Elsevier Editorial System(tm) for Neuroscience
Manuscript Draft

Manuscript Number:

Title: Novel distribution of CD200 adhesion molecule in glial cells of the peripheral nervous system of rats and its modulation after nerve injury

Article Type: Research Paper

Section/Category: Cellular and Molecular Neuroscience

Keywords: OX2; immunosuppression; Schwann cell; axonal degeneration; culture

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Abstract: This study examined CD200 expression in different peripheral nerves and ganglia. Intense CD200 immunoreactivity was consistently localized in unmyelinated nerve fibers as opposed to a faint immunostaining in the myelinated nerve fibers. By light microscopy, structures resembling the node of Ranvier and Schmidt-Lanterman incisures in the myelinated nerve fibers displayed CD200 immunoreactiity. Ultrastructural study revealed CD200 expression on the neurilemma of Schwann cells whose microvilli and paranodal loops at the node of Ranvier were immunoreactive. The CD200 immunoexpression was also localized in the satellite glial cells of sensory and autonomic ganglia and in the enteric glial cells. Double labeling of CD200 with specific antigens of satellite glia or Schwann cells in the primary cultures of dorsal root ganglia had shown a differential expression of CD200 in the peripheral glial cells. The existence of CD200 in glial cells in the peripheral nervous system (PNS) was corroborated by the expression of CD200 mRNA and protein in a rat Schwann cell line RSC96. Using the model of crush or transected sciatic nerve, it was found that CD200 expression was attenuated or diminished at the site of lesion. A remarkable feature, however, was an increase in incidence of CD200-labelled Schmidt-Lanterman incisures proximal to the injured site at 7 days postlesion. Because CD200 has been reported to impart immunosuppressive signal, we suggest that its localization in PNS glial cells may play a novel inhibitory role in immune homeostasis in both normal and pathological conditions.

Suggested Reviewers:

Opposed Reviewers:

Professor S.G. Lisberger
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December 6, 2010

Dear Professor Lisberger,

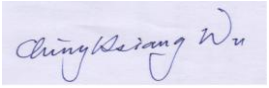
I submit herewith a paper entitled "**Novel distribution of CD200 adhesion molecule in glial cells of the peripheral nervous system of rats and its modulation after nerve injury**" for consideration to be published in *Neuroscience*.

I have read and have abided by the statement of ethical standards for manuscripts submitted to Neuroscience.

I declare that the work described has not been submitted for publication, in whole or in part, elsewhere and all the authors listed have approved the manuscript that is enclosed.

Author contribution
Chiu-Yun Chang: conception and design, data analysis and interpretation, manuscript writing
Yi-Hsuan Lee: collection and/or assembly of data, revising it critically for important intellectual content
Ya-Fen Jiang-Shieh: collection and/or assembly of data
Hsiung-Fei Chien: collection and/or assembly of data
Man-Hui Pai: data analysis and interpretation
Hui-Min Chen: data analysis and interpretation
Tsorng-Harn Fong: conception and design, final approval of manuscript
Ching-Hsiang Wu: conception and design, final approval of manuscript

Yours sincerely,



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Novel distribution of CD200 adhesion molecule in glial cells of the peripheral nervous system of rats and its modulation after nerve injury

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Abbreviations:

CAMs	cell adhesion molecules
CD200	cluster of differentiation 200
CNS	central nervous system
DAB	3,3' -diaminobenzidine
DMEM	Dulbecco's Modified Eagles Medium
DRG	dorsal root ganglion
EGCs	enteric glial cells
FBS	fetal bovine serum
GAPDH	glyceraldehydes-3-phosphate dehydrogenase
GFAP	glial fibrillary acidic protein
IgSF	immunoglobulin gene superfamily
MAG	myelin-associated glycoprotein
NCAM	neural cell adhesion molecule
NgCAM	neuron-glia cell adhesion molecule
PGP9.5	protein gene product 9.5
PNS	peripheral nervous system
PVDF	polyvinylidene fluoride
RSC96	rat Schwann cell line
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SGC	satellite glial cell
SLIs	Schmidt-Lanterman incisures
TBS	tris-buffer saline

Abstract

This study examined CD200 expression in different peripheral nerves and ganglia. Intense CD200 immunoreactivity was consistently localized in unmyelinated nerve fibers as opposed to a faint immunostaining in the myelinated nerve fibers. By light microscopy, structures resembling the node of Ranvier and Schmidt-Lanterman incisures in the myelinated nerve fibers displayed CD200 immunoreactiity. Ultrastructural study revealed CD200 expression on the neurilemma of Schwann cells whose microvilli and paranodal loops at the node of Ranvier were immunoreactive. The CD200 immunoexpression was also localized in the satellite glial cells of sensory and autonomic ganglia and in the enteric glial cells. Double labeling of CD200 with specific antigens of satellite glia or Schwann cells in the primary cultures of dorsal root ganglia had shown a differential expression of CD200 in the peripheral glial cells. The existence of CD200 in glial cells in the peripheral nervous system (PNS) was corroborated by the expression of CD200 mRNA and protein in a rat Schwann cell line RSC96. Using the model of crush or transected sciatic nerve, it was found that CD200 expression was attenuated or diminished at the site of lesion. A remarkable feature, however, was an increase in incidence of CD200-labelled Schmidt-Lanterman incisures proximal to the injured site at 7 days postlesion. Because CD200 has been reported to impart immunosuppressive signal, we suggest that its localization in PNS glial cells may play a novel inhibitory role in immune homeostasis in both normal and pathological conditions.

Key words: OX2, immunosuppression, Schwann cell, axonal degeneration, culture

Members of the immunoglobulin gene superfamily (IgSF) of cell adhesion molecules (CAMs) are widely expressed throughout the nervous system, and mediate their diverse functions, in part, through their specific membrane-linkages and cell-surface distributions (Barclay et al. 2002). Sequence of cDNA clones indicates that CD200 is a member of the IgSF and contains two extracellular immunoglobulin domains and one short cytoplasmic region unlikely to signal (Barclay et al. 1986). Disruption of CD200 and its ligand interaction precipitated vulnerability to collagen-induced arthritis in mice (Gorczynski et al. 2002). In a separate study, it was demonstrated that CD200 was up-regulated in rodent transplantation models where successful inhibition of rejection was accomplished, and believed to signal immunosuppression following engagement of its receptor, CD200R (Gorczynski et al. 2001). In the nervous system, lack of CD200 resulted in a more rapid onset of experimental autoimmune encephalomyelitis (Hoek et al. 2000). Recent evidence showed that tissue expression of CD200 generated inhibitory or down-regulatory signals to macrophages and microglia within the central nervous system (CNS) and retina (Banerjee et al. 2004). In the light of the above, it was surmised that CD200 may play a more general immunoregulatory role via the interactions with its ligand. One of the reasons for the common immunomodulatory function of CD200 may be attributed to its widespread distribution on a variety of cell types, such as activated T cells, B cells, follicular dendritic cells and some smooth muscle cells (Barclay et al. 1986). While it is generally accepted that CD200 is constitutively expressed by neurons, there is only a modicum of information of its localization in specific neural structures such as neuronal cell bodies and axons in the cerebellum (Morris et al. 1987). Localization of CD200 in the peripheral nervous system (PNS) has remained elusive except for a study by Bartolomé et al. (2002) who reported CD200 expression in the afferent and efferent fibers of rat auditory nerve.

Among cell adhesion molecules of IgSF, myelin-associated glycoprotein (MAG), myelin protein zero (P0), neuron-glia cell adhesion molecule (NgCAM), neuron-glia-related cell

adhesion molecule (NrCAM), neural cell adhesion molecule (NCAM) and neural adhesion molecule L1 were specifically distributed in all major structures and cellular elements of the PNS (Nieke and Schachner, 1985; Mirsky et al. 1986; Grumet, 1992; Krushel et al. 1993; Inuzuka et al. 1993; Haney et al. 1999; Bartsch, 2003). In mice with eliminated-expression of P0 (P0-/-), uncompacted myelin in nerves was predominantly developed and led to a severe dysmyelinating neuropathy (Xu et al. 2000). Similarly, Schwann cells in sensory nerves of L1-deficient mice did not succeed in maintaining normal axonal ensheathment (Haney et al. 1999). In the co-culture system treated with antibodies to L1 or NCAM, the differentiation of Schwann cell or its processes interdigitation into neurite bundles was eliminated respectively (Seilheimer et al. 1989). Following nerve injury, more Schwann cells expressed L1 and NCAM in the distal stump of transected sciatic nerves (Nieke and Schachner, 1985) and NgCAM expression was also significantly augmented (Grumet, 1992). It is evident from the abovementioned findings that PNS CAMs play a significant role in neurite outgrowth promotion and fasciculation, target recognition and myelination. An immediate question is whether PNS CD200 would exert similar functions. We report here a unique distribution of CD200 in major glial elements of the PNS, in particular the enteric glia in the gut, satellite glia in the peripheral ganglia and Schwann cells along the myelinated and un-myelinated axons. CD200 expression in PNS glia was confirmed in an immortalized Schwann cell line RSC96 using reverse transcription-polymerase chain reaction. Our results suggest that Schwann cell CD200 may be regulated in a manner similar to the responses of Schwann cell to nerve injury such as nerve crush or transection at the sciatic nerve.

1 Experimental procedures

1.1 Tissue processing and sciatic nerve injuries

Male Wistar rats weighing 200-250 g were used. All animals had free access to food and water at all times. For animal care and surgical procedures, the Guide for the care and use of

laboratory animals published by National Institutes of Health was followed. All surgical protocols were approved by the University Laboratory Animal Care and Use Committee of Taipei Medical University. The rats including the normal and those subjected to sciatic nerve axotomy were divided into three groups (n = 6 per group): intact/normal, crush and transection groups. They were anesthetized with an intraperitoneal injection of 7% chloral hydrate. The sciatic nerve was exposed in the gluteal region and crushed with a 2-mm diameter bulldog to hold tightly for 30 seconds. The nerve crush and nerve transection were made at the midpoint of the sciatic nerve emerging from the greater sciatic foramen to its bifurcation. The distal stump of the transected sciatic nerve, 1 mm in length was displaced from the proximal stump to prevent nerve reconnection and regeneration. The operated animals were then allowed to survive for 7d. For immunohistochemistry, the rats were fixed by intracardiac perfusion with 50 ml of 0.9% normal saline followed by 300 ml of 4% paraformaldehyde. The dorsal root ganglia (L4~L6) and sciatic nerve were removed and immersed in the same fixative for 2h and kept in 0.1 M phosphate buffer containing 30% sucrose overnight at 4ºC. For electron microscopy, animals were sacrificed by intracardiac perfusion with a fixative containing 4% paraformaldehyde and 0.05% glutaraldehyde in 0.1 M phosphate buffer.

