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Aberrant remyelination of axons after heat injury in the dorsal funiculus of rat spinal cord

M. Sasaki and C. Ide

Department of Anatomy, Iwate Medical University School of Medicine, Uchimaru 19-1, Morioka 020, Japan

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Summary. We studied the course of demyelination and subsequent remyelination of nerve fibers after heat injury in the dorsal funiculus of the rat spinal cord. Four weeks after heat treatment, we observed, in addition to normally remyelinated axons, a few aberrantly remyelinated axons which had both CNS- and PNS-type myelin sheaths: the CNS-type myelin sheaths were always situated inside the PNS-type sheaths. This finding indicates that in some conditions Schwann cells can form myelin sheaths around those formed by oligodendrocytes.

Key words: Heating – Remyelination – Schwann cell – Oligodendrocyte – Spinal cord

Recently the morphological changes of normal CNS tissues caused by clinical or experimental heat treatments have been studied by light microscopy to analyze the adverse effects of therapeutic hyperthermia [1, 9, 10]. According to these studies, destructive changes such as demyelination, axon degeneration, vasogenic edema, and hemorrhage occur in the white matter when temperatures over 42 °C are sustain ed for about 60 min [1, 7, 12, 17, 18]. In a previous study [20], we reported that heat treatment of the spinal cord using a hot water-perfused tube can produce a confined lesion in the dorsal funiculus of the rat. In the affected area most myelinated nerve fibers were completely demyelinated, while the axons themselves remained almost unaffected. These denuded axons began to be remyelinated by Schwann cells or oligodendrocytes about 2 weeks after treatment, and were almost fully remyelinated by 1 month after treatment. Four weeks after heat treatment, among axons normally remyelinated by Schwann cells or oligodendrocytes, we noticed some axons with aberrant "double" myelin sheaths, i.e., the axon was surrounded internally by a CNS-type myelin sheath and externally by a PNS-type one. In the present study, we describe in detail the morphology of such aberrant myelin sheaths with special reference to the relationship between CNS- and PNS-type myelin sheaths.

Offprint request to: M. Sasaki (address see above)

Materials and methods

Adult male rats (Wistar strain), weighing 200 to 300 g, were used. The animals were anesthetized by intraperitoneal injection of Nembutal (sodium pentobarbital, 100 mg/kg body weight), and the thoracolumbar region of the spinal cord was exposed by laminectomy. To produce the heat injury to the spinal cord, the bent end of a polyethylene tube of 2 mm in diameter was placed on the dura mater for 60 min using a stereotaxic device. Hot water warmed by a water bath was perfused through the tube, and the temperature of the tube surface was maintained at 48 °– 50 °C as monitored by a thermocouple probe of 1.0-mm diameter located approximately 1 cm from the bent end. After such treatment, the wound was closed in anatomical layers. The details of the operation and treatment have been described previously [20].

The animals were fixed 4 weeks after the surgery by perfusion through the heart with a fixative containing 2.0 % paraformaldehyde and 2.5 % glutaraldehyde in 0.1 M cacodylate buffer. The lesioned area together with the adjacent intact portion was excised and stored overnight in the same fixative. These specimens were postfixed in a 1.0 % osmium tetroxide solution in 0.1 M cacodylate buffer for 2 h, dehydrated through a graded series of ethanol, and embedded in Epon 812. Ultrathin sections were prepared using an LKB Ultrotome and observed in a JEOL-100B electron microscope after double staining with uranyl acetate and lead citrate. Semithin sections, 1 μm thick, were stained with 1 % toluidine blue and observed by light microscopy.

Results

The rats showed no gait or other behavior disturbances after the operation. The lesion produced by heat treatment was confined within the dorsal funiculus of the spinal cord. Sequential changes in the affected area over 4 weeks were described in detail at the ultrastructural levels as in a previous study [20].

