Spinal cord injury (SCI) causes immune dysfunction, increasing the risk of infectious morbidity and mortality. Since bone marrow hematopoiesis is essential for proper immune function, we hypothesize that SCI disrupts bone marrow hematopoiesis. Indeed, SCI causes excessive proliferation of bone marrow hematopoietic stem and progenitor cells (HSPC), but these cells cannot leave the bone marrow, even after challenging the host with a potent inflammatory stimulus. Sequestration of HSPCs in bone marrow after SCI is linked to aberrant chemotactic signaling that can be reversed by post-injury injections of Plerixafor (AMD3100), a small molecule inhibitor of CXCR4. Even though Plerixafor liberates HSPCs and mature immune cells from bone marrow, competitive repopulation assays show that the intrinsic long-term functional capacity of HSPCs is still impaired in SCI mice. Together, our data suggest that SCI causes an acquired bone marrow failure syndrome that may contribute to chronic immune dysfunction.
In adults, mature immune cells develop from a pool of hematopoietic stem and progenitor cells (HSPCs) through a process known as hematopoiesis. Hematopoiesis occurs primarily in bone marrow, where complex cellular interactions and molecular signaling pathways regulate the renewal of millions of immune cells each day. Ultimately, the maintenance of bone marrow hematopoiesis is essential for effective host defense and tissue repair.

Under physiological conditions, the proliferation, differentiation, and retention/release of bone marrow cells, including HSPCs, are controlled by neuroendocrine hormones and the autonomic nervous system, specifically sympathetic neurones12–18. After spinal cord injury (SCI), brain and brainstem connections that normally control spinal sympathetic preganglionic neurones are lost, creating a decentralized spinal autonomic network that includes aberrant sympathetic and neuroendocrine reflexes13. Uncontrolled sympathetic reflexes have been implicated in the overstimulation and cytotoxicity of mature leukocytes14–18 and disruption of leukocyte homing after SCI19.

A decentralized bone marrow may also impair hematopoiesis and contribute to the chronic immune dysfunction that plagues individuals with SCI20–27. Indeed, data from two independent studies in which bone marrow aspirates were analyzed from small cohorts of SCI and control subjects indicate that SCI impairs human bone marrow stem cell function21,22. Notably, SCI increased the overall proliferation and total numbers of HSPCs in bone marrow of human SCI subjects; however, the ability of these stem cells to form mature immune cells was also impaired21,22.

The goal of the current study is to determine the extent of hematopoietic dysfunction after acute and chronic SCI, and to identify molecular, cellular, and physiological mechanisms that may explain any SCI-induced impairments that develop in the bone marrow. We hypothesize that SCI will induce acute and chronic bone marrow failure in mice, similar to what has been described after SCI in humans. Our data show that SCI causes a rapid and chronic bone marrow failure syndrome characterized by excessive HSPC proliferation, accumulation, and impaired function. Importantly, post-injury injections of Plerixafor, an FDA-approved drug that blocks CXCR4, liberates HSPCs from bone marrow and partly reverses bone marrow failure by promoting extramedullary hematopoiesis. Treating bone marrow failure after SCI may help to reverse chronic immune dysfunction and anemia that persist indefinitely after SCI in humans.

Results

SCI increases bone marrow cell proliferation. Hematopoiesis, or the formation of new red and white blood cells, requires that HSPCs proliferate and differentiate in the marrow of all bones, including long bones (i.e. femur/tibia) and sternum. To investigate how SCI generally affects cell proliferation in bone marrow, we monitored post-injury changes in the sternum and femur/tibia of transgenic female mice that express luciferase in dividing cells (Mito-luc mice28,29; Fig. 1a). We used peak (max radiance) and average (total flux) bioluminescence as representative measures of bone marrow proliferation. To control for potential effects of surgical stress on cell proliferation, SCI mice were compared with sham-injured mice receiving a laminectomy only (Lam). Within 72 h of surgery, cellular proliferation increased in the sternum and femur of sham-injured and SCI mice; however, enhanced proliferation was maintained only in SCI mice, peaking within the first 7 days post-injury (dpi) and persisting up to 1-month post-injury (last time evaluated) (Fig. 1b, c).

To determine whether these changes could be specifically attributed to enhanced proliferation of HSPCs, multi-color flow cytometry was used to quantify the proportion of proliferating lineage−, Sca-1+, c-Kit+ (LSK) cells in the femoral bone marrow

SCI causes chronic expansion of bone marrow HSCs. The LSK fraction of bone marrow cells characterized in Fig. 1 includes all stem and multipotent progenitor cells31,32. To better define how SCI affects this pool of phenotypically and functionally heterogeneous cells, multi-color flow cytometry was used to analyze cells from another group of sham-injured and SCI mice (Fig. 2a, Supplementary Fig. 1c). Consistent with data in Fig. 1, the total number of bone marrow cells (Fig. 2b), including total LSK cells (Fig. 2c), increased after SCI. The increase in cell number affects multiple subsets of stem and progenitor cells including long-term repopulating stem cells (LT-HSCs; CD150+/CD48+/CD135− LSKs), short-term HSCs (ST-HSCs; CD150−/CD48+/CD135− LSKs), multipotent progenitor subset 2 cells (MPP2s; CD150+/CD48+/CD135− LSKs), myeloid-primed MPP3s (CD150+/CD48+/CD135− LSKs), lymphoid-primed MPP4s (CD135+ LSKs), and granulocyte–monocyte progenitors (GMPs; CD16/32+ LK cells) (Fig. 2d–i). Importantly, SCI did not cause HSPCs to increase expression of yH2AX, a marker of double-stranded DNA breaks (Fig. 2). These data demonstrate that SCI causes excessive proliferation of all HSPCs but without evidence of long-term replication stress.

