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Stem Cell Therapy for Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is a chronic inflammatory, autoimmune, and neurodegenerative disease of the central nervous system (CNS). It is characterized by demyelination and neuronal loss that is induced by attack of autoreactive T cells to the myelin sheath and endogenous remyelination failure, eventually leading to functional neurological disability. Although recent evidence suggests that MS relapses are induced by environmental and exogenous triggers such as viral infections in a genetic background, its very complex pathogenesis is not completely understood. Therefore, the efficiency of current immunosuppression-based therapies of MS is too low, and emerging disease-modifying immunomodulatory agents such as fingolimod and dimethyl fumarate cannot stop progressive neurodegenerative process. Thus, the cell replacement therapy approach that aims to overcome neuronal cell loss and remyelination failure and to increase endogenous myelin repair capacity is considered as an alternative treatment option. A wide variety of preclinical studies, using experimental autoimmune encephalomyelitis model of MS, have recently shown that grafted cells with different origins including mesenchymal stem cells (MSCs), neural precursor and stem cells, and induced-pluripotent stem cells have the ability to repair CNS lesions and to recover functional neurological deficits. The results of ongoing autologous hematopoietic stem cell therapy studies, with the advantage of peripheral administration to the patients, have suggested that cell replacement therapy is also a feasible option for immunomodulatory treatment of MS. In this chapter, we overview cell sources and applications of the stem cell therapy for treatment of MS. We also discuss challenges including those associated with administration route, immune responses to grafted cells, integration of these cells to existing neural circuits, and risk of tumor growth. Finally, future prospects of stem cell therapy for MS are addressed.

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Abbreviations

AD-MSCs	Adipose tissue-derived MSCs
AHSCT	Autologous hematopoietic stem cell transplantation
APC	Antigen-presenting cells
ASC	Adult stem cells
BBB	Blood–brain barrier
CNS	Central nervous system
Cy	Cyclophosphamide
DC	Dendritic cells
DMDs	Disease-modifying drugs
Dpi	Days of post immunization
EAE	Experimental autoimmune encephalomyelitis
EDSS	Expanded Disability Status Scale
ESC	Embryonic stem cells
G-CSF	Granulocyte colony-stimulating factor
GWAS	Genome-wide association studies
HLA	Human leukocyte antigen
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplantation
IDO	Indoleamine 2,3-dioxygenase
IFNγ	Interferon gamma
IL-10	Interleukin-10
IL-1β	Interleukin-1beta
iNSC	Induced neural stem cell
iOL	Induced oligodendrocyte
iOPC	Induced oligodendrocyte progenitor cell
iPSC	Induced pluripotent stem cell
MBP	Myelin basic protein
MHC	Major histocompatibility complex
MOG	Myelin oligodendrocyte glycoprotein
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
MSC	Mesenchymal stem cell
NK	Natural killer
NPC	Neural progenitor cells
NSC	Neural stem cell
OPC	Oligodendrocyte progenitor cell
PBMC	Peripheral blood mononuclear cells
PMS	Progressive MS
RRMS	Relapsing-remitting multiple sclerosis
SCT	Stem cell transplantation
SNP	Single nucleotide polymorphism
SPMS	Secondary progressive multiple sclerosis
SVZ	Subventricular zone
Th	T helper
TNF	Tumor necrosis factor
Tregs	T cell regulatory
TRM	Transplantation related mortality

1 Multiple Sclerosis

1.1 Overview of Multiple Sclerosis

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating, and autoimmune disease of the central nervous system (CNS). Myelin sheaths of neurons are attacked by autoreactive T and B cells, specific to myelin autoantigens such as myelin basic protein (MBP). MS was described in 1868 by Jean-Martin Charcot who observed multiple lesions and glial scar (plaque) areas in the white matter of the brain and medulla spinalis Gomes Mda and Engelhardt 2013). MS is characterized with segmental demyelination, axonal injury, and neuron and oligodendrocyte loss leading to neurological dysfunction and disability (Kawachi and Lassmann 2017). MS affects approximately 2.5 million people worldwide. High prevalence of MS is seen in northern parts of Europe and North America (Browne et al. 2014). MS is a major economical and social burden for modern societies, because of its progressive, chronic, and debilitating nature, lack of any cure, and its target age group, who are young adults, the most productive members of the population.

