Indian Journal of Experimental Biology
Vol 48, February 2010, pp. 110-116

Iba1 expressing microglia in the dorsal root ganglia become activated following peripheral nerve injury in rats

Nisha Patro, Aarti Nagayach & I K Patro*

School of Studies in Neuroscience, Jiwaji University, Gwalior 474 011, India

Received 5 October 2009; revised 17 November 2009

The presence of microglia in dorsal root ganglia (DRG) has not been reported earlier. The dorsal root ganglia contain satellite glial cells (SGCs) and macrophages, which are considered to have infiltrated from the systemic blood. An attempt was made to investigate whether microglia as found in the central nervous system are also present in the dorsal root ganglia of untreated rats and following experimental peripheral nerve injury. Female adult Wistar rats were subjected to sciatic nerve transection injury on the right hand side. The DRGs of the right side were studied with the contralateral DRGs of the left side serving as controls. The tissues, harvested at different time points after injury, following intracardial perfusion fixation, and frozen sections were immunolabeled with anti-GFAP as a marker for SGCs and anti-Iba1 and OX-6 as markers for microglia and activated macrophagic microglia, respectively. These antibodies were also used in combination to ascertain if Iba1+ cells are the SGCs or otherwise and also if macrophagic OX-6+ cells are Iba1 positive microglia. The results indicate that Iba1 positive microglial cells are different from the SGCs in the DRGs. The Iba1 positive microglial cells respond to the sciatic nerve injury becoming activated and macrophagic and express MHCII molecules. Such activated microglia apparently may serve as neurosupportive cells, providing neuroprotection and scavenging cellular debris in response to the injury.

Keywords: Dorsal root ganglia, Iba1, Injury, MHC II, Microglia, Rats, Satellite glial cells

The central nervous system (CNS) is protected by the blood brain barrier, in contrast to dorsal root ganglia (DRGs) which lack a similar blood nerve barrier[1]. The DRGs consist of perikarya of pseudounipolar afferent sensory neurons together with surrounding glial cells, known as satellite glial cells (SGCs). These SGCs, which are amply present in DRGs completely ensheath the DRG perikarya following injury[2-4] and are believed to control the neuronal microenvironment in the DRG[5]. It is widely considered that microglia are restricted to the CNS. In view of the lack of a blood nerve barrier in DRGs, it is presumed that small populations of T-lymphocytes perform the immune surveillance in the DRGs. Experimental peripheral nerve injury initiates satellite cell proliferation[6,7] and delay death of small neurons[8] in adult rat DRGs. In addition, there are increased numbers of cells with positive macrophage markers[9,10]. It is widely believed that the additional MHCII immunopositive cells in the lesioned DRGs may be either intrinsic SGCs or hematogeneous monocytes[9]. As far as the central nervous tissue is concerned, it always depends on the intrinsic system rather than the systemic system with microglia involved in the process.

Ionized calcium binding adapter molecule 1 (Iba1) gene in the major histocompatibility complex III region encoding an EF handed protein was identified by Imai et al[11]. Iba1, with 147 amino acid residues, is a calcium binding protein believed to be a key factor in membrane ruffling, which is a typical function of microglia[12-15]. In the brain, the Iba1 gene is specifically expressed in microglia. Initially Iba1 signal was detected in rat embryonic brain primary-cultures with co-existence of Iba1 and ED1 (macrophage marker) positive cells[11]. Iba1 protein in the nervous system was then confirmed to be expressed exclusively by microglial cells and not by neurons, astrocytes or oligodendrocytes[16]. Iba1 is considered to play a key role in cell migration and phagocytic activity of microglial function[17]. The expression of Iba1 is upregulated in activated microglia following facial nerve axotomy[16], ischemia[18], inflammatory conditions[19] and viral infections[20], thereby implicating it in the activated

*Correspondent author
Telephone: +91 751 2442789
Fax: +91 751 2442789
E-mail: ishanpatro@gmail.com

phenotypes of microglia. Thus immunolabeling glia following injury with anti-Iba1 and OX-6 antibodies may shed light on the possible presence of microglia in DRGs. Anti-Iba1 antibody was used to evaluate the presence of microglia in DRGs and also to investigate if Iba1 expression occurs in DRGs. By using anti-Iba1antibody as a specific marker for microglia, the aim was to detect the possible presence of microglia type cells expressing Iba1 in the DRGs following peripheral nerve injury, and also to ascertain if they differed from the SGCs.