SCI prevents HSPC mobilization from bone marrow. Enhanced HSPC proliferation after sham injury or SCI may represent a compensatory response to the stress of blood loss and trauma. Indeed, both psychological and physical stressors enhance bone marrow hematopoiesis and extramedullary hematopoiesis in secondary lymphoid tissues, including the spleen33–35.

We confirmed in the present study an acute effect of surgical stress on bone marrow hematopoiesis. Three days after sham surgery, extensive numbers of HSPCs were found in blood and spleen of sham-injured mice (occurring above circulating numbers of HSPCs in naïve mice) as assessed by MethoCult (Fig. 3a, b) and flow cytometry (Fig. 3d, e) assays, leading to an increase in spleen size (Fig. 3c). In contrast, 3 days after SCI, fewer HSPCs entered the circulation (Fig. 3a) or colonized the spleen in SCI mice (Fig. 3b, d, e). Additionally, fewer c-Kit+ HSPCs were proliferating in the spleen of SCI mice (Fig. 3e), likely as fewer proliferating HSPCs exit the bone marrow and enter the circulation after SCI.

The effects of SCI on HSPC sequestration in bone marrow were independent of sex; SCI abolished HSPC mobilization in both female and male SCI mice (compare Fig. 3a–c and g–i). Further, SCI-dependent effects on HSPCs were not influenced by either injury severity or spinal injury level; HSPCs fail to enter into the circulation after complete spinal transection injuries at either L6/S1, T9, or T3 spinal levels (Fig. 4a) and after an incomplete spinal contusion injury at either T3 or T9 spinal levels (Fig. 4b).

To determine if the SCI-dependent effects on HSPCs were species-specific, male and female mice with human HSPCs and immune systems (i.e., humanized mice) were generated38–40. Similar to wild-type mice, SCI prevented human HSPCs from entering the circulation and trafficking to the spleen (Fig. 4c, d).
Histograms of LSK proliferation at 3 dpi (closest to the mean). Quantification Data that these cells are unable to leave the bone marrow niche, naïve mice. dpi days post-injury, Lam laminectomy (sham injury). Source data are provided as a Source Data file.

Fig. 1 T3 transsection SCI protracts HSPC proliferation and causes HSPC accumulation in bone marrow. a Bioluminescent imaging of sternum and femur/tibia regions of interest (ROIs; red ellipses) pre-injury and 7 dpi. b, c Bioluminescence expressed as max radiance (peak intensity within ROI) and total flux (average intensity within ROI) for each region, with left and right femur/tibia averaged; data also expressed as the total (sum) of signals from 1 to 28 dpi. d Proportion of proliferating lineage low, Sca-1−, c-Kit+ (LSK) bone marrow cells, as indicated by the S-G2-M phase of the cell cycle, at 3 dpi; representative histograms of LSK proliferation at 3 dpi (closest to the mean). Quantification of LSKs (e), c-Kit+ progenitors (f), and total bone marrow cells (g) at 3, 7, and 28 dpi. h Images and heatmaps of tibia pairs from Lam and SCI mice demonstrating increased optical density (artifact) in SCI mice. Dotted lines represent average data from naïve mice. dpi days post-injury, Lam laminectomy (sham injury). Source data are provided as a Source Data file.

Fig. 2 T3 transsection SCI causes the chronic accumulation of hematopoietic stem cells and differentiated progenitors. a Hematopoietic hierarchy of HSPCs and their cell surface markers. Total number of bone marrow cells (b), LSKs (c), long-term HSCs (d), short-term HSCs (e), multipotent progenitors 2–4 (f–h), and granulocyte-monocyte progenitors (i) at 28 dpi. j Mean fluorescence intensity of γH2AX expression (i.e. replication stress) within c-Kit+ HSPCs at 28 dpi. All data are mean ± SEM, two-sample t-test; n = 9 and 10/group (b–d), n = 10/group (3 dpi), 5/group (7 dpi), and 5 and 6/group (28 dpi) in e–g. Dotted lines represent average data from naïve mice. dpi days post-injury, Lam laminectomy (sham injury). Source data are provided as a Source Data file.

Together, these results, when combined with data in Figs. 1–3, indicate that SCI triggers excessive proliferation of HSPCs but that these cells are unable to leave the bone marrow niche, causing HSPCs to progressively accumulate within the bone marrow. Importantly, HSPC sequestration in bone marrow, although SCI-dependent, is independent of sex, injury level, injury severity, or species (both mouse and human HSPCs respond identically to SCI).
SCI impairs B cell development and mobilization. The decentralized bone marrow that develops after SCI may sequester cells other than HSPCs. Indeed, in both animal models and human subjects, total numbers of circulating leukocytes decrease after SCI and stroke\(^{19,24,41-44}\). Here, we confirmed and expanded those data using a model of complete SCI (T3 spinal level). Specifically, at 3 dpi, T3 transection SCI reduced circulating blood lymphocytes (Fig. 5a, b) with a concomitant increase in the proportion of mature lymphocytes found in bone marrow, including B220\(^{\text{hi}}\) B cells, CD3\(^{+}/\text{CD4}^{-}\) T cells, and CD3\(^{+}/\text{CD4}^{+}\) T cells (Fig. 5c–e). Further, SCI reduced numbers of B220\(^{\text{low}}\) immature B cells (confirmed to be enriched for ProB, PreB, and IgM\(^{\text{low}}\) immature B cells; Supplementary Fig. 1e) in the bone marrow (Fig. 5c), similar to previous data showing impaired B lymphopoiesis after SCI and stroke\(^{19,44,45}\).

SCI increases CXCL12-CXCR4 signaling in bone marrow HSPCs. Immunoregulatory cytokines and chemokines, including IL1\(^{\beta}\), TNF\(\alpha\), TGF\(\beta\), CCL2, CXCL12, and CXCR4 can affect the development and function of HSPCs\(^{46,47}\). To determine if the excessive proliferation and retention of HSPCs in bone marrow after SCI is associated with changes in these cytokines, we prepared mRNA from whole-bone marrow cells isolated from T3 transection SCI mice and sham-injured mice. Real-time PCR analyses revealed that, with the exception of Ccl2, SCI increased the expression of all bone marrow cytokines and chemokines assessed (Fig. 6a). Notably, Cxcl12, a chemokine produced by bone marrow stromal cells, and its receptor Cxcr4 were increased in parallel (fourfold and threefold, respectively).

