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The changes in systemic monocytes in humans undergoing surgical decompression for degenerative cervical myelopathy may influence clinical neurological recovery

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ABSTRACT

Background: Degenerative cervical myelopathy (DCM) is the most common cause of non-traumatic spinal cord injury worldwide. Surgical decompression is recommended as the preferred treatment strategy for DCM as it halts disease progression and improves neurologic symptoms. We previously demonstrated that neuroinflammation, including monocytes, plays a critical role in the pathobiology of DCM and in ischemic-reperfusion injury (IRI) following surgical decompression. Monocytes are able to enter the spinal cord and brain tissues due to damage to the blood spinal cord and blood brain barrier following injury. Studies have demonstrated that stroke patients and individuals undergoing hip replacement surgery have increased systemic levels of monocytes. Additionally, changes in the signalling responses of monocytes are associated with post-surgical recovery or with ischemic neural tissue damage. Herein, we investigated the role of systemic monocytes as a predictive biomarker for clinical recovery following decompressive surgery for DCM.

Findings: There was a 2-fold increase in the number of monocytes in DCM patients at 24 h following decompression as compared to baseline levels, which was associated with a significant improvement in the modified Japanese Orthopedic Association scale (mJOA) at 6-months after surgery ($p < .0001$). In a mouse model of DCM, depleting acute monocytes reduced the non-classical ($Ly6C^{low}$) subset from circulation ($p < .05$) and resulted in a 1.8-fold increase in CD11b expression in the spinal cord at 5 weeks following decompression. Acute monocyte depletion was accompanied by a modest decline in long-term overground locomotion, as evidenced by significantly reduced hindlimb swing speed.

Conclusions: This work demonstrated that decompressive surgery leads to an acute increase in peripheral monocytes in human DCM patients, which is modestly associated with clinical recovery. We anticipate that this work could contribute to the implementation of routine measurements of blood monocyte subsets, their activation state, and production of cytokines following decompressive surgery. This information could help to select perioperative anti-inflammatory treatments that can enhance the beneficial effects of decompressive surgery and reduce the incidence of post-operative complications, while avoiding a reduction in systemic monocytes.

1. Introduction

Degenerative Cervical Myelopathy (DCM) is the most common form of non-traumatic spinal cord injury (SCI) worldwide (Nouri et al., 2015). The pathophysiology of DCM is complex, with chronic cervical spinal cord compression resulting in neuroinflammation, alterations to the micro- and macro-vasculature, ischemic injury and apoptosis of neurons and oligodendrocytes (Hirai et al., 2013; Karadimas et al.,

Abbreviations: CSM, cervical spondylotic myelopathy; DAPI, 4′,6-Diamidino-2-phenylindole; DCM, degenerative cervical myelopathy; H&E, hematoxylin and eosin; IRI, ischemia-reperfusion injury; LFB, luxol fast blue; mJOA, modified Japanese Orthopedic Association scale; PBS, phosphate buffered saline; FBS, fetal bovine serum; PFA, paraformaldehyde; RT, room temperature; SEM, standard error of the mean; SD, standard deviation

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2013; Tetreault et al., 2015; Yu et al., 2011). DCM symptoms are highly variable and include loss of manual dexterity, pain and gait impairment (Fehlingas et al., 2017a, Tetreault, Goldstein, 2015). Current treatment for DCM consists of decompressive surgery, which is effective in halting disease progression and leads to functional improvements for > 90% of patients (De la Garza Ramos et al., 2019; Fehlings et al., 2017b; Fehlings et al., 2013). However, there is a subset of patients (approximately 9.7%) who undergo surgical decompression and develop perioperative neurological decline, including worsening of myelopathy and delayed C5 palsy (De la Garza Ramos, Nouri, 2019). Our DCM mouse model has demonstrated that post-decompression neurological decline is associated with the presence of ischemia-reperfusion injury (IRI) and increased activation of the immune system, collectively exacerbating post-operative locomotor complications and hindering long-term functional recovery (Karadimas et al., 2015; Vidal et al., 2017).

There is growing evidence that neuroinflammation, occurring during both DCM progression and after surgical decompression, plays a critical role in recovery outcome (Vidal, Karadimas, 2017, Yu, Liu, 2011). Monocytes are a subset of myeloid cells present in the peripheral and central nervous system that are able to enter tissues during active disease states (Garcia-Bonilla et al., 2016; Gordon and Taylor, 2005) and facilitate tissue clearance and repair (Olingy et al., 2017). Three distinct monocyte subsets have been identified: classical (inflammatory), non-classical (anti-inflammatory) and intermediate (Wong et al., 2011; Ziegler-Heitbrock, 2014). In mice, monocyte subsets are mainly identified by the differential expression of $Ly6C^{hi}$ (classical), $Ly6C^{int}$ (intermediate) and $Ly6C^{low}$ (non-classical) (Olingy, San Emeterio, 2017). In a mouse model of delayed decompression for DCM, we recently demonstrated that an increased frequency of classical monocytes was associated with a smaller improvement in locomotor function at two weeks following decompressive surgery (Vidal, Karadimas, 2017). These findings suggest that monocytes may play a role in recovery following surgery. Previous studies in patients undergoing hip replacement surgery or ischemic stroke have shown that the number of monocytes is generally increased after surgery, and changes in their signalling responses correlate with post-surgical recovery and/or the extent of tissue damage (Gaudilliere et al., 2014; Kaito et al., 2013). Prompted by these observations, we sought to investigate whether monocyte levels were altered following surgical decompression in DCM patients and to identify their association with long-term functional recovery as well as the development of complications.

In this study, we examined the association between peripheral monocyte numbers obtained from DCM patients before and after decompressive surgery and each patients' long-term functional recovery. The study aimed to evaluate the potential for monocyte levels to be used as an accessible and minimally invasive predictor of each patients' long-term functional recovery. DCM patients were found to exhibit a 2-fold increase in the number of peripheral monocytes following decompression, as compared to pre-surgery levels. There was a correlation between the change in monocytes and patients' functional recovery at 6-months following decompressive surgery. Furthermore, acute systemic monocyte depletion in a mouse DCM model associated with severe neuroinflammation (Vidal, Karadimas, 2017), before decompressive surgery, led to an acute reduction in the non-classical monocyte subset. These changes were accompanied by a modest worsening of overground locomotion long-term recovery.

