



Review

Immune effects of mesenchymal stem cells: Implications for Charcot–Marie–Tooth disease [☆]

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ABSTRACT

Mesenchymal stem cell (MSC) therapy is the most clinically advanced form of cell therapy, second to hematopoietic stem cell transplants. To date, MSC have been used for immune modulation in conditions such as Graft Versus Host Disease (GVHD) and Crohn's Disease, for which Phase III clinical trials are currently in progress. Here, we review the immunological properties of MSC and make a case for their use in treatment of Charcot–Marie–Tooth disease type 1 (CMT1), a group of inherited peripheral neuropathies. CMT1 is characterized by demyelination and aberrant immune activation making this condition an ideal target for exploration of MSC therapy, given the ability of these cells to promote sheath regeneration as well as suppress inflammation. Studies supporting this hypothesis will be presented and placed into the context of other cell-based approaches that are theoretically feasible. Given that MSCs selectively home to areas of inflammation, as well as exert effects in an allogeneic manner, the possibility of an “off the shelf” therapy for CMT1 will be discussed.

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1. Mesenchymal stem cells

Mesenchymal stem cells (MSC) were initially described as an adherent population isolated when bone marrow mononuclear cells are cultured in vitro. In 1968 Friedenstein and colleagues provided a morphological description of what we now know to be MSC, and also identified their osteogenic ability [1]. MSC are identifiable as lacking expression of hematopoietic markers, expressing CD29, CD73, CD90, CD105, and CD166, and having ability to differentiate into bone, cartilage, and adipocytes when cultured under defined conditions [2]. Although originally identified from bone marrow, MSC or cells having MSC-like properties have subsequently been found in muscle [3], menstrual blood [4], cord blood [5], and a variety of other tissues [6]. The ability of MSC to differentiate into various tissue-specific cell types has been demonstrated in vitro and in animal models [7,8]. An MSC-like cell, termed Multipotent Adult Progenitor Cell (MAPC) has been described as having near-totipotency, even being able to generate hematopoietic lineage cells [9,10]. However, despite the various multilineage differentiation ability of MSC, their main therapeutic application has been immune modulation. Unlike other types of

stem cells, MSC not only “escape” immune responses but also the ability to mediate active suppression. For example, numerous investigators have reported MSC produce various immune suppressive mediators such as soluble HLA-G [11], prostaglandin E2 [12], IL-10 [13], and TGF- β [14]. From a functional standpoint, it is well-established that MSC also indirectly inhibit various immune responses through activation of T regulatory cells [15,16] and suppression of dendritic cell maturation [17]. Interestingly, pro-inflammatory cytokines such as TNF- α and IFN- γ profoundly inhibit hematopoiesis [18–20], therefore MSC which are located in the bone marrow niche may have a functional role in protecting the hematopoietic stem cell compartment from unrestrained inflammatory responses.

Indeed, it was the anti-inflammatory, and not the regenerative “stem cell” properties of the MSC that brought about their first clinical use. GVHD, a major complication associated with bone marrow transplantation, arises as a result of homeostatic expansion of newly-formed (or graft contaminating) lymphocytes that mediate an immune response against antigens of the transplant recipient, resulting in systemic inflammation and sometimes death [21,22]. While mild GVHD may be controlled by conventional immune suppressants, acute GVHD is refractory to most interventions and is associated with a high level of mortality. In 2004, Katherine Leblanc's group from the Karolinska Institute reported successful intervention and one year relapse-free follow up of a patient with treatment refractory grade-IV GVHD using third-party

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MSC [23]. Subsequently, these studies were repeated both by academic groups [24], as well as in industry [25] with positive results. MSC are currently in pivotal Phase III registration trials in the US for GVHD by the company Osiris Therapeutics. Preclinical models of autoimmune/inflammatory conditions such as rheumatoid arthritis [16], multiple sclerosis [26], transplant rejection [27,28], and autoimmune myocarditis [29] have been demonstrated to respond to treatment with MSC. Collectively, these studies point toward the potential utility of MSC in the treatment of other inflammatory disorders.