1.2 Etiology

The main cause of MS is not fully understood, but several genetic and environmental factors such as smoking contribute to the development of disease (Olsson et al. 2017). MS is not considered a hereditary disease; however genome-wide association studies (GWAS) have shown that several single nucleotide polymorphisms (SNPs) in immune system-related genes associate with predisposition to MS. Immune-related gene variants, of both adaptive and innate systems, are associated with MS (Parnell and Booth 2017). It has been confirmed by different studies that the human leukocyte antigen (HLA) *DRB1*1501* is associated with MS susceptibility in many populations (Olsson et al. 2017). Various infectious agents, especially Epstein-Barr virus, have been suspected in the etiology of MS for over a century (Mentis et al. 2017).

1.3 Clinical Presentation of Multiple Sclerosis

The specific signs and symptoms of MS are dependent on the neuroanatomical localization of the lesions in the CNS. Typical signs and symptoms include optic neuritis, diplopia, muscle weakness, sensory deficits, and ataxia (Compston and Coles 2008). Cognitive, behavioral, and emotional problems are also commonly seen in later stage of the disease. Clinical course of MS is categorized into four subtypes. "Clinically isolated syndrome" is the first episode of neurologic symptoms lasting at least 24 h that are suggestive of MS (Tsang and Macdonell 2011).

Clinically isolated syndrome (CIS) refers to a single clinical attack of central nervous system (CNS) inflammatory demyelinating symptoms that are suggestive of multiple sclerosis (MS).

"Relapsing-remitting multiple sclerosis" (RRMS) is the most common form of MS that is characterized by recurrent attacks or increasing neurologic symptoms and are followed by remission periods (Lublin et al. 2014). After 20–25 years, 90% of patients with RRMS turn into "secondary progressive multiple sclerosis" (SPMS) which is characterized by progressive neurological decline without relapses. Approximately 10–15% of patients with MS are diagnosed with "primary progressive multiple sclerosis" (PPMS) which is characterized by the steady progressive worsening neurologic function from the onset of symptoms, without relapses (Lublin et al. 2014). Some MS patients show rapid progression in a very short time period, and this particular type is called "aggressive MS" (Rush et al. 2015). To know the course and type of MS is essential to predict prognosis and to make treatment decisions both in DMTs and stem cell therapies.

1.4 Diagnosis of MS

The diagnosis of MS is based on neurologic findings supported by magnetic resonance imaging (MRI), cerebrospinal fluid, and evoked potential analyses (Garg and Smith 2015). The most recently revised McDonald criteria allowed safe and early diagnosis of MS (Polman et al. 2011). MRI has rapidly become a leading diagnostic tool in MS. Brain MRI shows T2 hyperintense white matter lesions in periventricular, juxtacortical, and infratentorial regions (Filippi et al. 2017b). Dissemination of MRI lesions in time and space are critically important for MS diagnosis.

1.5 Histopathology

Pathological hallmarks of MS are inflammation, gliosis, demyelination, axonal injury, and synaptic loss (Garg and Smith 2015). The MS plaques are localized demyelination areas with different degrees of inflammatory cell infiltrations predominantly located in the white matter of the brain, spinal cord, and optic nerves (Ransohoff et al. 2015). Demyelinated plaques can also locate in the cortical and subcortical gray matter. The inflammatory infiltrates are composed of activated T cells, activated macrophages/microglia, plasma cells, and B cells. While active plaques are characterized by myelin degradation,

reactive astrocytes, and perivascular and parenchymal inflammation, more extensive demyelination, axonal loss, oligodendrocyte injury, and less active inflammation are observed in chronic plaques (Popescu et al. 2013). Blood-brain barrier (BBB) disruption is another pathological feature of MS and allows immune cell infiltration into the brain. Remyelination following acute inflammatory episodes contributes to functional recovery, but it is frequently insufficient to restore neurological functions. The cortical brain atrophy is a characteristic histopathological feature of MS and is associated with cognitive impairment in progressive MS (PMS) (Filippi et al. 2017b).