1.2 Immunohistochemistry

After perfusion, the sciatic nerve, phrenic nerve and peripheral ganglia including the dorsal root ganglion (DRG), superior cervical ganglion, submandibular ganglion and intestine plexuses (myenteric and submucous) were removed. Frozen tissue sections were cut at 30 μm in thickness in a cryostat. Free-floating sections were then incubated with CD200 monoclonal antibody (1:100, MCA44G, AbD Serotec, Kidlington, Oxford, UK). Prior to incubation with the antibody, the sections were pretreated with 1% H_2O_2 for 1h to block any possible endogenous peroxidase and then with 2% horse serum for 1h. The sections were incubated in

primary antibody that was diluted 1:100 with 0.05 M Tris-buffer saline (TBS, pH 7.4) containing 0.1% Triton X-100 for 24h at 4ºC. Subsequent antibody detection was carried out using biotinylated horse anti-mouse IgG (1:300, BA-2001, Vector Laboratories, Burlingame, CA, USA), and then incubated with peroxidase-conjugated streptavidin for 1h (1:300, P0397, Dako Cytomation, Carpinteria, CA, USA). Immunoreaction was visualized using 0.025% 3, 3'-diaminobenzidine tetrahydrochloride (DAB, D5637, Sigma Chemical Co., St. Louis, MO, USA) and 0.02% hydrogen peroxide. The sections were examined and photographed under a light microscope.

For double immunofluorescence staining, the sections or cultured cells were blocked for nonspecific IgG binding with normal horse (S-2001, Vector Laboratories, Burlingame, CA, USA) and goat (S-1000, Vector Laboratories, Burlingame, CA, USA) sera for 1h and then incubated with mouse anti-rat CD200 antibody combined with rabbit anti-PGP 9.5 antibody (1:1000, P09936, AbD Serotec, Kidlington, Oxford, UK) for nerve fibers, or rabbit anti-galactocerebroside antibody (1:1000, AB142, Millipore, Billerica, MA, USA), rabbit anti-glial fibrillary acidic protein antibody (1:1000, Q28115, AbD Serotec, Kidlington, Oxford, UK) or rabbit anti-vimentin antibody (1:100, ab7783, abcam, Cambridge, UK) for satellite glia and Schwann cells overnight at 4°C. Following rinsing, the secondary antibodies: goat anti-rabbit IgG conjugated rhodamine (1:300, 111-025-003, Jackson Immunoresearch laboratories, West Grove, PA, USA) and goat anti-mouse IgG conjugated FITC (1:300, 115-095-003, Jackson Immunoresearch laboratories, West Grove, PA, USA) were applied and incubated for 2h at room temperature. After a brief rinsing in buffer, cell nuclei were stained with TOTO-3 iodide (1:5000; T3604, Molecular Probes, Inc. Leiden, Netherlands) for 15 min at room temperature. Sections were then examined and photographed under a Zeiss LSM 510 confocal microscope.

1.3 Quantitative study and statistical analysis

At least three rats from each group (sham/control or crush injury) were used for quantification. Following immunohistochemical staining, five to six sections of the sciatic nerve cut longitudinally were examined at a magnification of 400x. The total number of the node of Ranvier or the Schmidt-Lanterman incisures positive for CD200 at the injury site and at regions 3 mm proximal and distal to the injury site of crushed nerves in a microscopic field (0.226 mm^2) was counted. Values were expressed as number of CD200-positive structures per mm^2. All quantitative data were given as mean ± S.E.M. from at least three rats in each group and analyzed using ANOVA and Student's *t*-test. A *p*-value of less than 0.05 was taken as statistically significant.

1.4 Primary DRG culture and rat Schwann cell line (RSC96)

Male rats 2–3 month old were sacrificed after anesthesia. Approximately 35~40 DRGs in one rat were isolated from the cervical to the sacral levels of the spinal column for *ex vivo* studies as follows. Isolated ganglia were chopped and digested with 1% collagenase and 0.25% trypsin. The cell mixture was centrifuged at 1000 rpm for 5 min. After discarding the supernatant, digested DRG mixture was added into 50-ml centrifuge tube containing 4 ml of 30% and 4 ml of 60% of Percoll gradients, and then centrifuged at 2500 x *g* for 10 minutes. The supernatant and 4 ml of 30% Percoll solution were further overlaid on 3 ml of 30% percoll solution in a 15-ml centrifuge tube and centrifuged at 2000 x *g* for 10 min. The pellet was plated onto poly-L-lysine-coated 35-mm culture dishes and cultured in F-12/10% FBS medium in a humidified tissue culture incubator with 5% CO_2 maintained at 37 ºC. The day of plating was counted as day 1 *in vitro* (1 DIV).

Rat Schwann cell line (RSC96) used in the present study was a kind gift from Dr Ouyan, College of Medicine, Chang Gung University and cultured in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 4 mM L-glutamate, 1.5 g/ml sodium bicarbonate and 1% non-essential amino acids in a humidified atmosphere of 5%

CO_2 and 95% air.

1.5 Electron microscopy

The tissue was sectioned into 50-μm thick slices using a vibratome. Sections were incubated with primary antibody, CD200, for immunoelectron microscopy as that for light microscopy with the substitution of 0.01% Triton X-100 used in the solutions. All sections were then rinsed in phosphate buffer, post-fixed with 1% OsO_4 dissolved in 0.1 M phosphate buffer, dehydrated with alcohol and embedded in Epon-Araldite mixture. Ultra-thin sections were obtained using a diamond knife. Tissue sections without routine double staining were examined and photographed under a Hitachi 600 electron microscope attached with CCD camera.

1.6 Reverse transcription-polymerase chain reaction for rCD200 mRNA

A semi-quantitative method of RT-PCR was used following the manufacturer's instructions. Lyse cells directly in a culture dish by adding 1 ml of trizol reagent to a 3.5-cm diameter dish, and passing the cell lysate several times through a pipette. Via chloroform extraction, total RNA was isolated with subsequent first strand cDNA synthesis being performed on 2 μg DNase treated RNA. The gene specific primers (MD Bio Inc.) were as the follows: sense 5'-GTA TTC AGG AGA CCT TTC TG-3' and anti-sense 5'-TTT CAT TCT TTG CAT CCC CT-3' for rCD200, 822 bp, and sense 5'-GAC CCC TTC ATT GAC CTC AAC-3' and anti-sense 5'-GAT GAC CTT GCC CAC AGC CTT-3' for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 591 bp as an endogenous control for the quality of the cDNA. The PCR conditions were initiated denaturing at 94^{o}C for 3 min followed by 34 cycles at 53^{o}C for 30 seconds, and 72^{o}C for 1 minute with a final extension 10 min. PCR reaction products were then separated on 2% agarose gel stained with ethidium bromide.

1.7 Western blot analysis

Cultured RSC96 cells were washed twice with PBS and scraped down. The cell suspension was spun down, and cell pellets were lysed in the lysis buffer (0.15% Triton X-100 (X-100, Sigma Chemical Co., St. Louis, MO, USA), 10 mM EGTA (E-4378, Sigma Chemical Co., St. Louis, MO, USA), 2 mM $MgCl_2$ (M-8266, Sigma Chemical Co., St. Louis, MO, USA), 60 mM piperazine-*N, N'*-bis 9 (2-ethanesulfonic acid) (PIPES; P-6757, Sigma Chemical Co., St. Louis, MO, USA), 2.5 mM HEPES (H-3375, Sigma Chemical Co., St. Louis, MO, USA), pH 6.9 and then centrifuged at 14000 x *g*, 4^{o}C for 30 min. The supernatants were removed and placed in new Eppendorf tubes for Western blot analysis. Prestained protein ladders (SM0671, Fermentas/Level Bio Inc. TW) were used to determine the molecular weight of the immunoreactive bands. Proteins (100 μg/each) from the RSC96 cells were separated in a 12% gradient SDS-PAGE and transferred onto PVDF membrane (162-0177, Bio-Rad, Laboratories, Inc. TW). The membrane was blocked with 5% non-fat milk powder in TBST (50mM Tris-base [T-1503, Sigma Chemical Co., St. Louis, MO, USA], 150 mM NaCl [S-3014, Sigma Chemical Co., St. Louis, MO, USA], 0.1% Tween 20 [S36374224, Merck, Whitehouse Station, N.Y., USA]), pH 8.2 at room temperature for 1h and incubated with monoclonal CD200 antibody at 4^{o}C overnight. After rinsing three times with TBST, the membrane was incubated with horseradish peroxidase-conjugated secondary anti-mouse antibody (1:5000, 115-035-146, Jackson Immunoresearch laboratories, West Grove, PA, USA) for 2h. CD200 immunoreactivity was visualized using an enhanced chemiluminescence system (WBKLS0500, Millipore, Billerica, MA, USA).

2 Results

2.1 *CD200 expressions in the peripheral ganglia and different types of peripheral nerves*

In the peripheral ganglia, the dorsal root ganglion (Fig. 1A), superior cervical ganglion (Fig. 1B) and submandibular ganglion (Fig. 1C) representing the sensory, sympathetic and parasympathetic ganglia, respectively, were examined. CD200 immunoreactivity was detected in some nerve cells and fibers (Fig. 1A-C), notably intense in the satellite glial cell (SGC) and its sheath lining the exterior surface of the neuronal soma (Fig. 1A-C). Double immunofluorescence staining revealed that the PGP 9.5 positive neuronal cell body was tightly enveloped by a ring of CD200-positive satellite glia (Fig. 1D). Colocalization of GFAP, a specific marker for satellite glia, with CD200 confirmed the expression of CD200 in SGC (Fig. 1D, inset). In the enteric ganglia such as the myenteric and submucous plexuses of the intestine, CD200 positive neural elements were observed in the plexuses (Fig. 1E-F). Double labeling showed that glia marked with GFAP in the plexuses exhibited CD200 immunofluorescence (Fig. 1G). In different types of peripheral nerves e.g. the sciatic nerve and phrenic nerve which contains mixed and predominantly myelinated fibers, respectively, immunoperoxidase staining showed weak CD200 immunoreactivity along the neurilemma. It was, however, more conspicuous at the node of Ranvier and Schmidt-Lanterman incisure of both nerves (Fig. 2A-B). In contrast, the parasympathetic postganglionic nerves in the submandibular gland composed mostly of unmyelinated fibers (Fig. 2C), nearly all nerve fibers were strongly stained with anti-CD200 antibody. This was confirmed by double immunofluorescence labeling with anti-CD200 and anti-PGP 9.5 antibodies (Fig. 2D-E). CD200 immunoreactivity was detected at the neurilemma, node of Ranvier and Schmidt-Lanterman incisures of myelinated nerves (Fig. 2D) and was prominently colocalized with PGP 9.5, a marker for nerve fibers in the unmyelinated nerves (Fig. 2E).