2. Charcot–Marie–Tooth disease

Charcot–Marie–Tooth disease (CMT), also known as hereditary motor and sensory neuropathy (HMSN) or peroneal muscular atrophy, affects approximately 1 in 2500 Americans. The disease is named for its discoverers: Jean-Martin Charcot (1825–1893) and his pupil Pierre Marie (1853–1940), independently, Howard Henry Tooth (1856–1925) described the condition in 1886. Although CMT is not considered a fatal disease, it significantly decreases quality of life, depending upon the severity. Patients with CMT suffer from loss of muscle tissue and sensation, particularly in the extremities. There are 5 main types of CMT, and each of these is composed of several subtypes. The main forms of CMT include: CMT1, which is associated with abnormalities in myelin formation and comprises approximately 60–80% of CMT cases; CMT2, which is caused by axonal cell death in absence of myelin abnormalities, comprising 20–40% of cases; and CMTX which has both demyelinating and axonal death features, comprising approximately 10% of cases.

CMT1B, the most common form of this disease, is inherited in an autosomal dominant fashion. Disease onset occurs in the first decade of life, characterized by distal weakness, and nerve conduction velocity of <20 m/s [30]. At the cellular level, this condition is mediated by peripheral demyelination, which leads to abnormal nerve conduction initially, and subsequently, axonal cell death. Genetically, CMT1B is characterized by mutations in the gene encoding the myelin protein zero (MPZ) which is located on chromosome 1q22. MPZ is an immunoglobulin-like adhesion molecule that is involved in compaction of peripheral myelin and also serves as an intercellular adhesion molecule [31]. MPZ plays a major role in the process of myelination and actually comprises 50% of the total protein found in the myelin sheath in the peripheral nervous system. MPZ is synthesized exclusively by Schwann cells and its expression autoregulates numerous activities of this cell type. The mutations in MPZ that are associated with CMT1B are most frequently localized to the extracellular protein domain which is responsible for cellular adhesion [32], although numerous mutations of MPZ have been described throughout the protein [33]. Overall, MPZ mutations can be categorized into those that disrupt myelination during development, and those that interfere with the axonal–glial communications. The fundamental causative aspects of MPZ abnormalities *in vivo* were demonstrated elegantly by Giese et al. who created an MPZ-knockout mouse and reported that the animals exhibited CMT1B-like pathology, including hypomyelination of peripheral nerves, as well as abnormalities in myelin spiraling, compaction, and continuity of the myelin sheath [34].

3. Mesenchymal stem cells as a remyelinating cell type

Experimental intervention in the process of myelination can be divided into administering cells that differentiate into Schwann cells, or conversely providing cells that can accelerate ability of Schwann cells to generate new myelin. Inherited genetic conditions such as CMT are caused by defective endogenous cells, accordingly, the use of autologous cell therapy, or administration

of cells for trophic support, theoretically would be a futile effort. The possibility of manipulating cells to express the proper genetic profile *ex vivo* would circumvent such issues, however, the current state of gene transfer technologies, as well as vast polymorphism in CMT mutations, make this approach undesirable at present.

Given the plasticity of embryonic stem cells, numerous investigators have demonstrated that this population is capable of differentiating into functioning myelinating cells. For example, Liu et al. demonstrated that human embryonic stem cells induced to differentiate into oligodendrocytes through retinoic acid treatment were capable of remyelinating CNS nerves *in vitro* and *in vivo* [35]. Additionally, it was demonstrated that embryonic stem cells can be differentiated into Schwann cells using a variety of differentiation reagents [36]. While theoretically promising, embryonic stem cell approaches are limited by several main factors. Firstly, embryonic stem cells are known to cause teratomas, while various differentiation protocols exist, the possibility of teratoma contamination is still a very realistic risk for clinical translation. Secondly, embryonic stem cells are by definition allogeneic. While the argument has been made that these cells are tolerogenic [37], and hence may be accepted with little or no immune suppression, this argument has only been made for undifferentiated cells. Once embryonic stem cells differentiate, it is known that they elicit rejection responses [38]. Ideally, what is required is a cell population that possesses ability to remyelinate injured nerves but can be used in an allogeneic, off the shelf, manner. This would allow for widespread usage, especially in conditions where extraction and expansion of autologous stem cells is unfeasible.

The ability of MSC to differentiate into neuronal tissue was previously reported [39]. Due to reported pluripotency of MSC and MSC-like cells [40], ability to differentiate into Schwann cells was examined. Indeed, Caddick et al. [41] have recently reported the *in vitro* generation Schwann cells by treatment of human bone marrow derived MSC with glial growth factor. The resulting cells were not only positive for the Schwann cell specific markers S100, P75, and GFAP, but were in fact superior to isolated primary Schwann cells in myelinating and enhancing neurite outgrowth of sensory neurons in culture. In other studies, it was demonstrated that administered bone marrow derived MSC could selectively accumulate in the epineurium layer of injured sciatic nerve and actually contributed to recovery and myelination [42,43].