1.6 Pathogenesis

Although the pathogenic factors and mechanisms which lead to MS are largely unknown, the current view is that environmental triggers such as infection initiate progress of disease in genetically predisposed individuals. A wide variety of genetic, clinical, pathological, and epidemiological studies support this hypothesis. In addition to the well-known HLA-DRB1 risk gene, GWAS studies showed presence of MS-associated SNPs in non-MHC immune regulatory genes that are involved both in innate and adaptive immunity in MS patients (Parnell and Booth 2017). Interestingly, MS-associated gene loci show little overlap with those of primary neurodegenerative diseases (Hemmer et al. 2015). In contrast, one-third of disease-associated risk genes are common in MS and other autoimmune diseases (Parnell and Booth 2017; Yadav et al. 2015). Both brain biopsy and postmortem autopsy studies show immune cell infiltration in MS brain. Histopathological investigations confirm this finding in animal models of MS. Another evidence suggesting that immune component plays an important role in MS pathogenesis is the recovery of MS attacks by immunosuppressive and immunomodulatory therapies (Yadav et al. 2015). Finally, immunological analyses performed with body fluids and peripheral blood mononuclear cells (PBMC) samples of MS patients show aberrant immune parameters.

In healthy individuals, the immune system plays a vital role in self-defense mechanisms against bacteria, virus, and other environmental hazardous factors, discriminating self and non--self-antigens. Following the removal of foreign antigens, immune homeostatic mechanisms provide the resolution of inflammation and fading of immune responses. Self-antigen recognizing autoreactive T cells are deleted in thymus. Several circulating autoreactive T cells that escaped from thymic deletion are suppressed by regulatory T cells (Tregs) and normally do not encounter myelin antigens because of BBB (Jones and Hawiger 2017). It is thought that Treg activity and proliferation decrease and/or autoreactive T cells resist immunoregulatory mechanisms in MS. Both cellular and humoral components of adaptive and innate immunity are involved in autoimmune responses that mediate neurotoxic and gliotoxic injury.

Either bacterial or viral infections frequently trigger MS attacks. Myeloid cells of innate immunity (dendritic cells, macrophages, and microglia) become activated and mature when pathogen-associated molecular patterns such as bacterial lipopolysaccharide or viral antigens bind to pattern recognition receptors including Toll-like receptors and NOD-like receptors. Upon activation, these cells secrete interleukin-1beta (IL-1β) and interleukin-18 (IL-18) cytokines that induce expression of chemokines and their receptors both by T cells and antigen-presenting cells (APC), enhancing T cell migration (Lin and Edelson 2017).

Some other cytokines secreted by mature dendritic cells (DC) polarize CD4+ T helper (Th) subtypes. IL-1β and interleukin-23 stimulate Th17 cells that secrete interleukin-17 (IL-17). Interleukin-12 induces interferon gamma (IFNγ)-secreting proinflammatory Th1 cells (Grigoriadis et al. 2015). DCs are also APCs and using MHC-II receptors recognize self- or non-self-antigens that show structural similarity to myelin antigens. The second mechanism is called molecular mimicry that can initiate autoimmune responses. CNS antigens can pass to systemic circulation through glymphatic drainage system and are presented to CD4+ T cells by