2.2 *CD200 immuno-electron microscopy*

In the dorsal root ganglia, CD200 immunoreaction product was observed in some sensory neurons. Irrespective of neurons labeled by CD200 or the lack of it, the satellite glia

associated with the soma showed intense CD200 immunoreactivity in the cytoplasm as well as the plasma membrane especially in areas juxtaposing the neuron (Fig. 3A-D). Schwann cells of unmyelinated axons showed a specific CD200 distribution that was confined to their plasma membrane facing the intensely immunoreactive unmyelinated fibers (Fig. 3C-F). In the myelinated axons, CD200 immunoreactive products were deposited at the neurilemma but not the myelin sheath and axonal fibers (Fig. 3C-F). CD200 immunoreactivity was evident at the nodes of Ranvier, microvilli and paranodal loops of Schwann cells (Fig. 3E-F).

2.3 *CD200 expression in the crushed or transected sciatic nerve*

At 7 days after nerve crush, a remarked reduction or disappearance of CD200 expression was observed at the neurilemma and nodes of Ranvier at the lesioned site (Fig. 4A). Interestingly, some ovoid structures positive for CD200 were observed (Fig. 4A). The most striking change was an increase in number of CD200 positive Schmidt-Lanterman incisures in the proximal stump of the lesioned nerve (Fig. 4B). At the distal stump characterized by swollen degenerating fibers, CD200 immunoreactivity appeared to diminish (Fig. 4C). However, some fibers showed increase in Schmidt-Lanterman incisures that were immunoreactive for CD200. Quantitative estimation confirmed an increased number of CD200-positive Schmidt-Lanterman incisures regardless of the regions examined in the crushed nerve (Fig. 4D). At the crushed site and the region distal to this, the frequency of node of Ranvier expressing CD200 was significantly decreased (Fig. 4E).

After sciatic nerve transection, CD200 immunoreactivity in the DRG was unaffected (Fig. 5A). In the proximal stump of the lesioned nerve 2 mm from the transected site, CD200 immunoexpression appeared relatively normal at 7 days after transection (Fig. 5B). Distal and adjacent to this, the number of CD200-positive Schmidt-Lanterman incisures was increased as in the crushed nerve (Fig. 5C). Nearer to the transected site whose tissue was disorganized both CD200 and PGP 9.5 immunoreactivity was reduced (Fig. 5D). At the transected site, the

disordered tissue containing cellular debris was traversed by sparsely distributed PGP 9.5 nerve fibers (Fig. 5E). In the same region, a few cells emitting processes exhibited intense CD200 immunofluorescence (Fig. 5E). In the distal stump of the transected nerve, denervated Schwann cells formed the band of Büngner that contained numerous ovoid lipid debris (Fig. 5F). These Schwann cells expressed moderate PGP 9.5 (Lin et al. 1997) but very weak CD200 immunoreactivities (Fig. 5F).

2.4 *CD200 exists in rat Schwann cell line, RSC96, and primary DRG cultures*

In order to confirm the existence of CD200 in Schwann cells, a rat Schwann cell line RSC96 was examined. Immunocytochemical staining revealed that RSC96 Schwann cells did express CD200 molecule and contained different amounts of the molecule based on their phenotypes (Fig. 6A-B). CD200 immunoreactive products were distributed throughout the cytoplasm, mainly at the perinuclear zone (Fig. 6A-B). The round cells displayed the most intensive labeling for CD200. The labeling intensity of CD200 was weak in processes bearing cells (Fig. 6A-B). Some cells lacked CD200 immunoexpressiion (Fig. 6A-B). CD200 mRNA and protein expression was further confirmed in RSC96 cells by Western blotting and reverse-transcriptase polymerase chain reaction, respectively (Fig. 6C-D).

In the primary DRG cultures, antibodies against rat galactocerebroside, vimentin or GFAP were used for marking of satellite glia and Schwann cells. It was found that anti-CD200 antibody marked DRG cultured cells of different morphological profiles (Fig. 7A, D, G, J). In double labeling with anti-CD200 antibody, satellite glia or Schwann cells marked by the abovementioned specific antigens (Fig. 7B, E, H) also expressed CD200 of varying immunoreactivity (Fig. 7C, F, I), indicating that cultured satellite glia or Schwann cells maintained various amounts of CD200 as *in vivo*. A feature worthy of note was the occurrence of some CD200-expressing cells that were intimately associated with neurofilament-labeled neuronal processes (Fig. 7K). In the latter, some appeared to be enveloped by segmental

structures bearing CD200 immunoreactivity (Fig. 7L).

3 Discussion

3.1 *Novel expression of CD200 on Schwann cells in the intact nerve*

Adhesion molecules and members of the immunoglobulin superfamily, ICAM-1, VCAM-1 and L-selectin, were normally not found on Schwann cells but could be induced by proinflammatory cytokines. The upregulation of such adhesion molecules on Schwann cells has been suggested to have a role in the pathogenesis of inflammation in the peripheral nerve (Lisak and Bealmear, 1997; Constantin et al. 1999). Other members of IgSF such as the myelin-associated glycoprotein (MAG), the neural cell adhesion molecule (NCAM) and the neural adhesion molecule L1 were differentially expressed on Schwann cells of either myelin-forming or non-myelin-forming. These cell adhesion molecules may function in the fasciculation, initiation of axon-glial cell interaction and myelination, formation of structurally intact myelin sheaths and/or maintenance of myelin and axon integrity (Martini and Schachner, 1986; Mirsky et al. 1986; Bartsch, 2003). The present results have shown a novel expression of CD200 on Schwann cells. It would appear that the CD200 expression on Schwann cells is dependent on their ability to form myelin sheaths, the most remarkable feature being the localization of intense CD200 immunoreactivity at the nodes of Ranvier and Schmidt-Lanterman incisures (SLIs). As the role of CD200 as a powerful immunosuppressant and the immunocompetence of Schwann cells (Meyer et al. 2008) are well recognized, Schwann cell CD200 may possess a novel immunoregulatory effect on myeloid cells and help sculpt a safe immune response in the normal nervous tissues. The present results would further strengthen the view that Schwann cells are endowed with a constitutive immunosuppressant system in the PNS (Jander et al. 1996).

3.2 *Differential expression of CD200 on Schwann cells in the injured nerve*

After nerve injury, Schwann cells are activated, lose their myelin sheaths and de-differentiate to adopt the phenotype of immature Schwann cells. The activated Schwann cells can proliferate, remove the degenerated axonal and myelin debris and migrated to form Schwann cell columns (Büngner's bands) that also produce various trophic factors and adhesion molecules and offer a constructive environment for axon regeneration (see review by Fu and Gordon, 1997; Stoll and Müller, 1999). In the present study using two models of nerve injury, we have shown a marked reduction in CD200 expression at the distal stump of transected nerve and the lesion sites of both injury models. Concurrently, there was an increase in number of CD200 positive SLIs in the entire crushed nerve examined and in the proximal stump of the transected nerve. Interestingly, some CD200 expressing cells (presumably Schwann cells) appeared in the injury centre region where injured nerve fibers underwent drastic degeneration along with accumulation of macrophages. The present results indicated that Schwann cells of various functional stages differentially expressed CD200 in different regions of lesioned nerve. It is suggested that the injury-induced CD200 attenuation of Schwann cells may suppress immunosupression thus facilitating macrophage infiltration into Schwann tube probably to eliminate degenerated axons and myelin debris. It has been reported that macrophage infiltration in injured nerve fibers was through the node of Ranvier (Schubert and Friede, 1981; Griffin et al. 1996). The loss of CD200 at the node of Ranvier in the crush and/or cut sites in the present experimental models may explain the preferential site of macrophage invasion through the node of Ranvier. It is speculated that transmission of CD200 inhibitory signaling to macrophages is diminished or downregulated after a nerve injury. A feature indicative of response of Schwann cells to nerve damage in increase in number of SLIs in the affected nerve fibers. Although there is controversy on the change of SLI number after nerve injury (Ghabriel and Allt, 1981), there is strong evidence showing an overproduction of SLIs in the peripheral nerves of shiverer mutant mice (Gould et al. 1995),

desert hedgehog-null mice (Sharghi-Namini et al. 2006), as well the mutant mice incapable of synthesizing galactocerebroside and sulfatide (Hoshi et al. 2007). All these findings suggest that SLIs are instrumental in the metabolic processes of the myelin sheath such as nerve sheath development and maintenance. The present results have shown an increased frequency of SLIs in injured nerves and added that they are intensely positive for CD200, a recognized immune-suppressive molecule. In the light of this, it is suggested that CD200-expressing SLIs may potentially play a novel function in modulating local immune activity in the peripheral nerves.

3.3 *Immunocompetence of satellite glial cells and enteric glia*

Each neuron in dorsal root ganglia is enwrapped by an envelope of satellite glial cells (SGCs) to form a neuron/glia unit. SGCs function primarily as the physical support for neurons. Their envelope constituted a mechanical/protective barrier to allow but slow down access of large molecules and chemicals. SGCs can respond to changes in nearby environment and signals from other cells. Taken together, SGCs played a major role in the maintenance of neuronal homeostasis (see review by Hanani, 2005). There is now emerging evidence that SGCs are immune cell-like in many respects. For example, SGCs either in intact or pathological condition can express TNF-alpha (Shimeld et al. 1997), TGF-beta (Stewart et al. 1995) and IL-1 beta (Takeda et al. 2007). Recently, van Velzen et al. (2009) further demonstrated that SGCs may act as resident antigen presenting cells with potential T cell modulatory properties in human trigeminal ganglia. Our present results have revealed an unequivocal constitutive expression of CD200 on the SGCs in the peripheral ganglia and provided additional evidence supporting the immunocompetence of SGCs. The significance of SGC CD200 expression awaits further investigation. CD200 is a broadly expressed adhesion molecule of IgG superfamily and its interaction with CD200 receptor has been shown to regulate functions of the macrophage lineage in different tissues (Hoek et al. 2000).

