Macrophages and dendritic cells treatment of spinal cord injury: from the bench to the clinic

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Summary

The failure of the spinal cord to recover after injury has been associated with the immune privilege mechanism that suppresses immune activity throughout the central nervous system. Primed macrophages and dendritic cells were shown to promote neurological recovery in preclinical models of spinal cord injury. A cell therapy consisting of autologous incubated macrophages is now being tested on spinal cord injury patients in clinical trials.

Keywords: Cell therapy; immune privilege; neuroprotection; neurodegeneration; neuroimmunology.

Introduction

Most tissues of the human body are able to regenerate and repair damage caused by injury or disease. This includes the tissues of the peripheral nervous system (PNS). In marked contrast, the central nervous system (CNS) has virtually no regenerative capacity. Moreover, the initial damage is amplified by the spread of a damage beyond the primary lesion causing extensive functional loss. Thus, a damage to the CNS causes permanent disability, in the case of spinal cord injury (SCI) paraplegia or tetraplegia.

A common strategy in the development of therapeutic options for treating SCI has been to seek to interrupt stages in the damage process. Some of the damage processes targeted are: glutamate toxicity, reactive oxygen and nitrogen species, trophic factor deprivation, growth inhibitors, ion imbalance, etc. None of these approaches have yet led to an effective pharmacological therapy for SCI [6], although since the early 1990’s, there has been widespread use of the anti-inflammatory drug methylprednisolone administered in the first hours following SCI, a marginally effective [4, 5, 26] but controversial treatment [10, 20, 22].

In our search for an alternative therapeutic strategy for SCI, our research has concentrated on finding the physiological difference between the CNS and other tissues that may account for their different regenerative capacities. The underlying assumption is that inducing an appropriate physiological response to injury in the CNS may lead to sustained, comprehensive wound healing that overcomes some of the neurological loss. One major difference found is in the activity of the immune system in the different tissues. In most tissues, the immune system is continuously active in countering foreign agents and in removing tissue debris. Immediately after injury, the innate immune response is activated, with blood-borne macrophages arriving at the lesion site where they begin to clear debris and toxic elements. The macrophages also activate the lymphocytes, creating an inflammatory environment that is conducive to tissue renewal. In contrast, immune activity in the CNS is suppressed by a mechanism known as immune privilege, with the resident immune cells (microglia) suppressed by the environment and the entry of blood-borne macrophages almost non-existent after injury. Furthermore, the microglia respond to the injury in a way that may exacerbate the damage. Thus, after injury the CNS environment remains hostile and unsupportive of tissue repair. With increasing recognition of the central role of the immune system in physiological tissue repair [9], the idea arose of developing therapeutic technologies based on introducing elements of the immune system into the injured CNS.
Rationale for using macrophages and dendritic cells for treatment of spinal cord injury

In most non-CNS tissues, macrophages are primary players in the immune response to injury, being the first cells to arrive at the lesion site. Here they remove toxic elements and debris, and release cytokines that induce the recruitment of lymphocytes. Additionally, the macrophages act as antigen presenting cells (APCs), presenting debris-derived antigens on the cell surface to cause the specific activation of matching lymphocytes. The activated macrophages and lymphocytes then secrete more cytokines and growth factors that support tissue survival and regrowth. Interferon-γ plays a central role in the process, being the main cytokine secreted by a T-cell after its activation by a matching antigen presented on the APC MHC-II cell surface complex. Interferon-γ then acts on macrophages and similar cells to stimulate their APC activity, thus providing an amplification mechanism.

Though similar to blood-borne macrophages in some respects, CNS-resident microglia are poor APCs. Experiments have shown that microglial cells are responsive to molecules in their environment and the effect of activated microglia can be either protective or destructive depending on the activator used. Microglia activated with molecules such as lipopolysaccharide, amyloid-β and zymosan, known activators of macrophages via the “classical pathway”, cause increased cell death in the hippocampus of brain slices (unpublished results). In contrast, microglial cells, activated with Interferon-γ, a major secretory cytokine of activated helper T cells, promote hippocampal cell survival. The two contrasting types of microglial activation are also seen in the resulting metabolic activities of the microglia, with lipopolysaccharide-activation causing enhanced release of nitric oxide but no effect on glutamate uptake, while activation with Interferon-γ has no effect on nitric oxide release but enhances the uptake of glutamate.

Interferon-γ would not normally be expected in the injured CNS, because even though lymphocytes arrive at the lesion site, there are no effective APCs present to activate them, since blood-borne macrophages are excluded and the resident microglia show insufficient APC activity. We suggest that the lack of APCs can be overcome either by supplementing the injured CNS with exogenous APCs, or by vaccination with a CNS-specific antigen to boost the population of suitable lymphocytes.

It has been established that macrophages take on APC-like characteristics after incubation with regenerative tissue such as peripheral nerve tissue or skin [3]. After incubation, the macrophages have elevated MHC-II and co-stimulating molecules (CD80, CD86 and ICAM-1), all molecules participating in antigen presentation. The macrophages secrete pro-inflammatory cytokines, notably Interleukins 1β and 6, but reduced amounts of tumor necrosis factor α. The macrophages also produce growth factors such as brain-derived neurotrophic factor.

Dendritic cells, a type of professional APC, can be produced by culturing bone-marrow cells with interleukin-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF). These cells express very high levels of MHC-II and costimulatory molecules [8].

Effects of macrophages and dendritic cells in animal models of axonal injury

Axonal transection

Optic nerve and spinal cord transection models were used to test macrophages that had been incubated ex vivo in environments typical of the injured PNS (co-incubation with sciatic nerve segments) or the injured CNS (co-incubation with optic nerve segments).

When injected into the transected optic nerve, PNS co-incubated macrophages were found to promote myelin clearance [11], considered a prerequisite for axon regeneration [24]. Treatment with the PNS co-incubated macrophages was also found to restore axon continuity [12]. In contrast, macrophages that had been co-incubated with CNS-tissue proved to be ineffective.

In experiments conducted using the rat spinal cord transection model, injection of PNS co-incubated macrophages to the severed spinal cord resulted in partial reversal of paraplegia, with meaningful motor recovery observed in 15 of 22 macrophage-treated animals, while none of the 47 control rats showed recovery [23]. Neurological recovery after spinal cord transection has also been achieved using skin co-incubated macrophages.

Spinal cord contusion

The rat contusion model is the most thoroughly investigated and frequently used model for SCI and was