Several published studies demonstrated that CXCL12 binding to CXCR4 on HSPCs influences the maintenance and retention of HSPCs in bone marrow, a phenomenon that is dependent, in part, on the sympathetic branch of the autonomic nervous system\(^{8,10,48}\). Therefore, we validated the effects of high-level complete (T3 transection) SCI on this chemokine/chemokine receptor pair. In a separate cohort of T3 SCI and sham-injured mice, we found that SCI increased the amount of secreted CXCL12 protein in bone marrow extracellular fluid (Fig. 6b) and expression of CXCR4 receptor on LSK cells (Fig. 6c).

To test whether the SCI-dependent increase in CXCL12/CXCR4 expression causes HSPC sequestration in bone marrow, mice were treated with Plerixafor (AMD3100), a small-molecule antagonist of CXCR4, or vehicle for 3 days, beginning 1 h post-SCI. In Plerixafor-treated mice, the number of HSPCs released into the blood increased >6-fold (Fig. 6d), with a corresponding increase in the number of HSPCs found in the spleen (Fig. 6e–g).
Plerixafor treatment also reversed the normal onset of post-SCI leukopenia: both total numbers of splenocytes (Fig. 6h) and all subsets of circulating WBCs increased (Fig. 6i, j). Importantly, releasing cells from bone marrow with Plerixafor did not ablate the bone marrow. In fact, Plerixafor increased total bone marrow cellularity (Fig. 6k). These data implicate sustained CXCL12/CXCR4 signaling within the bone marrow as a potential mechanism underlying SCI-induced sequestration of HSPCs. Importantly, post-SCI sequestration of HSPSCs and mature immune cells can be overcome using the FDA-approved drug, Plerixafor.

**Chronic SCI impairs bone marrow responses to inflammatory stimuli.** Data above indicate that SCI causes an acquired bone marrow failure syndrome characterized by protracted HSPC proliferation with sequestration and associated peripheral lymphopenia. Bone marrow failure develops within 72 h post-injury and persists until at least 1 month post-injury. To determine whether failed bone marrow in SCI mice is able to respond to physiologically relevant stimuli, mice with chronic SCI were challenged with endotoxin (1 mg kg\(^{-1}\); lipopolysaccharide (LPS)), a potent inducer of HSPC proliferation and mobilization\(^{34,49}\). Collectively, these data indicate that SCI causes chronic, and perhaps permanent, bone marrow failure.

**SCI impairs the long-term function of bone marrow HSPCs.** Data in Fig. 6 indicate that the sequestration and accumulation of HSPCs in bone marrow after SCI are due, in part, to aberrant cytokine and chemokine signaling in the bone marrow niche. To determine whether SCI also affects the intrinsic capacity of bone marrow HSPCs, notably their ability to restore hematopoiesis to a depleted bone marrow niche, we performed in vivo competitive repopulating assays.

Bone marrow HSPCs were removed from SCI mice 3 days after T3 transection SCI or sham surgery. In SCI mice, this timing corresponds with a period of enhanced HSPC proliferation (Fig. 1) and sequestration (Fig. 3), but no difference in total HSPC numbers (Fig. 1). When bone marrow cells were injected into unjured mice that had received lethal doses of irradiation (Supplementary Fig. 2a, b), SCI-derived donor cells exhibited faster engraftment in recipient mice at 8 weeks (Fig. 8a, b). By 19 weeks post-engraftment, stable engraftment was achieved in all recipient mice, regardless of the donor cell source (Fig. 8b).

By 7 days after LPS injections, basal cell proliferation is restored and maintained in sham-injured mice, presumably because HSPCs have repopulated the depleted bone marrow\(^{49}\). In contrast, LPS-induced proliferation continues in the bone marrow of SCI mice; the luciferase signal overshoots baseline proliferation by >200% between 9 and 14 days post-LPS (Fig. 7b, c). Remarkably, the same characteristics that define bone marrow failure early after SCI are recapitulated in chronic SCI animals after LPS injections (compare Fig. 7 to Figs. 1–5). Collectively, these data indicate that SCI causes chronic, and perhaps permanent, bone marrow failure.

![Fig. 4 Complete and incomplete SCI impairs acute mouse and human HSPC mobilization.](image)

**Fig. 4 Complete and incomplete SCI impairs acute mouse and human HSPC mobilization.** a Number of CFCs per mL whole blood after T3 and T9 contusion injury. b Number of CFCs per mL whole blood after T3 or L6/S1 transection injury. c Number of hCFCs per mL whole blood. d Total numbers of splenic hCFCs, proportion of splenic hCD34\(^{hi}\)/hCD38\(^{low}\) human HSPCs, and spleen weight of hNSG mice 3–5 dpi. All data are mean ± SEM, one-way ANOVA with Tukey multiple comparison test (a, b), two-sample t-test (c, d); n = 20, 15, 14, and 5/group in a, n = 6, 10, and 5/group in b; n = 8/group in c, n = 6, 6, 4, 8 and 9/group in d. dpi days post-injury, Lam laminectomy (sham injury). Source data are provided as a Source Data file.
HSPC engraftment was identical between groups 4 weeks after secondary transplantation (Fig. 8e, f). However, no significant change in engraftment occurred throughout the evaluation period (6 months) in mice receiving SCI bone marrow; only mice receiving HSPCs that were originally derived from sham-injured (6 months) in mice receiving SCI bone marrow; only mice 24 weeks after secondary transplantation, engraftment was significantly reduced in the bone marrow of mice receiving SCI donor cells compared with mice receiving sham-injured donor cells (Fig. 8h). These data indicate that SCI negatively affects the long-term self-renewal capacity of bone marrow HSPCs.

