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Increased Cx32 expression in spinal cord TrkB oligodendrocytes following peripheral axon injury

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Abstract

Following injury to motor axons in the periphery, retrograde influences from the injury site lead to glial cell plasticity in the vicinity of the injured neurons. Following the transection of peripherally located preganglionic axons of the cervical sympathetic trunk (CST), a population of oligodendrocyte (OL) lineage cells expressing full length TrkB, the cognate receptor for brain derived neurotrophic factor (BDNF), is significantly increased in number in the spinal cord. Such robust plasticity in OL lineage cells in the spinal cord following peripheral axon transection led to the hypothesis that the gap junction communication protein connexin 32 (Cx32), which is specific to OL lineage cells, was influenced by the injury. Following CST transection, Cx32 expression in the spinal cord intermediolateral cell column (IML), the location of the parent cell bodies, was significantly increased. The increased Cx32 expression was localized specifically to TrkB OLs in the IML, rather than other cell types in the OL cell lineage, with the population of Cx32/TrkB cells increased by 59%. Cx32 expression in association with OPCs was significantly decreased at one week following the injury. The results of this study provide evidence that peripheral axon injury can differentially affect the gap junction protein expression in OL lineage cells in the adult rat spinal cord. We conclude that the retrograde influences originating from the peripheral injury site elicit dramatic changes in the CNS expression of Cx32, which in turn may mediate the plasticity of OL lineage cells observed in the spinal cord following peripheral axon injury.

Keywords

retrograde neuronal signaling; gap junction plasticity in spinal cord; oligodendrocyte plasticity in spinal cord; CNS plasticity following peripheral injury; cervical sympathetic trunk; brain derived neurotrophic factor (BDNF) in spinal cord

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INTRODUCTION

Understanding how neurons and glial cells communicate, particularly following injury, is the fundamental basis for understanding neuronal survival. Following injury to motor axons in the periphery, retrograde influences from the injury site lead to plasticity in the centrally located cell bodies. In addition to exhibiting robust neurotransmitter and morphological plasticity, the injured cell bodies release factors into the local environment [1], which in turn serve to activate nearby glial cells [2, 3, 4,5]. These glial cell changes appear to contribute to neuronal survival and regeneration [4,6], yet the specific roles served by the activation of astrocytes, microglia as well as oligodendrocytes (OLs) following peripheral axon injury are poorly understood. In particular, the plasticity of cells in the OL lineage is not well studied, yet the dysregulation of OLs contributes to demyelinating disorders [7,8], mood disorders [9], and lack of recovery following both traumatic brain injury and spinal cord injury [10,11]. Therefore a better understanding of the many factors that influence these cells has important clinical implications.

We recently reported the novel finding that a population of OLs expressing full length TrkB, the cognate receptor for brain derived neurotrophic factor (BDNF), was increased in the vicinity of injured sympathetic preganglionic neuronal cell bodies in the intermediolateral cell column following the transection of the axons in the cervical sympathetic trunk (CST) [5]. Such robust oligodendrocyte plasticity in the spinal cord following CST transection suggested that cell-cell communication in the spinal cord is influenced by the peripheral injury.

Glial cells communicate with each other via gap junction channels that allow for intercellular transfer of ions and small signaling molecules [12]. Gap junctions are comprised of a family of connexin (Cx) membrane proteins which form hemichannels that dock with compatible hemichannels on adjacent cells to form gap junctions [12]. Cx32 (based on MW of 32kDa) is exclusive to cells of the OL lineage and associates mainly with Cx32 on other OL lineage cells to form OL-OL homotypic channels, or with astrocyte Cx26 or Cx30 [13] to form heterotypic channels to communicate with astrocytes.

The observed plasticity of OL lineage cells in the spinal cord following CST transection led to an investigation of whether Cx32 expression in the spinal cord was influenced by the injury. Here, we show that Cx32 expression in the spinal cord is increased following peripheral axon injury and that the increased expression was localized specifically to TrkB OLs rather than other cell types in the OL cell lineage.