2. Methods

2.1. Patient blood sample collection

The study was approved by the Research Ethics Board at the Toronto Western Hospital and the University Health Network (UHN; Toronto, Canada), and was carried out in accordance with The Code of Ethics of the World Medical Association. Informed consent was obtained from each patient enrolled in the study. One hundred and seventy-five patients were prospectively enrolled in either the CSM-North America (December 2005 to September 2007) or International (October 2007 to January 2011) studies at Toronto Western Hospital, Canada. Patients were eligible to participate in these studies if they met the following inclusion criteria: (1) aged 18 years or older; (2) presenting with symptomatic DCM (numb hands, clumsy hands, impaired gait, bilateral arm parasthesias, L'Hermitte's phenomena, weakness) with at least one clinical sign of myelopathy (atrophy of the intrinsic hand muscles, corticospinal deficits, hyperreflexia, positive Hoffman sign, lower limb spasticity, broad-based unstable gait, upgoing plantar responses); (3) imaging evidence of cervical cord compression; and (4) no previous cervical spine surgery. Patients were excluded if they were asymptomatic or if they had only radicular signs and symptoms, active infection, neoplastic disease, rheumatoid arthritis, ankylosing spondylitis or concomitant lumbar spinal stenosis. All participants underwent surgical intervention on their cervical spine. The attending surgeon decided what approach to use (anterior and/or posterior), the number of levels to decompress, and whether or not to use instrumentation and fusion. One hundred and seven of the one hundred and seventy-five patients received anterior discectomy and fusion, thirty-one out of one hundred and seventy-five received anterior corpectomy, seventy-one out of one hundred and seventy-five laminectomy with fusion and two out of one hundred and seventy-five had laminoplasty. At the discretion of the attending surgeon, patients may or may not have received perioperative anti-inflammatory treatment. Patients were evaluated preoperatively and at 6-months following surgery using several assessment tools, including the modified Japanese Orthopedic Association (mJOA) scale, Nurick grade, Neck Disability Index (NDI) and Short-Form-36 (SF-36), as previously described (Fehlings et al., 2015; Fehlings et al., 2013). Based on the mJOA score, the severity of myelopathy can be classified as mild (15–17), moderate (12–14) and severe ($0-11$) (Tetreault et al., 2017). Investigators were required to record all adverse events that occurred throughout the study period. Surgeons could select from a list of anticipated complications (e.g. dysphagia, C5 nerve root palsy, non-union) or specify the details of the event in a textbox. All adverse events were then adjudicated by an external panel and classified as either related to DCM, related to surgery, or unrelated. Perioperative complications were defined as a surgery-related event occurring within 30 days of the operation. Further details of the CSM-NA and CSM-I studies are summarized in the primary publications (Fehlings et al., 2015; Fehlings et al., 2013). Retrospectively, electronic patient records were accessed to obtain data on the number of monocytes before decompression and within 24-h after surgery. Monocyte levels and differentials were computed using a variety of quantitative and qualitative techniques. Briefly, a drop of blood was placed on a glass slide, air dried and then stained with Wright or May-Grunewald-Giemsa stain. Two hundred cells were counted and classified either manually or with an automated machine. Absolute numbers were computed using the differential count and the total number of white blood cells per volume.

2.2. Patient clinical outcomes

SAS v9.4 software (SAS Institute, Cary, NC, USA) was used to conduct all statistical analyses. Continuous variables were summarized using means, standard deviations and ranges. Categorical variables were described using frequencies and percentages. Patients were excluded from this analysis if they did not have a complete blood count performed within 24 h of surgery. A paired *t*-test was conducted to evaluate the change in monocyte levels before and after surgery. Change in monocyte count was computed by subtracting preoperative levels from levels obtained within 24 h after surgery. Change in mJOA was calculated by subtracting preoperative scores from scores achieved at 6-months postoperative. A linear regression analysis was conducted to evaluate the association between change in monocyte counts and change in mJOA scores. If this association yielded a p-value < .2, a

multivariate regression analysis was performed to control for previously identified covariates (Tetreault et al., 2013). Patients were defined as having a perioperative neurological complication if they experienced new radiculopathy, C5 nerve root palsy or progression of myelopathy within 30 days of surgery. Logistic regression analyses were used to determine the association between change in monocyte counts and the presence of any perioperative complications or a neurological complication.

2.3. Animals

We used adult 8-week old C57BL/6 female mice purchased from the Ontario Council Institute (Canada). All experiments were carried out in accordance with the recommendations of the Animal Use Committee of the University Health Network (UHN; Toronto, Canada). Evaluations were performed by investigators blinded to the treatment groups for the duration of the study.

2.4. Spinal cord compression and decompression

DCM was induced in mice by implanting an aromatic polyether material underneath C5-C6 laminae to cause progressive compression of the cervical spinal cord, as previously described (Vidal et al., 2017; Vidal et al., 2018). At 12 weeks post-DCM, mice underwent decompressive surgery using a microdrill to remove the osteoid formation between the aromatic polyether and laminae (Vidal et al., 2017; Vidal et al., 2018). All surgical procedures were performed under anaesthesia using 2% isofluorane. Mice received buprenorphine post-operatively for analgesia, and were sacrificed at 5 weeks after decompression, following deep anaesthesia with isofluorane.

2.5. Experimental groups

At 12 weeks following DCM, animals were randomly assigned into one of two experimental groups by receiving an intravenous (i.v) injection of either: 1) liposomes loaded with PBS (herein referred to as control), or 2) liposomes loaded with clodronate treatment (0.01 ml/g, herein referred to as clodronate). Treatments were given 30 min before decompressive surgery and two weeks following decompressive surgery (Fig. 2A). Treatments were purchased from Liposome B·V (The Netherlands).

2.6. Flow cytometric analysis of mouse monocytes

Repeated blood sampling from mice was performed via the saphenous vein without anaesthesia, as previously described (Vidal et al., 2018). Blood samples were collected at 12 weeks following DCM (herein referred to as baseline), and at 24 h, 2 and 5 weeks after decompressive surgery. In addition, cells from the spleen and the bone marrow were isolated and assessed at 5 weeks after decompression (end point of the study) as previously described (Ulndreaj et al., 2016). Red blood cells were lysed in red blood cell lysis buffer (Vidal et al., 2017) and 0.5×10^6 viable cells were stained with viability dye (Fixable viability dye eFluor 780, Biosciences) for 20 min. Next, cells were stained with a cocktail of primary antibodies. The following antibodies were used: Ly6C-Pacific blue (clone HK1.4; BioLegend), Ly6G-PerCP/Cy5.5 (clone 1A8; BioLegend) and CD11b- FITC (clone M1/70; BioLegend). Matching isotype controls were used to set the gates during data acquisition and analysis. Data were acquired using a BD LSR II flow cytometer (BD Biosciences) and analyzed using FlowJo X 10 (Trestar).