Discussion

Prior studies in mice and humans found that SCI increases the total number of cells in bone marrow. Data in this report extend those observations and also provide new insight to help explain how/why after SCI, both humans and rodents exhibit prolonged hematological abnormalities marked by leukopenia and chronic immunologic dysfunction. Specifically, data in this report reveal that traumatic SCI causes a bone marrow failure syndrome marked by excessive proliferation and sequestration of HSPCs, altering of cytokine and chemokine signaling within the bone marrow niche, failure to generate and mobilize mature lymphocytes, and chronic impairments in the clonogenic potential of HSPCs (Fig. 9). Bone marrow failure diseases develop when the bone marrow is unable to produce appropriate numbers of healthy mature white and red blood cells. Normal aging and various diseases including diabetes, warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM) syndrome, glioblastoma, and chemotherapy cause sequestration of mature and immature cells in the bone marrow. In each case, sequestration of bone marrow cells either causes or is associated with hematopoietic dysfunction. Both neural and humoral mechanisms undoubtedly participate in causing the aberrant sequestration phenomenon, although disease-specific mechanisms are likely.

HSPCs reside in a specialized perivascular bone marrow niche (i.e. microenvironment). It is in the niche that complex cellular interactions between hematopoietic cells and the microenvironment, including soluble factors, extracellular matrix components, and cellular components, are thought to orchestrate hematopoiesis.
and molecular cues orchestrated between HSPCs, CXCL12-expressing perivascular stromal cells and the autonomic nervous system, particularly sympathetic noradrenergic nerve fibers, control HSPC proliferation, regeneration, and differentiation\(^8,10,11,65\). Specifically, post-ganglionic sympathetic neurons release norepinephrine into the bone marrow, causing bone marrow stromal cells to decrease their expression of CXCL12 which, in turn, unethers CXCR4\(^+\) HSPCs and mature leukocytes from the niche, facilitating their egress into the circulation\(^8,10,36\). Loss of proper sympathetic tone in bone marrow, whether by experimental manipulation or by aging, causes hematopoietic dysfunction\(^12,64\). A similar neurogenic mechanism may cause bone marrow failure after SCI.

After SCI, much or all of the tonic supraspinal control over the sympathetic nervous system is lost. Previously, using models of thoracic SCI, we showed that normal physiological stimuli (e.g., visceral afferent input from bladder/bowel) trigger exaggerated or uncontrolled sympathetic reflexes in spinal autonomic circuits\(^14-17\). However, similarly aberrant sympathetic reflexes may be triggered even when SCI occurs below the caudalmost sympathetic preganglionic neurons located in lower thoracic/upper lumbar spinal cord. Anatomical tracing studies indicate that femoral bone marrow is innervated by both sympathetic and sensory nerves originating as far rostral in the spinal cord as T8-9 and as far caudal as the sacral spinal cord\(^7,66,67\). This is a large segment of spinal cord through which sensory input from bone marrow could activate propriospinal relay neurons and multi-segmental sympathetic reflexes\(^13\). Depending on which relay neurons are activated and the relative integrity (anatomical or functional) of the intersegmental circuitry, bone-specific intersegmental sympathetic reflexes may be exaggerated or silenced after SCI. At the level of the bone marrow niche, either outcome would be perceived by stromal cells and HSPCs as a break in homeostasis. Under pathological conditions, when sympathetic tone to bone marrow is disrupted, CXCL12 and CXCR4 levels increase and HSPC mobilization is impaired\(^10,12,39,64\). Since proper descending modulation of spinal sympathetic reflexes is never restored after traumatic SCI and also intersegmental neuronal circuitry continues to undergo structural and functional plasticity, neurogenic control of bone marrow function may never be restored after SCI. Evidence for permanent bone marrow failure is apparent from data in Fig. 7, which
revealed that a potent hematopoietic stimuli (endotoxin) does not effectively mobilize HSPCs and mature lymphocytes from the bone marrow of mice at chronic post-injury periods.

SCI-induced changes in circulating hormones, glucocorticoids in particular, are also likely culprits underlying acute and chronic bone marrow failure. Normally, circulating glucocorticoids act on diverse cell types in the periphery and the nervous system to help maintain HSPCs and the bone marrow niche. Glucocorticoids increase within 24 h post-injury then remain at supraphysiological concentrations indefinitely. This may explain why, in the present study, SCI always causes HSPC sequestration, regardless of injury level (see Fig. 4). Future studies are needed to explore in-depth how SCI affects neural–humoral signaling in bone marrow and to what extent glucocorticoids and other blood-borne factors (e.g., microbial metabolites) contribute to acquired bone marrow failure.

An important observation made in this report, and one that may have an immediate impact on people affected by SCI, is that it is possible to overcome certain aspects of SCI-induced bone marrow failure. Specifically, we found that the FDA-approved drug and CXCR4 antagonist Plerixafor, when injected post-injury, effectively liberates HSPCs and mature leukocytes from the bone marrow of SCI mice (Fig. 6). In SCI patients, Plerixafor could be a potentially safe and effective way to mobilize cells from the bone marrow niche to help restore immune function.
immune system and exacerbat ing neuroinflammatory-mediated injury or trauma-induced autoimmunity, both of which can impair neurological recovery, more research is needed to define the optimal therapeutic conditions for Plerixafor treatment after SCI.

Another intriguing aspect of SCI-induced bone marrow failure was revealed during the competitive repopulating unit (CRU) assays (Fig. 8). Specifically, we found evidence that the long-term clonogenic potential of HSPCs is impaired and that these long-term effects are imprinted in HSPCs early after injury. Indeed, bone marrow cells isolated from SCI donor mice 3-day post-injury, when transplanted into irradiated naive mice, engrafted the irradiated bone marrow faster than bone marrow cells obtained from sham-injured mice. This early advantage could be explained by improved homing to the bone marrow by HSPCs from SCI donors, perhaps because of glucocorticoid-mediated enhancement of CXCR4 on bone marrow LSK cells (Fig. 6). However, this repopulation advantage was transient as deficits in the long-term repopulation or clonogenic potential of SCI bone marrow became obvious after secondary transplantation (Fig. 8).