MATERIALS AND METHODS

1. Surgery and tissue collection

Young adult (3 months of age) female Sprague Dawley rats (Harlan Labs, Indianapolis, IN) were housed in the Miami University Animal Facilities in a 12:12 light:dark environment at regulated temperature. A 3 cm ventral incision was made on the neck region of the animal. The CST was exposed and gently separated from surrounding tissue and transected approximately 2 mm from the entry into the SCG [14]. After the cut the proximal stump was

placed carefully back into original position in close proximity to the distal stump. The procedure was repeated on the other side. The incision was closed using sutures and tissue glue (Nexaband, Phx, AZ). The CST was exposed, but not transected, in sham animals. The CST was bilaterally transected and animals survived for 1 week (n=6 animals) and compared with shams in which the CST was dissected and exposed but was not cut (n=6 animals). All methods used in this study were approved by the Miami University Institutional Animal Care and Use Committee and efforts were taken to minimize discomfort and pain to the animals and to minimize the number of animals used in the study.

Because the robust glial plasticity following CST transection observed in a previous study was confined primarily to T1 of the spinal cord [5, 6], all analyses in this study were carried out at the T1 level, which was identified by counting the nerve roots extending from the cord and was verified by noting its location immediately caudal to the cervical enlargement.

After the one week survival, animals were transcardially perfused with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB). Spinal cord and SCG tissues were removed, and stored in 0.1M PB, cryoprotected by infiltrating with 30% sucrose in 0.1M PB, embedded in optimal cutting temperature medium (Ted Pella Inc.), and cut using a MICROM HM 550 series cryostat. Coronal sections of T1 spinal cord (18 μm) were thaw mounted onto Superfrost microscope slides in series across approximately 15 slides, with each slide containing a complete representation of the T1 segment with sections approximately 270 μm apart. Sections then were incubated overnight in 0.1M PBS-0.2% Triton-X solution, blocked with normal donkey serum, and then incubated for 48 hrs at 4°C with a cocktail of rabbit anti-TrkB (1:200; SC-12; Santa Cruz Biotechnologies; directed against full length TrkB), mouse anti-Cx32 (1:200; Invitrogen, 358900), rabbit anti-Cx32 (1:200; Invitrogen, 710600), mouse anti-CC1 (1:500; Abcam, ab16794), and mouse anti-NG2 (1:200; Millipore, MAB5384) using the following combinations: TrkB(rabbit)/ Cx32(mouse), NG2(mouse)/Cx32(rabbit), and CC1(mouse)/Cx32(rabbit). Following a series of rinses, sections were incubated for 2 hours in AlexaFluor conjugated antibodies (1:200; Molecular Probes) directed against the primary antibody hosts. Sections then were coverslipped using fluorescent mounting medium with 4',6-diamidino-2-phenylindole (DAPI; Vectashield). Images were captured with a Zeiss 710 laser scanning confocal microscope.

2. Data collection and analysis

Confocal Z-stacks were acquired from the intermediolateral cell column (IML; location of the injured neuronal cell bodies) and the lateral funiculus (LF; white matter area immediately adjacent to the IML which contains axons of the injured neurons) from the left and right side of the T1 spinal cord from each animal. Images were acquired using the 40x oil objective and by taking optical slices at 3 μm intervals, typically obtaining approximately 9 scans per section. Each side of the cord was treated as an individual experiment since the left and right CST were injured independently, resulting in the analysis of 12 different Z-stacks per treatment. The images were collapsed for data collection to form a composite image using Zen 2000 software (Zeiss). Separate composite images were obtained of the

IML and the LF from each side of the cord and each composite was enlarged to 800x on a computer monitor and analyzed using Image Pro 6.3 software.

The analysis of Cx32 was based on methodology described previously by Markoullis and colleagues [15] where a Cx32 plaque was defined as a focal accumulation of connexin 32 immunoreactivity with a size between 0.01 and 1 mm^2. The number of Cx32 plaques in the IML and the adjacent LF was determined by counting all immunolabeled profiles in the field of view, which encompassed an area of approximately 0.2 mm^2 and then expressed as plaques per mm^2. For the overall density of plaques the numbers from the IML and LF were combined. To estimate whether injury affected the number of Cx32 plaques expressed per oligodendrocyte, the number of Cx32 plaques per cell was determined by dividing the total number of Cx32 plaques by the total number of DAPI nuclei within that region. The number of DAPI nuclei was obtained by extracting all DAPI positive nuclei from the area of interest using Image Pro 6.3 software. Neuronal nuclei were excluded based on size and pale staining intensity using DAPI.