For the treatment of peripheral neuropathies, MSCs appear to possess certain benefits in addition to direct ability to transdifferentiate into Schwann cells, or Schwann-like cells [44]. In CMT1B patients, as a result of continued demyelination, axons and their respective neurons, undergo damage and in some cases apoptosis [45]. Numerous anti-apoptotic factors with neuroprotective activities are known to be produced by MSCs. For example, it was demonstrated that both bone marrow and adipose-derived MSC secrete anti-apoptotic factors such as VEGF, HFG and IGF in response to pro-apoptotic mediators such as TNF- α [46]. Numerous studies have shown that these cytokines play a protective role in ischemia and inflammation induced neuronal damage [47–49]. While little work has been performed in the area of MSC therapy for CMT, the positive influence of these cells on neural regeneration in conditions such as ALS, multiple sclerosis, and various neuropathies, has been documented [24,26].

4. Localization of mesenchymal stem cells

While from the above discussion MSCs appear to a promising cell type for the induction of remyelination, the next question arises of how one would administer these cells for therapeutic benefit. Specifically, in situations of peripheral neuropathy such as CMT1B, it is very difficult to directly inject cells into every nerve

that is affected. The advantage of MSCs is that in contrast to other types of remyelinating cells, these cells possess migratory activity towards injured/inflamed tissue when administered systemically. Selective localization of systemically infused MSC into injured tissue has been demonstrated in models of kidney failure [50], heart failure [51,52], and brain injury [53,54]. It is known that inflammatory mediators such as TNF- α and the IFN- γ Inducible Protein 10 (IP-10) act as selective chemoattractants for MSC [55]. Additionally, mediators associated with tissue degradation such as hyaluronic acid degradation products, bind to CD44 on MSC and act as chemoattractant factors, selectively calling the regenerative cells to areas of tissue injury [56]. Therefore, there is scientific rationale supporting the concept that intravenous administration of mesenchymal stem cells is feasible in conditions associated with inflammation. However, it is currently unclear whether the localized inflammation in CMT neurons is sufficient to mediate a significant homing of MSC to exert a therapeutic effect. Below we will discuss the quality of this localized inflammation.

5. Innate immune activation in pathogenesis of cmt animal models

In peripheral demyelination associated with autoimmune attack, such as Guillain–Barre syndrome, it is natural to anticipate immunological cell infiltration in the epineurial area [57], however, immunological infiltrates have also been detected in demyelinating diseases not conventionally believed to be associated with autoimmunity, such as various subtypes of CMT [58]. Due to the heterogeneity of CMT diseases, objective scientific evaluation of the role of inflammation in CMT1B can be assessed through studies of the animal model of CMT1b, the P0 (MPZ) heterozygous knockout mouse. It was demonstrated that preceding demyelination in this model, a wave of immune cells, including macrophages and lymphocytes infiltrate epineurial spaces [59]. The question of inflammatory contribution to disease progression was evaluated in studies breeding the heterozygous P0 knockout with mice deficient in mature macrophages. These mice, the osteopetrotic (*op/op*) strain, are known to possess a defect in the M-CSF receptor which does not allow for proper macrophage development. Crossing the heterozygous knockout with the *op/op* mice resulted in animals with reduced demyelination as compared to controls [60]. Further support for the role of macrophages in demyelination comes from studies in which mice deficient in macrophage adhesion molecules necessary for migration exhibit resistance to demyelination as a result of heterozygous MPZ deletion [61]. The involvement of immune responses as a co-factor in clinical development of disease is not limited to the innate arm of the immune system. Studies breeding T cell deficient mice with MPZ heterozygous knockouts have demonstrated that these cells also play a critical role in the demyelination process [62]. It can not be said that inflammation is only associated with demyelination as a result of MPZ deficiency, since the PMP22 overexpressing model of CMT1A, as well as connexin-32 knockout models, also have demonstrated a contributing immunological component to the demyelination process [63]. Clinically, some advanced cases of CMT disease have been described to respond to anti-inflammatory treatment such as administration of corticosteroids [64,65]. It is possible that in the typical presentation of CMT, there is an underlying inflammation, however, it usually is present below the threshold of detection using clinical parameters that are used in practice. Such a low grade underlying inflammation may be in some ways comparable to the chronic inflammation associated with atherosclerosis which is only detectable using specialized techniques such as high sensitivity C-reactive protein testing [66].