Currently, we do not know why SCI impairs the long-term function of HSPCs or how this occurs within only 3 days. Since we did not see an increase in the DNA damage marker γH2AX after chronic SCI (Fig. 2), it is unlikely that excessive HSPC proliferation after SCI causes DNA damage. However, HSPC exhaustion is possible as cell cycle number has been shown to inversely correlate with long-term HSPC function. It is also possible that aberrant sympathetic–neuroendocrine reflexes influence the clonogenic potential of HSPCs; both catecholamines and glucocorticoids can cause epigenetic modifications in cells, imprinting them with new functional identities. In the context of monocytes/macrophages, for example, exposure of these cells to certain stimuli endows them with enhanced microbicidal functions against secondary infections. This trained immunity in monocytes/macrophages is orchestrated by epigenetic reprogramming, which also occurs in HSPCs. Perhaps SCI creates an environment in the bone marrow niche that favors the induction of a form negative trained immunity. Regardless of mechanism, our data are consistent with those showing that the clonogenic potential of hematopoietic and stromal cells isolated from SCI patient bone marrow is impaired.

In conclusion, data in this report reveal that SCI-induced bone marrow failure is caused by cell-intrinsic and extrinsic mechanisms; impaired control of HSPC proliferation and sequestration is likely a cell-extrinsic phenomenon regulated in the niche while deficits in HSPC clonogenic potential are likely the result cell-intrinsic changes. Bone marrow failure develops soon after injury but has long-lasting adverse effects on the host HSPCs. For example, impaired bone marrow function may limit the development of an effective immune system, perhaps explaining the higher incidence of infectious morbidity and mortality in SCI patients. Also, SCI-induced bone marrow failure may preclude the use of bone marrow cells from SCI donors as a transplantation source. A similar limitation was recently described for bone marrow isolated from mice with experimental CNS autoimmune disease. Together, these data highlight the bone marrow as a previously underappreciated therapeutic target for improving health outcomes and quality of life after SCI.
human immune systems (hNSG mice) was performed as follows. The Il2rgtm1Wjl/SzJ mice (NSG mice) were bred in-house from adult breeding pairs originally purchased from The Jackson Laboratory (strain #005557). Animals were fed commercial food pellets and chlorinated reverse osmosis water ad libitum and housed in ventilated microisolator cages layered with corn cob bedding. Hair was shaved at the region of the thoracic spinal cord and skin was treated with a sequence of betadine, 70% ethanol, and betadine. A small midline incision was made to expose the vertebra and then a partial laminectomy was performed. The meninges were cut using micro-scissors and then spinal cord tissue was removed. The wound was then closed with sutures or staples. After surgery, mice were placed in cages on heating pads and monitored frequently until they recovered consciousness. Bladders were expressed at least twice daily to maintain urinary function, and urine underwent periodic pH testing to identify bladder infections. Gentocin (5 mg kg$^{-1}$, s.q.) was given until mice were moving spontaneously. Mice were given 5% cage ventilation and monitored frequently until they recovered consciousness.

**Methods**

**Mice and housing.** The Institutional Animal Care and Use Committee of the Office of Responsible Research Practices at The Ohio State University approved all animal protocols for this study. All experiments were performed in accordance with the guidelines and regulations of The Ohio State University and outlined in the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health. Female and male C57BL/6 mice (strain #000664; CD45.2) were purchased from The Jackson Laboratory (Bar Harbor, ME), female B6.SJL-Ptprca Pepcb/BoyJ mice (C57BL/6-CD45.1; BoyJ) were bred in-house from adult breeding pairs purchased from The Jackson Laboratory (strain #00014), and male and female NOD.Cg-Prkdcscid Il2rgtm1Wj/SjJ mice (NSG mice) were bred in-house from adult breeding pairs purchased from The Jackson Laboratory (strain #005557). Animals were fed commercial food pellets and chlorinated reverse osmosis water ad libitum and housed in a 12-h light–dark cycle at a constant temperature (20 ± 2 °C) and humidity (50 ± 20%). All mice were housed in a specific pathogen-free housing facility with routine testing of sentinel mice for specific pathogens. Generation of NSG mice with human immune systems (hNSG mice) was performed as follows. Newborn NSG pups (24–72 h postnatal) received 1 Gy whole-body X-ray irradiation (RS 2000, Rad Source, Suwanee, GA), followed immediately by engrafting 1 × 10$^8$ human umbilical cord CD34$^+$ stem cells (Lonza Incorporated, Walkersville, MD or Stemcell Technologies, Vancouver, BC) via intrathecal injection. Body temperature was maintained at 37 °C using a heating pad until pups were returned to their dams for normal maturation and weaning 21–24 days postnatal.

**SCI and animal care.** Adult C57BL/6 mice (16–24 weeks old) were used for SCI experiments. Mice were subjected to a complete spinal cord transection injury at the third thoracic, ninth thoracic, or sixth lumbar spinal levels. Mice subjected to laminectomy (Lam or sham injury) only served as controls, and naive mice were used as a reference for outcomes. Mice were anesthetized with ketamine (120 mg kg$^{-1}$, i.p.) and xylazine (10 mg kg$^{-1}$, i.p.) for all surgical procedures and provided prophylactic antibiotic treatment with gentamicin sulfate (5 mg kg$^{-1}$, s.q.). Aseptic conditions were maintained during all surgical procedures and mice were placed on a warming pad to regulate body temperature. Hair was shaved at the region of the thoracic spinal cord and skin was treated with a sequence of betadine, 70% ethanol, and betadine. A small midline incision was made to expose the vertebra and then a partial laminectomy was performed. The meninges were cut using micro-scissors and then spinal cord tissue was removed using micro-scissors and a sterile glass aspiration tube for suction of fluid/blood, stabilization of the spinal cord during transection, and to confirm completion of injury. Muscle overlaying the injury site was sutured, followed by closure of the wound with sutures or staples. After surgery, mice were placed in cages on heating pads and monitored frequently until they recovered consciousness and were moving spontaneously. Mice were given fluids (1–2 mL 0.9% sterile saline) to maintain hydration and soft food to eat ad libitum as they recovered.