2.7. Immunohistological analysis of the spinal cord

Animals were transcardially perfused with phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA) in PBS. The spinal cords were dissected out (0.3 cm rostral and 0.3 cm caudal from the compression epicenter), post-fixed and cryoprotected in 30% sucrose/PBS for 48 h. Coronal sections (30 μm thick) were prepared and blocked (5% normal serum, 1% bovine serum albumin, 0.3% triton X-100 in PBS) for 1 h at room temperature (RT). Incubation with the primary antibody CD11b (1:300, CBL1313, Millipore) was performed overnight (at 4 °C), followed by 1-h incubation with 4′,6-diamidino-2-phenylindole (DAPI, 1:200, Sigma), and the corresponding secondary antibody at RT. Sections were systematically sampled every 360 μm over 3240 μm (9 sections per animal). The fluorescence intensity/positive area of acquired images was automatically quantified by Image J software using a customized script. Lesion morphometry was assessed using Luxol fast blue (LFB) as well as hematoxylin and eosin (H&E) staining. The unbiased Cavalieri probe using Stereo Investigator (MBF Bioscience, Williston, VT) was used for quantification of white and gray matter sparing. Results are expressed as a percentage of the total spinal cord area. In both cases, sections were systematically sampled every 360 μm over 3240 μm (9 sections per animal). CD11b images were acquired using a 20× objective lens with a Nikon eclipse Ti $C2^+$ inverted confocal microscope with NIS element imaging software version 4.20. LFB/H&E images were acquired using a 10× objective lens on the Zeiss Axioplan 2 fluorescence microscope.

2.8. Neurobehavioral assessments

Locomotion of decompressed animals was assessed at 1, 3 and 5 weeks following decompressive surgery (Fig. 2A) using the CatWalk XT 10.6 system (Noldus, The Netherlands), as previously described (Vidal et al., 2017; Vidal et al., 2018). Stance phase, stride length and swing speed were analyzed in both forepaws and hindlimbs. Analysis was carried out averaging three runs per animal, without significant differences in speed between runs (Machado et al., 2015; Vidal et al., 2017). Mechanical allodynia was assessed using a 0.4 g von Frey hair monofilament, as previously described, at 5 weeks following decompressive surgery (Vidal et al., 2017). A group undergoing DCM without surgical decompression was included for reference in the behavioral assessments.

2.9. Statistical analysis

The results were analyzed using Prism 5.0 (GraphPad Software, La Jolla, California, USA), SPSS version 22 (IBM, Armonck, New York, USA) software, and SASv9.4 software (as described above for patient samples). Rodent flow cytometry data were analyzed using either a one-way ANOVA or an unpaired *t*-test. Confocal microscopy data were analyzed using a one-way ANOVA with Holm-Sidak correction for multiple comparisons. CatWalk and von Frey results were analyzed using a two-way ANOVA with Tukey's post-hoc test and an unpaired t-test, respectively. All data are presented as mean ± standard error of the mean (SEM). Results were considered significant at a p-value $\leq .05$.

3. Results

3.1. Monocyte levels increase following decompressive surgery in DCM patients

Preoperative and postoperative blood counts were available for 78 patients. Of these, nine were obtained outside the time window (i.e. within 24 h of surgery) and were excluded from the analysis. The sample consisted of 44 (63.77%) men and 25 (36.23%) women, with ages ranging from 34 to 86 years (mean age: 59.59 ± 12.55). The average preoperative mJOA score was 11.94 ± 2.88 (range: 3 to 17) and the average duration of symptoms was 32.75 ± 41.27 months (range: 1 to 240 months). Nineteen (27.54%) patients smoked and 46 (66.67%) had co-morbidities. Thirty-two (46.38%) patients were treated anteriorly, thirty-four (49.28%) posteriorly, and three (4.35%) with a combined anteroposterior approach. The mean number of levels

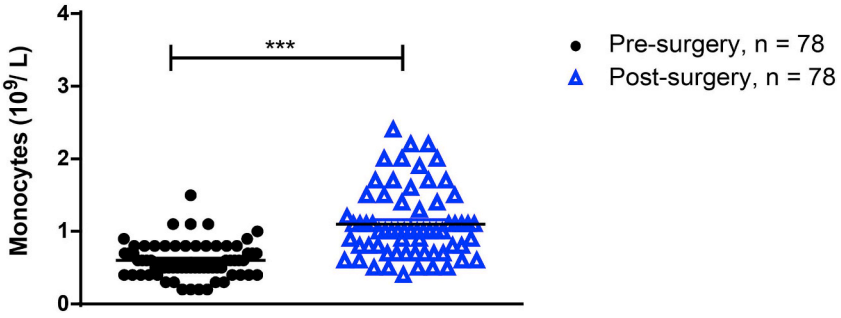

Fig. 1. Decompressive surgery increases acute monocyte levels in DCM patients. Monocyte counts in DCM patients pre-surgery and 24 h following decompressive surgery (post-surgery). A 2-fold increase was observed between pre- and post-surgery (***$p < .001$). Data were analyzed using a paired Student's *t*-test and are presented as mean ± SEM.

decompressed was 4.26 ± 1.38 (range: 2 to 7). Twenty-two patients (31.88%) experienced a complication within 30 days of surgery (range: 0 to 22 days). The most common complications were dural tear ($n = 4$, 5.80%), dysphagia ($n = 3$, 4.35%), and progression of myelopathy (n = 3, 2.90%). Only one subject each (1.45%) experienced a superficial or deep wound infection, C5 nerve root palsy, new radiculopathy or dysphonia. The average preoperative monocyte level was 0.60 ± 0.24 (range: 0.20 to 1.50); this was not associated with preoperative mJOA score ($r = 0.085$, $p = .49$). These levels increased to ~2-fold (1.10 ± 0.48, range: 0.40 to 2.60) by 24 h post-surgery (Fig. 1A). The difference was statistically significant (mean: 0.50, 95%CI: 0.40 to 0.60, $p < .0001$).

3.2. Increased monocyte levels correlate with surgical recovery

To gain insight into the relationship between the rise in monocytes and clinical outcomes for DCM patients following decompressive surgery, a univariate analysis was performed. There was no relationship between the increase in monocytes after surgery and the change in mJOA at 6-months (parameter estimate: 1.34, 95%CI: −0.37 to 3.05, $p = .122$). However, following the addition of preoperative mJOA score and age as co-variants, an increase in monocyte level after surgery was observed and while modest, this significant change was associated with a greater improvement in mJOA scores (Table 1). There was no association between change in monocyte levels and the presence of perioperative complications (OR: 2.68, 95%CI: 0.69 to 10.43, $p = .15$) or neurological complications (OR: 2.87, 95% CI: 0.29 to 28.39, $p = .37$). Rates of complications, however, were low and these results should be interpreted cautiously.