The number of Cx32 cells in the IML and in the LF was determined by counting the number of cells (identified by DAPI nucleus) in the field of view of each region that showed two or more Cx32 plaques in association. The number of cells coexpressing TrkB and Cx32 was determined by counting the number of DAPI profiles which showed both TrkB immunoreactivity and Cx32 plaques in association. Similar analyses were carried out for the number of cells co-expressing CC1 and Cx32, and NG2 and Cx32.

In order to provide information regarding the extent of variability within the control sets, a mean was obtained for each set of controls and the values from each cord in the data set (both controls and treatments) were expressed as the proportion of control. These proportional data were square root transformed to stabilize the variance. The transformed values were subjected to statistical analysis using the Mann-Whitney test with significance reported at $p<0.05$ and were used for presentation in the figures.

RESULTS

Cx32 plaques were observed throughout both the IML and the LF of the spinal cord (Fig. 1A.–F.) and resembled the plaques reported in Sargiannidou et al. [16]. The overall density of Cx32 plaques (Fig. 1.G.) throughout the IML and LF averaged 2,793/mm^2 in the shams and 6,314/mm^2 at one week after injury, corresponding to a significant 126% increase ($p=0.009$). This overall increase could be attributed to a significant 108% increase in plaques in the IML ($p=0.03$), where the density of plaques in the sham controls averaged 3,976 plaques/mm^2 and 9,063/mm^2 at one week post-injury. Though there was a trend for an increase in the LF, this value did not reach significance (Fig.1.G.).

The increase in Cx32 plaques could be due to an increased number of cells expressing Cx32 or an increased number of plaques per cell. No significant difference was observed between sham and injury in the number of Cx32 cells in the IML (sham, 240 cells/mm^2; injury, 312 cells/mm^2) or LF (290 cells/mm^2; injury, 335 cells/mm^2). When the number of plaques per cell was taken into account (Fig. 1.H.), the overall number of Cx32 plaques per cell averaged

1.7 plaques per cell in the control. This value was increased by 70% to 2.9 plaques per cell at one week after injury (p=0.007). The overall increase in plaques per cell was attributed to a significant 80% increase in plaques per cell specifically in the IML (p=0.013), which increased from 2.0 plaques per cell in control to 3.6 plaques per cell at one week after injury. Though there was a trend for an increase in the LF, this value did not reach significance (Fig. 1.H.).

In an effort to determine which cell type in the OL lineage was responsible for the increased number of plaques per cell, double labeling of Cx32 with the OL markers TrkB [17,18], CC1 [19], or NG2 [20] was carried out. In a recent study from our lab, the majority of TrkB cells (>80%) were shown to colocalize CC1, indicating their mature phenotype [18], yet a proportion of TrkB cells may represent a separate pool of OL linage cells [18] and thus TrkB cells were considered separately. Cx32 immunofluorescent plaques that associated with TrkB as well as CC1 cells were observed predominantly around the cell body (Figs. 2.A.–B.) while Cx32 plaques associated with NG2 cells were primarily associated with the cellular processes (Fig. 2.C.). The overall proportion of cells that expressed both Cx32 and TrkB was approximately 23% of the Cx32/TrkB population and this proportion was not significantly increased postinjury. However in the IML specifically, the proportion of Cx32/TrkB cells was increased by 59% at one week post-injury (p=0.02; Fig. 2.D.). Overlap in the Cx32/CC1 population was ~20% and no changes were observed following injury (Fig. 2.E.). The overlap in Cx32/NG2 population was approximately 10% in the controls and the overall number of cells that expressed both Cx32 and NG2 was significantly decreased (p=0.003). The decrease was specific to the LF (p=0.02; Fig 2.F.), while no changes were observed in the IML.

