that there was direct contact between the microglia/macrophages and synaptic structures, immune-electron microscopic examination was performed. Consistent with the confocal imaging, this analysis showed that the microglia/macrophages made direct contact with both presynaptic and postsynaptic structures (Figure 6B). These observations indicated that classically activated microglia/macrophages had migrated to the compressed spinal cord and eliminated synaptic terminals.

Discussion

In the present study, we showed that severe chronic progressive spinal cord compression in *twy* mice caused more body weight loss and neurological deficits in motor function than milder spinal cord progression. Furthermore, M1 macrophage-dominant inflammation was present in mice with a severely compressed spinal cord. In agreement, Cyr61, an inducer of M1 macrophages, was also markedly upregulated in these spinal cords. Furthermore, immunostaining and electron microscopic analyses indicated that the inflammatory C1q complement cascade eliminated synapse formation, resulting in neurodegeneration.

Macrophages are typically divided into classically activated (M1) and alternatively activated (M2) macrophages [32]. M1 macrophages, activated via TLRs, produce proinflammatory cytokines and oxidative metabolites [33]. Here we found that the M1 macrophage and TLR signals were activated in the chronically compressed spinal cord. These results were consistent with the distribution of M1 macrophages in traumatic spinal cord injury that continues even during the chronic phase [34,35]. The shift to M1 macrophages, which have deleterious and cytotoxic effects [36], may represent the main pathology of the neurodegeneration that accompanies chronic spinal cord compression.

Although the extracellular matrix has been classically viewed as an inert scaffold, recent studies have revealed that it influences diverse aspects of cellular behavior and function [37]. Cyr61 is a matricellular protein that is highly expressed at sites of inflammation, where its ability to regulate gene expression in macrophages plays an important role [25,38]. In addition, various mechanical stresses induce Cyr61 expression in cartilage/bone tissues and periodontal ligaments [26,39]. Our present data indicated that Cyr61 is significantly upregulated in the chronically, severely compressed spinal cord and colocalizes extensively with reactive astrocytes. These findings suggest that Cyr61 engages in a distinct intracellular signaling cascade in microglia/macrophages and promotes M1 macrophage recruitment in the compressed spinal cord.

Microglia/macrophages were recently identified as the phagocytes responsible for eliminating tagged synapses, via classical complement signaling [40], and the complement cascade is strongly induced in the brain tissues of patients with various neurodegenerative diseases [41]. Interestingly, in a mouse model of glaucoma, a relatively common neurodegenerative disease related to high intraocular pressure, the classical complement pathway is upregulated long before retinal ganglion cell death occurs [28]. Yet another study suggested that initiation of the classical complement pathway via C1q is detrimental to recovery after spinal cord injury [42]. The present microarray and immunohistochemical analyses showed that the classical complement pathway via C1q was significantly upregulated in the severely compressed spinal cord. Our findings raise the intriguing possibility that Clq may also be involved in synapse elimination in the chronically compressed spinal cord. Future studies should examine whether the inhibition of C1q in animal models of chronic spinal cord compression hinders the associated neurodegenerative changes.

Previous studies on the surgical outcomes of CCM patients demonstrated that the postoperative recovery was poor in those with severe canal stenosis, because irreversible changes had occurred in the spinal cord [5]. Recent studies have revealed that neural stem cell therapy can be an effective treatment for previously incurable nervous system disorders, such as spinal cord injury [43-47]. Therefore, an appropriate stem cell treatment for CCM should be examined in future studies.

To our knowledge, these data are the first to document the detailed pathophysiology of the inflammatory response in an animal model of chronic spinal cord compression. The clinical implications are noteworthy, because manipulation of the classical complement cascade in the chronically compressed spinal cord could be a strategy for minimizing synapse loss and secondary neurodegeneration due to inflammation. We believe that our findings are valuable for future research on the alterations taking place in the inflammatory environment in CCM.

Abbreviations

CCM: cervical compressive myelopathy; Cyr61: cysteine-rich protein 61; FOV: field of view; RT-PCR: reverse transcriptase polymerase chain reaction; TLR: toll-like receptor; *twy*: tip-toe walking Yoshimura.