3.3. Systemic monocyte depletion reduces $Ly6C^{low}Ly6G^-$ subsets following decompressive surgery in mice

To better understand the effects of increased systemic monocytes following decompressive surgery in humans, we used a mouse model of DCM associated with severe neuroinflammation (Vidal et al., 2017, 2018) where we injected liposomes loaded with PBS (control group) or clodronate (to deplete peripheral blood monocytes before decompression). Clodronate treatment has been shown to effectively deplete 90% of circulating monocytes (Sunderkotter et al., 2004). This approach abolished the rise of monocytes typically observed following decompressive surgery in order to better understand the relationship between monocytes and long-term locomotor recovery. At 24 h after decompressive surgery, injection of clodronate depleted 70% of circulating monocytes as compared to control ($p \leq .05$) or baseline groups (prior to injection) (Fig. 2B, C; $p \leq .001$). Of note, liposomes loaded with PBS (control group) reduced systemic monocytes compared to both baseline (Fig. 2B, C; $p \leq .05$) and a group of mice receiving only decompressive surgery (orange dotted line). As a result, all comparisons were performed between control and clodronate groups. Monocytes are a heterogeneous cell population with diverse functions and phenotypes. Here we identified three monocyte subsets: $CD11b^+Ly6C^{low}Ly6G^-$, $CD11b^+Ly6C^{int}Ly6G^-$, $CD11b^+Ly6C^{hi}Ly6G^-$ (Auffray et al., 2009), and investigated which subsets would be affected by clodronate treatment. Monocyte depletion significantly decreased the $CD11b^+Ly6C^{low}Ly6G^-$ (herein referred to as $Ly6C^{low}$) subset compared with the control group (Fig. 2 D; $p \leq .01$). Of note, clodronate reduced subsets $Ly6C^{low}$ and $CD11b^+Ly6C^{int}Ly6G^-$ (herein referred to as $Ly6C^{int}$) when compared to baseline levels (Fig. 2 D; $p \leq .05$, $p \leq .001$). The different depletion effect of clodronate on the monocyte populations seen in our study could be explained by the rapid kinetic (< 24 h for $Ly6C^{hi}$ subset) of monocytes in circulation following one single dose of clodronate (Sunderkotter, Nikolic, 2004), as well as their different endocytotic and replenishment capacity (Edin et al., 2013; Tarique et al., 2015).

Furthermore, the incidence of postoperative locomotor complications (reduced ankle movement and plantar stepping, forepaw palsy and upper or lower extremity stiffness) was increased 7.2% following monocyte depletion within the first 24 h after decompressive surgery. However, these results were not significant (Table 2, $p = .78$).

3.4. Clodronate treatment does not lead to long-term changes in bone marrow, splenic or blood monocytes

We assessed the effect of acute monocyte depletion on the blood, bone marrow and spleen, as these organs are the primary reservoirs from which monocytes are recruited after injury (Blomster et al., 2013; Hammond et al., 2014; Swirski et al., 2009). At 24 h after the second clodronate injection (i.e. 2 weeks following decompressive surgery), we observed a 45% reduction in monocyte levels in blood (Fig. 3; $p \leq .05$). However, by week 5 of decompression, no clodronate-dependent differences were observed in the blood (Fig. 3B). Similarly, at 5 weeks, bone marrow monocyte levels did not differ between the two treatment groups (Fig. 3C). Of note, the clodronate treatment did not alter monocyte subsets in the blood, bone marrow or spleen at this time point. Splenic monocytes experienced a 1.2-fold increase in the clodronate treated group (Fig. 3D; $p = .054$), suggesting that the decline in systemic monocytes seen at 2 weeks could be partially due to an

Table 1
Difference in mJOA associated with change in systemic monocytes levels

	Parameter Estimate	95% Confidence Interval	p-value
Change in monocytes	1.37	0.076 to 2.66	0.038
Preoperative mJOA score	−0.72	−0.93 to −0.51	< 0.0001
Age	−0.096	−0.14 to −0.049	0.0001