Both polyclonal and monoclonal anti-Iba1 antibodies were used against activated microglia in the same set of animals in the spinal cord of L4-L5 following sciatic nerve injury[21,22]. Although it is believed that microglial activation in the CNS provides both neurodegenerative and neuro-degenerative roles[19,21-26], the presence of such cells in the DRGs has still not been shown. The present study is aimed to determine if DRGs contain microglia phenotypically similar to those in CNS and if they upregulate Iba1 and express MCH II upon activation following peripheral nerve injury.

Materials and Methods

Three months old healthy female Wistar rats (12) weighing150±10g from our stock were randomly selected and used in the study. The animals were kept under standard animal house conditions and were subjected to sciatic nerve transection (SNT) of the right hind limb under deep anesthesia by opening the skin over a length of 2 cm in the proximal half of the line between trochanter major and knee joint. The m. vastus lateralis and m. biceps femoris were separated by blunt preparation techniques and the sciatic nerve could now be seen from where it emerges from under the m. gluteus maximus and runs over the m. semimembranous and m. semitendinous[22,27]. Then the nerve was cut (transection) with a fine scissor and the cut ends of nerve were placed close to each other and then the wound was sutured. The L4 and L5 DRGs of both right (injured) and left (uninjured) side were harvested following transcardial perfusion initially with phosphate buffered saline (0.9% NaCl in 0.01*M* phosphate buffer, *p*H 7.4) followed by 2% paraformaldehyde in 0.01*M* phosphate buffer, *p*H 7.4 on 3, 7, 14 and 21 days post-operation (DPO). The L4 and L5 DRG's were washed with phosphate buffer and then cryopreserved in sucrose gradients, i.e., 10, 20 and 30% (prepared in 0.01*M* phosphate buffer) at 4°C, till the tissues settled at the bottom. Frozen sections were cut at 15µm thickness with Leica cryotome (CM 1900, Leica Germany). Cryocut sections were collected on chromalum gelatin coated slides and stored at -20°C till further use. Left hand side DRG's served as controls. All the experimental studies were carried out as per the guidelines of CPCSEA and the procedures were pre-approved by the Institutional Animal Ethics Committee.

Immunohistochemistry—Tissues were immuno-labeled using standard immuno-fluorescence methods for labeling SGCs with rabbit polyclonal anti-GFAP antibody (diluted 1:400, Dako, Denmark) and microglial localization and Iba1 expression using rabbit polyclonal anti-Iba1 antibody (diluted 1:300, Wako, Japan) and mouse monoclonal anti-Iba1 antibody (diluted 1:50, Santa Cruz, CA, USA). To assess whether the MHC II expressing antigen presenting cells also appear in DRG following injury, mouse monoclonal OX-6 antibody (Serotec, UK) was used alone and as a cocktail with rabbit polyclonal Iba1 to confirm whether they show a microglial phenotype. These markers were finally visualized with Fluorescein isothiocyanate (FITC) and Tetramethyl Rhodamine Isothiocyanate (TRITC) respectively. To evaluate if the SGCs differ from the Iba1 expressing cells, the adjacent sections were co-labeled with a mixture of rabbit polyclonal anti-GFAP antibody and mouse monoclonal anti-Iba1 antibody and visualized with FITC and TRITC, respectively, under a fluorescence microscope (Leica DM 6000) fitted with a digital camera DFC 420. The images were acquired using Leica Application Suite software. The co-localized sections were also visualized with a fluorescence microscope and the images were grabbed using I3 and N2.1 filters for FITC and TRITC respectively. The images were overlaid using Image Overlay function of Leica Application Suite software (Version 2.6.0 R1, Leica Microsystems, GmbH). At every immunolabelling points, respective controls were used. For example sections from the spinal cord of the same animals (L4, L5 region) were used as they contained activated astrocytes and microglia (Saxena et al., 2007). Negative controls for various antibodies were also performed by omitting the primary or the secondary antibody incubation in the same set of sections.

