauma for the development of HO. The link between the dual injury process of CNS injury and muscle trauma is further discussed below.

The Nexus Between CNS Injury and Tissue Inflammation

We have previously established that a dual insult of SCI and muscle damage is required for SCI-NHO as NHO usually does not develop in mice with only SCI or only muscle injury [56•]. A dual-insult effect is also seen in models of TBI-NHO, where TBI alone does not induce NHO; however, the combination of TBI with multiple traumas (fracture or fracture + muscle crush) increases NHO prevalence accordingly [59]. Therefore, the development of pathological NHO following CNS injury requires a combined insult from both the CNS and tissue trauma (Fig. 3). These observations are consistent with patient data where there is a higher prevalence of NHO (up to 60%) in army personnel victims of combat inflicted blast and gunshot injuries with concomitant spinal damage [6–8]. Likewise, NHOs are more prevalent in SCI/TBI patients with concomitant bed sores, tracheostomy, pneumonia, smoking, systemic, or urinary tract infections, all signs of local or general inflammation [11, 12, 57].

An appropriate inflammatory response is also essential for muscle repair [58, 77, 78]. Macrophages are highly plastic and can direct the regeneration process toward either normal regeneration or pathological scar formation and fibrosis. In injured muscles without CNS injury, infiltration of monocytes/macrophages peaks 3–4 days post injury in mice and switches from a $Ly6C^{+}/CX3CR1^{low}$ to a $Ly6C^{-}/CX3CR1^{hi}$ phenotype with a change in expression of pro-inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin-1β (IL1β) to pro-regenerative transforming growth factor-β (TGF-β) [79–81]. Macrophages also produce various cytokines that enhance myogenesis such as interleukin-6 (IL6) and insulin-like growth factor 1 (IGF1) [82]. The recruitment of macrophages during muscle repair is mediated by CC chemokine ligand 2 (CCL2) and its receptor CC chemokine receptor 2 (CCR2). Inhibition of myeloid cell infiltration by depleting intramuscular $CD11b^{+}$ cells or deletion of *Ccl2* or *Ccr2* genes significantly reduced intramuscular monocyte/macrophages and subsequently delayed clearance of necrotic muscle and muscle regeneration [83–86]. In addition, emerging evidence suggests macrophages are important in preventing fibrosis through inducing the apoptosis and clearance of muscle fibro-adipogenic progenitors (FAPs) via TNF [87]. Blocking TNF or myeloid-specific knockout of *Tnf* gene or *Ccr2* gene deficiency all resulted in FAP accumulation and fibrosis in injured muscles [87].

Unlike dystrophic calcification post muscle injury, which is resolved over time via TNF-mediated mechanisms [88], SCI-NHOs at 21 days post-surgery contain osteocalcin-expressing osteoblasts and osterix-expressing osteocytes embedded within NHO foci in injured muscles [56•]. Importantly, we have illustrated that monocyte/macrophages infiltration into muscles is higher at 4 days post-surgery in mice with a SCI and CDTX muscle injury compared to mice without SCI, and this persists up to day 28 when NHO are mature [89•]. This is also observed in human NHO with $CD68^{+}$ macrophages accumulating in the muscle at the periphery of the NHO [56•]. The key role of phagocytic macrophages in NHO, FOP, and traumatic HO pathogenesis is highlighted by the fact that their depletion by intravenous injection of clodronate-loaded liposomes significantly reduces HO formation in various animal models including NHO, FOP, burn and tenotomy HO model, and HO induced by exogenous BMP2 plus injury [51, 56, 68, 90]. Importantly we have found that neutrophils, unlike macrophages, do not play an important role in SCI-NHO pathogenesis. Mice with loss-of-function mutations in the gene encoding the granulocyte colony-stimulating factor (G-CSF) receptor are profoundly neutropenic yet still develop NHO in response to SCI and muscle injury similar to their wild-type controls with similar density of osterix-expressing osteoblasts [91•]. Likewise twice-daily administration of recombinant G-CSF, a treatment suggested to favor neuroregeneration after SCI or TBI, did not alter the course of NHO development [91•]. Therefore while G-CSF administration suppresses osteoblasts on endosteal surfaces of skeletal bones [92], it has no effect on osteoblasts forming NHOs in muscle [91•], similar to osteoblasts present on periosteal surfaces [92]. This further illustrates that the suppressive effect of G-CSF on endosteal osteoblasts is not direct but mediated by the adjacent bone marrow macrophages [92, 93].