DISCUSSION

The results of the present study suggest that retrograde factors released by neurons following peripheral axon injury [2, 3, 4] can modulate the expression of Cx32 in the vicinity of the injured neurons, and that injury-induced changes in Cx32 are specific to different OL subpopulations. To our knowledge, we are the first to show that peripheral axon injury can lead to an increase in Cx32 protein expression in the central nervous system and that, in the spinal cord, OL progenitor cells express Cx32. Similar to previous reports [8, 15] we observed Cx32 expression in both GM and WM subregions of the spinal cord with Cx32 expression was found in the processes and cell bodies of OPCs and on the perimeter of mature OLs [21,22] expressing CC1 or TrkB.

The use of the CST transection model, particularly the CNS glial plasticity that follows peripheral axon injury, provides a unique model for understanding how injured neurons can directly influence glial cells without the complications of secondary injury. Here we observed that injured preganglionic neurons can promote an increase in Cx32 expression specifically in a population of OL lineage cells that expresses the full length TrkB receptor.

Further, we have shown that the majority (86%) of the TrkB cells responding to the injury are mature OLs and typically express CC1 [18], but that a pool of TrkB cells does not express either NG2 or CC1. These TrkB expressing cells, which are increased in number by

the peripheral axon injury, may represent a population of OL lineage cells that has upregulated TrkB expression while 'in transition' from a progenitor to a mature OL state. We are confident that the TrkB cells are of the OL cell lineage since all non-neuronal cells expressing full length TrkB were shown to also express Olig2 (18), and astrocytes express only the truncated isoform of TrkB (23, 24).

The expression of TrkB typically suggests responsiveness to the neurotrophin BDNF. In a previous study, we observed that the mature BDNF isoform was decreased in the spinal cord at 7 days following injury to preganglionic axons [6], a decrease that was attributed to a reduced retrograde supply of target derived BDNF. Yet while BDNF was decreased, full length TrkB protein was significantly increased in the spinal cord 1 week post-injury [6] and paralleled the increase in TrkB OLs observed in the vicinity of the injured neuronal cell bodies at the same post-injury time point [5].

Due to the role of connexins in facilitating the propagation of signaling molecules [25], we propose that the increased Cx32 in TrkB cells during a time of decreased BDNF availability might help to propagate and/or maximize the effects of BDNF on these cells. This would be an important function due to the low availability of BDNF. If OL-OL or OL-astrocyte coupling is increased following the injury, calcium and IP3 in one cell can be propagated throughout the interacting OLs and astrocytes, thereby maximizing the effects of BDNF and possibly inducing the release of survival factors in the vicinity of the injured neurons, and promoting their survival. Astrocyte activation in the facial nucleus following facial nerve injury has been shown to contribute to neuronal survival (26) and it may be that the increased Cx32 expression in TrkB OL lineage cells could facilitate the survival-promoting activities of astrocytes. Indeed we have previously shown a close interaction between astrocytic processes and the injured neuronal cell bodies in the IML of the spinal cord at 7 days following injury [5].

Our results also demonstrated that Cx32 is decreased in the OL progenitor pool following injury, indicating a possible decrease in intercellular communication by NG2 cells. A recent study demonstrated that Cx32 in hippocampal OPCs is down-regulated prior to re-entering the cell cycle [21]. The decreased Cx32 expression in OPCs observed in our model also is suggestive of increased OPC proliferation. In fact, preliminary studies have demonstrated an increase in the number of NG2 cells in the IML at one week following injury [27]. Whether this increase is the result of proliferation has not been investigated, but our findings regarding reduced Cx32 in OPCs would support a proliferative role.

In summary, although the physiological importance of the changes in intercellular communication is not yet fully understood, the results of this study provide evidence that peripheral axon injury can differentially affect the expression of gap junctions in OL lineage cell subpopulations in the spinal cord. The retrograde influences originating from the peripheral injury site elicit dramatic changes in the CNS expression of Cx32, which in turn may mediate the plasticity of OL lineage cells observed in the spinal cord following peripheral axon injury and possibly contribute to survival of the injured neurons.