In DRG, most resident macrophages were found to be closely associated with the neuron/SGC complexes (Olsson, 1990; Lu and Richardson, 1993). It is therefore suggested that SGCs may regulate macrophage function via the engagement of immunosuppressive molecule CD200. As the counterpart of PNS glia to SGC, enteric glial cells (EGCs) also play a fundamental role in the mechanical support, neurotransmitter and homeostatic functions in the gut (Cabarrocas *et al.,* 2003; Rühl *et al.,* 2004). Several lines of evidence have also showed that intestine proinflammatory stimuli may activate EGCs that contributed to the initiation and/or the progression of inflammatory diseases via cytokine synthesis such as IL-6 (Rühl *et al.,* 2001) and antigen presentation (Hirata *et al.,* 1986; Cabarrocas *et al.,* 2003; Rühl *et al.,* 2004). The MHC class II-expressing EGCs could actually make close contact with infiltrating T-cells in intestinal lesions (Geboes *et al.,* 1992; Rühl *et al.,* 2004). All these evidences implicated a significant immune capability of EGCs in a manner similar to SGCs. Indeed, our double immunofluorescence staining showed a constitutive expression of CD200 in EGC identified by its GFAP immunoreactivity. Our present findings thus offer further evidence to support the immunocompetence of EGC that may impart a novel immunosuppressive signal leading to restraining myeloid cell functions after CD200-CD200R interaction. In conclusion, besides its known expression in nerve cells and fibers, we have shown a novel distribution of CD200 in Schwann cells enwrapping different types of nerve fibers, satellite glial cells of the sensory and autonomic ganglia and enteric glial cells. These peripheral glial cells are diverse in their structure, biochemistry and functions (Jessen, 2004). The immune suppressive CD200 seems to be a specific marker shared by the PNS glial cells that may exert a common immunomodulatory function in the PNS.

4 Acknowledgements

This work serves as the doctoral thesis of CY Chang. The study was supported in part by a grant NSC98-2320-B-016-007-MY3 from the National Science Council, Taiwan.

5 Refferences

Banerjee D, Dick AD (2004), Blocking CD200-CD200 receptor axis augments NOS-2 expression and aggravates experimental autoimmune uveoretinitis in Lewis rats. Ocul Immunol Inflamm 12(2):115-125.

Barclay AN, Clark MJ, McCaughan GW (1986), Neuronal/lymphoid membrane glycoprotein MRC OX-2 is a member of the immunoglobulin superfamily with a light-chain-like structure. Biochem Soc Symp 51:149-157.

Barclay AN, Ward HA (1982), Purification and chemical characterisation of membrane glycoproteins from rat thymocytes and brain recognised by monoclonal antibody MRC OX 2. Eur J Biochem 129:447–458.

Barclay AN, Wright GJ, Brooke G, Brown MH (2002), CD200 and membrane protein interactions in the control of myeloid cells. Trends Immunol 23(6):285-290.

Bartolomé MV, Ibáñez-Olías MA, Gil-Loyzaga P (2002), Transitional expression of OX-2 and GAP-43 glycoproteins in developing rat cochlear nerve fibers. Histol Histopathol 17(1):83-95.

Bartsch U (2003), Neural CAMS and their role in the development and organization of myelin sheaths. Front Biosci 8:d477-490.

Cabarrocas J, Savidge TC, Liblau RS (2003), Role of enteric glial cells in inflammatory bowel disease. Glia 41(1):81-93.

Constantin G, Piccio L, Bussini S, Pizzuti A, Scarpini E, Baron P, Conti G, Pizzul S, Scarlato G (1999), Induction of adhesion molecules on human schwann cells by proinflammatory cytokines, an immunofluorescence study. J Neurol Sci 170(2):124-130.

Dolapchieva S, Eggers R, Kuhnel W (2001), Expression of neural cell adhesion molecule (NCAM) in the peripheral nerve fibers demonstrated by postembedding immunogold method on ultrathin sections. A preliminary study. Ann Anat 183(2):129-133.

Doran JF, Jackson P, Kynoch PA, Thompson RJ (1983), Isolation of PGP 9.5, a new human neurone-specific protein detected by high-resolution two-dimensional electrophoresis. J Neurochem 40(6):1542-1547.

Fu SY, Gordon T (1997), The cellular and molecular basis of peripheral nerve regeneration. Mol Neurobiol 14(1-2):67-116.

Geboes K, Rutgeerts P, Ectors N, Mebis J, Penninckx F, Vantrappen G, Desmet VJ (1992), Major histocompatibility class II expression on the small intestinal nervous system in Crohn's disease. Gastroenterology 103(2):439-447.

Ghabriel MN, Allt G (1981), Incisures of Schmidt-Lanterman. Prog Neurobiol 17(1-2):25-58.

Gorczynski RM, Chen Z, Hu J, Kai Y, Lei J (2001), Evidence of a role for CD200 in regulation of immune rejection of leukaemic tumour cells in C57BL/6 mice. Clin Exp Immunol 126(2):220-229.

Gorczynski RM, Chen Z, Lee L, Yu K, Hu J (2002), Anti-CD200R ameliorates collagen-induced arthritis in mice. Clin Immunol 104(3):256-264.

Gould RM, Byrd AL, Barbarese E (1995), The number of Schmidt-Lanterman incisures is more than doubled in shiverer PNS myelin sheaths. J Neurocytol 24(2):85-98.

Griffin JW, Li CY, Macko C, Ho TW, Hsieh ST, Xue P, Wang FA, Cornblath DR, McKhann GM, Asbury AK (1996), Early nodal changes in the acute motor axonal neuropathy pattern of the Guillain-Barré syndrome. J Neurocytol 25(1):33-51.

Grumet M (1992), Structure, expression, and function of Ng-CAM, a member of the immunoglobulin superfamily involved in neuron-neuron and neuron-glia adhesion. J Neurosci Res 31(1):1-13.

Hanani M (2005), Satellite glial cells in sensory ganglia: from form to function. Brain Res Brain Res Rev 48(3):457-476.

Haney CA, Sahenk Z, Li C, Lemmon VP, Roder J, Trapp BD (1999), Heterophilic binding of L1 on unmyelinated sensory axons mediates Schwann cell adhesion and is required for

axonal survival. J Cell Biol 146(5):1173-1184.

Hirata I, Berrebi G, Austin LL, Keren DF, Dobbins WO 3rd (1986), Immunohistological characterization of intraepithelial and lamina propria lymphocytes in control ileum and colon and in inflammatory bowel disease. Dig Dis Sci 31(6):593-603.

Hoek RM, Ruuls SR, Murphy CA, Wright GJ, Goddard R, Zurawski SM, Blom B, Homola ME, Streit WJ, Brown MH, Barclay AN, Sedgwick JD (2000), Down-regulation of the macrophage lineage through interaction with OX2 (CD200). Science 290(5497):1768-1771.

Hoshi T, Suzuki A, Hayashi S, Tohyama K, Hayashi A, Yamaguchi Y, Takeuchi K, Baba H (2007), Nodal protrusions, increased Schmidt-Lanterman incisures, and paranodal disorganization are characteristic features of sulfatide-deficient peripheral nerves. Glia 55(6):584-594.

Inuzuka T, Sato S, Fujita N, Baba H, Nakano R, Ishiguro H, Miyatake T (1993), Expression of myelin-associated glycoprotein isoforms after sciatic nerve crush injury in mice. Mol Chem Neuropathol 20(3):191-196.

Jander S, Pohl J, Gillen C, Stoll G (1996), Differential expression of interleukin-10 mRNA in Wallerian degeneration and immune-mediated inflammation of the rat peripheral nervous system. J Neurosci Res 43(2):254-259.

Jessen KR (2004), Glial cells. Int J Biochem Cell Biol 36(10):1861-1867.

Jessen KR, Thorpe R, Mirsky R (1984), Molecular identity, distribution and heterogeneity of glial fibrillary acidic protein: an immunoblotting and immunohistochemical study of Schwann cells, satellite cells, enteric glia and astrocytes. J Neurocytol 13(2):187-200.

Jiang-Shieh YF, Chien HF, Chang CY, Wei TS, Chiu MM, Chen HM, Wu CH (2010), Distribution and expression of CD200 in the rat respiratory system under normal and endotoxin-induced pathological conditions. J Anat 216(3):407-416.

Krushel LA, Prieto AL, Cunningham BA, Edelman GM (1993), Expression patterns of the

cell adhesion molecule Nr-CAM during histogenesis of the chick nervous system. Neuroscience 53(3):797-812.

Lin WM, Hsieh ST, Huang IT, Griffin JW, Chen WP (1997), Ultrastructural localization and regulation of protein gene product 9.5. Neuroreport 8(14):2999-3004.

Lisak RP, Bealmear B (1997), Upregulation of intercellular adhesion molecule-1 (ICAM-1) on rat Schwann cells in vitro: comparison of interferon-gamma, tumor necrosis factor-alpha and interleukin-1. J Peripher Nerv Syst 2(3):233-243.

Lu X, Richardson PM (1993), Responses of macrophages in rat dorsal root ganglia following peripheral nerve injury. J Neurocytol 22(5):334-341.

Martini R, Schachner M (1986), Immunoelectron microscopic localization of neural cell adhesion molecules (L1, N-CAM, and MAG) and their shared carbohydrate epitope and myelin basic protein in developing sciatic nerve. J Cell Biol 103:2439-2448.

Meyer zu Hörste G, Hu W, Hartung HP, Lehmann HC, Kieseier BC (2008), The immunocompetence of Schwann cells. Muscle Nerve 37(1):3-13.

Mirsky R, Jessen KR, Schachner M, Goridis C (1986), Distribution of the adhesion molecules N-CAM and L1 on peripheral neurons and glia in adult rats. J Neurocytol 15(6):799-815.

Mirsky R, Winter J, Abney ER, Pruss RM, Gavrilovic J, Raff MC (1980), Myelin-specific proteins and glycolipids in rat Schwann cells and oligodendrocytes in culture. J Cell Biol 84(3):483-494.

Morris RJ, Beech JN (1987), Sequential expression of OX2 and Thy-1 glycoproteins on the neuronal surface during development. An immunohistochemical study of rat cerebellum. Dev Neurosci 9(1):33-44.

Nieke J, Schachner M (1985), Expression of the neural cell adhesion molecules L1 and N-CAM and their common carbohydrate epitope L2/HNK-1 during development and after transection of the mouse sciatic nerve. Differentiation 30(2):141-151.

Olsson Y (1990), Microenvironment of the peripheral nervous system under normal and

pathological conditions. Crit Rev Neurobiol 5(3):265-311.

Rühl A, Franzke S, Collins SM, Strem mel W (2001), Interleukin-6 expression and regulation in rat enteric glial cells. Am J Physiol Gastrointest Liver Physiol 280(6):G1163-1171.

Rühl A, Nasser Y, Sharkey KA (2004), Enteric glia. Neurogastroenterol Motil 16 Suppl 1:44-49.

Schubert T, Friede RL (1981), The role of endoneurial fibroblasts in myelin degradation. J Neuropathol Exp Neurol 40(2):134-154.

Seilheimer B, Persohn E, Schachner M (1989), Antibodies to the L1 adhesion molecule inhibit Schwann cell ensheathment of neurons *in vitro*. J Cell Biol 109:3095-3103.

Sharghi-Namini S, Turmaine M, Meier C, Sahni V, Umehara F, Jessen KR, Mirsky R (2006), The structural and functional integrity of peripheral nerves depends on the glial-derived signal desert hedgehog. J Neurosci 26(23):6364-6376.