Competing interests

H. Okano is the scientific consultant of San Bio, Inc; Eisai Co Ltd; and Daiichi Sankyo Co Ltd. The remaining authors declare that they have no competing interests.

Authors' contributions

MT, HO, and MN conceived and designed the experiments. MT, SK, YK, SS, and KH performed the experiments. MT, SK, and SS analyzed the data. YT, HO, and MN contributed the reagents, materials, and analysis tools. MT, HO, and MN wrote the paper. All authors read and approved the final manuscript.

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References

1. Bohlman HH, Emery SE: The pathophysiology of cervical spondylosis and myelopathy. *Spine (Phila Pa 1976)* 1988, 13:843–846.
2. Fehlings MG, Skaf G: A review of the pathophysiology of cervical spondylotic myelopathy with insights for potential novel mechanisms drawn from traumatic spinal cord injury. *Spine (Phila Pa 1976)* 1998, 23:2730–2737.
3. Kameyama T, Hashizume Y, Ando T, Takahashi A, Yanagi T, Mizuno J: Spinal cord morphology and pathology in ossification of the posterior longitudinal ligament. *Brain* 1995, 118:263–278.
4. Mizuno J, Nakagawa H, Chang HS, Hashizume Y: Postmortem study of the spinal cord showing snake-eyes appearance due to damage by ossification of the posterior longitudinal ligament and kyphotic deformity. *Spinal Cord* 2005, 43:503–507.
5. Baba H, Imura S, Kawahara N, Nagata S, Tomita K: Osteoplastic laminoplasty for cervical myeloradiculopathy secondary to ossification of the posterior longitudinal ligament. *Int Orthop* 1995, 19:40–45.
6. Iwasaki M, Okuda S, Miyauchi A, Sakaura H, Mukai Y, Yonenobu K, Yoshikawa H: Surgical strategy for cervical myelopathy due to ossification of the posterior longitudinal ligament: part 1: clinical results and limitations of laminoplasty. *Spine (Phila Pa 1976)* 2007, 32:647–653.
7. Okawa A, Nakamura I, Goto S, Moriya H, Nakamura Y, Ikegawa S: Mutation in Npps in a mouse model of ossification of the posterior longitudinal ligament of the spine. *Nat Genet* 1998, 19:271–273.
8. Uchida K, Baba H, Maezawa Y, Kubota C: Progressive changes in neurofilament proteins and growth-associated protein-43 immunoreactivities at the site of cervical spinal cord compression in spinal hyperostotic mice. *Spine (Phila Pa 1976)* 2002, 27:480–486.
9. Yamaura I, Yone K, Nakahara S, Nagamine T, Baba H, Uchida K, Komiya S: Mechanism of destructive pathologic changes in the spinal cord under chronic mechanical compression. *Spine (Phila Pa 1976)* 2002, 27:21–26.
10. Takano M, Komaki Y, Hikishima K, Konomi T, Fujiyoshi K, Tsuji O, Toyama Y, Okano H, Nakamura M: In vivo tracing of neural tracts in tiptoe walking Yoshimura mice by diffusion tensor tractography. *Spine (Phila Pa 1976)* 2013, 38:E66–E72.
11. Yu WR, Baptiste DC, Liu T, Odrobina E, Stanisz GJ, Fehlings MG: Molecular mechanisms of spinal cord dysfunction and cell death in the spinal hyperostotic mouse: implications for the pathophysiology of human cervical spondylotic myelopathy. *Neurobiol Dis* 2009, 33:149–163.
12. Yato Y, Fujimura Y, Nakamura M, Watanabe M, Yabe Y: Decreased choline acetyltransferase activity in the murine spinal cord motoneurons under chronic mechanical compression. *Spinal Cord* 1997, 35:729–734.
13. Yu WR, Liu T, Kiehl TR, Fehlings MG: Human neuropathological and animal model evidence supporting a role for Fas-mediated apoptosis and inflammation in cervical spondylotic myelopathy. *Brain* 2011, 134:1277–1292.
14. Uchida K, Baba H, Maezawa Y, Furukawa S, Furusawa N, Imura S: Histological investigation of spinal cord lesions in the spinal hyperostotic mouse (twy/twy): morphological changes in anterior horn cells and immunoreactivity to neurotropic factors. *J Neurol* 1998, 245:781–793.
15. Baltes C, Radzwill N, Bosshard S, Marek D, Rudin M: Micro MRI of the mouse brain using a novel 400 MHz cryogenic quadrature RF probe. *NMR Biomed* 2009, 22:834–842.
16. Bosshard SC, Baltes C, Wyss MT, Mueggler T, Weber B, Rudin M: Assessment of brain responses to innocuous and noxious electrical forepaw stimulation in mice using BOLD fMRI. *Pain* 2010, 151:655–663.
17. Baba H, Furusawa N, Fukuda M, Maezawa Y, Imura S, Kawahara N, Nakahashi K, Tomita K: Potential role of streptozotocin in enhancing ossification of the posterior longitudinal ligament of the cervical spine in the hereditary spinal hyperostotic mouse (twy/twy). *Eur J Histochem* 1997, 41:191–202.
