

Wallerian Degeneration: Morphological and Molecular Changes

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Abstract

Wallerian degeneration is a process that follows damage to the nerve fiber. Instantly after the initial injury, Wallerian degeneration begins at the distal stump. The axon breaks down, retraction of the myelin sheath happens and the axoplasm is surrounded within ellipsoids of myelin. In response to loss of axons by disruption of their myelin sheaths, myelin genes are down regulated and Schwann cells dedifferentiated. Schwann cells start multiplying, and macrophages continue to digest debris. By the end of the first week, Schwann cells form a chain within the endoneurium. After about 2 weeks, macrophages disappear, leaving behind endoneurial tubes filled with Schwann cells. Here, we discussed morphological changes and sub cellular events during Wallerian degeneration.

Keywords: Hypoglossal Nerve; Lysosomal Enzymes; Medulla; Catabolic; Chromatolysis; Phenotype; Depolymerization; Schwann Cells; Neurotrophin-3

Abbreviations: MAPK: Mitogen-Activated Protein Kinase; AAD: Acute Axonal Degeneration; PNS: Peripheral Nervous System; CNS: Central Nervous System; mRNA: Messenger RNA; BDNF: Brain-Derived Neurotrophic Factor.

Introduction

Augustus Waller

Dr. Augustus Waller (1816-1870), British physiologist, defined morphological alterations together with the study of axons insulated from their cellular bodies; therefore its description as wallerian degeneration [1]. Waller conducts experiment on frogs in 1850, by cutting their glossopharyngeal and hypoglossal nerves. He then detected the distal nerves from the site of injury, which were disconnected from their cell bodies which is located in the brain stem. Waller nominated the fragmentation of myelin, which he mentioned to as "medulla", into discrete units of different sizes. The disintegrated axons shaped droplets that could be stained, consequently allowing studies of the development of individual nerve fibers [2].

Morphological Changes during Wallerian Degeneration

Wallerian degeneration is the trophic process of disintegration of a neuron or axon that happens in the neuron at the site of the injury and moves in a distal course from the cell body as follows [3]. The axon breaks down through a process of inflammation and following granulation that lasts about 3 to 4 days. In this process, extrusion of the myelin sheath happens and leads to surround the axoplasm within ellipsoids of myelin and hydrolysis happens by lysosomal enzymes within these ellipsoids. Ellipsoids or digested chambers are defined as secondary demyelination process and described as follows. Myelin fragmented into droplets. This swelling which forms beside the internodes contains axonal granules. Degeneration of axons and myelin happens together. In fact, because of a close collaboration between the axon and its myelin, and myelin dies if the axon disappears [4]. Consequently, the motor end-plate degenerates. Proliferation of the Schwann cells which contain nuclei and cytoplasmic organelles occur and subsequently, a column of Schwann cells form and recognize as a B ngner band. Simultaneously, proliferation of the adjacent endoneurial cells also happens. These B ngner's band or columns of Schwann cells provide corridors for regenerating axons to reach to

their targets that were denervated. Also, growth factors which are secreted by Schwann cells enforce the outgrowth of axonal sprouts from the proximal cut ends of neuron [5]. Concomitantly, in the injured distal cut end, Schwann cells release growth factors that entice new axonal buds recruiting from the proximal stump after complete cut. Therefore, possible reinnervation of the target cell or organ happens. Of course, the regeneration is not complete, as possible confusing happens during reinnervation of the proximal axons to designate cells [6]. This regeneration initiates in about 7 days. Each axon emits a number of processes called axonal sprouts that grow into the B ngner bands. The speed of growth is about 1 to 4 mm per day. This process is dependent on close immediacy of the axonal sprouts and the B ngner bands [7]. With this regard, the estimation of healing period by measuring the distance from the site of the injury to the middle of the denervated muscles. With regard to the slowest speed (1 mm/day), the healing period can be estimated as the distance in millimeters. When a nerve has been completely cut, it is important to suture the cut ends to provide the least inhibition to the axonal sprouts to regenerate. If an inhibition such as fibrosis stops the axonal sprouts to reach the close B ngner band, the axonal sprouts will regenerate in a random manner and form swellings called as a neuroma. These neuromas can be a source of significant distress. The resultant neuroma may then be an additional source of irritation to the patient [8]. Similar changes that happen in the neuron distal to the injury, other changes take place proximal to the injury. Following an injury, the injury will lead to degenerate of a few internodes proximal to the injury in a same rate comparable to the catabolic degeneration that happens distal to the injury [9]. A reaction happens in the cell body which is located in the spinal cord, named the axonal reaction as follows. An insignificant enlargement of the cell body, a dislocation of the nucleus to one side of the cytoplasm (an eccentric nucleus), and a distribution of the granular endoplasmic reticulum of the Nissle substance within the cytoplasm. The mentioned even it is termed as central chromatolysis and signifies the efforts of the cell body to create more products for the axoplasmic flow necessary to promote regeneration [10].

