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Impaired regeneration in aged nerves: Clearing out the old to make way for the new

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ABSTRACT

Although many observational studies have shown that peripheral nerve regeneration is impaired with aging, underlying cellular and molecular mechanisms have remained obscure until recently. A series of recent genetic, live imaging and heterochronic parabiosis experiments are providing new insights into the underlying mechanisms of reduced regenerative capacity with aging. These studies show that Schwann cells pose a primary impediment to axon regeneration in older animals as they fail to support regenerating axons, while the contribution from macrophages remains an unresolved issue. Neurons do not appear to have an intrinsic defect of axonal elongation with aging but are impaired when they encounter an inhibitory environment, suggesting that therapeutic approaches to improve intrinsic neuronal regeneration capacity across inhibitory environments, as it is being done in central nervous system regeneration, can improve peripheral nerve regeneration as well. As in many aspects of neuroscience therapeutics development, a combinatorial approach may yield the best outcomes for nerve regeneration in aged individuals.

Contents

1. Introduction . . . 79
2. Initial studies . . . 79
3. Recent studies . . . 80
   3.1. Schwann cells . . . 80
   3.2. Macrophages . . . 80
   3.3. Circulating factors . . . 81
4. Conclusion . . . 82
Acknowledgements . . . 82
References . . . 82

1. Introduction

It has been known for a long time that advanced age contributes to poor regeneration after peripheral nerve injury. A multitude of earlier studies described this finding in animal models of nerve injury, and although a host of possible explanations were examined, no molecular or mechanistic insights were gained. After decades of research and no significant advances, the field was mostly quiet until recently. Improved imaging, heterochronic parabiosis models, and molecular studies have awakened this topic again, allowing us to finally begin teasing apart this complex system to uncover how aging impairs nerve regeneration.

2. Initial studies

Peripheral nerves are capable of regeneration after injury, but functional recovery is often limited. Several factors that contribute to poor prognosis include size and location of injury, as well as timing and type of surgical intervention (Scheib and Hoke, 2013). Clinical investigations have revealed that advanced age correlates with poor prognosis (Kovacic, et al., 2009, Nagano, 1998, Wang and Casolaro, 2014), and several animal studies have supported this. Around 1940 through 1990, a variety of injury models in rodents and rabbits were performed,

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including nerve crush, transection, freezing, and botulism toxin (Black and Lasek, 1979, Kovacic, et al., 2010, Navarro and Kennedy, 1988, Pestronk, et al., 1980, Vaughan, 1992, Verdu, et al., 1995, Verdu, et al., 2000, Weddell, et al., 1941). Regeneration was analyzed by measuring functional recovery and/or counting regenerating axons and collateral sprouting. Taken together, the data reveal that regeneration is slower and less complete in aged animals than young animals.

Initially, impaired regeneration in aged animals was attributed to aged neuron's inability to extend axons. In support of this, axon transport was found to be slower in aged neurons (Brunetti, et al., 1987, McQuarrie, et al., 1989, Milde, et al., 2015, Parhad, et al., 1995), with a delay in transporting materials to the growth cone seeming like an obvious explanation for the impaired regeneration. In addition, it was suggested that aged neurons might be less able to respond to trophic factors (Kovacic, et al., 2009, Parhad, et al., 1995, Uchida and Tomonaga, 1987). However, the nerve is a complex multicellular environment, and other studies started to analyze cell types other than neurons. It was thought that, with aging, distal nerve could become less supportive of regenerating axons. In fact, multiple studies noted a delay in clearance of debris after injury in aged animals (Tanaka and Webster, 1991, Tanaka, et al., 1992, Vaughan, 1992). This lead to further analysis of nerve degeneration and two of the cell types involved: Schwann cells and macrophages.

3. Recent studies

In vivo time-lapse imaging of fluorescent motor axons, regenerating after crush, allowed for a comparison of growth rates in young and aged mice (Kang and Lichtman, 2013). Surprisingly, the peak growth rates of young and aged axons were similar, suggesting that neuronal age does not directly affect the intrinsic ability of neurons to extend an axon. However, the average growth rate was slower for aged axons, and although both young and aged axons would temporarily slow down at times, the aged axons had more moments of slowing and temporarily stopping, as if they were coming in contact with more obstructions. Indeed, aged nerves contained more Schwann cells with vacuoles of myelin debris. When axons of any age came in contact with myelin debris, they decelerated and grew around the obstruction. Taken together, the authors concluded that delayed clearance of debris in aged mice was causing slowed growth rates of axons.