18. Takano M, Hikishima K, Fujiyoshi K, Shibata S, Yasuda A, Konomi T, Hayashi A, Baba H, Honke K, Toyama Y, Okano H, Nakamura M: MRI characterization of paranodal junction failure and related spinal cord changes in mice. *PLoS One* 2012, 7:e52904.
19. Ogura H, Matsumoto M, Mikoshiba K: Motor discoordination in mutant mice heterozygous for the type 1 inositol 1,4,5-trisphosphate receptor. *Behav Brain Res* 2001, 122:215–219.
20. Mistry DS, Chen Y, Sen GL: Progenitor function in self-renewing human epidermis is maintained by the exosome. *Cell Stem Cell* 2012, 11:127–135.
21. Kigerl KA, Lai W, Rivest S, Hart RP, Satoskar AR, Popovich PG: Toll-like receptor (TLR)-2 and TLR-4 regulate inflammation, gliosis, and myelin sparing after spinal cord injury. *J Neurochem* 2007, 102:37–50.
22. Long HQ, Li GS, Hu Y, Wen CY, Xie WH: HIF-1alpha/VEGF signaling pathway may play a dual role in secondary pathogenesis of cervical myelopathy. *Med Hypotheses* 2012, 79:82–84.
23. Kele J, Simplicio N, Ferri AL, Mira H, Guillemot F, Arenas E, Ang SL: Neurogenin 2 is required for the development of ventral midbrain dopaminergic neurons. *Development* 2006, 133:495–505.
24. Tanabe F, Yone K, Kawabata N, Sakakima H, Matsuda F, Ishidou Y, Maeda S, Abematsu M, Komiya S, Setoguchi T: Accumulation of p62 in degenerated spinal cord under chronic mechanical compression: functional analysis of p62 and autophagy in hypoxic neuronal cells. *Autophagy* 2011, 7:1462–1471.
25. Bai T, Chen CC, Lau LF: Matricellular protein CCN1 activates a proinflammatory genetic program in murine macrophages. *J Immunol* 2010, 184:3223–3232.
26. Chaqour B, Goppelt-Struebe M: Mechanical regulation of the Cyr61/CCN1 and CTGF/CCN2 proteins. *FEBS J* 2006, 273:3639–3649.
27. Kivela R, Kyrolainen H, Selanne H, Komi PV, Kainulainen H, Vihko V: A single bout of exercise with high mechanical loading induces the expression of Cyr61/CCN1 and CTGF/CCN2 in human skeletal muscle. *J Appl Physiol* 2007, 103:1395–1401.
28. Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, Nouri N, Micheva KD, Mehalow AK, Huberman AD, Stafford B, Sher A, Litke AM, Lambris JD, Smith SJ, John SW, Barres BA: The classical complement cascade mediates CNS synapse elimination. *Cell* 2007, 131:1164–1178.
29. Zhang Z, Pinto AM, Wan L, Wang W, Berg MG, Oliva I, Singh LN, Dengler C, Wei Z, Dreyfuss G: Dysregulation of synaptogenesis genes antecedes motor neuron pathology in spinal muscular atrophy. *Proc Natl Acad Sci U S A* 2013, 110:19348–19353.
30. Stephan AH, Madison DV, Mateos JM, Fraser DA, Lovelett EA, Coutellier L, Kim L, Tsai HH, Huang EJ, Rowitch DH, Berns DS, Tenner AJ, Shamloo M, Barres BA: A dramatic increase of C1q protein in the CNS during normal aging. *J Neurosci* 2013, 33:13460–13474.
31. Naito AT, Sumida T, Nomura S, Liu ML, Higo T, Nakagawa A, Okada K, Sakai T, Hashimoto A, Hara Y, Shimizu I, Zhu W, Toko H, Katada A, Akazawa H, Oka T, Lee JK, Minamino T, Nagai T, Walsh K, Kikuchi A, Matsumoto M, Botto M, Shiojima I, Komuro I: Complement C1q activates canonical Wnt signaling and promotes aging-related phenotypes. *Cell* 2012, 149:1298–1313.
32. Gordon S: Alternative activation of macrophages. *Nat Rev Immunol* 2003, 3:23–35.
33. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M: The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol* 2004, 25:677–686.
34. Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG: Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. *J Neurosci* 2009, 29:13435–13444.
35. Guerrero AR, Uchida K, Nakajima H, Watanabe S, Nakamura M, Johnson WE, Baba H: Blockade of interleukin-6 signaling inhibits the classic pathway and promotes an alternative pathway of macrophage activation after spinal cord injury in mice. *J Neuroinflammation* 2012, 9:40.
36. Laskin DL: Macrophages and inflammatory mediators in chemical toxicity: a battle of forces. *Chem Res Toxicol* 2009, 22:1376–1385.
37. Aszodi A, Legate KR, Nakchbandi I, Fassler R: What mouse mutants teach us about extracellular matrix function. *Annu Rev Cell Dev Biol* 2006, 22:591–621.
38. Jun JI, Lau LF: The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. *Nat Cell Biol* 2010, 12:676–685.
39. Li Y, Li M, Tan L, Huang S, Zhao L, Tang T, Liu J, Zhao Z: Analysis of time-course gene expression profiles of a periodontal ligament tissue model under compression. *Arch Oral Biol* 2012, 58:511–522.