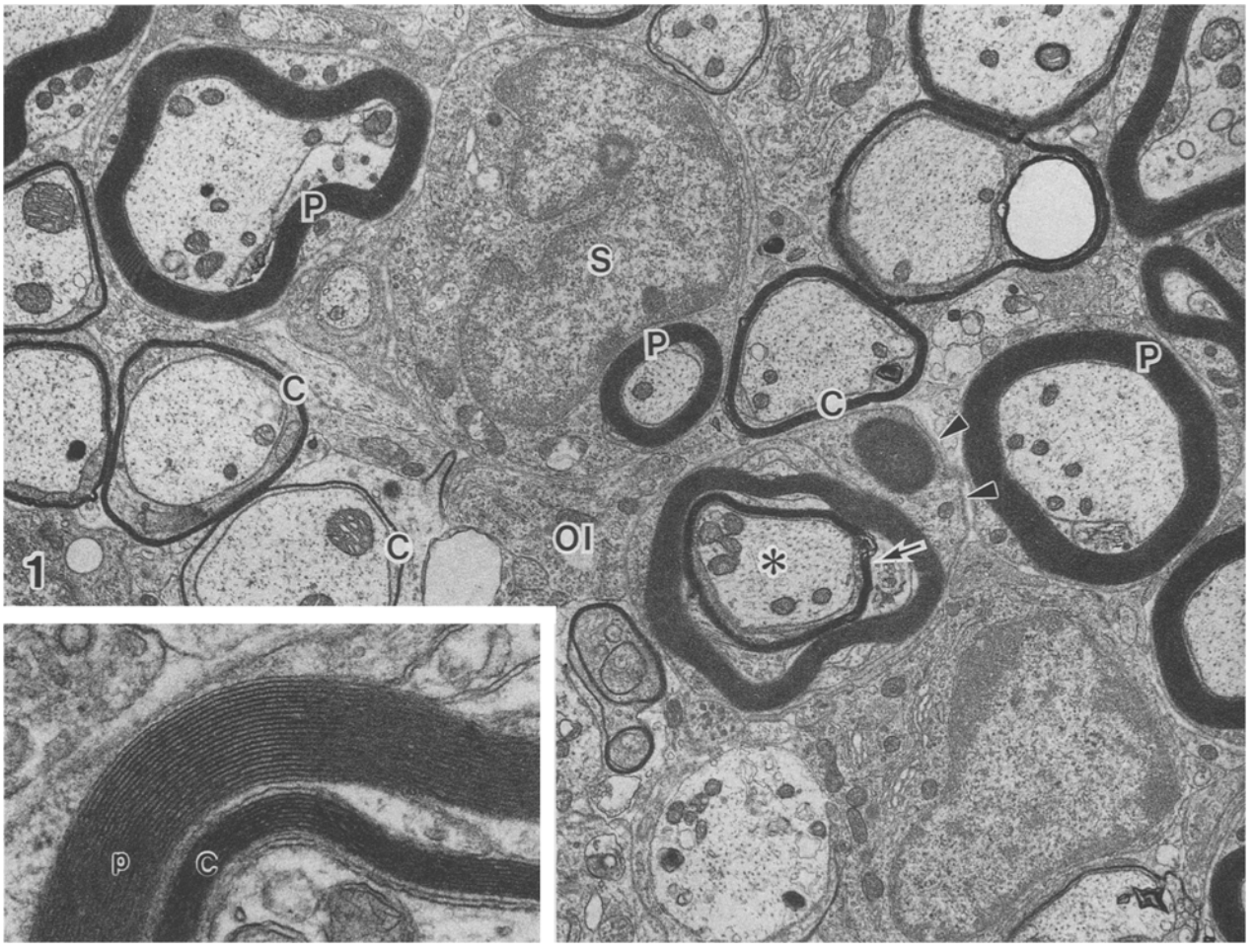
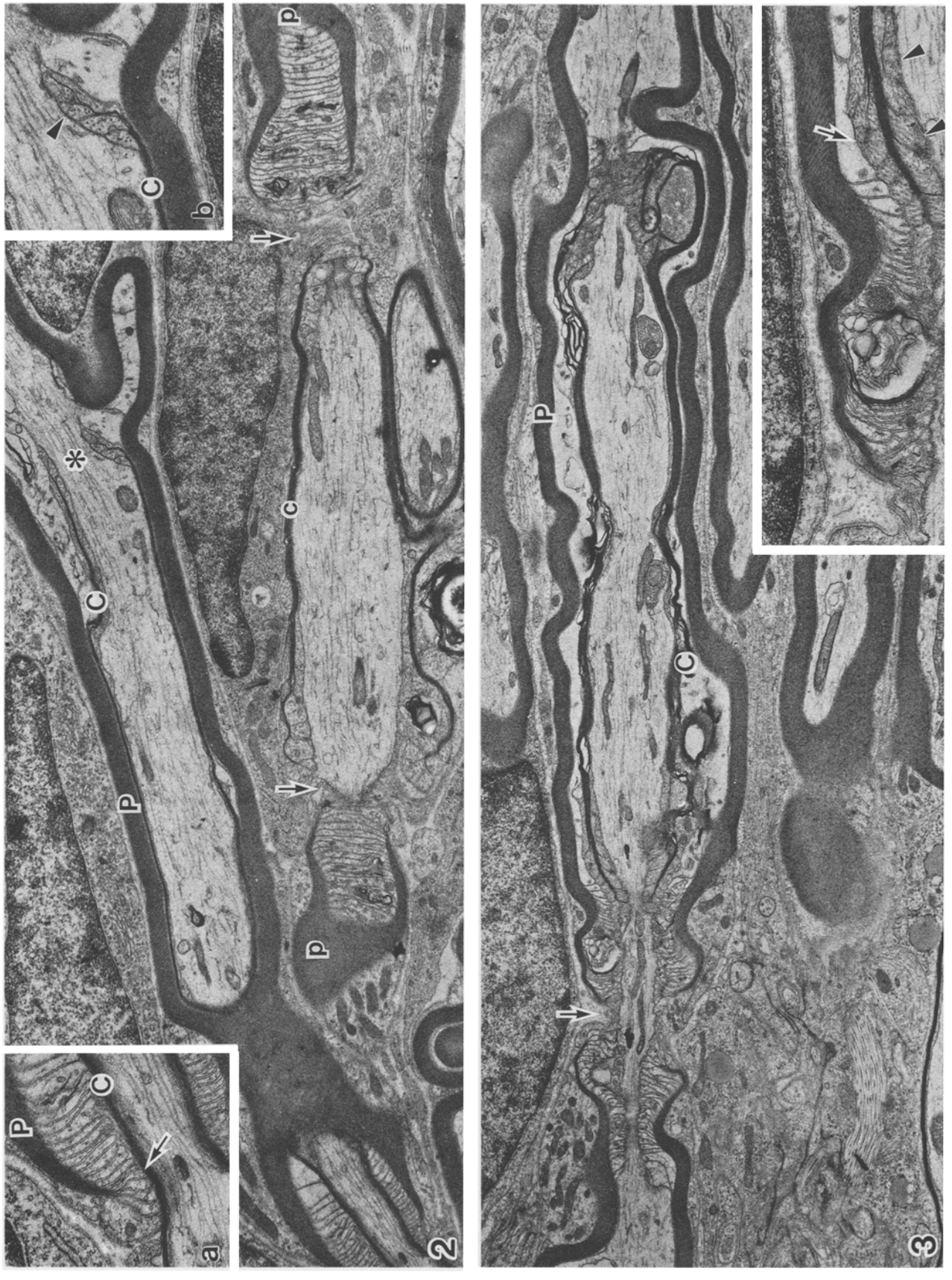