**IVIS imaging.** Mito-luc mice were injected with 80 mg kg$^{-1}$ (i.p.) D-luciferin potassium salt (Cayman Chemical, Ann Arbor, MI), anesthetized with isoflurane (2.5–4% vaporized in oxygen, delivered at 1 L min$^{-1}$), and placed on a heated surface within the IVIS Lumina II system (Caliper Life Sciences, Hopkinton, MA) for image acquisition. Imaging of long bones in limbs required securing forelimbs at 90° from midline, and hindlimbs 45° from midline, with small pieces of translucent medical tape. Mice were kept on 1.5–2% isoflurane throughout the duration of imaging. Baseline levels of mitosis were determined prior to SCI, and then mice were imaged at 1, 3, 7, 14, 21, and 28 dpi. Data were analyzed with the Living Image® software (v.4.3.1; Caliper Life Sciences). Bioluminescence was measured by defining regions of interest (ROIs) of defined sizes around the sternum, left femur/tibia, and right femur/tibia. Total flux (photons s$^{-1}$) and the maximum radiance for each ROI was determined, and an average was calculated for both left and right femur/tibia ROIs. Data were plotted for each individual animal as either raw values (post-SCI study; Fig. 1) or percent of pre-LPS (chronic SCI plus LPS study; Fig. 7).

**Plerixafor treatment.** After SCI or sham surgery, mice were injected subcutaneously with 5 mg kg$^{-1}$ Plerixafor (AMD3100; Sigma-Aldrich) in 0.9% sterile saline. First dose was given 1 h post-injury, and then once a day until 3 dpi. Mice were terminally anesthetized with ketamine and xylazine 1 h after a final dose of AMD and tissues were collected as described below.

**Stimulating hematopoiesis with systemic endotoxin.** Mito-luc transgenic and wild-type mice underwent SCI or sham surgery as previously described. Approximately 6–8 weeks after injury mice were injected i.p. with 1 mg kg$^{-1}$ LPS (E. coli O55:B5, Sigma-Aldrich) in 0.9% sterile saline once per day for 3 days. Mito-
luc mice underwent IVIS imaging at 2, 4, 7, 9, 11, and 14 days post-LPS (first dose). Wild-type mice underwent submandibular bleeds prior to LPS, 1-h post-LPS, 4 days post-LPS, and 11 days post-LPS. Blood was collected into an EDTA-coated capillary tube (Sarstedt Inc.; Thermo Fisher Scientific, Waltham, MA).

### Tissue collection and processing
Mice were terminally anesthetized with ketamine and xylazine for euthanasia and tissue collection. Blood was then collected via cardiac puncture and placed in blood collection tubes coated with EDTA. Blood was then treated with ammonium chloride-based red blood cell (RBC) lysis buffer and resuspended in Iscove’s Modified Dulbecco’s Medium (IMDM) with 2% fetal bovine serum (FBS) or 0.1% phosphate buffered saline (PBS) with 2% FBS (flow buffer) for flow cytometry. Spleens were rapidly isolated, weighed, and placed in Hank’s Balanced Salt Solution (HBSS). Spleens were minced with sterile dissection scissors, smashed through a 40-μm cell strainer, and placed in a small volume of HBSS. Bone marrow cells were isolated by either flushing bones with 10 mL of HBSS or crushing in a mortar and pestle and washed with media. Cell counts were obtained by a standard hemocytometer (bone marrow and spleen), or with a Coulter counter. Cell fractions were stained for lymphocytes, platelets, and RBCs.

**Immunolabeling and flow cytometry.** 2-10 × 10^6 bone marrow cells and spleenocytes, or approximately 50 μL RBC-lysed blood, were allocated for flow cytometry analysis. All antibodies were used at a 1:100 dilution for staining purposes. BD StemFlow™ Mouse Hematopoietic Stem Cell Isolation Kit (BD Biosciences, cat #560492) was used to label lineages and Ki67. Sca-1+ HSPCs. Mouse lineage cocktail (BD Biosciences; cat #558074) contained the following APC-conjugated antibodies: CD4 (145-2C11), CD11b (M1/70), CD45R/B220, and TER-119, and Ly6G/C (R6-80C5). Fc receptors were blocked for 15 min using rat anti-mouse CD16/32 antibody (BD Biosciences, cat #553142), followed by labeling with antibodies for 60 min. Dead cells were labeled with eFluor780 (eBioscience, cat #65-0888-82) and analyzed with an LSR Fortessa (BD, San Jose, CA). Cell viability was assessed using 7AAD (BD Biosciences, cat #560425) or propidium iodide. All data were acquired for at least 10,000 events, and later analyzed using the FlowJo software.

### Statistics and reproducibility
All values were represented as mean ± standard error of the mean (SEM), with individual data points representing independent biological replicates. Group sizes were determined by analyzing preliminary and published datasets (Fig. 1b). Recombinant CXCL12-SDF-1α (100 ng/mL) and chemokines (100 nM) were used to detect a 1.25-fold change with a coefficient of variation of 20% and 80% power for flow cytometry and MethoCult assays. To compensate for unexpected morbidity/mortality, an n/group = 3 was used as the minimum number of mice per experimental group. Data were excluded only if identified as a statistical outlier by Grubbs’ test with a probability of >0.05. For the experiment comparing the effect among sham, thoracic, and lumbar hemorrhage (Fig. 4g), one-way ANOVA was performed followed by Tukey post hoc comparisons. For the longitudinal data (Fig. 5b, c, d) and survival data (Fig. 4g), a two-tailed t-test was used to detect a significant increase in mortality. Data were analyzed using GraphPad Prism version 8 (GraphPad Software, La Jolla, CA).