DCs in deep cervical lymph nodes (Simon and Iliff 2016). Th1 and Th17 cells disrupt the structure of BBB and increase its permeability by cytokines, chemokines, and matrix metalloproteinases. These soluble factors also mediate the recruitment and infiltration of other immune cells (macrophages, B cells, CD8+ T cells, and natural killer (NK) cells) to the CNS (Dargahi et al. 2017). All of these cells and their products lead to immune-mediated glial and neuronal injury and cell death. Upon activation, B lymphocytes convert to autoantibody-secreting plasma cells. Myelin-reactive autoantibodies result in myelin injury and antibody- and complement-dependent death of oligodendrocytes (OLs) and neurons (Compston and Coles 2008; Disanto et al. 2012). Recruited CD8+ T cells bind to MHC-I receptor-expressing cells including oligodendrocytes and neurons and secrete cytotoxic granules containing perforin and granzyme B that lyse cells by pore formation (Salou et al. 2015). Activated T cells can also kill oligodendrocytes or neurons through Fas-Fas ligand and tumor necrosis factor (TNF)-α-TNF-α receptor interactions (Connick et al. 2012). Autoreactive T cells activate microglia and polarize them to M1 proinflammatory phenotype by TNF-α and IFNɣ (Yadav et al. 2015). Microglia can phagocytose oligodendrocytes or kill these cells indirectly by soluble factors including nitric oxide, reactive oxygen species, reactive nitrogen species, glutamate, and proinflammatory cytokines (Kawachi and Lassmann 2017). Liberation of iron from dead oligodendrocytes amplifies oxidative injury (Kawachi and Lassmann 2017). Autoregulatory immune mechanisms move in to resolve inflammation in acute MS attacks. Inflammation-resolving lipid mediators such as resolvin D, anti-inflammatory cytokines such as interleukin-10 (IL-10), Treg cells and microglia which are polarized to M2 phenotype, and neurotrophic factors secreted from glial cells control autoimmune responses and repair damaged CNS tissue (Guo 2016; Miron 2017).

In contrast, uncontrolled autoimmune responses amplify and increase tissue damage in chronic phase. Activated microglia are predominant in cerebral parenchyma, and B cells constitute meningeal ectopic foci. Main property of chronic neuroinflammation in MS is its compartmentalization.

1.7 Current Treatment

There is no cure for MS. Currently available treatments for MS help improve patient's overall quality of life and minimize long-term disability by preventing the frequency of relapses and severity of acute MS attacks (Garg and Smith 2015). In recent years, many new disease-modifying drug (DMD) options have become available. These drugs primarily target the underlying immunologic etiology of the disease. Interferon-ß and glatiramer acetate have been used as first-line DMDs for RRMS over two decades (Comi et al. 2017). Teriflunomide, fingolimod, and dimethyl fumarate are other moderately effective immunomodulators. Teriflunomide and fingolimod suppress activated T and B lymphocytes through different mechanisms (Grigoriadis et al. 2015). Natalizumab specifically inhibits cell migration via integrin blockage (Delbue et al. 2017). Alemtuzumab, which is newly introduced in MS treatment, reduces CD52+ T and B cells. While these drugs significantly reduce the frequency and severity of MS attacks, they have serious side effects including progressive multifocal leukoencephalopathy, hypertension, leukemia, viral infections, teratogenesis, and cardiac arrhythmias (Comi et al. 2017). These adverse effects hinder their long-term use. They also have limited long-term effectiveness, high cost, and inability to reverse disease (Dargahi et al. 2017). Eventually the majority of RRMS patients turn to progressive MS. DMDs do not prevent progressive neurodegenerative processes. Thus, new drugs and treatment regimens are required to effectively treat both primary and secondary PMS (Dargahi et al. 2017). As MS enters progressive phase, inflammation continues, but it evolves to a more CNS-restricted pattern without BBB leakage (Grigoriadis et al. 2015). Hence, new CNS-targeted immunomodulatory drugs that can

cross repaired BBB and reach to ectopic B cell follicles are still needed.

However, DMDs in multiple sclerosis target the immune system and do not have regenerative effect. Regenerative treatment approaches promoting remyelination are promising for MS treatment (Plemel et al. 2017).

2 Basics of Stem Cell Biology

Stem cells are undifferentiated cells that are capable of self-renewal and have the ability to give rise to specialized cells through asymmetric division (Tabansky and Stern 2016). Stem cells are categorized into two main parts: embryonic stem cells and adult stem cells.