Fig. 1. This electron micrograph shows an affected part of the dorsal funiculus 4 weeks after heat treatment. Axons, denuded of myelin by heat treatment, are either thickly remyelinated (*P*) by Schwann cells (*S*), or thinly myelinated (*C*) by oligodendrocytes (*OI*). There is an axon (*asterisk*) which is surrounded by a CNS-type myelin sheath internally (*arrow*), and by a PNS-type myelin sheath externally. The Schwann cell cytoplasm (*arrowheads*) is present around this PNS-type myelin sheath. *Inset:* Enlargement of the part shown by an *asterisk*. The outer myelin sheath (*P*) surrounded by Schwann cell cytoplasm on the outer surface is the PNS type, whereas the inner one (*C*) is the CNS type, with a smaller interlamellar periodicity than that of the outer one. × 9,600; *inset* × 42,700

Fig. 2. This electron micrograph shows a longitudinal section obtained from the boundary zone, i.e., the area bordering the Schwann cell-remyelination and oligodendrocyte-remyelination regions. In the *upper part* of the micrograph, the axon is surrounded by a thick PNS-type myelin sheath (*P*) externally and thin CNS-type myelin sheath (*C*) internally. In the *lower part*, there is an axon with a thin CNS-type myelin sheath (*c*) interposed between two thick PNS-type ones (*p*). Nodes of Ranvier (*arrows*) are formed at both ends. *Inset a:* Higher magnification of the nodal part seen at the *lower left* of the aberrantly myelinated fiber. Lateral loops of the PNS-type myelin sheath (*P*) abut the CNS-type one (*C*) (*arrows*). *Inset b:* Part of the seminode of CNS-type myelin sheath (*asterisk*) surrounded by PNS-type myelin sheath. Lateral loops of the CNS-type myelin sheath (*C*) directly attach themselves to the axolemma (*arrowhead*). × 9,200; *insets* **a, b** × 19,300