### Quantification of CXCL12 in bone marrow extracellular fluid
Bone marrow was isolated from two tibiae and two femurs from mice using 1 ml ice cold PBS. By light microscopy, trituration, and cell debris were separated from extracellular fluid by centrifugation for 5 min at 400 × g. Fluid was aliquoted and frozen in liquid nitrogen. Mouse CXCL12/SDF-1α quantitation was determined using Luminex xMAP technology and was performed with an independent experimental replicate included in the data presented (Fig. 1a).

### MethoCult CFC assay
The MethoCult™ GM M3434 was used to culture bone marrow cells from wild-type (C57BL/6) and HSPC-depleted (Mx1-Cre; R26R) mice. HSPCs were isolated from bone marrow as described above. Cells were cultured in MethoCult H4034 Optimum media for 14 days before the assay was performed.
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experiential replications. Data demonstrating that PlexiXafor liberates HSPCs from bone marrow after SCI (Fig. 6d–k) were independently verified in two additional experiments not included in the manuscript. Data were analyzed using GraphPad Prism software v5.0 (GraphPad Software Inc., San Diego, CA). Illustrations were created with BioRender scientific illustration software paid sub

reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Source data are provided with this paper as a Source Data file, including all data for Figs. 1–8 (and Supplementary Fig. 2) which support the findings of the current study. All other information is available within the manuscript, supplementary information file, or by reasonable request from the corresponding author. Source data are provided with this paper.

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References

1. Pinto, S. & Frenette, P. S. Haematopoietic stem cell activity and interactions with the niche. Nat. Rev. Mol. Cell Biol. 20, 303–320 (2019).
2. Kunz, A. & Richins, C. A. Innervation of the bone marrow. J. Comp. Neurol. 83, 213–222 (1945).
3. Takase, B. & Nomura, S. Studies on the innervation of the bone marrow. J. Comp. Neurol. 108, 421–443 (1957).
4. Courties, G. et al. Ischemic stroke activates hematopoietic bone marrow stem cells. Circ. Res. 116, 407–417 (2015).
5. Maestroni, G., Conti, A. & Pedrinis, E. Effect of adrenergic agents on hematopoietic bone marrow stem cells. Exp. Hematol. 23, 666–710 (1995).
6. Denes, A. et al. Central autonomic control of the bone marrow: multisynaptic tract tracing by recombinant pseudorabies virus. Neuroscience 134, 947–963 (2005).
7. Katayama, Y. et al. Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. Cell 124, 407–421 (2006).
8. Nance, D. M. & Sanders, V. M. Autonomic innervation and regulation of the immune system (1987–2007). Brain Behav. Immunol. 21, 736–745 (2007).
9. Méndez-Ferrer, S., Lucas, D., Battista, M. & Frenette, P. S. Haematopoietic stem cell release is regulated by circadian oscillations. Nature 452, 442–447 (2008).
10. Heidt, T. et al. Chronic variable stress activates hematopoietic stem cells. Nat. Med. 20, 754–758 (2014).
11. Maryanovich, M. et al. Adrenergic nerve degeneration in bone marrow drives aging of the hematopoietic stem cell niche. Nat. Med. 24, 782–791 (2018).
12. Taylor, R. F. & Schramm, L. P. Differential effects of spinal transection on sympathetic nerve activities in rats. Am. J. Physiol. 253, R611–618 (1987).
13. Lucin, K. M., Sanders, V. M., Jones, T. B., Malarek, W. B. & Popovich, P. G. Impaired antibody synthesis after spinal cord injury in mice alters wound healing and worsens neurological outcome. J. Immunol. 29, 753–764 (2009).
14. Courties, G. et al. Haematopoietic stem cell activity maps proliferation sites in live animals. Mol. Biol. Cell 23, 1467–1474 (2012).
15. Lucin, K. M. et al. Chronic spinal cord injury impairs primary antibody responses but spares existing humoral immunity in mice. J. Immunol. 188, 5257–5266 (2012).
16. Boettcher, S. & Manz, M. G. Regulation of inflammation- and infection-driven hematopoiesis. Trends Immunol. 38, 345–357 (2017).
17. Chavakis, T., Mitroulis, I. & Hajishengallis, G. Hematopoietic progenitor cells as integrative hubs for adaptation to and fine-tuning of inflammation. Nat. Immunol. 20, 802–811 (2019).
18. Méndez-Ferrer, S. et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature 466, 829–834 (2010).
19. Nagai, Y. et al. Toll-like receptors on hematopoietic progenitor cells stimulate innate immune system replenishment. Immunity 24, 801–812 (2006).
20. Liu, Q. et al. CXCR4 antagonist AMD3100 redistributes leukocytes from primary immune organs to secondary immune organs, lung, and blood in mice. Eur. J. Immunol. 45, 1855–1867 (2015).
21. Skreeci, T. et al. Mobilization of stem and progenitor cells in septic shock patients. Sci. Rep. 9, 3289 (2019).
22. Chernykh, E. R. et al. Characteristics of bone marrow cells under conditions of impaired innervation in patients with spinal trauma. Bull. Exp. Biol. Med. 141, 117–120 (2006).
23. Campagnolo, D. L., Dixon, D., Schwartz, J., Bartlett, I. A. & Keller, S. E. Altered innate immunity following spinal cord injury. Spinal Cord 46, 477–481 (2008).
24. Rieger, T. et al. Immune depression syndrome following human spinal cord injury (SCI): a pilot study of hematopoietic stem cells and multipotent progenitors. Cell Stem Cell 12, 102–116 (2013).
25. Pietras, E. M. et al. Functionally distinct subpopulations of hematopoietic stem cells and multipotent progenitors. Cell Stem Cell 13, 83–96 (2013).
26. Maryanovich, M. et al. Adrenergic nerve degeneration in bone marrow drives hematopoietic stem cell release by reasonable request from the corresponding author. Source data are provided with this paper.
53. Monahan, R., Stein, A., Gibbs, K., Bank, M. & Bloom, O. Circulating T cell subsets are altered in individuals with chronic spinal cord injury. *Immunol. Res.* 63, 3–10 (2015).