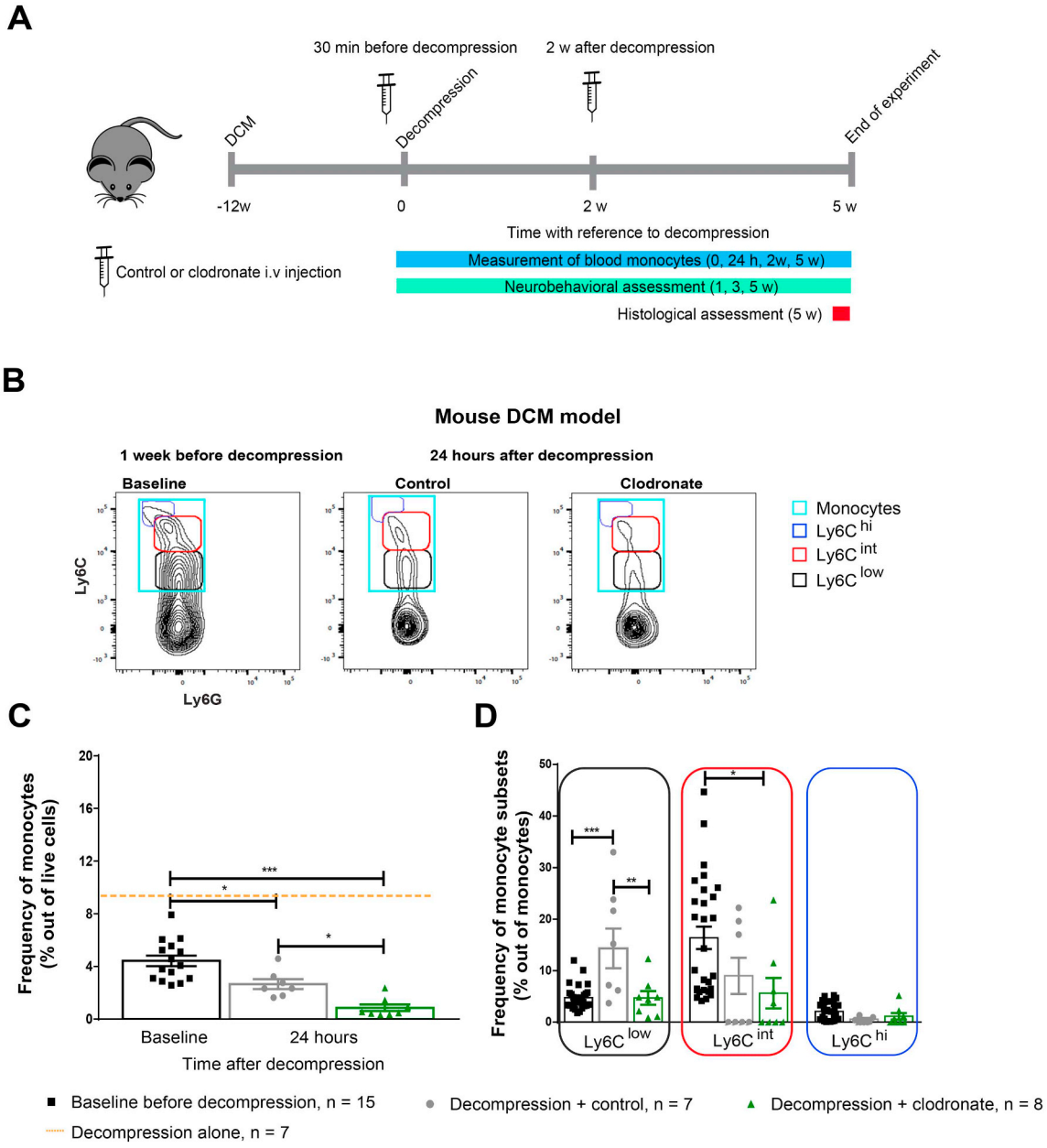

Fig. 2. Clodronate treatment decreases non-classical ($Ly6C^{low}Ly6G^{-}$) monocyte subsets following decompressive surgery in mice. (A) Scheme of the experimental design, where the two time points selected for clodronate or control liposome (i.v) injection are depicted along with the readouts used. The color code indicates the readouts used at selected time points. (B) Blood samples were longitudinally collected and assessed by flow cytometry at two time points, baseline (1 week before decompressive surgery) and 24 h following decompression. Representative flow charts showing a color code for monocytes and each of the three population subsets analyzed. (C) The frequency of monocytes (out of total live white blood cells) was significantly decreased in the control (*$p < .05$) and clodronate (*p < .001) groups as compared with baseline levels. Clodronate treatment led to a 70% depletion of monocytes compared with the control group (*p < .05). Decompression alone also increased monocyte levels compared with all the groups. (D) The $Ly6C^{low}$ monocyte subset was significantly decreased in the clodronate treated group (**$p < .01$), without significant changes observed in the other two subsets at 24 h following decompression. Compared with baseline levels, the control group had a significant increase of the $Ly6C^{low}$ subset (***p < .001), whereas the $Ly6C^{int}$ subset was significantly reduced (*$p < .05$). Data were analyzed using a one-way ANOVA with Tukey's post hoc test and are presented as mean ± SEM.

Table 2
Motor complications after decompressive surgery

Groups	Day 1	Day 2	No complications
Decompression + control	2 animals (28.6%)	1 animal (14.3%)	4 animals (57.1%)
Decompression + clodronate	3 animals (37.5%)	1 animal (12.5%)	4 animals (50%)

accumulation of monocytes in the spleen, as previously reported (Swirski, Nahrendorf, 2009).

3.5. Clodronate treatment does not promote myeloid cell infiltration nor tissue injury

Circulating monocytes are known to differentiate into macrophages when they infiltrate the spinal cord after injury; thus, we investigated whether clodronate treatment decreased the level of infiltrating monocytes in the spinal cord. Histological assessment for CD11b, a