Fig. 3. This electron micrograph shows another example of the axon which has an aberrant remyelination. An internal CNS-type myelin sheath (*C*) is covered over its entire length by a PNS-type one (*P*). Lateral loops of this externally formed PNS-type myelin sheath make a node of Ranvier with a neighboring PNS-type one (*arrow*). *Inset:* A high-power micrograph showing an intimate contact of lateral loops in the paranodal region of PNS- and CNS-type myelin sheaths. Relatively electron-dense lateral loops of the internal CNS-type myelin sheath are divided into inwardly and outwardly directed components; the former attach themselves to the surface of the axon (*arrowheads*), while the latter to lateral loops of the PNS-type myelin sheath (*small arrow*). × 6,800; *inset* × 19,300


a
P
C
b
C
2
P
C
*
P
c
P
3
P
C

Light microscopic findings

Four weeks after heat-treatment, almost all the axons that had once been denuded were completely remyelinated by Schwann cells or oligodendrocytes. Axons located near the pial surface exhibited thick myelin sheaths which had a 1:1 relationship with the associated Schwann cells. On the other hand, in the boundary zones to the surrounding intact area, axons had thin myelin sheaths exhibiting no cytoplasmic components on the external surface, the characteristic feature of myelination by oligodendrocytes. A thick layer of spindle-shaped dark pial cells covered the surface of the affected dorsal funiculus. There were no signs suggesting degeneration of axons, edema or hemorrhage in the CNS tissue of the affected region at this time.

Electron microscopic findings

Most axons located in the sub pial and perivascular areas of the affected region were thickly myelinated by Schwann cells. Such PNS-type myelin sheaths were about 0.5 to 1 μm in diameter and had an interlamellar periodicity of about 15 nm. A few collagen fibers were seen, but no astrocyte processes were present in the spaces between these nerve fibers. On the other hand, most axons were remyelinated by oligodendrocytes in the areas bordering on the intact zones. Such CNS-type myelin sheaths were as yet very thin (about 0.1 μm in thickness), displaying an interlamellar periodicity of about 13 nm. A few astrocyte processes were found among these axons.

The aberrant myelin sheaths were found in the transitional zones between these PNS- and CNS-type myelination areas: axons were surrounded internally by thin CNS-type, and externally by thick PNS-type myelin sheaths (Fig. 1).

Longitudinal sections clearly demonstrated the relationships between the two types of myelin sheaths. While normally remyelinated axons had CNS- and/or PNS-type myelin sheaths which were arranged in line, aberrantly myelinated axons had double myelin sheaths of the CNS and PNS type, as if the former was telescoped into the latter (Fig. 2). Overlapping of the two kinds of myelin sheath ranged from 8 to 20 μm in length. Among them, one PNS-type myelin sheath covered the full length of the internodal segment of the internal myelin sheath (Fig. 3). This inner myelin sheath showed the following morphological characteristics: (1) interlamellar periodicity was about 13 nm; (2) it had a short and thin internode similar to some other regenerated CNS-type myelin sheath; and (3) no cytoplasmic component suggesting a Schwann cell channel and no basal lamina were found. These findings support the idea that inner mylin sheath is the CNS type, because these findings are adequete to the morphological criteria of CNS-type regenerated myelin sheath shown in our recent paper [20]. In this case, lateral loops of the PNS-type myelin sheath extended beyond the termination of the internal CNS-type one to form a node of Ranvier with the neighboring PNS-type myelin sheath (Fig. 3).

The inner and lateral loops of the PNS-type myelin sheath usually abutted directly on the surface of CNS-type myelin sheath (Fig. 2). The inner loop of the PNS-type sheaths seemed to be easily detached from the CNS-type sheath at its paranodal portion during tissue preparation (Fig. 3). However, the lateral loops of PNS-type myelin sheath appeared to be able to attach themselves firmly to the CNS-type myelin sheath with about 15-nm spacing (Fig. 2 inset). The lateral loops of CNS-type myelin sheaths exibited unusual features in the attachment with the PNS-type one: half of them on the adaxonal side abutted on the axons as in the case of normal CNS myelin sheaths, while the remaining abaxonal half were turned outwardly to attach themselves to the inner and lateral loops of the PNS-type myelin sheath of the paranodal region (Figs. 2 inset, 3 inset).