Results

The satellite glial cells (SGCs) in the DRGs were positive for anti-GFAP immunolabeling (GFAP+). In

control ganglia, only a few neurons were masked/surrounded by GFAP+ satellite glial cells. The neuronal soma were found together in groups in the DRG sections and the GFAP+ cells lay scattered in the tissue, small in size and expressing GFAP only at detectable level (Fig. 1a). Immediately after sciatic nerve injury (within 24h of nerve transection) GFAP expression was upregulated in SGCs of the injured DRGs from day 3 onwards. The density of GFAP + cells increased almost 5 to 6 fold by 7 DPO. These intensely GFAP+ satellite glial cells formed a ring of tightly apposed cells all around the perikarya, more prominently around the larger perikarya (Fig. 1b and c). No such activation of SGCs was observed in controls. However, both the intensity and number of GFAP+ cells decreased gradually in 14 and 21 day preparations.

In the adjacent sections Iba1 immuno-histochemistry was performed with rabbit polyclonal anti-Iba1 antibody known to selectively label activated microglia in nervous tissue. In the control sections a few Iba1 positive (Iba1+) cells could be seen (Fig.1d). On the injured side, an appreciable number of the glia, closely apposed to the perikarya were Iba1+. Such Iba1+ cells were prominently recorded on 3 and 7 DPO (Fig. 1e and f) after which