40. Aguzzi A, Barres BA, Bennett ML: **Microglia: scapegoat, saboteur, or something else?** *Science* 2013, **339**:156–161.
41. Stephan AH, Barres BA, Stevens B: **The complement system: an unexpected role in synaptic pruning during development and disease.** *Annu Rev Neurosci* 2012, **35**:369–389.
42. Galvan MD, Luchetti S, Burgos AM, Nguyen HX, Hooshmand MJ, Hamers FP, Anderson AJ: **Deficiency in complement C1q improves histological and functional locomotor outcome after spinal cord injury.** *J Neurosci* 2008, **28**:13876–13888.
43. Amoh Y, Li L, Campillo R, Kawahara K, Katsuoka K, Penman S, Hoffman RM: **Implanted hair follicle stem cells form Schwann cells that support repair of severed peripheral nerves.** *Proc Natl Acad Sci U S A* 2005, **102**:17734–17738.
44. Amoh Y, Li L, Katsuoka K, Hoffman RM: **Multipotent hair follicle stem cells promote repair of spinal cord injury and recovery of walking function.** *Cell Cycle* 1865–1869, **2008**:7.
45. Amoh Y, Li L, Katsuoka K, Penman S, Hoffman RM: **Multipotent nestin-positive, keratin-negative hair-follicle bulge stem cells can form neurons.** *Proc Natl Acad Sci U S A* 2005, **102**:5530–5534.
46. Li L, Mignone J, Yang M, Matic M, Penman S, Enikolopov G, Hoffman RM: **Nestin expression in hair follicle sheath progenitor cells.** *Proc Natl Acad Sci U S A* 2003, **100**:9958–9961.
47. Liu F, Uchugonova A, Kimura H, Zhang C, Zhao M, Zhang L, Koenig K, Duong J, Aki R, Saito N, Mii S, Amoh Y, Katsuoka K, Hoffman RM: **The bulge area is the major hair follicle source of nestin-expressing pluripotent stem cells which can repair the spinal cord compared to the dermal papilla.** *Cell Cycle* 2011, **10**:830–839.

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