3.1. Schwann cells

In one of the most thorough studies on this topic to date, Painter et al. analyzed neurons, Schwann cells, and macrophages after injury of sciatic nerve in mice (Painter, et al., 2014). Using transcriptional profiling, they found that young and aged dorsal root ganglia (DRG) neurons did not differentially express regeneration-associated genes (RAGs) after injury, which supports previous data that aged neurons are not intrinsically less able to regenerate. However, distal to injury, several genes were found to be deregulated after injury, including the transcription factor c-Jun. Recent work found that c-Jun. is required for Schwann cells to take on a repair cell phenotype after injury (Arthur-Farraj, et al., 2012). In a conditional knock-out mouse, where c-Jun was absent in Schwann cells, Schwann cells were less able to maintain Bands of Bungner or break down myelin debris, and regeneration was impaired (Arthur-Farraj, et al., 2012, Fontana, et al., 2012). In addition, 172 molecules were transcriptionally deregulated, including growth factors, adhesion molecules, and other transcription factors (Arthur-Farraj et al., 2012). Therefore, therapeutic c-Jun expression in denervated Schwann cells is currently a prominent candidate to increase peripheral nerve regeneration.

Painter et al. (2014) also revealed that aged mice had better functional recovery after receiving young nerve grafts, supporting the idea that nerve environment is the critical factor that alters regeneration in aging. In agreement with previous studies, the authors found a delay in macrophage recruitment and debris clearance with aging. To determine if young macrophages could rescue delayed regeneration in aged mice, heterochronic parabiosis was used to combine the circulatory systems of young and aged mice, and then one mouse in each parabiotic pair underwent sciatic nerve crush. Since young (injured): young pairs had better functional recovery compared to old (injured): young pairs, it was concluded that young macrophages could not fully rescue the phenotype. The shortcoming of this experiment was that since the Schwann cells in the injured nerve were aged, it is possible that the recruitment of young macrophages from the parabiotic pair was impaired due to age of the Schwann cells rather than inability of the young macrophages to rescue the phenotype. Nevertheless, this study identified age of the Schwann cells as a critical factor in regeneration in older nerves.

3.2. Macrophages

In addition to Schwann cells, macrophages play an important role in clearance of debris and regeneration. Since myelin contains inhibitory molecules (Filbin, 2003, Gaudet, et al., 2011, Winzeler, et al., 2011), it needs to be efficiently removed after injury. When macrophages were prevented from entering injured sciatic nerve, inhibitory myelin debris was not cleared, neurotrophins were not upregulated, and regeneration was impaired (Barrette, et al., 2008, Tanaka, et al., 1992). However, in C-C chemokine receptor type 2 (CCR2) knock-out mice, in which macrophages do not enter injured nerves, there was normal clearance of myelin 7 days after axotomy implying that in the absence of blood derived macrophages endogenous resident macrophages of the nerve and activated Schwann cells can clear myelin debris (Niemi, et al., 2013). Although these results are contradictory, macrophages remain a key therapeutic target for nerve repair.

In a recent publication, our group found that debris clearance was impaired in young rat sciatic nerves when grafted into aged rat nerves, yet the clearance defect was not fully rescued in aged rat nerves when grafted into young rat nerves (Scheib and Hoke, 2016). These data suggested that Schwann cell age in the graft was not the only factor contributing to delayed clearance; rather, age of host rat was also having an effect. Possibly, macrophages or other circulating factors in aged rats were not supporting efficient clearance. We found that aged rats had an attenuated immune response after nerve injury (Fig. 1). Interestingly, we also found that healthy, uninjured nerves had an increased number of activated macrophages. This may not be surprising, considering that the immune system is known to be less effective in aged individuals, yet there is an increased basal level of inflammation, sometimes referred to as “inflammaging” (Franceschi, et al., 2007). This chronic sterile inflammation includes increased circulating levels of pro-inflammatory mediators, such as interleukin 6 (IL-6), interleukin 1 beta (IL-1β), and tumor necrosis factor (TNFα) (Franceschi, et al., 2000). In addition, macrophages from aged animals are less phagocytic and have altered cytokine and chemokine secretion (Ferrandez and De la Fuente, 1999, Izgut-Uysal, et al., 2004, Li, 2013, Plowden, et al., 2004).

In response to injury, aged macrophages may show altered M1/M2 polarization. Recently, studies have tried to describe microglia/macrophages as being either a classically activated M1 or alternatively activated M2 phenotype. Although this is an oversimplification of a spectrum of multiple activation states (Martinez and Gordon, 2014, Miron and Franklin, 2014), it allows us to begin characterizing the complexities of immune response to injury. M1 macrophages produce pro-inflammatory cytokines and oxidative metabolites, and thus damaging tissues, and they are often characterized by their expression of inducible nitric oxide synthase (iNOS), TNFα, IL-1β, and IL-6. M2 macrophages are anti-inflammatory and express arginase-1, YM1, mannose receptor CD206, and interleukin 10 (IL-10). There are three subtypes of M2 macrophages. M2a and M2c are healing and release anti-inflammatory cytokines, promoting tissue repair. M2b produce both pro- and anti-inflammatory cytokines.