Few processes or cell bodies of astrocytes were found in the transitional zones between PNS- and CNS-type remyelinated areas. Therefore, unlike the normal PNS-CNS junctions in the spinal dorsal root, areas of the nodes of Ranvier formed by the two types of myelin sheath were not covered by astrocyte endfeet.

Discussion

We found that almost all the axons were demyelinated in the heat-treated dorsal funiculus and that such denuded axons were well remyelinated by Schwann cells or oligodendrocytes by 1 month after injury. Occasionally, single axons were remyelinated by Schwann cells and oligodendrocytes forming alternate PNS- and CNS-type myelin sheaths. An unexpected finding was that some axons were aberrantly remyelinated, i.e., the PNS-type myelin sheath covered the CNS-type one. Such an aberrant remyelination was found in the both proximal and distal border zones of the lesion.

The present study is the first that has described an aberrant double remyelination in the CNS. On the other hand, in the normal PNS-CNS transitional zone of the dorsal root, a similar aberrant arrangement of two types of myelin sheath was reported by Berthold [2, 3]. This so-called "transitional internode" showed the insertion of the CNS-type myelin sheaths into the PNS-type ones in the same manner as described in the present study [2].

In the present study the longitudinal sections clearly showed the relationships of the two different types of myelin sheaths in the aberrant remyelination. Absence of the astrocyte processes at the site of apposition of Schwann cells and oligodendrocytes might be considered one of basic factors for the genesis of such an aberrant remyelination. In lesions made by treatments other than heat [6, 8, 19] nerve fibers with new CNS-type myelin sheaths are always surrounded by astrocyte processes, thus being definitely separated from PNS-type myelinated fibers. This means that Schwann cells cannot invade the CNS compartment. Similarly, in the case of normal dorsal root entry, astrocyte processes are always present between the PNS- and CNS-type myelin sheaths, thus keeping them from being in direct contact

with each other [2, 3]. However, in the case of the aberrant transitional internode as reported by Berthold [2], cytoplasm of the Schwann cells was partly in direct contact with the CNS-type myelin sheath. This aberrant transitional internode is considered to be formed by accidental direct contact of Schwann cells with oligodendrocytes at an early myelination during normal development.

On the other hand, in lesions caused by irradiation [5, 6] or by lysophosphatidylcholine injection [22] only a few astrocyte processes were found at the junctional spaces between Schwann cells and the myelin sheaths formed by oligodendrocytes. In this case Schwann cells can occasionally abut on oligodendrocytes without astrocyte intervention [13, 22]. Nevertheless, no overlapping of PNS- and CNS-type myelin sheaths has been reported in these studies, indicating that in addition to the primary requisite of the direct contact of Schwann cells and oligodendrocytes, there are some other unknown factors necessary for the formation of aberrant remyelination as described in the present study.

Why does the PNS-type myelin always cover the CNS-type one? Concerning the PNS "double myelination" in the sympathetic nervous system, Kidd and Heath [15, 16] have demonstrated that the outer Schwann cell failed to make contact with an axon, yet its myelin sheath remained intact [17]. This result suggests the ensheathning cell of the PNS-type myelin does not necessarily attach itself to the axons. In the present study lateral loops of PNS-type myelin firmly attached themselves to outer surface of the CNS-type myelin with a spacing of about 15 nm. Since the distance between these two kinds of myelin is characteristic to the glia-axonal interaction mediated by myelin-associated glycoprotein (MAG) [23], this phenomenon suggests an adhesion molecule of the PNS-type myelin, i.e., MAG, can form tight interaction to the CNS-type myelin as well as to the axonal surface. In addition, considering that the CNS-type remyelination occurs earlier than the PNS-type after heat injury, it would be highly unlikely that oligodendrocytes migrate to cover the PNS-type myelin sheath.

The aberrant remyelination as observed in the present study might occur under such a condition in which astrocytes or their processes do not form an effective barrier to the invading Schwann cells. Accordingly, Schwann cells come in direct contact with oligodendrocytes, and such Schwann cells are considered to migrate along the newly formed CNS-type myelin sheaths and eventually form PNS-type myelin sheaths on them.

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