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REFERENCES

1. Abe N, Cavalli V. Nerve injury signaling. Cur. Opin. Neurobiol. 2008; 18:276–283.
2. Jones KJ, Serpe CJ, Byram SC, Deboy CA, Sanders VM. Role of the immune system in the maintenance of mouse facial motoneuron viability after nerve injury. Brain, Behav, Immun. 2005; 19:12–19. [PubMed: 15581733]
3. Vallejo R, Tilley DM, Vogel L, Benyamin R. The role of glia and the immune system in the development and maintenance of neuropathic pain. Pain Prac. 2010; 10:167–184.
4. Pekny M, Pekna M. Astrocyte reactivity and reactive astrogliosis: costs and benefits. Physio. Rev. 2014; 94:1077–1098.
5. Coulibaly AP, Isaacson LG. Transient changes in spinal cord glial cells following transection of preganglionic sympathetic axons. Auton. Neurosci. 2012; 168:32–42. [PubMed: 22289358]
6. Coulibaly AP, Gannon SM, Hawk K, Walsh BF, Isaacson LG. Transection of preganglionic axons leads to CNS neuronal plasticity followed by survival and target reinnervation. Auton. Neurosci. 2013; 179:49–59. [PubMed: 23891533]
7. Lasiene J, Yamanaka K. Glial cells in amyotrophic lateral sclerosis. Neurol. Res. Inter. 2011 Article ID 718987, 7 pp.
8. Markoullis K, Sargiannidou I, Schiza N, Roncaroli F, Reynolds R, Kleopa KA. Oligodendrocyte gap junction loss and disconnection from reactive astrocytes in multiple sclerosis gray matter. J. Neuropathol. Exp. Neurol. 2014; 73:865–879. [PubMed: 25101702]
9. Karoutzou G, Emrich HM, Dietrich DE. The myelin-pathogenesis puzzle in schizophrenia: a literature review. Mole. Psych. 2008; 13:245–260.
10. Dewar D, Underhill SM, Goldberg MP. Oligodendrocytes and ischemic brain injury. Cereb. Blood Flow Metab. J. 2003; 23:263–274.
11. McTigue DM, Horner PJ, Stokes BT, Gage FH. Neurotrophin-3 and brain-derived neurotrophic factor induce oligodendrocyte proliferation and myelination of regenerating axons in the contused adult rat spinal cord. J. Neurosci. 1998; 18:5354–5365. [PubMed: 9651218]
12. Bedner P, Steinhäuser C, Theis M. Functional redundancy and compensation among members of gap junction protein families? Biochim. Biophys. Acta. 2012; 1818:1971–1984. [PubMed: 22044799]
13. Theis M, M, Giaume C. Connexin-based intercellular communication and astrocyte heterogeneity. Brain Res. 2012; 1487:88–98. [PubMed: 22789907]
14. Sun Y, Zigmond RE. Involvement of leukemia inhibitory factor in the increases in galanin and vasoactive intestinal peptide mRNA and the decreases in neuropeptide Y and tyrosine hydroxylase mRNA in sympathetic neurons after axotomy. J. Neurochem. 1996; 67:1751–1760. [PubMed: 8858962]
15. Markoullis K, Sargiannidou I, Gardner C, Hadjisavvas A, Reynolds R, Kleopa KA. Disruption of oligodendrocyte gap junctions in experimental autoimmune encephalomyelitis. Glia. 2012; 60:1053–1066. [PubMed: 22461072]
16. Sargiannidou I, Vavlitou N, Aristodemou S, Hadjisavvas A, Kyriacou K, Scherer SS, Kleopa KA. Connexin32 mutations cause loss of function in Schwann cells and oligodendrocytes leading to PNS and CNS myelination defects. J. Neurosci. 2009; 29:4736–4749. [PubMed: 19369543]
17. McCartney AM, Abejuela VL, Isaacson LG. Characterization of trkB immunoreactive cells in the intermediolateral cell column of the rat spinal cord. Neurosci. Lett. 2008; 440:103–108. [PubMed: 18550280]
18. Coulibaly AP, Deer MR, Isaacson LG. Distribution and phenotype of TrkB oligodendrocyte lineage cells in adult rat spinal cord. Brain Res. 2014; 1582:21–33. [PubMed: 25072185]