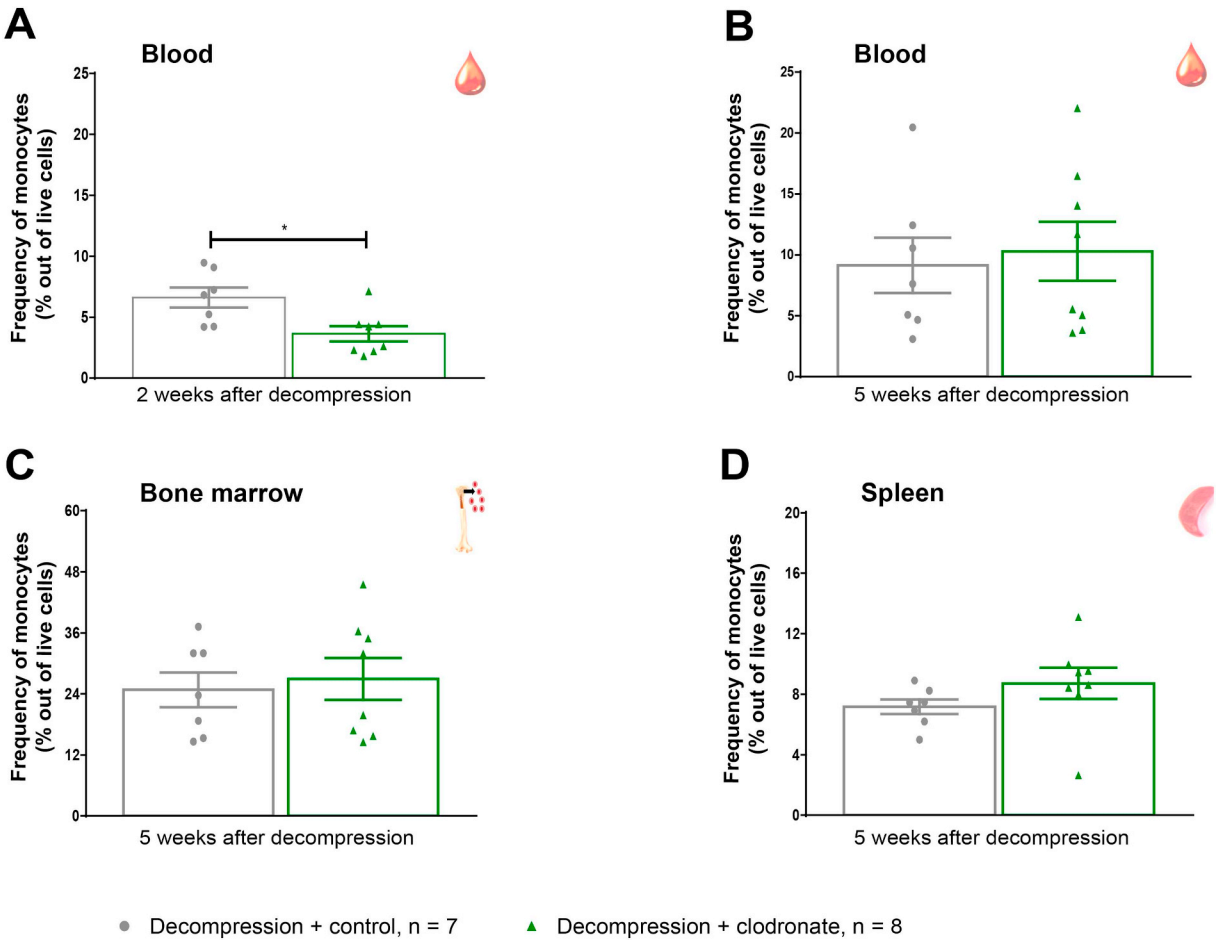

Fig. 3. Clodronate treatment does not induce long-term changes in monocytes. The frequency of monocytes was assessed by flow cytometry at 2 and 5 weeks following decompressive surgery in blood. (A) A 45% reduction in monocyte frequency was observed at 2 weeks following decompressive surgery ($p = .04$), whereas at 5 weeks monocyte levels were not significantly affected in blood (B), bone marrow (C) or the spleen (D) between the two groups. Data were analyzed using an unpaired Student's *t*-test and are presented as mean ± SEM.

common myeloid cell-surface marker for microglia, monocytes and blood-derived macrophages (Saijo and Glass, 2011), revealed an overall modest increase in expression (Fig. 4A-B, p = .08) in the clodronate treated group, reaching significance only at 720 µm rostral from the decompression area (Fig. 4C, $p \leq .05$).

Spinal cord gray and white matter degenerates during progression of the disease in both DCM patients and experimental rodent models of DCM (Karadimas et al., 2013; Yu et al., 2011). Treatment with clodronate to deplete monocytes has been shown to preserve functional white matter tracts in the brain following traumatic brain injury (Makinde et al., 2018). However, clodronate treatment following decompressive surgery did not lead to an overall increase in white or gray matter area compared with the control group (Fig. 4D–F). Of note, clodronate treated animals presented with some areas of high cell infiltration, possibly monocytes, in the spinal cord (Fig. 4D).

3.6. Clodronate treatment leads to modest impairment in long-term functional recovery

Overground locomotor assessments were performed for 5 weeks following decompressive surgery, using the CatWalk system (Vidal et al., 2017, 2018) (Fig. 5A). At 5 weeks after decompressive surgery, swing speed significantly worsened in the hindlimbs of clodronate-treated animals compared with the control group (Fig. 5B; $p \leq .05$). Neither stride length nor stance phase was affected by clodronate administration (Fig. 5C–D). To assess mechanical allodynia in the forelimbs and hindlimbs, we measured the frequency of response to a von Frey hair filament at 5 weeks following decompressive surgery, as previously described (Vidal et al., 2017). However, clodronate treatment did not significantly affect pain development compared with the control group (Fig. 5E). Of note, the frequency of response to the von Frey hair filament was significantly increased in the forepaws of both decompressed groups ($p \leq .05$, $p \leq .001$), as well as in the hindlimbs of the control group undergoing decompression as compared to the DCM only group ($p \leq .05$) (Fig. 5E).

4. Discussion

In this study, we show for the first time that an association exists between an increased number of monocytes and clinical recovery in DCM patients following decompressive surgery. To further examine the role of monocytes after decompressive surgery, we injected clodronate to deplete systemic monocytes in a mouse model of DCM. We found that peri- and post-operative clodronate injections reduced systemic monocyte levels by 70% and 45%, respectively, leading to a 7.2% increase in acute post-operative locomotor complications and modest long-term neurobehavioral impairments.

Monocytes are particularly important for wound healing after central nervous system injury and are needed for proper brain function (Zhan et al., 2014), regulating inflammation and combating infections (Herz et al., 2017). Herein, we observed high systemic monocyte levels following decompressive surgery in DCM patients. A similar situation