Shaw G, Osborn M, Weber K (1981), Arrangement of neurofilaments, microtubules and microfilament-associated proteins in cultured dorsal root ganglia cells. Eur J Cell Biol 24(1):20-27.

Shimeld C, Whiteland JL, Williams NA, Easty DL, Hill TJ (1997), Cytokine production in the nervous system of mice during acute and latent infection with herpes simplex virus type 1. J Gen Virol 78:3317-3325.

Stewart HJ, Rougon G, Dong Z, Dean C, Jessen KR, Mirsky R (1995), TGF-betas upregulate NCAM and L1 expression in cultured Schwann cells, suppress cyclic AMP-induced expression of O4 and galactocerebroside, and are widely expressed in cells of the Schwann cell lineage *in vivo*. Glia 15(4):419-436.

Stoll G, Müller HW (1999), Nerve injury, axonal degeneration and neural regeneration: basic insights. Brain Pathol 9(2):313-325.

Takeda M, Tanimoto T, Kadoi J, Nasu M, Takahashi M, Kitagawa J, Matsumoto S (2007), Enhanced excitability of nociceptive trigeminal ganglion neurons by satellite glial

cytokine following peripheral inflammation. Pain 129(1-2):155-166.

van Velzen M, Laman JD, Kleinjan A, Poot A, Osterhaus AD, Verjans GM (2009), Neuron-interacting satellite glial cells in human trigeminal ganglia have an APC phenotype. J Immunol 183(4):2456-2461.

Xu W, Manichella D, Jiang H, Vallat JM, Lilien J, Baron P, Scarlato G, Kamholz J, Shy ME (2000), Absence of P0 leads to the dysregulation of myelin gene expression and myelin morphogenesis. J Neurosci Res 60(6):714-724.

6 Figure legends

Fig. 1A-G. CD200 expression in dorsal root ganglion (A), superior cervical ganglion (B) and submandibular ganglion (C). CD200 immunoreactivity is distributed in the satellite cell (A-C, double arrows) and its extending sheath (A-C, arrows) investing the neurons some of them also express weak CD200 immunoreactivity (X). Sections double stained with anti-CD200 (green) and PGP 9.5 (red) antibodies show CD200 positive satellite cells (D, double arrows) and their capsules (D, arrows) enveloping DRG neurons that may also exhibit CD200 immunoexpression (D, X). The CD200 expression in satellite cells is confirmed by the colocalization of GFAP, a specific marker of satellite cell, with CD200 (D, inset). In the intestine, CD200 positive neural structures (E, F, double arrows) are evident in the periphery of myenteric (E) and submucous (F) plexuses. Double immunofluorescence staining of CD200 with glial marker GFAP (G) confirms myenteric glia expressing CD200 that is also found at the intestine mesothelium (F-G, arrowhead). The sections are counterstained with TOTO-3 that marks cell nucleus (blue). Scale bars: A-C = 50 μm; D-G, inset in D = 40 μm.

Fig. 2A-E. CD200 expression in different types of peripheral nerves. In nerves composed of mixed (A, sciatic) and mostly myelinated (B, phrenic) nerve fibers, CD200 immunoreactivity is distributed along the neurilemma (A, B, inset, arrows), at the nodes of Ranvier (A-B, inset, double arrows) and Schmidt-Lanterman incisures (A-B, inset, double-head arrows). Remarkably, most unmyelinated parasympathetic postganglionic nerves (C) are intensely labeled with CD200. A similar feature is seen in the same nerves double stained with CD200 (green) and PGP 9.5 (red) antibodies (data not shown). In (D), CD200 immunofluorescence is evident at the neurilemma (arrows), Schmidt-Lanterman incisures (double-head arrows) and node of Ranvier

(double arrows) in most of the myelinated nerve fibres. In a transverse (E) and longitudinal (E, inset) sections of unmyelinated nerves, CD200 immunoreactivity is colocalized with PGP 9.5 immunoreactive nerve fibers (yellow). TOTO-3 marks all cell nuclei (blue). Scale bars: A-C, insets in A and B = 20 μm; D = 25 μm; E, inset in E = 60 μm.

Fig. 3A-F. Electron microphotographs showing CD200 immunoreactivity in the dorsal root ganglia. CD200 immunoreactivity is detected in the cytoplasm of satellite cells (A, Sa) and on their plasma membrane (A-B, arrows) encasing sensory neurons either positive for CD200 (SN+) or lack of it (SN). Heavy deposits of immunoreactive products are found in the axoplasm of unmyelinated fibers (C-D, UA) enveloped by Schwann cells (Sc) that express CD200 exclusively on their plasma membrane apposing to the axons. In the myelinated axons (C-D, MA), CD200 immunoreactivity appears at the neurilemma (C-D, arrows). Additionally, the immunoreactivity is localized at the nodes of Ranvier where the microvilli (E-F, arrows) and paranodal loops (F, arrowheads) of Schwann cells are positive for CD200. Scale bars: A = 2 μm; B-F = 0.5 μm.

Fig. 4A-E. CD200 expression in the sciatic nerve with crush injury. Note a reduction or disappearance of CD200 immunoreactivity at the neurilemma and nodes of Ranvier in the lesioned sites at 7 days after crush (A). Some vacuolated ovoid structures expressing CD200 (A, arrows) occur in the lesioned fibers. The most drastic change after crush injury is an increase in number of CD200-positive Schmidt-Lanterman incisures in the proximal stump (B) of crushed nerve. A similar diminution in CD200 immunoreactivity is observed at the distal stump (C) of crushed nerve with swollen degenerating fibers. Some fibers with a small diameter show increased Schmidt-Lanterman incisures (C, arrows) that are positive for CD200. Quantitative estimation confirms an increased number of CD200-positive Schmidt-Lanterman

incisures at the crushed site and at regions proximal and distal to it (D). On the other hand, nodes of Ranvier with CD200 immunoreactivity are notably decrease in number at the injury site and at the distal stump (E). *, $p < 0.01$; #, $p < 0.05$ when compared with the sham operation/control group. Scale bar = 20 μm applied to A-C.

Fig. 5A-F. Representative confocal micrographs illustrating the immunoreactivity of CD200 (green) and PGP 9.5 (red) in different regions either proximal or distal to the transected site of the sciatic nerve. When compared with normal DRG, denervated DRG (A) shows no noticeable alteration in CD200 immunoreactivity at 7 days after axotomy. At a distance 2 mm proximal from the transected site, the tissue exhibits normal expression of CD200 (B). Immediately adjacent and distal to the transected site, the tissue exhibits an increased number of CD200-positive Schmidt-Lanterman incisures (C, double-head arrows). Immediately proximal to the transected site, the disorganized tissue shows reduced CD200 and PGP 9.5 immunoreactivities (D). The transected site is filled with cells and PGP 9.5-positive debris (E, arrowheads). Nerve fibers positive for PGP 9.5 are rare (E, arrows) and unexpectedly some process-bearing cells are positive for CD200 (E, double-head arrows). In the distal stump of the transected nerve, very weak CD200 immunoreactivity can still be observed in Schwann cell cords (bands of Büngner) that contain ovoid debris and also express PGP 9.5 (F, arrows). Cell nuclei are counterstained with TOTO-3 (blue). CD200 immunoreactivity is constantly observed in blood vessels (A-F, v). Scale bars: A-E = 25 μm, F = 15 μm.

Fig. 6A-D. Expression of CD200 mRNA and protein in Schwann cell line RSC96. Note that RSC96 cells show different CD200 immunoreactivity dependent on their morphological profiles. The immunoreactive product is concentrated around the cell nucleus (A-B, arrows). The round cells (A-B, arrowheads) appear to display a stronger immunoreactivity compared to the process-bearing cells (A-B, double-head arrows).

In the latter, CD200 immunoreactivity is weak or absent. CD200 mRNA and protein expression in RSC96 cells is further confirmed by Western blot analysis (C) and RT-PCR from 2 replicates (D, S1, S2). M, marker; GAPDH as an internal control; Scale bar = 50 μm.

Fig. 7A-L. CD200 expression in the primary DRG cultures. The satellite glia and Schwann cells are marked with galactocerebroside (B, GC), vimentin (E, Vim) or GFAP (H) antibody and then double labeled with anti-CD200 antibody (A, D, G). Double labeling demonstrates that CD200-positive cells display different external morphological profiles and immunoreactive intensities (A, D, G), and they also express the specific antigen of satellite glia and Schwann cells (C, F, I, arrows). When DRG neuron is marked with anti-neurofilament antibody (NF, K), its process is closely apposed by the processes or somata of cells expressing CD200 (J, L, arrows). Some neurites are invested by CD200-positive segment-like structures (J, L, double-head arrows). TOTO-3 is applied to mark cell nuclei (blue). Scale bars = 20 μm.

Figure

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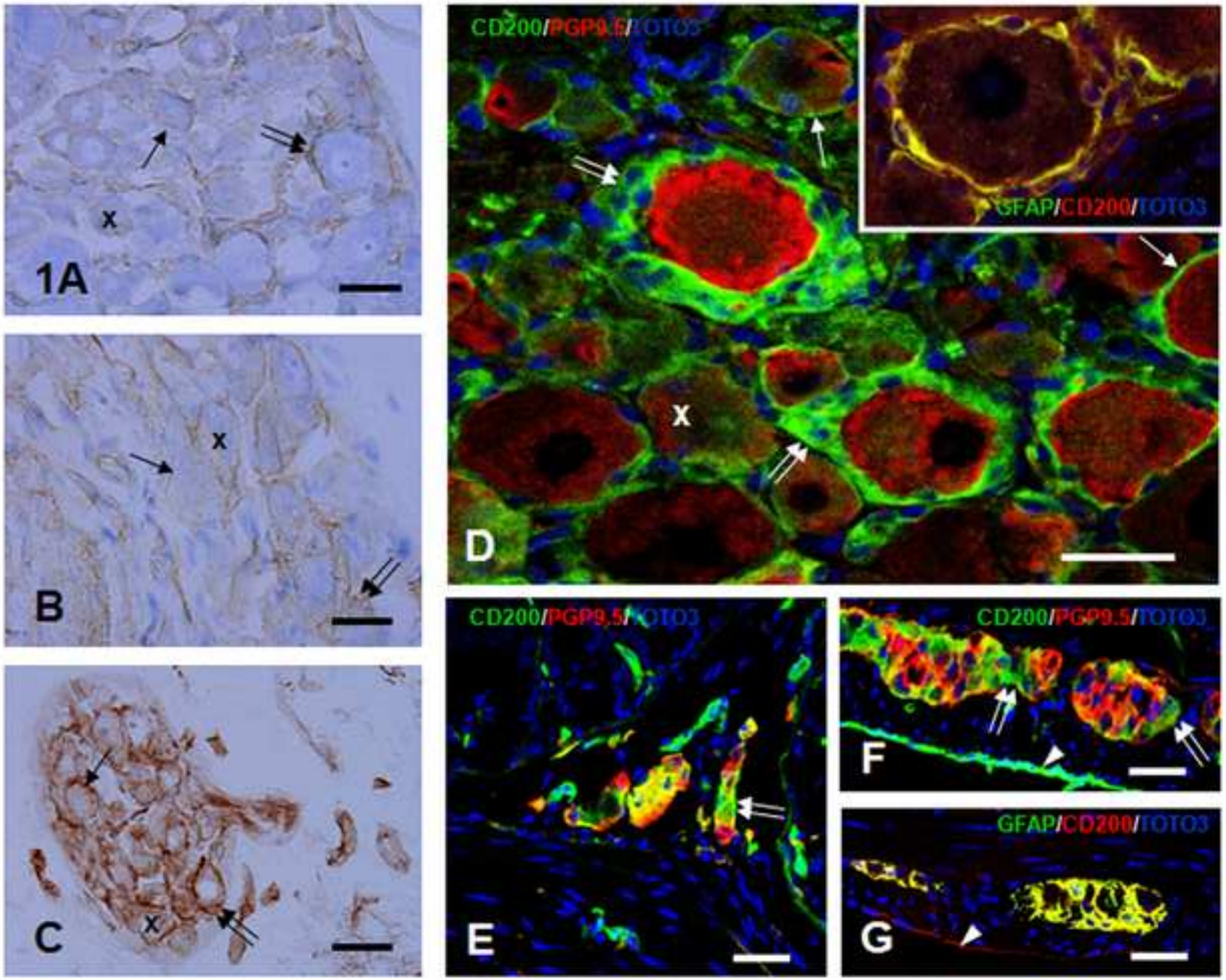