54. Rouleau, P., Ung, R.-V., Lapointe, N. P. & Guertin, P. A. Hormonal and immunological changes in mice after spinal cord injury. *J. Neurotrauma* 24, 367–378 (2007).

55. Stirling, D. P. & Yong, V. W. Dynamics of the inflammatory response after murine spinal cord injury revealed by flow cytometry. *J. Neurosci.* 19, 1944–1958 (2008).

56. Kopp, M. A. et al. Long-term functional outcome in patients with acquired infections after acute spinal cord injury. *Neurology* 89, 892–900 (2017).

57. Pang, W. W. et al. Human bone marrow hematopoietic stem cells are increased in frequency and myeloid-biased with age. *Proc. Natl Acad. Sci. USA* 105, 20312–20317 (2008).

58. Hu, P. et al. CNS inflammation and bone marrow neuropathy in type 1 diabetes. *Am. J. Pathol.* 183, 1608–1620 (2013).

59. Albiero, M. et al. Diabetes causes bone marrow autonomic neuropathy and impairs stem cell mobilization via dysregulated p66Shc and Sirt1. *Diabetes* 63, 1533–1545 (2014).

60. Galino, A. V. et al. Altered leukocyte response to CXCL12 in patients with warts hypogammaglobulinemia, infections, myelokathexis (WHIM) syndrome. *Blood* 104, 444–452 (2004).

61. Majumdar, S., Murphy, P., Majumdar, S. & Murphy, P. M. Adaptive immunodeficiency in WHIM syndrome. *Int. J. Mol. Sci.* 20, 3 (2018).

62. McDermott, D. H. et al. Plerixafor for the treatment of WHIM syndrome. *N. Engl. J. Med.* 380, 163–170 (2019).

63. Chongthaidkiet, P. et al. Sequestration of T cells in bone marrow in the setting of gliblastoma and other intracranial tumors. *Nat. Med.* 24, 1459–1468 (2018).

64. Lucas, D. et al. Chemotherapy-induced bone marrow nerve injury impairs hematopoietic regeneration. *Nat. Med.* 19, 695–703 (2013).

65. Dar, A. et al. Rapid mobilization of hematopoietic progenitors by AMD3100 and catecholamines is mediated by CXCR4-dependent SDF-1 release from bone marrow stromal cells. *Leukemia* 25, 1286–1296 (2011).

66. Thai, J., Kylohi, M., Travis, L., Spencer, N. J. & Ivanusic, J. J. Identifying spinal afferent (sensory) nerve endings that innervate the marrow cavity and bone marrow periosteum using anterograde tracing. *J. Comp. Neurol.* 528, 1903–1916 (2020).

67. Wee, N. K. Y., Lorentz, M. R., Bekirov, Y., Jaccquin, M. F. & Scheller, E. L. Shared autonomic pathways connect bone marrow and peripheral adipose tissues across the central neuraxis. *Front. Endocrinol. (Lausanne)* 10, 668 (2019).

68. Parce, H. et al. Cholinergic signals from the CNS regulate G-CSF-mediated HSC mobilization from bone marrow via a glucocorticoid signaling relay. *Cell Stem Cell* 20, 648–658.e4 (2017).

69. Guo, B., Huang, X., Cooper, S. & Broxmeyer, H. E. Glucocorticoid hormone-induced chromatin remodeling enhances human hematopoietic stem cell homing and engraftment. *Nat. Med.* 23, 424–427 (2017).

70. Walter, D. et al. Exit from dormancy provokes DNA-damage-induced attrition in hematopoietic stem cells. *Nature* 520, 549–552 (2015).

71. Qiu, J., Papatsenko, D., Niu, X., Schaniel, C. & Moore, K. Divisional history and hematopoietic stem cell function during homeostasis. *Stem Cell Rep.* 2, 473–490 (2014).

72. Bernitz, J. M., Kim, H. S., MacArthur, B., Sieburg, H. & Moore, K. Hematopoietic stem cell self-renewal divisions. *Cell* 167, 1296–1309.e10 (2016).

73. Maity, S., Jarome, T. J., Blair, J., Lubin, F. D. & Nguyen, P. V. Noradrenaline goes nuclear: epigenetic modifications during long-lasting synaptic potentiation triggered by activation of β-adrenergic receptors. *J. Pharmacol. Exp. Ther.* 354, 863–881 (2016).

74. Novakovic, B. et al. β-Glucan reverses the epigenetic disfate of LPS-induced immunological tolerance. *Cell* 167, 1354–1368.e14 (2016).

75. Kaufmann, E. et al. BCG educates hematopoietic stem cells to generate protective innate immunity against tuberculosis. *Cell* 172, 176–190.e19 (2018).

76. Mitroulis, I. et al. Modulation of myelopoiesis progenitors is an integral component of trained immunity. *Cell* 172, 147–161.e12 (2018).

77. de Laval, B. et al. C/EBPβ-dependent epigenetic memory induces taints Immunity in hematopoietic stem cells. *Cell Stem Cell* 26, 657–674.e8 (2020).

78. Wright, K. et al. The cell culture expansion of bone marrow stromal cells from humans with spinal cord injury: implications for future cell transplantation therapy. *Spinal Cord* 4677, 811–817 (2008).