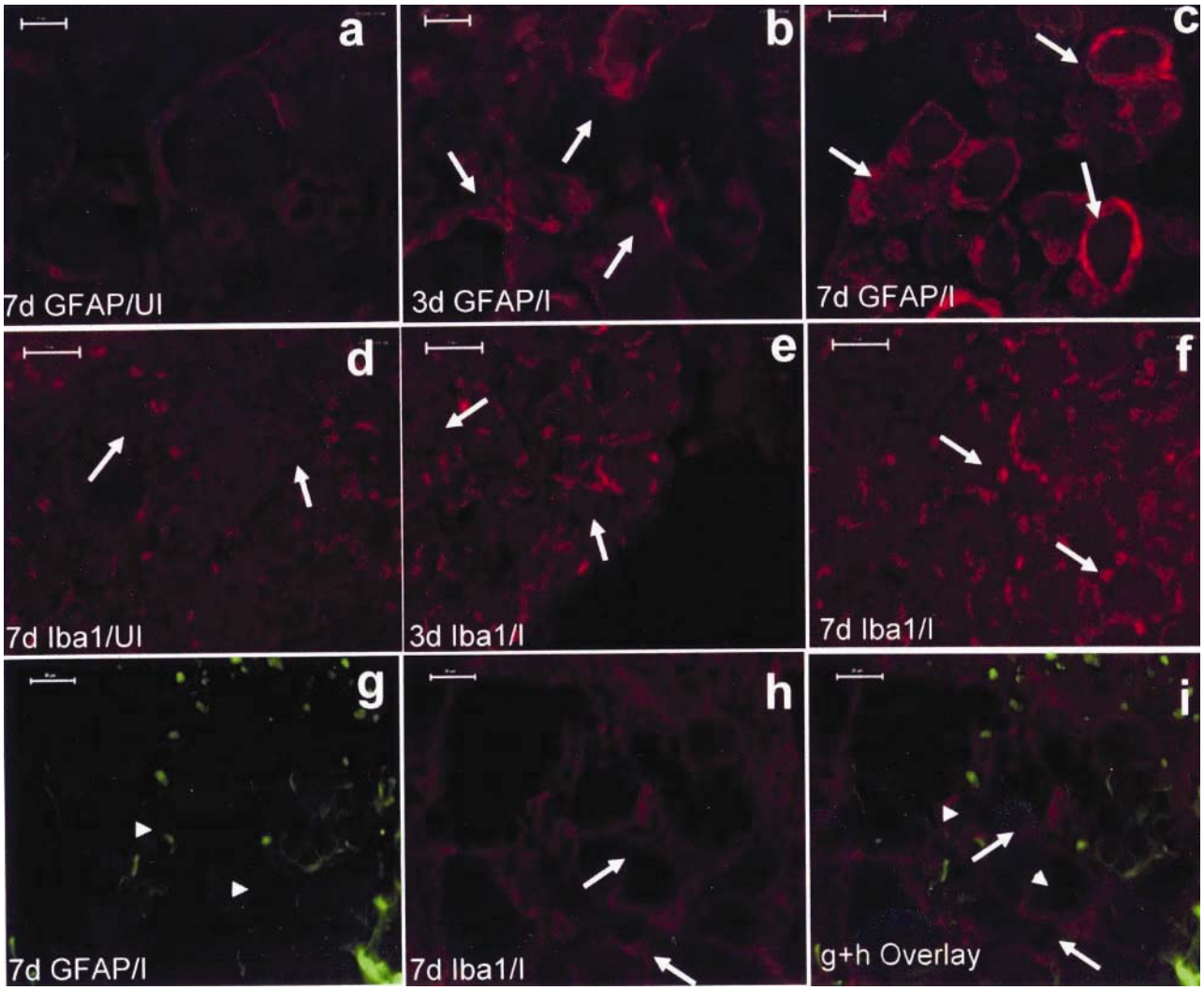

Fig. 1 — Satellite glial cells and microglia in DRGs – **[**Satellite glial cells (SGCs) labeled with anti-GFAP antibody showing their gradual activation and upregulation of GFAP expression (arrows) following injury at 3DPO (b) and 7DPO (c). No such activation of SGCs was visible in controls (a). Iba1 labeled microglia (arrows) in the vicinity of the DRG neurons in controls (d), after 3d (e) and 7d (f) transection injury. These Iba1+ cells were seen closely packing the DRG neurons following injury. Colocalization using anti-GFAP (g) and Iba1 (h; monoclonal) antibodies establishes that Iba1+ cells (labeled red; arrows) are different than SGCs (labeled green; arrowheads) as evident in the g+h overlay (i)]. Scale bar= 50 µm; UI-Uninjured; I-Injured

their number and expression both were downregulated. Both the GFAP+ and Iba1+ cells closely packed the neurons and appeared some what flattened and wrapping the neurons under stress. The Iba1+ cells were always in the vicinity of the neurons surrounding them along with the satellite cells. Iba1 specificity was ascertained with the help of mouse monoclonal anti-Iba1 antibody (Fig. 1h). In the injured and control sections GFAP and Iba1 were co-labeled. The controls had only mildly GFAP+ SGCs and very few Iba1+cells. The injured DRGs contained both GFAP+ SGCs and Iba1+ cells in close apposition to the neurons (Fig. 1g and h). None of the GFAP+ cells were co-labeled with anti-Iba1 (Fig. 1i), thus establishing that Iba1+ microglial cells exist in DRGs, but become activated following injury. In both polyclonal and monoclonal labeling of these cells, a marked upregulation of Iba1 could be seen at 3 and 7 DPO and then receded.

Only a few OX-6 positive, MHC II expressing cells were seen in 3 DPO preparations, which gradually increased both in number (almost 4-5 fold) and expression in 7 and 14 DPO preparations (Fig. 2b and e). All the MHC II expressing cells (OX-6+) also co-labeled with Iba1+, thus depicting clearly that these were microglial phenotypes (Fig. 2c and f). No such co-labeled cells were visible in 21 day preparations.

Discussion

In the central nervous system microglia are resident cells and become activated following any insult to the nervous tissue along with the astrocytes. Because the CNS is a privileged organ owing to blood brain barrier (BBB), infiltration of immunological cells occurs only in conditions of disruption or damage to the BBB. Microglia become activated and adopt the immunological functions of the tissue following damage. In both L4 and L5 DRG's the Iba1 positive cells seen were in line with earlier report on the L4 and L5 spinal cord dorsal and ventral horn following sciatic nerve injury[22] which is at its peak at 7DPO. With the lack of blood nerve barrier[1], it is believed that invasion of macrophages and T cells into DRGs occur following injury[28]. Hu and associates have reported the involvement of immune cells in DRG after sciatic nerve transection[29]. Such immune cells were specifically positive for macrophage markers such as ED2, MHC-II and OX-42. However, in the present study using a well known microglial marker i.e., Iba-1, the microglia were clearly localized,