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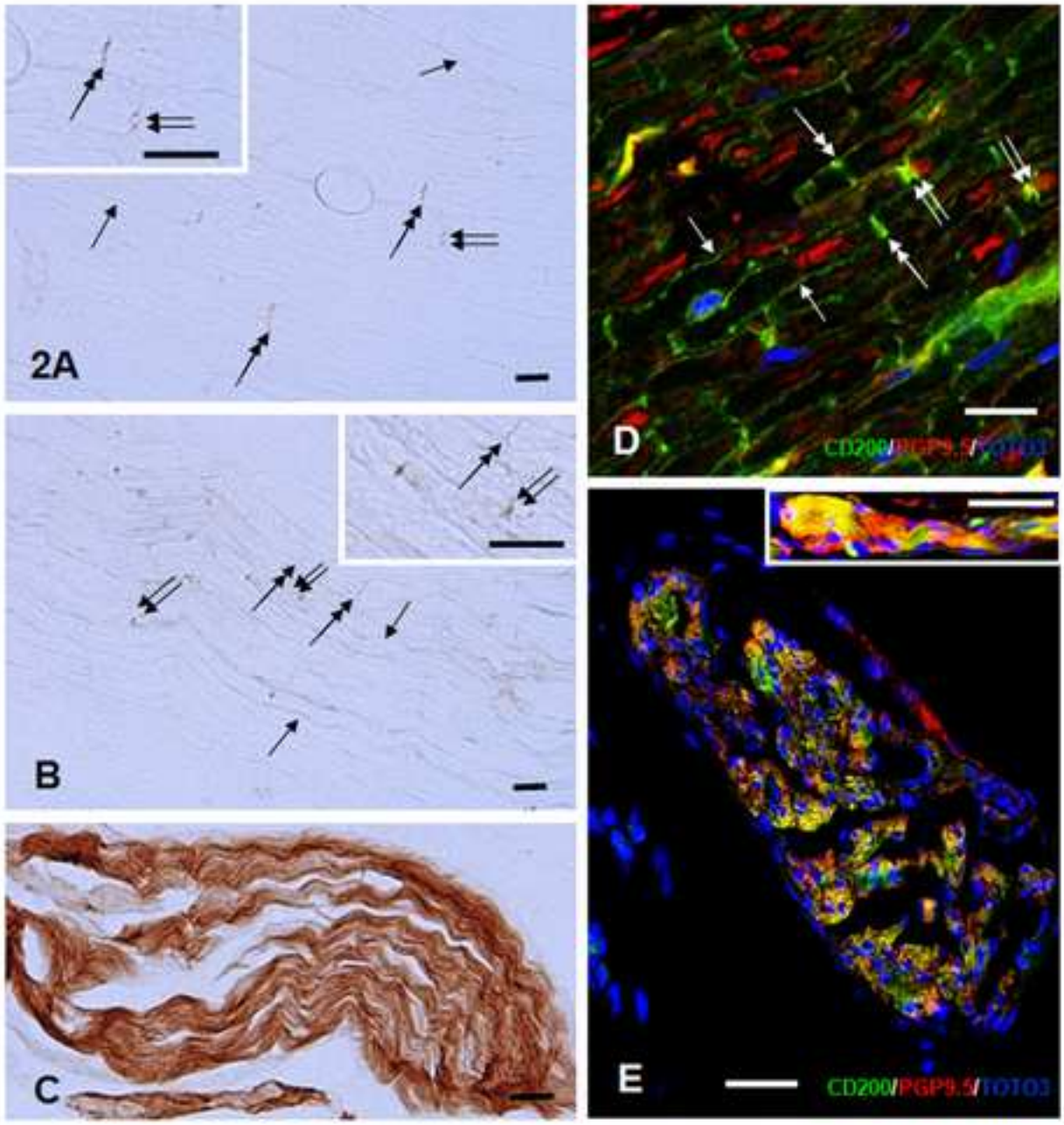

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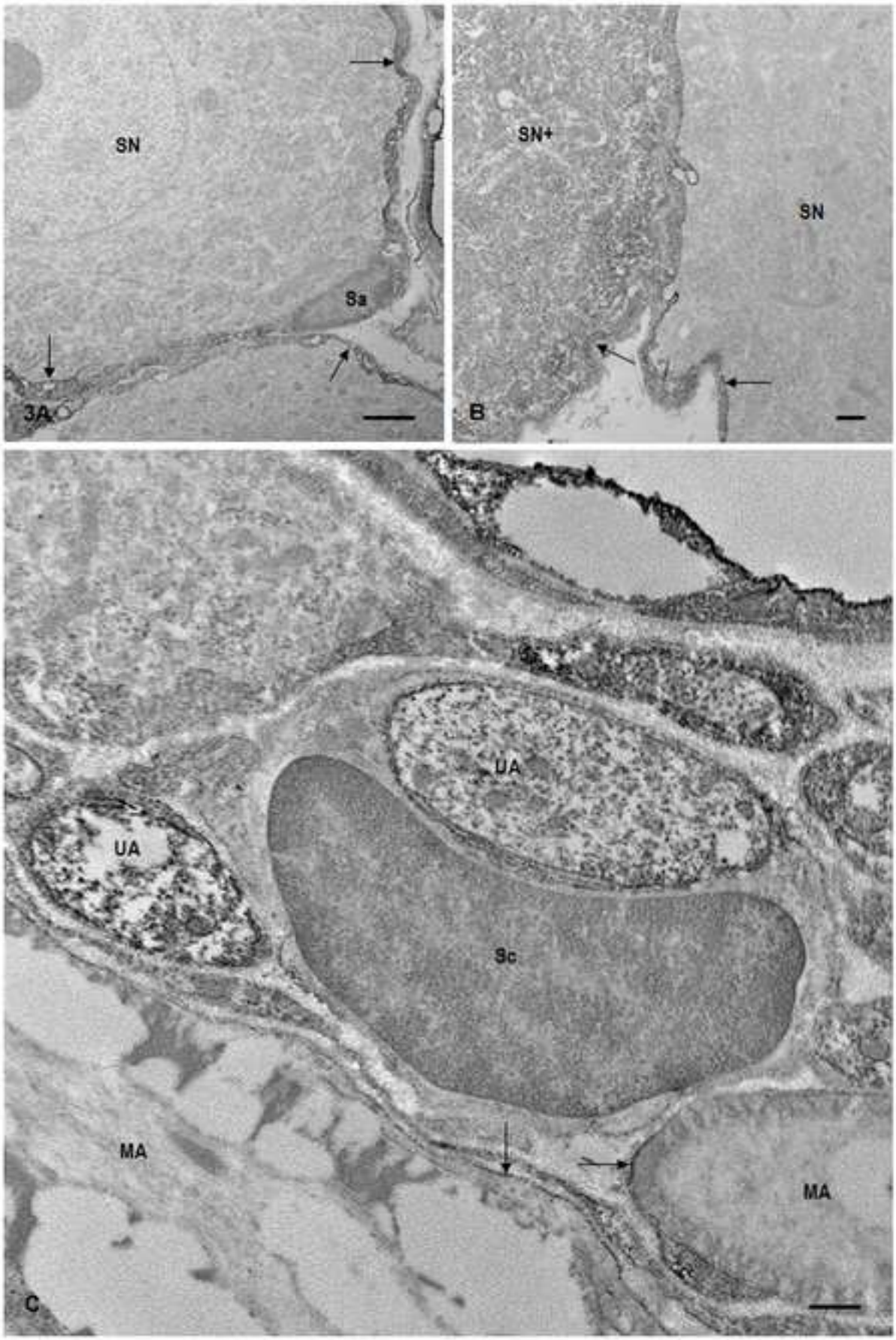