Distal End Changes

After damage, morphological changes occur not only at the damage site, but also in the proximal and distal regions. Above-mentioned, this process refers to events that occur after nerve injuries, during which a conductive micro-environment is re-created for axonal growth and

neuronal regeneration. This process occurs during the first week after injury and when axonal damage markers such as the lack of cell membrane integrity and the breakdown of the axonal skeletal system occur. Axonal changes at the distal nerve cut end result in the breakdown of the nerve endings to create a way to repair the axon. The marked sign of this phase is the granulation of the cellular skeleton. This occurs after the flow of extracellular ions such as calcium and sodium, causing a cascade of events such as apoptosis. Calcium influx signaling promotes resealing of cut ends. Axonal injuries initially lead to acute axonal degeneration (AAD), which is due to rapid separation of the proximal cut end (the part nearer the cell body) and distal cut end (the part farther the cell body) within 30 minutes of injury [11]. Granular disintegration of the axonal cytoskeleton and inner organelles occurs after axolemma degradation. Early changes include accumulation of mitochondria in the paranodal regions at the site of injury. Endoplasmic reticulum degrades and mitochondria swell up and eventually disintegrate. The depolymerization of microtubules occurs and is soon followed by degradation of the neurofilaments and other cytoskeleton components [12]. The disintegration is dependent on Ubiquitin and Calpain proteases (caused by influx of calcium ion), suggesting that axonal degeneration is an active process and not a passive one as previously misunderstood [13]. Thus the axon undergoes complete fragmentation. The rate of degradation is dependent on the type of injury and in the PNS is also slower than in the CNS. Another factor that affects degradation speed is the diameter of the axon: larger axons require a longer time for degradation of the cytoskeleton and thus takes a longer time to degenerate and macrophages are recruited by a signal from the Schwann cell. It has been shown that the distal cut end is capable of transmitting the potential of action hours after interruption. Recently, it has been seen that there is an increase in the BDNF [14] and GDNF [15] mRNA expression [16]. Also, mRNA expression of neurotrophin-3 and ciliary neurotrophic factor decrease [14,17].

Proximal End Changes

Changes occur in proximal cut end vary according to the location of damage with regard to the nerve trunk and severity of injury. Apoptosis occurs if the injury is near the trunk. In severe injuries; the nerve trunk is affected by chromatolysis and is restored by changing the genetic program of the cell body to the regenerative phenotype [18]. During this process, associated-growth factors such as GAP-43, tubulin, and actin will increase, and neurofilaments which are involved in the formation of axon diameter, will decrease [15,19,20]. Nerve protectors,