Embryonic stem cells (ESC) give rise to all cell types and differentiate into tissue types apart from extraembryonic tissues (Tabansky and Stern 2016). This feature is defined as pluripotency. Furthermore, they are able to pass unlimited and symmetric mitotic divisions without being specialized. ESCs are derived from blastocyst, which is a premature embryo that forms 5 days after fertilization. ESCs are obtained from the blastocyst's inner cell mass that consists of 30 cells (Tabansky and Stern 2016). Zygote and the cells at very early stages of fertilization are defined as totipotent (Wu et al. 2016). They have ability to constitute all the cells needed to generate a complete organism. Usage of ESCs for research has started to create ethical controversies because blastocyst is destroyed and loses its ability to generate a human (King and Perrin 2014).

Adult stem cells (ASCs) differentiate into limited types of cell of tissue or organ where they are located (Mariano et al. 2015). They are characterized as multipotent cells because of their capacity to produce tissue-specific cell types. Their main function is to repair or produce specialized cells when wear and tear, injury, or disease occurs. ASCs have been found in many different tissues or organs and are divided into categories depending upon their locations or diversified features such as hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), and neural stem cells (NSCs). HSCs can be found in the bone marrow (BM), peripheral blood, and umbilical cord blood and generate all types of blood cells (Ng and Alexander 2017). MSCs are also located in the BM and develop into different types of cells, including fat cells, cartilage, bone, tendon and ligaments, muscles cells, skin cells, and even nerve cells (Volkman and Offen 2017). NSCs are located in the brain and constitute neurons and glial cells (Nam et al. 2015). ASCs' isolation is very hard and impractical. In 2006, Yamanaka and his co-workers found a solution to this problem by converting somatic cells into pluripotent stem cells (Takahashi and Yamanaka 2006). These reprogrammed pluripotent stem cells are called as induced pluripotent stem cells (iPSCs). iPSCs have similar features to embryonic stem cells, such as giving rise to many varied tissue types, and are able to divide indefinitely in culture. However, the risk of tumor formation of iPSCs is very high (Heslop et al. 2015). This risk is reduced by a procedure called transdifferentiation (Cieslar-Pobuda et al. 2017), in which a cell type is converted into another cell type in different tissues without going through a pluripotent cell state. Progenitor cells such as induced neural stem cells (iNSC), induced oligodendrocyte progenitor cells (iOPC), and induced oligodendrocytes (iOL) can be derived from a somatic cell type by transdifferentiation (An et al. 2016).

3 Hematopoietic Stem Cell Therapy

Hematopoietic stem cells are rare cells with characteristics of pluripotentiality and self-renewal ability and constitute about 0.01% all total nucleated cells in the BM. HSCs are capable of generating all hematopoietic cell lineages including erythrocytes, megakaryocytes, platelets, and innate and adaptive immune system cells (Ng and Alexander 2017). HSCs undergo self-renewal when transplanted to humans and are able to differentiate into all of the hematopoietic cell types. HSC transplantation (HSCT) has been used for about half a century in the clinic as an

effective therapeutic approach in cancer. In the early 1990s, preclinical studies showed that HSCT is also effective in various experimental models of autoimmunity including experimental autoimmune encephalomyelitis (EAE) (Karussis and Slavin 2004). During the last two decades, HSCT became an alternative therapy option to immunosuppressive and immunomodulatory drugs in autoimmune diseases such as MS. Here, the rational for HSCT is based on the concept of rebooting the aberrant immune system through the elimination of autoantigen-reactive T and B lymphocytes, increase of Treg population, and reconstitution of self-tolerance (Swart et al. 2017).