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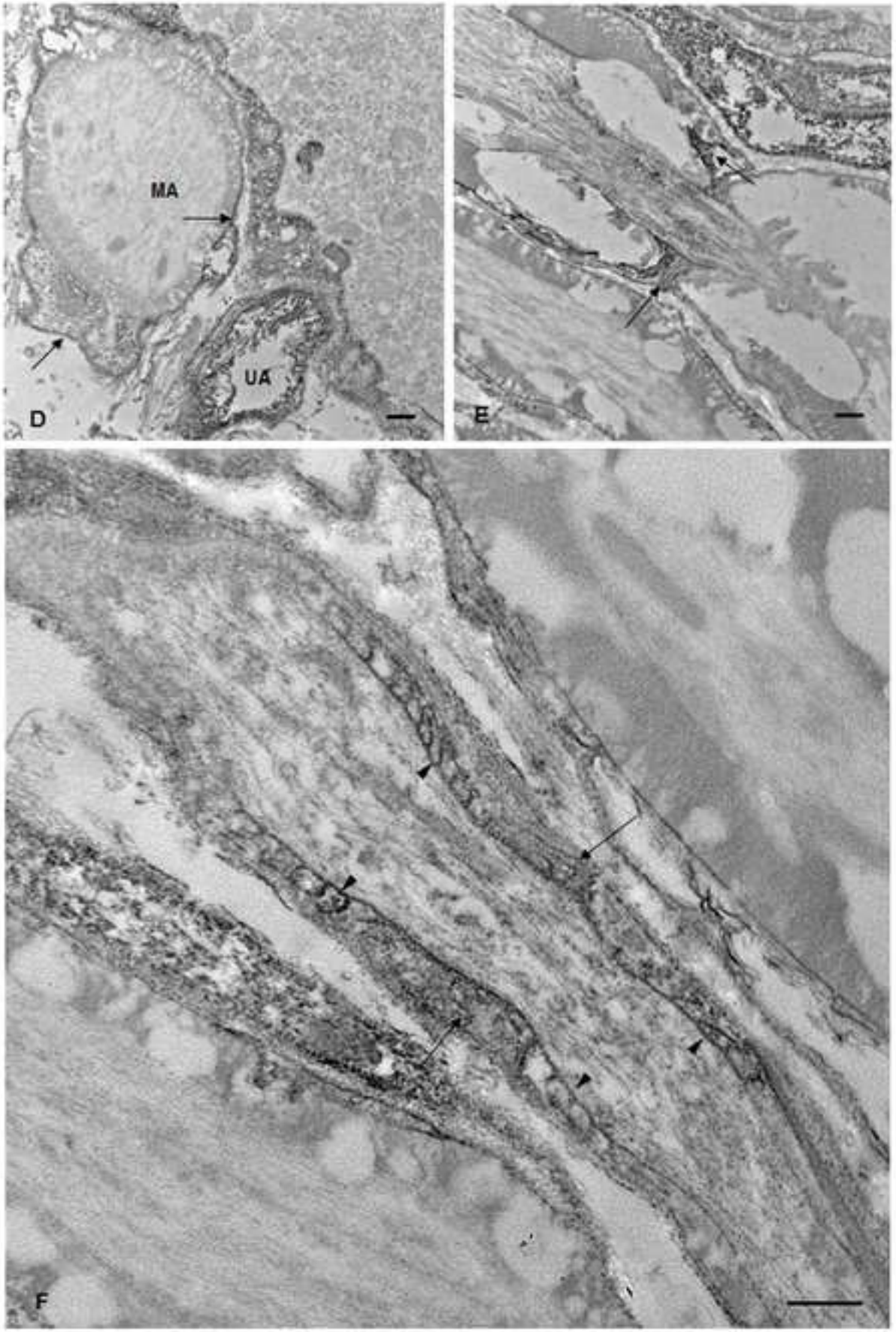

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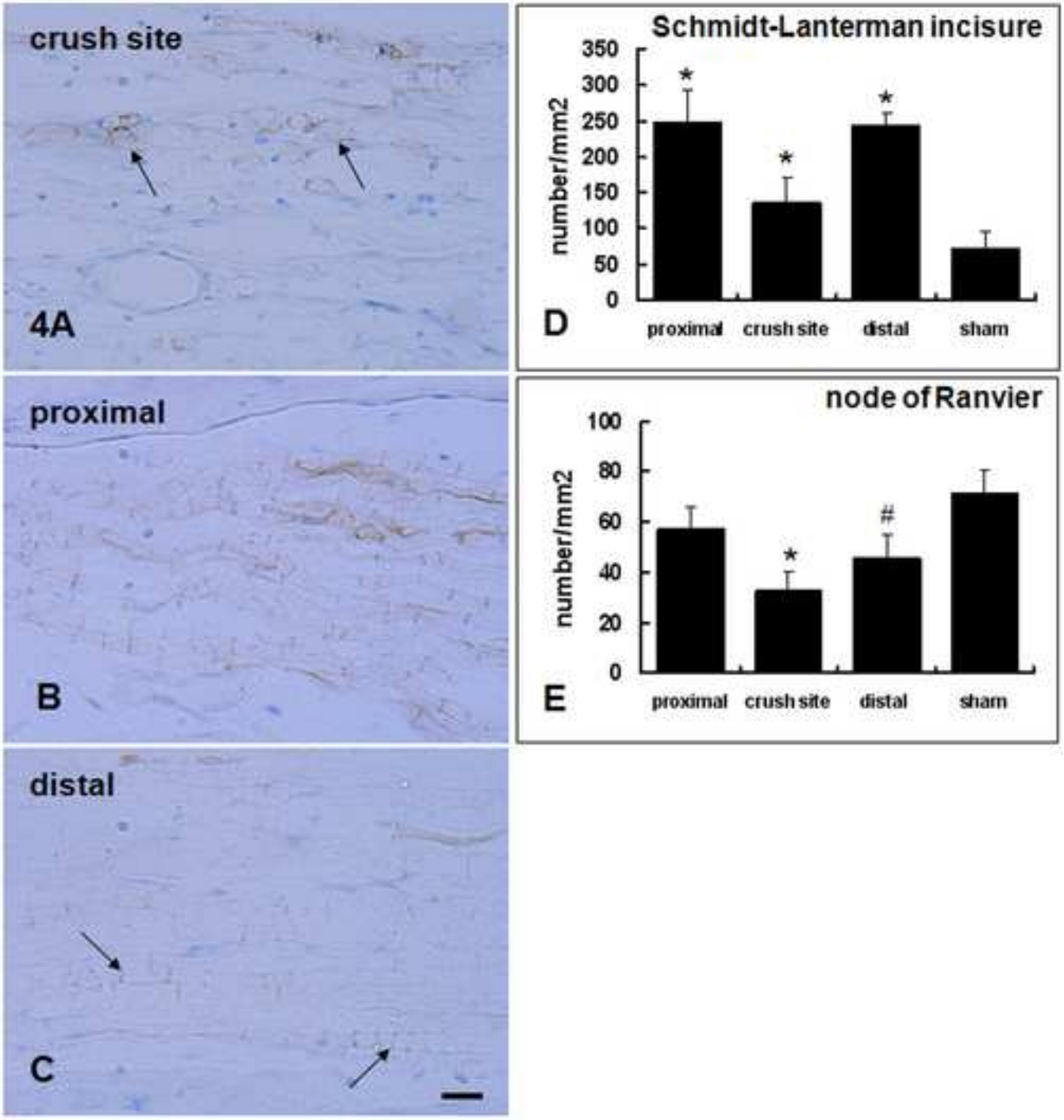

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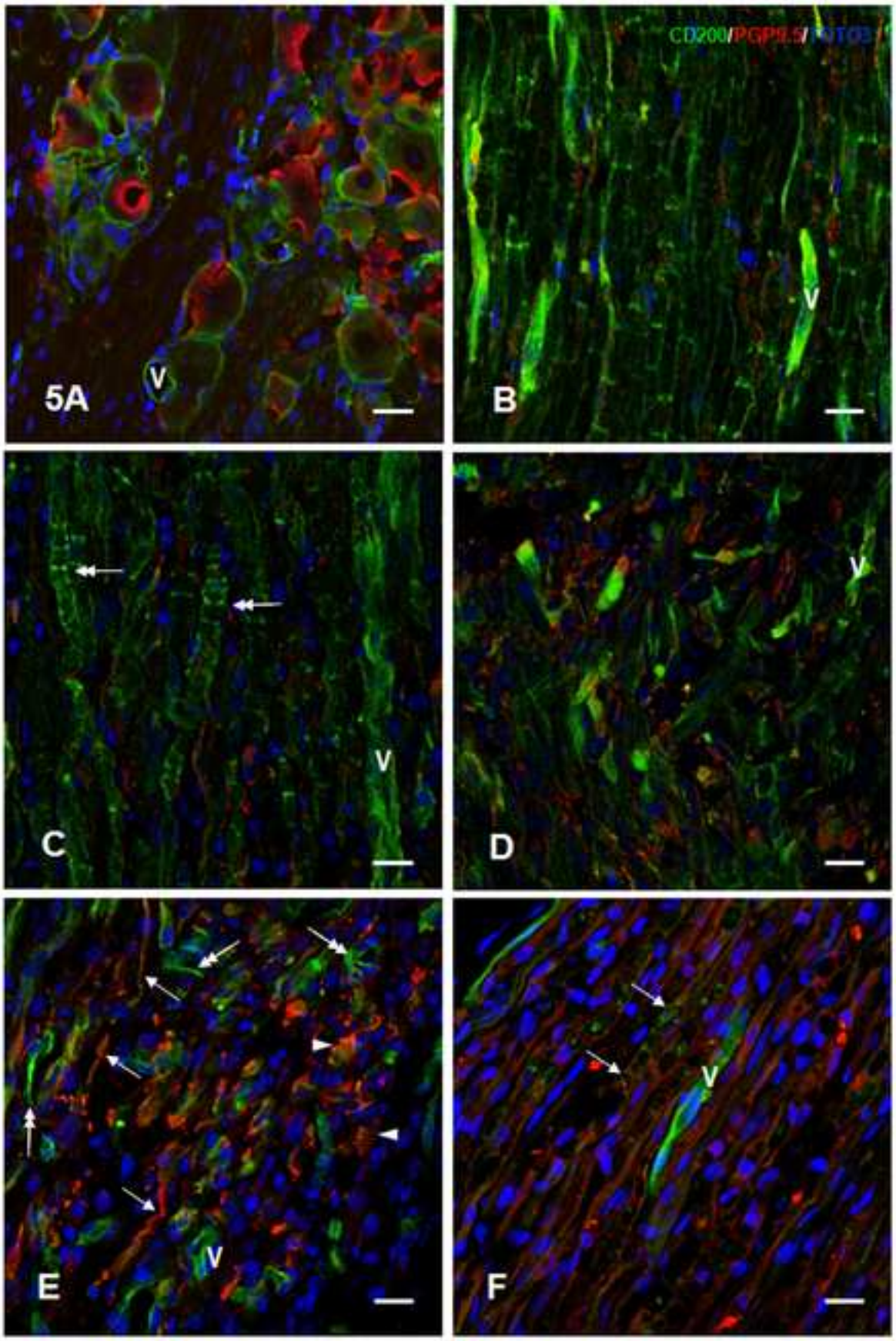