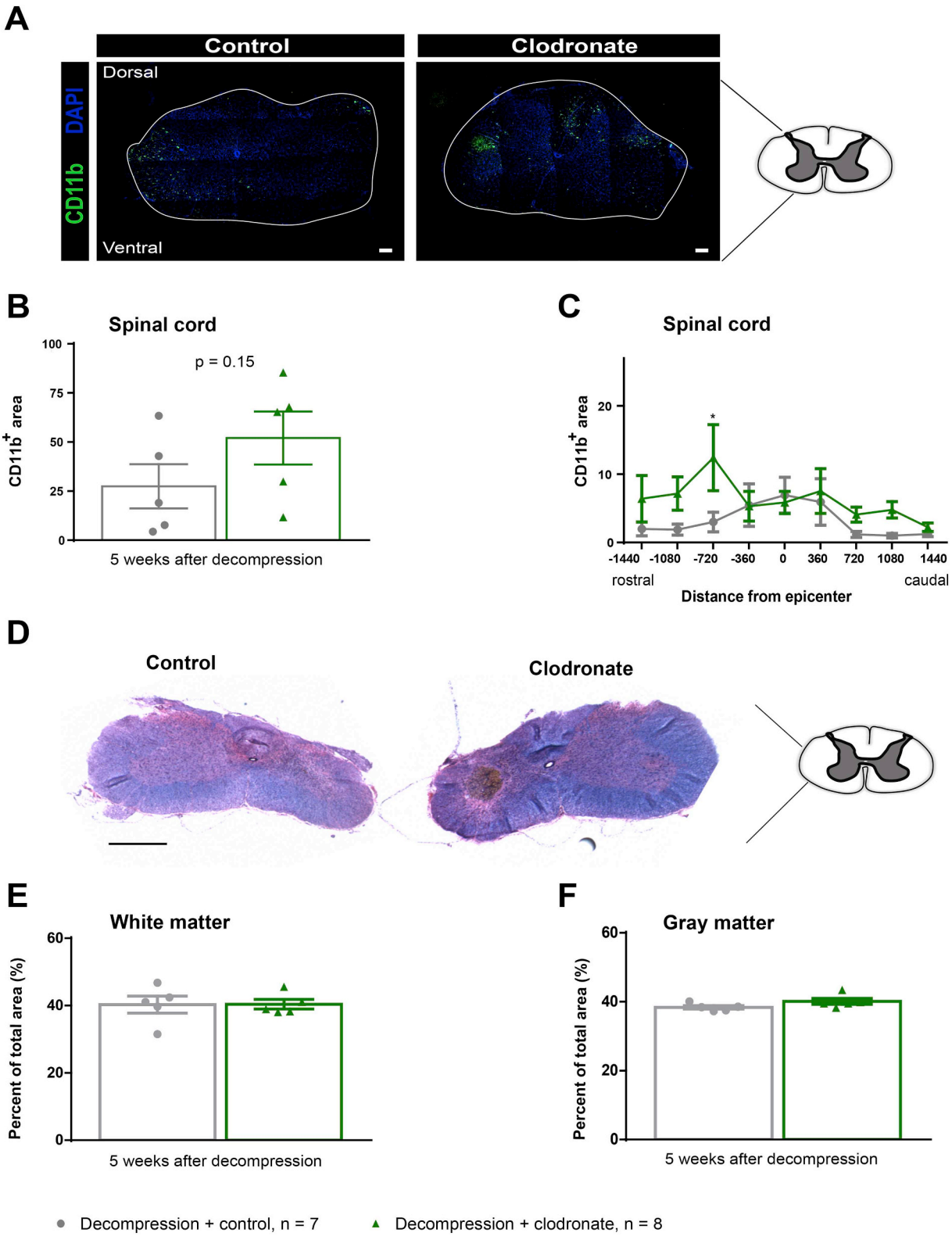

Fig. 4. Clodronate treatment does not promote tissue injury. Immunohistochemical analysis of the decompressed spinal cord around the C5-C7 region at 5 weeks following decompressive surgery. (A) Representative images for each treatment group and a schematic of the spinal cord sections depicting the areas analyzed are shown. A non-significant increase in CD11b$^+$ area was observed after clodronate treatment (B), which reached significance only at -720 µm rostral to the decompressed area (C) (*$p < .05$). (D) Representative cross-sectional serial spinal cord sections stained with LFB/H&E from control and clodronate groups. Unbiased measurements were made with the Cavalieri estimator and showed non-significant differences in white and gray matter percent area between the two treatment groups (E). Data were analyzed using an unpaired Student's t-test (B, *E*-F), and two-way ANOVA with Holm Sidak correction for multiple comparisons (C), and are presented as mean ± SEM. Scale bars = 25 µm (A) and 500 µm (D).

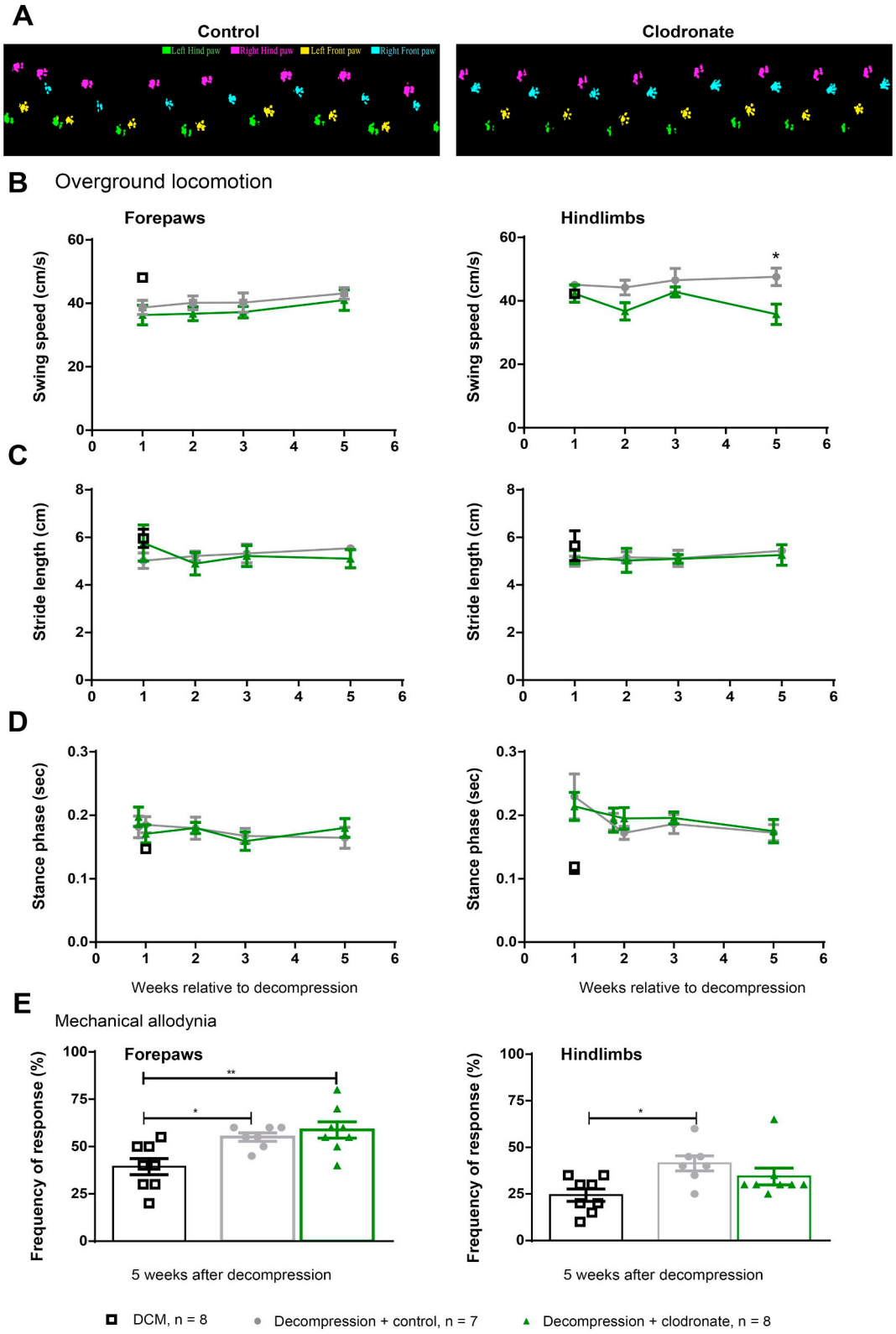

Fig. 5. Systemic monocyte depletion impaired long-term neurobehavioral recovery. Locomotor recovery was quantified using the CatWalk system at 1, 2, 3 and 5 weeks following decompressive surgery. (A) Representative footprints of control- and clodronate-treated groups at 5 weeks after decompression. (B) Swing speed was significantly reduced at 5 weeks following clodrante treatment (*p < .05), without significant changes in the forepaws, stride length (C) and stance phase (D). As a reference the open squares represent the gait of DCM animals without undergoing surgical decompression. (E) Mechanical allodynia was analyzed using the von Frey hair filament test at 5 weeks after decompressive surgery. Significant differences were observed between the DCM and the control (*p < .05), or clodronate treated group (*p < .05, **p < .01) in the forepaws and hindlimbs. No significant differences were observed between the two treatment groups in the forepaws and hindlimbs. Data were analyzed using a two-way ANOVA with Tukey's post hoc (B-D) and one-way ANOVA with Tukey's post hoc (E), and are presented as mean ± SEM.