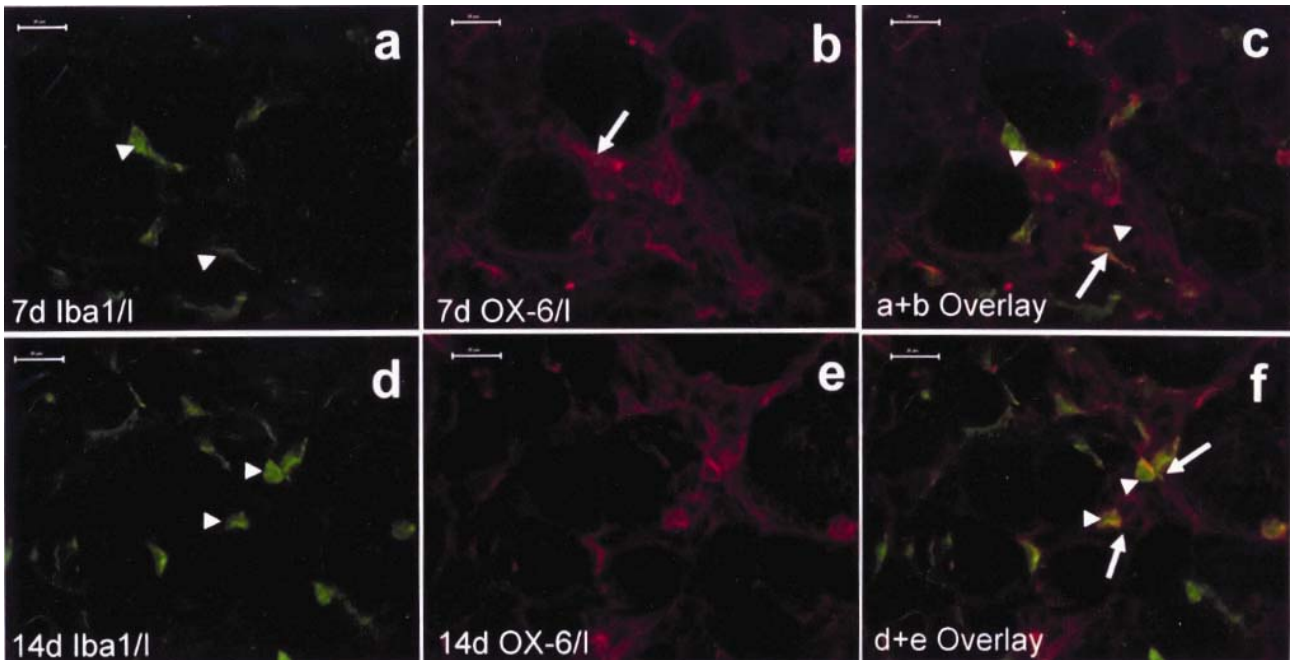

Fig. 2 — OX-6+ MHC II expressing cells and Iba-1+ cells are microglia only [7DPO: Colocalization using anti-Iba1 (a) and Ox-6 (b) antibodies establishes that all the MHC II expressing OX-6+ cells (labeled red; arrows) also colabeled with Iba1 (labeled green; arrowheads), thus depicting clearly that these were microglial phenotypes. This is clearly evident from the a+b overlay (c). 14DPO: Colocalization using anti-Iba1 (d) and Ox-6 (e) antibodies establishes that all the MHC II expressing OX-6+ cells (labeled red; arrows) also colabeled with Iba1 (labeled green; arrowheads), thus depicting clearly that these were microglial phenotypes. This is clearly evident from the d+e overlay (f)]. Scale bar= 50 µm; UI-Uninjured; I-Injured

stripping the dorsal root ganglia neurons after peripheral nerve transection. All the MHCII positive cells appeared to be the microglia as they co-localized with Iba-1 antibody in the injured DRGs which was completely missing in the uninjured side, although Iba1+ cells in lower number do exist in the uninjured DRGs. MHCII expression has been reported in the activated microglia in the L4 and L5 spinal cord of the same animal[22,24] at about 7DPO similar to the present observations with DRGs. Initially, the macrophagic Iba1 and OX-6+ microglia formed a circle around the satellite glia that closely pack the injured neuron but at later stages they take an inner position. Microglia have been noted to make such movements in the vicinity of the spinal cord motor neurons (*vide supra*). Because these injured animals also recovered by 60 DPO[24], it may be believed that such MHCII expression may be associated with neuronal survival[30,31], while their role in degeneration cannot be discussed. Although the invasion of the peripheral macrophages in DRGs following any injury in the absence of blood nerve barrier cannot be denied, the presence of Iba1+ve cells even in controls (in the absence of any injury) strongly supports the presence of resident microglia in the DRGs similar to CNS.