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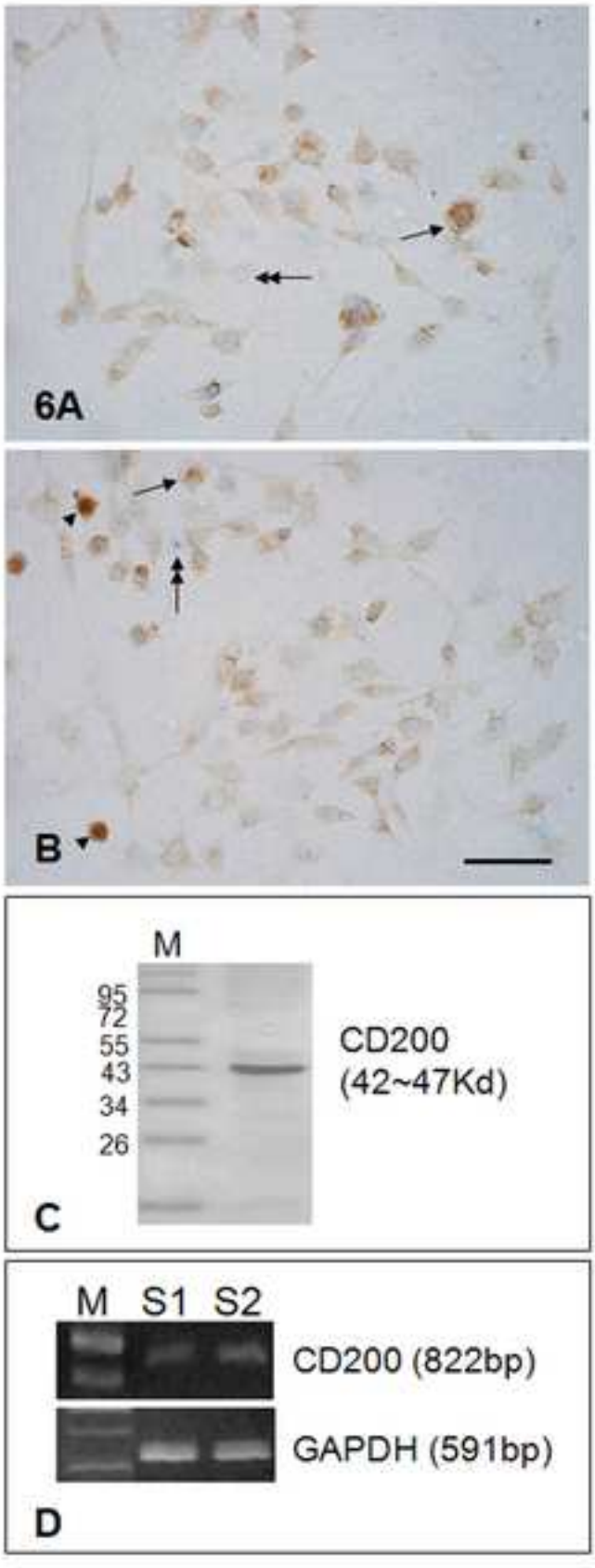

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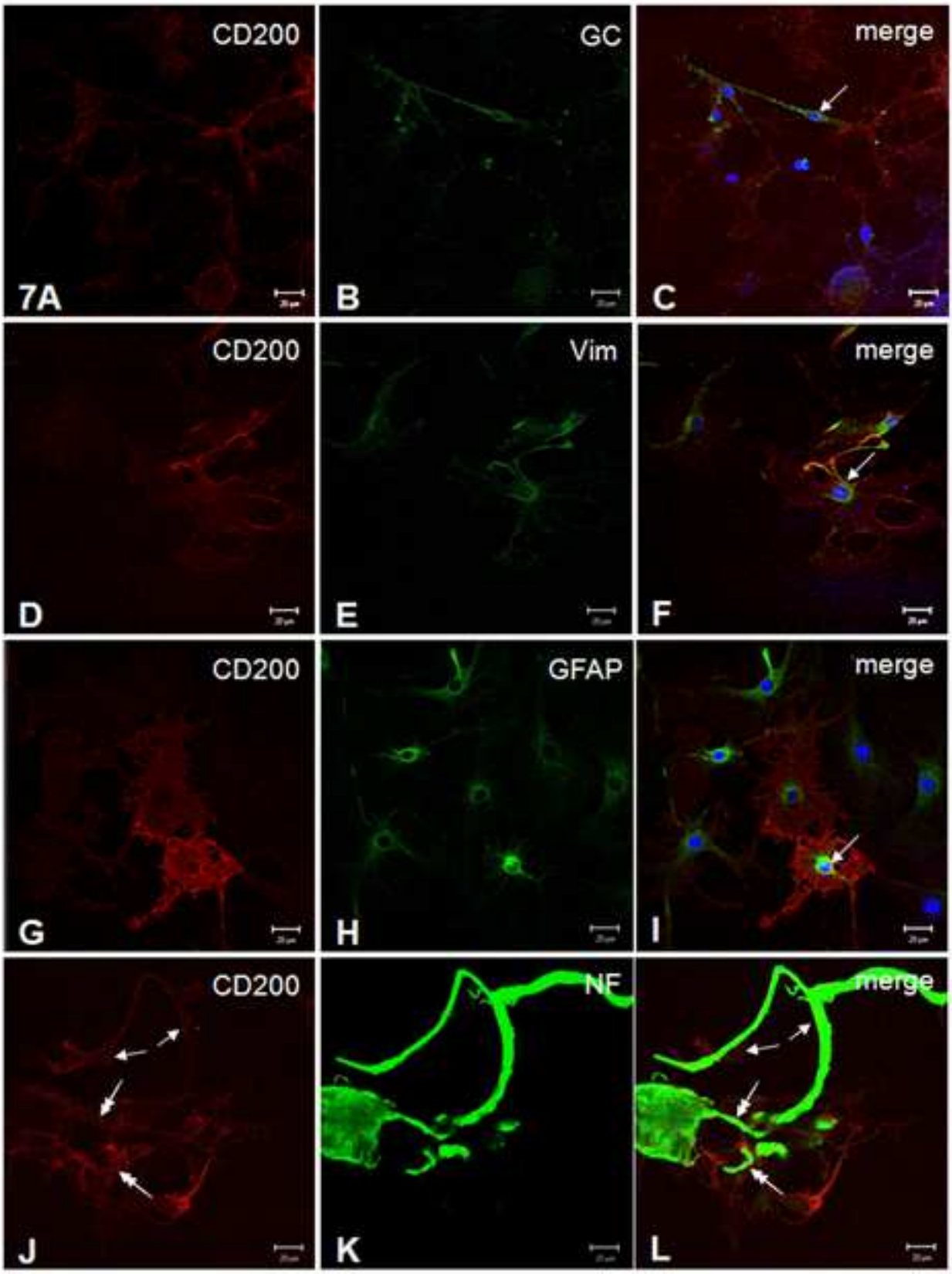

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Research highlights

CD200 exists in Schwann cells of different peripheral nerves, the satellite glial cells of sensory and autonomic ganglia and the enteric glial cells.

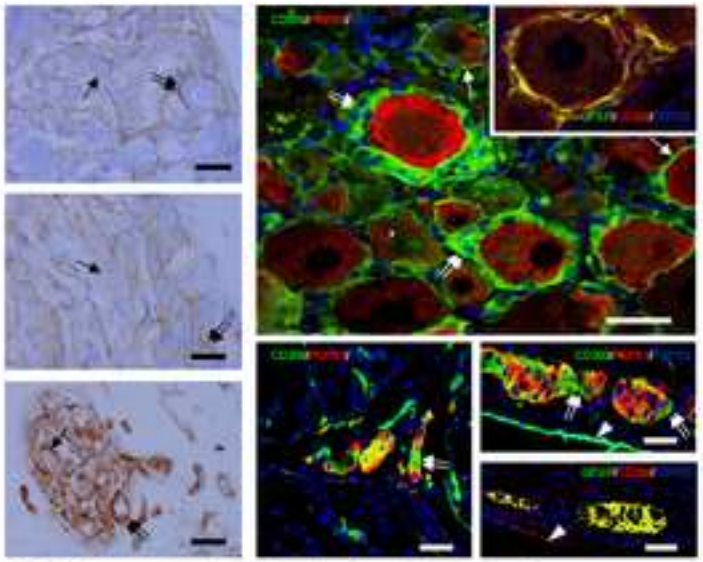
CD200 expression is found on the neurilemma of Schwann cells whose microvilli and paranodal loops at the node of Ranvier are immunoreactive.

Satellite glia or Schwann cells in the primary cultures of dorsal root ganglia has shown a differential expression of CD200.

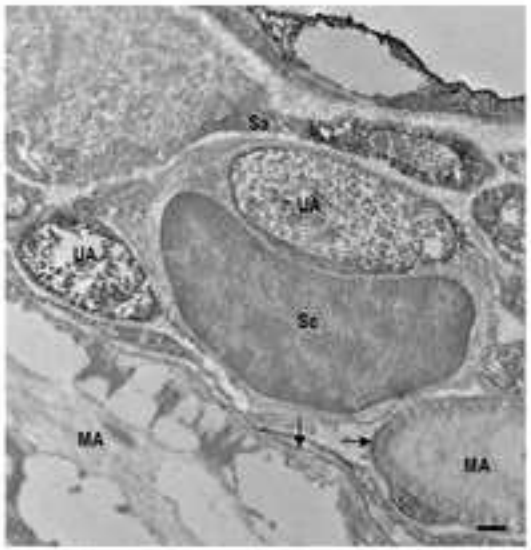
The existence of CD200 in PNS glial cells is corroborated by the expression of CD200 mRNA and protein in a rat Schwann cell line RSC96.

CD200 expression is attenuated at the site of crush or cut lesion proximal to which an increase in incidence of CD200-immunoreactive Schmidt-Lanterman incisures is evidenced.

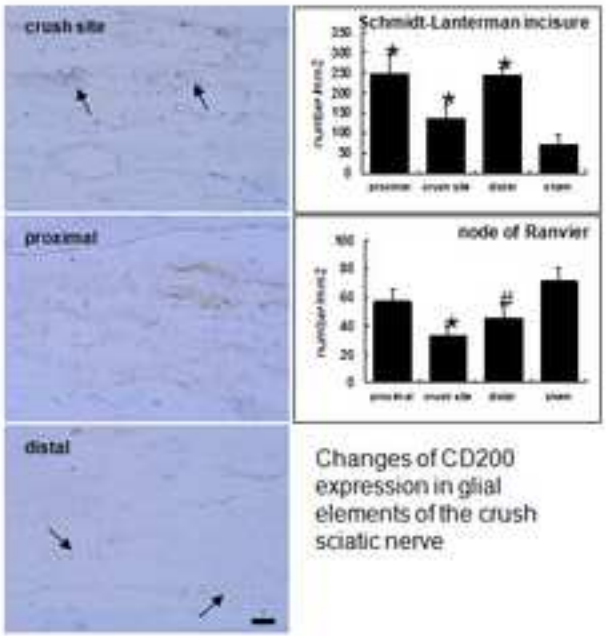
Graphical Abstract



CD200 expression in glial cells of peripheral ganglia



Subcellular distribution of CD200 in satellite glial cell (Sa)and Schwann cell (Sc) associated with myelinated (MA) and unmyelinated (UA)axons



Changes of CD200 expression in glial elements of the crush sciatic nerve