19. Bhat RV, Axt KJ, Fosnaugh JS, Smith KJ, Johnson KA, Hill DE, Baraban JM. Expression of the APC tumor suppressor protein in oligodendroglia. Glia. 1996; 17(2):169–174. [PubMed: 8776583]
20. Nishiyama A, Komitova M, Suzuki R, Zhu X. Polydendrocytes (NG2 cells): multifunctional cells with lineage plasticity. Nature Rev: Neurosci. 2009; 10:9–22. [PubMed: 19096367]
21. Melanson-Drapeau, Beyko S, Dave S, Hebb ALO, Franks DJ, Sellitto C, Bennett SAL. Oligodendrocyte progenitor enrichment in the connexin32 null-mutant mouse. J Neurosci. 2003; 23:1759–1768. [PubMed: 12629180]
22. Kleopa KA, Orthmann JL, Enriquez A, Paul DL, Scherer SS. Unique distributions of the gap junction proteins connexin29, connexin32, and connexin47 in oligodendrocytes. Glia. 2004; 47:346–357. [PubMed: 15293232]
23. Rose CR, Blum R, Pichler B, Lepier A, Kafitz KW, Konnerth A. Truncated TrkBT1 mediates neurotrophin-evoked calcium signalling in glia cells. Nature. 2003; 426:74–78. [PubMed: 14603320]
24. Colombo, Emanuela; Cordiglieri, Chiara; Melli, Giorgia; Newcombe, Jia; Krumbholz, Markus; Parada, Luis F.; Medico, Enzo; Hohlfeld, Reinhard; Meinl, Edgar; Farina, Cinthia. Stimulation of the neurotrophin receptor TrkB on astrocytes drives nitric oxide production and neurodegeneration. J Experimental Medicine. 2012; 209:521–535.
25. Maglione M, Tress O, Haas B, Karram K, Trotter J, Willecke K, Kettenmann H. Oligodendrocytes in mouse corpus callosum are coupled via gap junction channels formed by connexin47 and connexin32. Glia. 2010; 58:1104–1117. [PubMed: 20468052]
26. Tyzack GE, Sitnikov S, Barson D, Adams-Carr KL, Lau NK, Kwok JC, Zhao C, Franklin RJM, Karadottir RT, Fawcett JW, Lakatos A. Astrocyte response to motor neuron injury promotes structural synaptic plasticity via STAT3-regulated TSP-1 expression. Nat. Commun. 2014; 5:4294–4309. [PubMed: 25014177]
27. Coulibaly, AP.; Isaacson, LG. Changes in mature and progenitor oligodendrocyte populations in the spinal cord following distal preganglionic axon injury, Program No. 234.15, 2012 Neurosci. Meeting Planner. New Orleans, LA: Soc. for Neurosci; 2012. Online

HIGHLIGHTS

- Gap junction protein connexin 32 (Cx32) was increased in spinal cord after peripheral axon injury.
- The increase in Cx32 in spinal cord at one week post-injury was localized to TrkB oligodendrocytes.
- Cx32 in oligodendrocyte precursor cells (OPCs) was decreased following peripheral injury.
- Retrograde influences from the peripheral injury site elicit changes in CNS Cx32 expression
- Cx32 may mediate the glial cell plasticity observed in spinal cord following peripheral injury