The presence of such MHCII expressing microglia close to the injured neurons may be assumed to be engaged in removal of degenerated cells, damaged dendrites and synapses, functionally not active. Such activity shall be useful for the DRG in creating new connection in the space so made available as proposed by Hanisch and Kettenmann[23].

If DRGs contain any microglia remains to be established. There exists only one report in which IB4 staining presented few IB4 positive cells in the control which could not be recorded following injury[9]. Isolectin B4 (IB4), lectin from *Bandeiraea simpilicifolia* is known for successfully staining microglia[32]. Microglia are supposed to express MHCII and become phagocytic in CNS following injury[21]. Hu and McLachlan[9] did not find any MHCII positive cells which were also positive for IB4 in both the control and lesioned DRGs.

Microglia type cells that become activated following injury exist in the DRGs. Microglial antigen heterogeneity in brain has been variously reported. However, Ito *et al.*[16] established that the polyclonal anti-Iba1 antibody developed by them (also used in this study) could recognize ramified microglia throughout the white and grey matter and perivascular microglia in the brain. Thus it is indicative that Iba1 expression in cells in DRG's following injury are a subpopulation of microglia.

RNA blot and immunoblot analysis has also established that in nervous tissue Iba1 is detectable only in the microglia[11]. The polyclonal antibody used in the study was raised against a synthetic peptide corresponding to the Iba1 carboxyl-terminal sequences that is completely conserved among human, rat and mouse (Iba1 antibody was generously provided by Dr. Imai, Japan). This antibody is thus capable of recognizing Iba1 protein in rat[33]. Taken together all the arguments point towards the existence of microglia in the DRGs and upregulation of Iba1 following injury. To add to this support, anti-Iba1 monoclonal antibody raised in mouse was also employed. The activated microglia, that we have located in the DRGs are positive for both polyclonal and monoclonal antibodies. That these are not the SGCs has been very well established with non-existence of Iba1 and GFAP co-labeling. Due to the sciatic nerve injury the dorsal root ganglion neurons may become hyperactive involving the astrocyte activation, which secretes signaling substances which trigger the microglial proliferation and early phase of their activation[34].

Moreover, Ito *et al.*[18] and Tonchev *et al.*[35] in rat brain following transient focal cerebral ischemia; Mori *et al.*[20] in herpes simplex virus infection, Imai and Kohsaka[36] following M-CSF induced microglia activation; Patro and Patro[19] following Poly I:C infection; Jinno and Kosaka[37] following electroconvulsive shock have all confirmed that the Iba1 positive cells in nervous tissue following experimental injury or insult are microglia. Similarly, we have reported activation of microglia and hyper-expression of Iba1 have been reported in the microglia in the proximity of the sensory and motor neurons in the L4, L5 spinal cord of the rats from which the DRGs for the present study have been harvested[22]. This suggests that Iba1 protein plays an important role in regulating the functioning of activated microglia and such Iba1 regulation is of importance as microglia are known to act as antigen presenting cells in brain[21,32,38]. In similar experiments with spinal cord Tawfik *et al.*[39] and Romero-Sandoval *et al.*[40] using Iba1 as microglial marker established that microglia play a role in the long-term maintenance of neuropathic pain. The microglial

activation (Iba1 positivity) in the DRGs following peripheral injury at 3 and 7 DPO, reported in the present communication, adds to the above and indicates an importance of detailed study of microglial marker expression at the spinal cord and DRGs and their pathophysiological role in response to peripheral injury. Activated microglia, as seen in present study, are thought to act as neurosupportive cells, providing neuroprotection and scavenging cellular debris resulting from injury and disease. Further studies on the role of microglia into the mechanism of action in the healing may prove to be helpful in developing strategies for cure following peripheral nerve injury.