Given that animal models of peripheral neuropathy are not associated with infectious disease, or immunization with autoantigens, one may ask what stimuli activate innate (macrophage) and subsequently adaptive (T cell) responses in these spontaneous genetically-associated settings? One possible explanation appears to be tissue injury resulting from abnormal myelin deposition. Specifically, in the MPZ heterozygous knockout, failure of myelin compaction is noted [34]. This abnormal deposition of myelin is believed to result in the release of various extracellular matrix degradation products [67]. One mechanistic hypothesis revolves around the concept of “sterile inflammation”. In various physiological conditions in which extracellular matrix fragments are released, inflammatory cells can be activated in the absence of viral/bacterial infection. It is known that various members of the toll like receptor (TLR) family, which are found on macrophages, are directly activated by the products of tissue remodeling or abnormal accumulation of structural proteins in situations similar to the abnormal myelination generated in response to genetic abnormalities [68]. TLR2 found on macrophages recognizes low molecular weight hyaluronan fragments that are released upon tissue injury. These fragments are known to be associated with demyelinating lesions similar to those found in CMT [69]. Activation of macrophages is associated with production of TNF- α which not only is found in animal models of CMT, but also contributes to inhibition of Schwann cell function [70]. Accordingly, one possible cause of the inflammatory processes associated with CMT is recognition of abnormal protein structures by innate immune mechanisms. It is possible that this trigger actually stimulates local inflammatory events that would chemoattract systemically administered stem cells.

6. Administration of mesenchymal stem cells for treatment of CMT1B

Mesenchymal stem cells have been administered systemically for treatment of a variety of inflammatory and degenerative diseases both in animal models and in clinical trials. Although MSC are considerably large cells, (2–3 times in size as compared to polymorphonuclear leukocytes), clinical trials have no reported adverse effects such as adherence to microcapillaries [71–73]. The positive aspects of this cell population in terms of remyelination ability, neuroprotective properties, and anti-inflammatory activity provides rationale for their use in peripheral neuropathies such as CMT1B. Given the established safety profile of mesenchymal stem cells clinical evaluation, it is safe to believe that minimal, if any, preclinical safety studies are needed before clinical entry for peripheral neuropathies.

We propose a dose escalating trial of allogeneic mesenchymal stem cells in patients with CMT1B in order to identify optimum cell numbers for subsequent efficacy identifying trials. Although *in vivo* proliferation of bone marrow derived allogeneic MSC is relatively low [74], other sources of MSC may be considered. For example, Meng et al. recently reported an MSC population derived from menstrual blood termed “Endometrial Regenerative Cells”, which have a doubling rate of approximately 19.6 h, as compared to other types of MSC which double approximately every 30–48 h [4]. As with any clinical investigation, success is critically linked to the selection of patients. Given the heterogeneity of CMT diseases, this issue becomes even more critical. Suitable inclusion criteria in such a trial would include presence of disease factors that can be quantified. Physical examination and electrophysiological tests must indicate the CMT1B clinical signs, and other causes of neuropathy must be excluded. Identification of the CMT causative mutation would be desirable. In addition, the presence of inflammatory markers, even if at a subclinical level

could be measured. Representative markers could include TNF- α , IL-1, IL-6, and C reactive protein. In terms of efficacy endpoints, loss of nerve conduction velocity is a typical objective measurement of demyelination. Similar objective endpoints could include quantitative muscle testing, and pegboard performance. Other parameters of investigation could include muscular myosin heavy chain content, and histological analysis of sural nerve biopsy. Several quality of life instruments have been developed for assessment of peripheral neuropathies. It will be necessary to include these assessment indexes in clinical studies. One potential tool is the Mayo Clinic Neuropathy Impairment Score (NIS), which was used in a previous study of regenerative therapeutic is for CMT disease [75].

7. Conclusion

At present no therapeutic intervention exists that addresses the causative elements of CMT1B disease. MSC are a worthy candidate for clinical investigation in this condition due to their abilities to: (a) cause remyelination; (b) exert neuroprotective/antiapoptotic effects on neurons; (c) home to injured areas after intravenous administration; and (d) inhibit inflammation. The feasibility of this approach would not only provide a possible solution to CMT1B, but would serve as a model for treatment of other neurodegenerative diseases associated with peripheral demyelination.

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