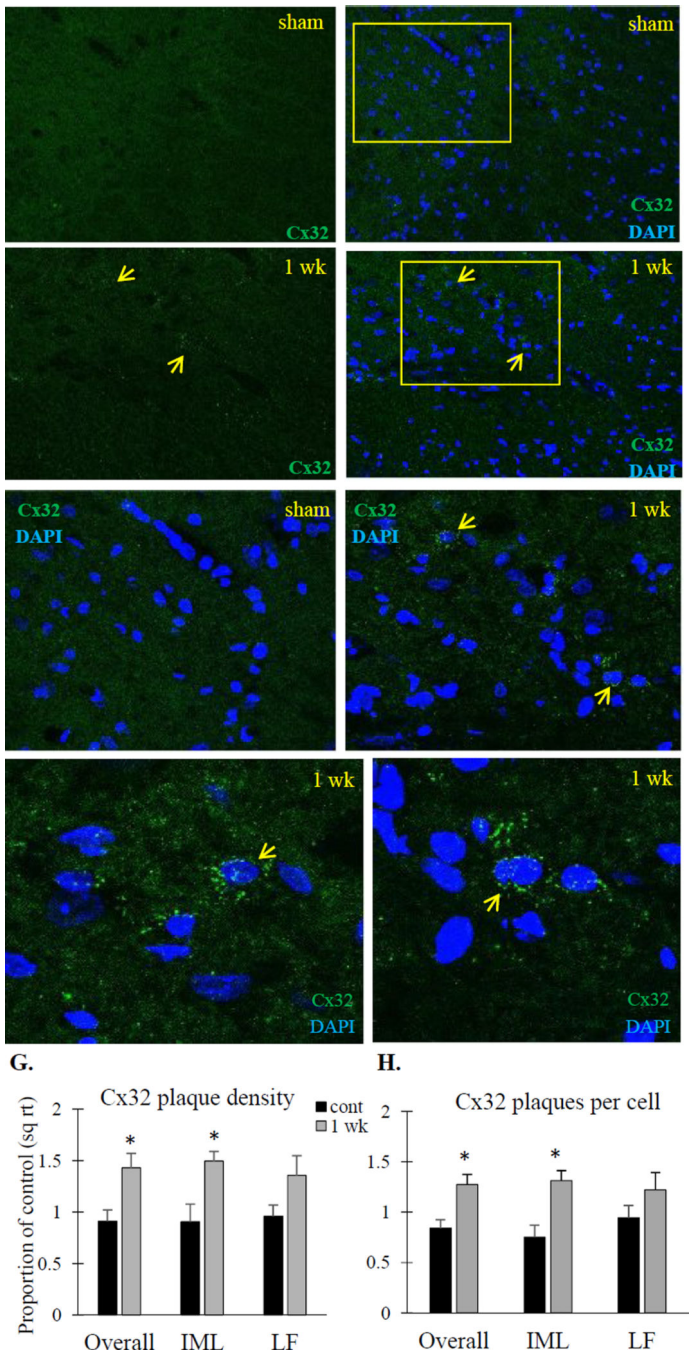

Figure 1.

Cx32 expression is increased in the IML following peripheral axon injury. Confocal micrographs of Cx32 (green) in the IML from rats following sham surgery (**A.-A'.**) or one week post-injury (**B.-B.'**) show increased plaques following injury. Dashed lines in A. and B. delineate IML (upper) and LF (lower) in the spinal cord. **C.-D.** Boxed areas in A. and B. shown at higher magnification reveal details of Cx32 plaques (yellow arrows) in the IML. **E.-F.** Higher magnification of two regions (arrows in D.) show Cx32 plaques associated with DAPI labeled nuclei (blue). Scale bar for A.-B.=50 μm; C.-D.=25 μm; E.-F.=10 μm. DAPI

(blue) shows cellular nature of labeled profiles. **G.** The number of Cx32 plaques overall and specifically in the intermediolateral cell column (IML) was increased at 1 week (1wk) post-injury. **H.** The number of Cx32 plaques per cell was increased overall and in the IML at 1 week post-injury. *, $p < 0.05$.