Acknowledgement

Thanks are due to the Department of Biotechnology (HRD and BIF scheme), Govt. of India for financial support to the School. AN was awarded studentship and thesis grant under this scheme.

References

1 Jacobs J M, Macfarlane R M & Cavanagh J B, Vascular leakage in the dorsal root ganglia of the rat studied with horseradish peroxidase, *J Neurol Sci*, 29 (1976) 95.

2 Devor M & Selzer Z, Pathophysiology of damaged nerves in relation to chronic pain, in *Textbook of pain*, 5th, volume edited by Wall P D & Malzack R (Churchill Livingstone, Edinburgh) 1999, 129.

3 Hanani M, Satellite glial cells in sensory ganglia: From form to function, *Brain Res Reviews*, 48 (2005) 457.

4 Pannese E, Gioia M, Carandente O & Ventura R A, Quantitative electron microscope study of the perikaryal projections of sensory ganglion neurons, cat and rabbit, *J Comp Neurol*, 214 (1982) 239.

5 Keast J R & Anderson T M, Glutamate and aspartate immunoreactivity in dorsal root ganglion cells supplying visceral and somatic targets and avoidance for peripheral axonal transport, *J Comp Neurol*, 424 (2000) 577.

6 Humbertson A, Zimmermann E &Leedy M, A chronological study of mitotic activity in satellite cell hypothesis associated with chromatolytic neurons, *Z Zellforsch*, 100 (1969) 507.

7 Pannese E, The satellite cells of sensory ganglia, *Adv Anat Embryol Cell Biol*, 65 (1981) 1.

8 Lekan H A, Chung K, Yoon Y W, Chung J M & Coggeshall R E, Loss of dorsal root ganglion cells concomitant with dorsal root axon sprouting following segmental nerve lesion, *Neuroscience*, 81 (1997) 527.

9 Hu P & McLachlan E M, Macrophages and lymphocyte invasion of dorsal root ganglia after peripheral nerve lesion in the rat, *Neuroscience*, 112 (2002) 23.

10 Lu X & Richardson P M, Responses of macrophages in rat dorsal root ganglia following peripheral nerve injury, *J Neurocytol*, 22 (1993) 334.

11 Imai Y, Ibata I, Ito D, Ohsawa K & Kohsaka S, A novel gene Iba1 in the major histocompatability complex class III region encoding an EF hand protein expressed in a monocytic lineage, *Biochem Biophys Res Commun*, 224 (1996) 855.

12 May R C & Machesky L M, Phagocytosis and the actin cytoskeleton, *J Cell Sci*, 114 (2001) 1061.

13 Mitchison T J & Cramer L P, Actin-based cell motility and cell locomotion, *Cell*, 85 (1996) 371.

14 Strossel T P, On the crawling of the animal cells, *Science*, 260 (1993) 1086.

15 Yamada M, Ohsawa K, Imai Y, Kohsaka S & Kamitori S, X-Ray structures of the microglia/macrophages-specific protein Iba1 from human and mouse demonstrate novel molecular conformation change induced by calcium binding, *J Mol Biol*, 364 (2006) 449.

16 Ito D, Imai Y, Ohsawa K, Nakajima K, Fukuuchi Y & Kohsaka S, Microglia-specific localization of a novel calcium binding protein, Iba1, *Mol Brain Res*, 57 (1998) 1.

17 Ohsawa K, Neo M, Matsuoka H, Akiyama H, Ito H, Kohno H & Nakamura T, The expression of bone matrix protein mRNAs around beta-TCP particles implanted into bone, *J Biomed Mater Res*, 52 (2000) 460.

18 Ito D, Tanaka K, Suzuki S, Dembo T & Fukuuchi Y, Enhanced expression of Iba1, ionized calcium-binding adapter molecule 1, after transient focal cerebral ischemia in rat brain, *Stroke*, 32 (2001) 1208.

19 Patro N & Patro I K, Effect of immuneactivator (Poly I: C) on the rat cerebral cortex, *J Cell Tissue Res*, 3 (2004) 71.

20 Mori I, Goshima F, Koshizuka T, Imai Y, Kohsaka S, Koide N, Sugiyama T, Yoshida T, Yokochi T, Kimura Y & Nishiyama Y, Iba1-expressing microglia respond to herpes simplex virus infection in the mouse trigeminal ganglion, *Brain Res Mol Brain Res*, 12 (2003) 52.

