Effect of modulating macrophage phenotype on peripheral nerve repair

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ABSTRACT

Peripheral nerve repair across long gaps remains clinically challenging despite progress made with autograft transplantation. While scaffolds that present trophic factors and extracellular matrix molecules have been designed, matching the performance of autograft-induced repair has been challenging. In this study, we explored the effect of cytokine mediated 'biasing' of macrophage phenotypes on Schwann cell (SC) migration and axonal regeneration in vitro and in vivo. Macrophage phenotype was successfully modulated by local delivery of either Interferon-gamma (IFN-γ) or Interleukin-4 (IL-4) within polymeric nerve guidance channels, polarizing them toward pro-inflammatory (M1) or pro-healing (M2a and M2c) phenotypes, respectively. The initial polarization of macrophages to M2a and M2c phenotype results in enhanced SC infiltration and substantially faster axonal growth in a critically-sized rat sciatic nerve gap model (15 mm). The ratio of pro-healing to pro-inflammatory population of macrophages (CD206+/CCR7+), defined as regenerative bias, demonstrates a linear relationship with the number of axons at the distal end of the nerve scaffolds. The present results clearly suggest that rather than the extent of macrophage presence, their specific phenotype at the site of injury regulates the regenerative outcomes.

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1. Introduction

Peripheral nerve defects are caused due to trauma or in the course of surgery [1]; however, bridging long gaps remains a clinical challenge [2,3]. The current clinical approach for repairing long nerve deficits (15 mm or longer) is to use nerve autografts; however, the paucity of donor nerves, the disparity of donor nerve sizes with the recipient sites and the modality mismatch has compelled a search for alternatives that can match or exceed autograft performance [1–4].

Several approaches to enhance regeneration have been explored including the design of novel nerve guidance channels [2], fillers within nerve guidance channels [5], local delivery of neurotrophic factors [6,7], transplantation of cells [8,9], and/or application of topographical cues [4,10,11]. While these approaches are promising, the overall success rate in matching autograft performance has been limited. Current approaches focus on enhancing axon growth by direct action on nerves, or glial cells, and here we investigate an alternative approach to influencing regenerative outcomes by modulating the initial inflammatory sequence via

macrophages [12]. Since the sequence of cellular and molecular events associated with nerve regeneration is influenced by immune cells [13,14], we hypothesize that modulating immune cells upstream of action on nerves or Schwann cells (SC) triggers endogenous repair mechanisms that can stimulate nerve repair across long gaps [12].

It is evident that the immune response plays an important role during regeneration in many tissues [15–17]. Macrophages are quite abundant and phenotypically diverse immune cell populations presented during nerve degeneration and regeneration [12,13,18]. Macrophages arrive at the site of injury within 24 h and their numbers at the site peak within 14–21 days [19], whereas it takes at least one week for lymphocyte influx to occur [13]. Macrophages, which are mainly recruited from circulation, account for the bulk of phagocytosis within days of peripheral nerve injury and play a critical role in debris removal, growth factor production, and remodeling of the extracellular matrix (ECM) of the distal nerve [20,21]. Thus macrophages might represent an upstream 'lever' to influence downstream axon and SC regeneration (Fig. 1a).

Recently, macrophages have been demonstrated to have a spectrum of activation states/phenotypes which has led to categorizing them broadly as classically activated (M1) and alternatively activated (M2) macrophages [22–24]. M1 macrophages, which are activated by injury-triggered endogenous inflammatory signals, such as T-helper 1 (Th1) cytokine IFN-γ, considered to be