such as heat shock proteins will be up-regulated to increase the survival of injured neurons [21,22]. When axon ultimately reaches their target organ [23], neuronal maturation is required, and the gene expression in the neurons switches from regenerative to supportive state. Schwann cells are the primary mediators and initiators of many events in Wallerian degeneration at the site of injury [24]. Schwann cells play as the key to restoring axons. When axons and Schwann cells loose connection, Schwann cells find a non-myelinating behavior, and several proteins such as PMP22, Krox-20 [25], P0 and Connexin-32 [26] are decreased [27]. Synthetic process stops, and differentiation process begins with production of C-jun [28] and neurotrophic factors such as NGF [29] and ciliary neurotrophic cell cycle factor [27]. These factors are involved in the formation of Schwann cells reservoir by aid of intrinsic compounds such as erythropoietin. These changes are regulated by the release of mitogens such as ATP and Neuregulin from the proximal cut end of the neuron. Neuregulins are believed to be responsible for the rapid activation [30,31]. They activate ErbB2 receptors in the Schwann cell microvilli, which results in the activation of the mitogen-activated protein kinase (MAPK) [24]. Although MAPK activity is detected, the injury *sensing* mechanism of Schwann cells is yet to be fully understood. The *sensing* is followed by decreased synthesis of myelin lipids and eventually stops within 48 hrs. The myelin sheaths separate from the axons at the Schmidt-Lanterman incisures first and then rapidly deteriorate and shorten to form bead-like structures. Above-mentioned factors together with acetylcholine help to maturation of Schwann cells in the development of myelin [32]. Schwann cells continue to clear up the myelin debris by degrading their own myelin, phagocyte extracellular myelin and attract macrophages to myelin debris for further phagocytosis [33].

Schwann Cells and Macrophages

However, the macrophages are not attracted to the region for the first few days; hence the Schwann cells have the major role in myelin cleaning. Schwann cells purify cell residues with the process of phagocytosis and macrophages. Schwann cells have been observed to recruit macrophages by release of cytokines and chemokines after *sensing* of axonal injury. This effect goes back to the MAC-2 protein that supports the Schwann cell phagocytosis [34]. Schwann cells are the source of monocyte-1 adsorbent protein that interferes with the recruitment of macrophages [35]. The macrophages are absorbed by the Schwann cells in the area of the damage, a process that is dependent upon the breakdown of the blood-brain barrier [35]. In peripheral nervous system,

macrophages attach their receptor called CCR2 which is necessary for Wallerian degeneration [36]. The recruitment of macrophages helps to improve the clearing rate of myelin debris. The resident macrophages present in the nerves release further chemokines and cytokines to attract further macrophages. Another source of macrophage recruitment factors is serum. Delayed macrophage recruitment was observed in B-cell deficient mice lacking serum antibodies³³. While Schwann cells mediate the initial stage of myelin debris clean up, macrophages come in to finish the process. Debris clean up done by macrophages are mediated by opsonins, which label debris for removal. The 3 major groups found in serum include complement, pentraxins, and antibodies. However, only complement has shown to help phagocytosis of myelin debris [27]. Macrophages are involved in the use of damaged nerve cholesterol and the production of apolipoprotein E from other lipoproteins [37,38]. After cleaning the myelin residues, dedifferentiated Schwann cells proliferate in the endoneurial tube of the in the extracellular matrix. Possible sources of proliferation signal are attributed to the ErbB2 receptors and the ErbB3 receptors [39]. Cellular candidates such as dedifferentiated Schwann cells, neutrophils, dendritic cells, fibroblasts, and endothelial cells have the capability to perform phagocytosis and clearance of debris which have shown that neutrophils play an impressive role in myelin removal during Wallerian degeneration [40]. It is reported that neutrophils phagocytosis is done by using lytic enzymes produced from their granule proteins to digest and remove foreign debris. Also, neutrophil granules are important for macrophage differentiation and cytokine production [41].

Conclusion

Wallerian degeneration or also called as Wallerian degeneration of the pyramidal tract is a process that results when a nerve fiber is cut or crushed and the part of the axon distal to the injury (i.e. farther from the neuron's cell body) degenerates. A related process known as 'Wallerian-like degeneration' occurs in many neurodegenerative diseases, especially those where axonal transport is impaired. In peripheral neuropathies such as Guillain-Barré syndrome and Charcot-Marie-Tooth, Wallerian degeneration is also studied. In addition, C57BL/*Wlds* mice which has delayed Wallerian degeneration opens new windows to better and precise study of cellular and molecular processes in accompany with Wallerian degeneration. Decreasing the onset of Wallerian degeneration increases the quality of nerve regeneration. Also, two main aims in managing peripheral

nerve injuries include modulation of inflammatory responses and upgrading of axonal regeneration. Understanding the mechanisms and differences of Wallerian degeneration between central nervous system and peripheral nervous system is a promising way to treat related disease and disorders.

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