The mechanism by which macrophages promote NHO development involves aberrant activation of the oncostatin M (OSM)/signal transducer and activator of transcription (STAT)-3 signaling pathway. SCI causes a persistent overexpression of OSM in injured muscles whereas in the absence of SCI, OSM expression normalizes over 3 weeks [94•]. Persistent OSM accumulation is an important driver of NHO pathogenesis as mice lacking the OSM receptor (OSMR) gene had a fourfold reduction in NHO volumes compared to wild-type controls. These results in mice were validated in humans as patients with NHO have higher OSM plasma concentration. OSM is also secreted by macrophages isolated from NHO biopsies, and this OSM promotes osteogenic differentiation of stromal cells derived from the muscles surrounding NHOs [94•]. OSM signals by binding to a cell surface receptor

made of OSMR complexed to GP130. This complex subsequently signals by activating Janus tyrosine kinases JAK1 and JAK2, which then phosphorylate the transcription factor STAT3 enabling its translocation to the nucleus and activation. Indeed persistent OSM overexpression in injured muscles after SCI leads to persistent phosphorylation of STAT3. The functional relevance of STAT3 activation is highlighted by the fact that treatment with ruxolitinib, a selective JAK1/2 tyrosine-kinase inhibitor used to treat myeloproliferative neoplasms, significantly reduced NHO formation in mice [89•].

The Role of the CNS Injury

In mice, SCI exacerbates the inflammatory response to muscle injury ultimately leading to NHO formation instead of muscle repair [56, 89, 90, 94]. The question of which mechanisms initiated by the SCI lead to the sequence of pathological events driving NHO formation remains unresolved and a subject of intense investigation. Several risk factors have been identified in retrospective studies on patients with severe CNS injuries. The advantage of animal models is that these "risk factors" can be tested for their potential to promote NHO formation. For instance, muscle spasticity was identified as a significant risk factor of developing NHO in patients with TBI, stroke, or cerebral anoxia [95]. However, in our mouse model of SCI-NHO, injection of botulinum toxin A in the CDTX-injured muscle, to block the neuromuscular junctions and prevent muscular spasticity, did not reduce but instead increased NHO volumes. This suggests that muscle spasticity is not a trigger of NHO but rather the consequence of heterotopic bones growing in the muscle [96, 97].

Another possibility is that the SCI triggers the secretion of neuromediators at the site of the SCI, which excite neurons that synapse in the injured muscle, and ultimately modify the inflammatory response in the injured muscle. For instance, substance P has been described to be released in the dorsal horn of the spinal cord after SCI in humans [98] and rats [99]. However, in the mouse model of SCI-NHO, NHO volumes were increased in denervated limbs in which sciatic and femoral nerves were both excised [100]. This contradicts a direct role of the afferent nerves in promoting NHO in the injured muscle.

Retrospective case studies in SCI and TBI patients have also shown that autonomic dysreflexia (AD) is a significant risk factor associated with enhanced prevalence of NHO [62, 63, 101]. AD is frequent in patients with high-level SCI typically at and above vertebra T6. It is caused by a loss of the central control of post-ganglionic sympathetic nerve flow below the SCI. Typically, prolonged stimulation of sympathetic sensory nerves imposed by visceral stressors, such as overfull bladder or fecal compaction, stimulates the sympathetic nervous system. These impulses cannot be regulated by preganglionic nerves below the SCI because they have lost central control. This initiates an uncontrolled sympathetic reflex that causes very high norepinephrine release which constricts arteries with a sudden elevation of arterial pressure. A parasympathetic negative feedback reflex takes place by which baroreceptors in the carotid sinus sense the arterial hypertension and signal back to the brain which responds via the uninjured parasympathetic vagus nerve to decrease the heart rate. A parallel sympathetic reflex also takes place to relieve the vasoconstriction by reducing sympathetic release of norepinephrine. However, this sympathetic negative feedback via preganglionic sympathetic nerves is disconnected due to the SCI, and as a result, the hypertension and norepinephrine release remain unopposed while heart rate decreases [102, 103]. Hypertension combined with bradycardia represents the clinical signs of AD. If not managed rapidly, AD can lead to seizures, stroke, coma, cardiac arrest, and death. Although the mechanistic link between AD and immunodepression observed in severe CNS injuries has been explored [104, 105], the potential of AD and more generally systemic complications of SCI to trigger NHO development remains to be explored in animals. This is clearly an area of interest.

The concept that systemic complications of CNS injury could trigger NHO is supported by a remarkable feature of our SCI-NHO mouse model in which NHOs develop in the CDTX-injected muscle regardless of whether CDTX is injected in the mobile non-paralyzed front limb or the paralyzed hind limb [56•]. NHO in non-paralyzed limbs are frequent following stroke and TBI but also observed (albeit quite rarely) in SCI patients with NHO in the non-paralyzed shoulder/elbow particularly if fractured [2]. These findings suggest that systemic factors promoting NHO are released in the circulation in response to SCI [2, 56]. In support of this, we have shown that plasma from mice that underwent both SCI and CDTX-mediated muscle injury had a greater osteogenic potential in vitro when cultured with mesenchymal progenitor cells and satellite cells isolated from mouse muscles, compared to plasma from mice that had no SCI [56•]. This is also consistent with the observation that TBI combined with bone fracture in rats increased calcitonin gene-related peptide (CGRP) plasma concentration, as well as CGRP expression in muscles, which correlated with accelerated fracture repair [61].