21 Patro I K, Pathak Seema & Patro N, Central response to peripheral nerve injury: Role of non-neuronal cells, in *Molecular and cellular neurobiology*, edited by M K Thakur and S Prasad (Narosa, Delhi) 2005, 217.

22 Saxena K, Patro N & Patro I K, FK506 protects neurons following peripheral nerve injury via immunosuppression, *Cell Mol Neurobiol*, 27 (2007) 1049.

23 Hanisch U K & Kettenmann H, Microglia: active sensor and versatile effector cells in the normal and pathologic brain, *Nature Neuroscience*, 10 (2007) 1387.

24 Patro I K, Saxena K, Tiwari S & Patro N, FK-506 helps motor coordination recovery following sciatic nerve transaction in the young but not in the senile rats, *Int J Neuroprotec Neuroregen*, 4 (2008) 145.

25 Schwartz M, Macrophages and microglia in central nervous system injury: Are they helpful or harmful?, *J Cerebral Blood Flow Metab*, 23 (2003) 385.

26 Streit W J, Microglia and neuroprotection: Implications for Alzheimer's disease, *Brain Res Rev*, 48 (2005) 234.

27 Patro I K, Chattopadhyay M &Patro N, Flunarizine enhances functional recovery following sciatic nerve crush lesion in rats, *Neurosci Lett*, 263 (1999) 97.

28 Bennett D L, Michael G J, Ramachandran N, Munson J B, Averill S, Yan Q, McMahon S B & Priestley J V, A distinct subgroup of small DRG cells express GDNF receptor components and GDNF is protective for theses neurons after nerve injury, *J Neuroscience*, 18 (1998) 3059.

29 Hu P, Bembrick A L, Keay K A, Mclachlan & E M, Immune cell involvement in dorsal root ganglia and spinal cord after chronic constriction or transection of the rat sciatic nerve, *Brain Behav Immun*, 21 (2007) 599.

30 Djukic M, Mildner A, Schmidt H, Czesnik D, Brück W, Priller J, Nau R & Prinz M, Circulating monocytes engraft in the brain, differentiate into microglia and contribute to the pathology following meningitis in mice, *Brain*, 129 (2006) 2394.
31 Schwartz M & Moalem G, Beneficial immune activity after CNS injury: Prospects for vaccination, *J Neuroimmunol*, 113 (2001) 185.
32 Streit W J, Graeber M B & Kreutzberg G W, Functional plasticity of microglia: A review, *Glia*, 1 (1988) 301.
33 Ohsawa K, Imai Y, Nakajima K & Kohsaka S, Generation and characterization of a microglial cell line, MG5, derived from a p53-deficient mouse, *Glia*, 21 (1997) 285.
34 Ji R R & Suter M R, P^{38} MAPK, microglial signaling and neuropathic pain, *Mol Pain*, 3 (2007) 3.
35 Tonchev A B, Yamashima T, Zhao L & Okano H, Differential proliferative response in the postischemic hippocampus, temporal cortex and olfactory bulb of young adult macaque monkeys, *Glia*, 42 (2003) 209.
36 Imai Y & Kohsaka S, Intracellular signaling in M-CSF induced microglia activation: Role of Iba1, *Glia*, 40 (2002) 164.
37 Jinno S & Kosaka T, Reduction of Iba1-expressing microglial process density in the hippocampus following electroconvulsive shock, *Exp Neurol*, 212 (2008) 440.
38 Thomas W F, Brain macrophages: Evaluation of microglia and their functions, *Brain Res Rev*, 17 (1992) 61.
39 Tawfik V L, Nutile-McMenemy N, Lacroix-Fralish M L & Deleo J A, Efficacy of propentofylline, a glial modulating agent, on existing mechanical allodynia following peripheral nerve injury, *Brain Behav Immun*, 21 (2007) 238.
40 Romero-Sandoval A, Chai N, Nutile-McMenemy N & DeLeo J A, A comparison of spinal Iba1 and GFAP expression in rodent models of acute and chronic pain, *Brain Res*, 1219 (2008) 116.