3.1 Procedure

HSCs are mobilized from BM by treatment with cyclophosphamide (Cy) and granulocyte colony-stimulating factor (G-CSF). The reason of Cy administration is to prevent a possible MS relapse due to G-CSF (Bell et al. 2017). After 4 or 5 days, HSCs are collected by peripheral vein leukapheresis. Following staining by anti-CD34 monoclonal antibody, cells are purified using either fluorescence-activated cell sorting or magnetic-activated separation and then are stored frozen until transplantation. The selection of CD34+ cells increases the purity excluding autoreactive lymphocytes. The minimum number required for autologous HSCT (AHSCT) is 3×10^6 CD34+ cell/kg/body weight. After 4 or 5 weeks, the thawed AHSCT are reinfused to the patient. Generally, 3–5% of all the cells in a graft are HSCs (Atkins and Freedman 2013). To prevent the expansion of autoreactive lymphocytes after transplantation, immune ablative conditioning regimens that consist of chemotherapeutics and immunosuppressive drugs are used. The doses and combinations of conditioning drugs determine the intensity of regimens which are positively correlated with the outcome of AHSCT, the frequency of side effects, and transplantation-related mortality (TRM). TRM is percentage of mortality in the first 100 days after transplantation. The most widely conditioned scheme is intermediate-intensity regimen which is called BEAM and includes BCNU, etoposide, AraC, and melphalan. Low-intensity regimens are used to reduce the toxicity related to intense immunosuppression. The conditioning regimen is followed by infusion of autologous CD34+ stem cells. Most patients are lymphopenic during several months after AHSCT while their immune system fully reconstitutes (Sarkar et al. 2017). Prophylactic acyclovir treatment is used for 1 year posttransplantation to prevent viral infections (Bell et al. 2017).

3.2 Clinical Studies

AHSCT has been applied to MS patients since 1997 (Fassas et al. 1997). The results of early clinical studies with MS patients who underwent AHSCT vary due to small sample sizes, different cohort characteristics, included populations with different proportions of RRMS and PMS, distinct conditioning regimes for AHSCT, and diverse toxic effects of different drugs, which make comparisons between studies difficult (Dorr 2016). The more recent clinical AHSCT studies in RRMS have reported beneficial effects based on Expanded Disability Status Scale (EDSS) score and MRI activity (Burman et al. 2014; Burt et al. 2015; Nash et al. 2015), with no TRM. In a more recent study, AHSCT without maintenance immunosuppressive therapy was effective for inducing long-term sustained remission of active RRMS at 5 years follow-up (Muraro et al. 2017). The only completed controlled randomized clinical study is the *A*utologous *S*tem *C*ell *T*ransplantation *I*nternational *M*ultiple *S*clerosis (ASTIMS) trial (Mancardi et al. 2015). This study, comparing mitoxantrone versus AHSCT, had both aggressive RRMS and SPMS patients. Although no difference was observed in EDSS score, results showed that AHSCT is superior with regard to MRI activity and relapse rate.

AHSCT is not effective in PMS despite aggressive immune ablation regime resulting in a posttransplant immune reset (Casanova et al. 2017). Neurological disability observed in

SPMS is mainly caused by neurodegenerative processes due to axonal atrophy and not inflammatory process. As a result, the progressive phase may not be treatable by neither immunomodulatory agents nor AHSCT.

3.3 Patient Selection Criteria for Autologous Hematopoietic Stem Cell Transplantation

Good candidates for AHSCT are patients in early phase of disease. The patient inclusion criteria for AHSCT in MS are as follows: RRMS, age between 18 and 45 years, duration of MS not exceeding 5 years, EDSS between 2.5 and 6.5 points, clinically active disease, and evidence of gadolinium enhancement on MRI (Bell et al. 2017).

3.4 Follow–Up and Outcome

Long-term follow-up (over years) is mandatory. In a recent long-term outcome study, Muraro et al. have reported that factors associated with neurological progression after AHSCT are older age, PMS form of MS, and more than two previous DMTs (Muraro et al. 2017) .

"No evidence of disease activity (NEDA)" status has emerged as a composite measure of RRMS treatment success and is defined as "no evidence of relapse," "no evidence of disability progression," and "no MRI activity, namely absence of new or enlarging T2 lesions or Gd-enhancing lesions" (Matta et al. 2016). NEDA is used as a primary endpoint in AHSCT. A recent meta-analysis study has reported that pooled proportion of NEDA patients in AHSCT was 83% (70%–92%) at 2 years and also was 67% (59%–70%) at 5 years (Sormani et al. 2017). Favorable outcome was seen in young patients with clinical and radiologically active disease, having short disease duration.