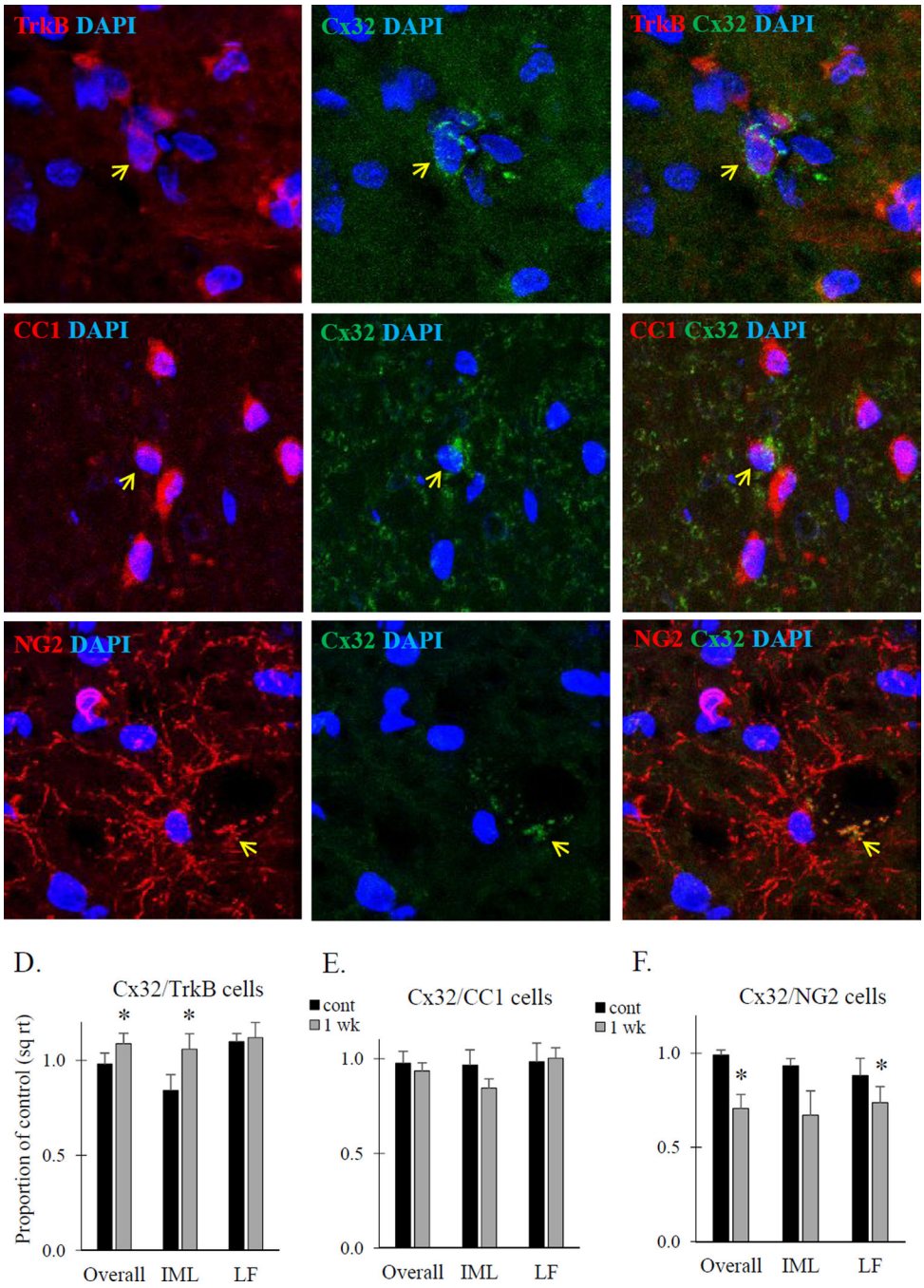

Figure 2.

Cx32 plaques in association with OL lineage cells in the IML. **A.-A".** Cx32 expression (green; arrows) was found on the perimeter of the cell body of TrkB cells (red). **B.–B''.** Similar to TrkB cells, Cx32 expression (green; arrows) was found on the perimeter of CC1 cells (red). **C.-C".** Cx32 (green) was found primarily associated with the processes of NG2 cells (yellow arrows). DAPI (blue) shows cellular nature of stained profiles. Scale bar for all images = 12.5 μm. **D.** Cx32/TrkB cells were increased overall and in the IML. **E.** No

changes were observed in Cx32/CC1 cells. **F.** Cx32/NG2 ce lls were decreased overall and in the LF. *, p <0.05.