Several osteogenic peptides have been found to be increased in the plasma of patients developing NHO such as substance P [56•] and OSM [94•]. Substance P increases mineralization of sorted muscle mesenchymal progenitor cells and satellite cells in vitro [56•]. The fact that administration of a selective inhibitor of the substance P receptor NK1R reduced NHO volumes following SCI in mice suggests that substance P plays an important role in NHO pathogenesis [56•]. In support of this, implantation of a bio-scaffold containing substance P against the Achilles tendon in mice was sufficient to induce HO formation whereas scaffolds containing CGRP had no such effect and even inhibited the promoting effect of

substance P [106•]. Of note, implantation of the scaffold required drilling of the calcaneus bone to anchor the implant where the HO subsequently developed [106•]. Therefore the inflammatory component necessary for HO formation could have been elicited from the inflamed calcaneus.

OSM is also increased in the plasma of NHO patients and stimulates mineralization of sorted muscle mesenchymal progenitor cells and satellite cells in vitro [94•]. Deletion of the OSM receptor gene significantly reduced NHO volumes after SCI in mice suggesting an important role in NHO pathogenesis [94•]. However OSM is abundantly produced locally by activated myeloid cells in the injured muscle [89, 94]; thus, its increase in plasma could be a consequence of exacerbated unresolved muscular inflammation rather than a direct consequence of the SCI.

Likewise, serum concentrations of TGF-β1 are increased in NHO patients particularly in the early osteogenesis phase [107•], and TGF-β1 is well known as an important regulator of bone formation and coupling with bone resorption [108]. TGF-β1 expression was also increased in percutaneously injured Achilles tendons forming HO in mice despite the absence of a CNS injury. Administration of a neutralizing anti-TGF-β1 antibody or conditional deletion of the *Tgfbr2* gene (encoding the main TGF-β receptor) in mesenchymal cells prevented HO development in this model [107•]. However, the fact that HOs were prevented in mice with conditional deletion of the *Tgfb1* gene specifically in myeloid cells [107•] suggests that TGF-β1 is not released in the circulation as a consequence of the SCI but rather locally by inflammatory myeloid cells infiltrating the damaged tissue developing HO.

While there is a plethora of reports on the role of BMPs and activin A in FOP pathogenesis, there is a very few in respect to their potential role in NHO pathogenesis. There is evidence that some BMPs such as BMP-2 and BMP-9 can be induced in non-neurogenic trauma-induced HO particularly when endochondral bone formation is involved [109]. Many BMPs (e.g., BMP-2, BMP-4, BMP-6, BMP-7, and BMP-9) are osteoinductive when administered in one form or another in muscles or tendons in vivo whereas others, such as BMP-3, inhibit HO induced by osteoinductive BMPs [110]. However, whether BMPs have any role in NHO pathogenesis remains to be demonstrated. We could find only one article in which BMP concentration was measured in the plasma of CNS-injured patients with and without NHO, however, which BMP was measured that was not specified [111]. It has been recently shown that SP7/osterix-expressing cells present in the endoneurium that surrounds myelinated and non-myelinated axons can exit the nerve and migrate to the site of HO formation induced by recombinant adenovirus producing BMP-2 [112]. However, the fact that HOs were induced by adenoviral BMP-2 questions the relevance of these findings to NHO. In the same study, osterix-expressing cells were also found in nerves entrapped in human NHO biopsies together with cells in which Smad1/5/8 was phosphorylated. This study however did not assess whether BMPs were produced in the surrounding muscle to induce osteogenic differentiation of these cells. We measured mRNA expression for BMP-2, BMP-4, and BMP-7 in mouse muscles in our NHO model 4 days after injury. The mRNA for all these BMPs was downregulated in CDTX-injured muscles in the presence of a SCI. Furthermore, daily injection of LDN-193189, a selective inhibitor of BMPR1a, BMPR1b, and ACVR1 kinase activity that blocks Smad1/5/8 activation downstream of BMP binding, had surprisingly no effect of SCI-NHO development in our mouse model (Tseng HW et al., submitted), whereas LDN-193189 treatment inhibits HO in mouse models of FOP driven by $ACVR1^{Q207D}$ [44]. Overall, a potential of BMPs and their receptors in NHO pathogenesis is possible but remains to be established unlike FOP which is clearly caused by modulating point mutations in the *AVCR1* gene, and BMP-type receptor kinase inhibitors may not be as effective at preventing NHO development as they are with FOP.

Conclusion

The recent development of animal model of NHO in the past 5 years has enabled the identification of some mechanisms involved in its pathogenesis. The nexus between the CNS injury and tissue trauma/inflammation is key in the development of NHO and explains why NSAIDs given early following CNS trauma have shown some success at reducing NHO in patients [27•]. Experiments in mice have shown that JAK1/2 inhibitors may also have this potential in patients by targeting the GP130/JAK pathway instead of the cyclooxygenase pathway. A better understanding of how systemic and autonomic deregulations following CNS injury cooperate with muscle inflammation to promote NHO development will no doubt provide novel targets to prevent NHO development in patients. These will also be likely to lead to the identification of novel biomarkers to predict the onset of NHO and to develop treatments for patients to prevent this debilitating condition. Finally this research area has revealed intriguing interactions between the nervous system and innate immunity that enables muscle regeneration while when deregulated leads to bone formation instead.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent All reported studies/experiments with human or animal subjects performed by the authors complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

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