has been observed in patients with stroke or undergoing hip replacement surgery, as well as in a mouse model for delayed decompressive surgery, suggesting that monocytes can be used as a biomarker to predict clinical recovery post-surgery (Gaudilliere et al., 2014; Kaito et al., 2013; Krieg et al., 2018; Liberale et al., 2017; Vidal et al., 2017). Furthermore, in animal models of stroke and SCI, monocytes are rapidly recruited to the injured area (Blomster et al., 2013; Garcia-Bonilla et al., 2016; Hammond et al., 2014; Wattananit et al., 2016), but their effects on recovery are inconclusive and confounded by various factors, including the injury model and post-injury time of assessment (Blomster et al., 2013; Hammond et al., 2014; Wattananit et al., 2016). Our results show that high acute monocyte levels are needed in order to achieve improvements in the mJOA score in DCM patients. Furthermore, in a preclinical mouse model there is a modest contribution of the early response of monocytes to the development of post-operative locomotor complications (first 48 h after decompression) as well as long-term reductions in swing speed following decompressive surgery for DCM.

There are at least four potential reasons for these findings. First, it is possible that early monocyte depletion led to impaired recovery compared to the control group. In an ischemic brain injury model, monocyte depletion has been associated with a shift from the inflammatory to anti-inflammatory monocyte phenotype during the first days after injury (Wattananit et al., 2016). A second possibility is that clodronate administration may have contributed to the vascular remodelling known to take place in DCM and after decompressive surgery (Vidal et al., 2017; Vidal et al., 2018). In a stroke model, clodronate administration triggered perilesional haemorrhage that led to a lack of functional improvement (Gliem et al., 2012). A third possibility is related to the heterogenous phenotype of monocytes, which may have contributed to the initial exacerbation of the immune response mediated by the interaction between monocytes and T cells. For example, in an autoimmune disease model of multiple sclerosis, $CCR2^{+}$ monocytic cells of peripheral origin were shown to mediate activation of T cells to promote development of the pathology (Jordao et al., 2019). A fourth possibility is associated with the decreased systemic levels of the $Ly6C^{low}$ subset within the first 24 h following decompression. This monocyte subset is involved in reparative processes in models of stroke (Gliem, Mausberg, 2012), SCI (Donnelly et al., 2011; Hansen et al., 2016), and skin injury due to their contribution to generate $CD206^{+}$ wound healing macrophages (Olingy et al., 2017), $Ly6C^{low}$/iNOS monocytes (Donnelly et al., 2011), and increased expression of L-selectin (McCreedy et al., 2018). Furthermore, the $Ly6C^{int}$ and $Ly6C^{low}$ monocyte subsets can be recruited to the intact gray matter distal to the injury site, where they have been thought to contribute to the maintenance of locomotor networks (Hansen et al., 2016).

Our neurobehavioral assessments show a modest worsening in hindlimb swing speed without significant changes at the histological level, which can be primarily attributed to: i) the non-linear relationship between histology and functional recovery, and ii) maladaptive plasticity. In traumatic SCI, it is well-established that the relationship between histology and functional recovery is non-linear for non-severe injuries (Fouad et al., 2013). To the best of our knowledge, a relationship between lesional content and neurological recovery in DCM has not been examined systematically. Maladaptive plasticity occurs at the spinal cord and/or the brain level following traumatic SCI (Ferguson et al., 2012; Huie et al., 2017) DCM or decompressive surgery (Green et al., 2015). Maladaptive plasticity can cause considerable neurological deficits despite preservation of gross histology. Microglia have been implicated in modulating and maintaining maladaptive functions in the spinal cord (Morara et al., 2015), as well as shaping neuronal postnatal circuitry in the central nervous system (Meyer, 2013; Schafer et al., 2012; Sellgren et al., 2019). Thus, it is possible that worse neurological outcomes in the clodronate group are driven by microglia (which we found to be significantly increased in the spinal cord following clodronate treatment) despite overall similar gray and white matter content between our groups. Specifically, recruitment and activation of the so-called “dark-microglia” (i.e. mainly characterized by exhibiting signs of oxidative stress and their highly active state) have been associated with pathological conditions (Bisht et al., 2016).

Our study has certain limitations that need to be acknowledged. First, we used a mixed cohort of DCM patients, with some patients receiving perioperative anti-inflammatory treatments that may have masked the effect of decompression on systemic monocytes levels. Thus, future studies should assess changes to monocyte levels in a homogeneous and larger patient population. Also, since our mouse research suggests that non-classical monocytes may drive pathological events after decompressive surgery, clinical studies should analyse different monocyte subsets, and examine any correlation between subsets and recovery after decompression. Our histology method is not detailed enough to provide specific information about the types of neurons and tracts that were affected following decompressive surgery, which could thoroughly explain the neurological recovery observed between the groups. A more detailed characterization of the neuronal circuits that affect locomotion during DCM or following decompressive surgery is outside the scope of this study, but we anticipate that this topic will be explored in future studies. Preliminary research in this area has shown that following decompressive surgery there is axonal sprouting, restoration of synapses and restoration of serotonergic fibers (Dhillon et al., 2016).

Taken together, our study provides preliminary evidence for the involvement of non-classical monocytes in promoting gait recovery and limiting the development of acute locomotor complications following decompressive surgery for DCM. Future research is needed to evaluate the effects of commonly administered perioperative anti-inflammatory treatments on monocyte numbers, activation status, and the potential for long-term locomotor recovery in DCM patients.

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Declaration of Competing Interest

The authors declare no conflicts of interest.

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Author's contributions

MGF and PMV conceived the study and the experimental design. AU and PMV performed sample collection and flow cytometry experiments. AU performed randomisation of the experimental groups. PMV performed neurobehavioral tests and statistical analysis. LT collected and analyzed patients' data. JH performed CD11b analysis and wrote script for assessment of $CD11b^{+}$ area. All authors contributed to writing the manuscript.

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