3.5 Parameters of Treatment Effectiveness

The parameters of treatment effectiveness are relapse-free survival, MRI event-free survival, and progression-free survival (Burman et al. 2014). Disease-free survival is determined by absence of both clinical and radiological disease sign and symptoms in a particular period. Sustained reduction in disability is defined as the improvement of 1.0 point in the EDSS score sustained 6 months.

3.6 Biomarkers

The combination of specific and selective biomarkers will contribute to a better and earlier selection of patients for ASHCT and follow-up process (Londono and Mora 2016). It is important to identify these patients using putative predictive markers as early as possible and to apply effective treatment strategies. Unfortunately, there are no predictive markers to identify patients who will develop aggressive MS.

3.7 Safety Profile and Adverse Effects

Early side effects of ASHCT are fever and viral infection, while a late adverse effect is the development of autoimmune thyroiditis (Zeher et al. 2017). Following AHSCT, some MS patients may show accelerated brain atrophy that is likely associated with busulfan neurotoxicity and neurodegeneration of committed tissues (Lee et al. 2017). The rate of determined brain atrophy declines to that of expected for age-matched healthy people. The same group found that busulfan dose was a significant predictor of white matter and gray matter atrophy. Long-term rates of gray and white matter atrophy were comparable to those of healthy controls at the same age. Cryopreservation is recommended against infertility (Bell et al. 2017).

The overall TRM (1995–2106) for AHSCT is 2%, and it has decreased to 0.2% over the last 5 years (2012–2016) (Muraro et al. 2017). Nowadays, TRM reaches 0% in several centers, which may result from standardized optimal transplant procedures being implemented and presence of effective collaboration between transplant hematologists and neurologists.

3.8 Immune Mechanisms

The exact mechanisms of AHSCT's therapeutic effect in RRMS are not fully understood. The early effect is instant and temporary radical depletion of autoreactive pathogenic immune cells due to ablative conditioning regimes. The use of AGT in conditioning regimen specifically may also play a role through complement-mediated lysis of autoantibody-producing plasma cells (Zand et al. 2005). In the late phase, adaptive immune system is reconstituted from newly formed naïve T and B cells by increased thymopoietic activity. Naïve B cell reconstitution restores the B cell repertoire and antibody diversity. Following AHSCT, Th1 and Th17 activities apparently decrease (Karnell et al. 2017). Clonal diversity of T cell receptor (TCR) improves, and subsequently new and diverse T cell repertoire develops (Muraro et al. 2005). After AHSCT, diverse immune cells are repopulated in a particular order. The immune repopulation process has very special dynamics. Firstly, innate immune system cells appear just within weeks following AHSCT. Monocytes are the first cells to engraft with subsequent population by neutrophils and NK cells. While CD8+ T lymphocytes return to normal values 3 months after transplantation, B lymphocytes reach a normal value at 6 months (Zeher et al. 2017).

Treg cells erase and suppress proinflammatory and autoimmune processes driven by Th17 cells and decrease the development of autoreactive T cell clones (Zeher et al. 2017). Restoration of gene expression changes takes longer time after AHSCT (Muraro et al. 2017).

Not all patients receiving autologous HSCT have achieved long-term clinical responses (Kelsey et al. 2016). When patients cannot develop a diverse repertoire of naive T cells after AHSCT, their response to treatment is likely to be less successful (Muraro et al. 2014). Further studies are necessary to determine which immune mechanisms contribute to the effect of AHSCT in MS (Muraro et al. 2017).

3.9 Cost-Effectiveness and Risk-Benefit Ratio of AHSCT

Risk-benefit profile and cost-effectiveness of AHSCT are comparable with those of current MS drugs. These drugs may lead to serious adverse effects such as PML (natalizumab), secondary autoimmune disease (alemtuzumab), and cardiac arrhythmias (fingolimod) (Bell et al. 2017; Burt et al. 2012; Curro and Mancardi 2016). Furthermore, these medications are expensive and are usually continued infinitely or until complications arise. In comparison, patients' compliance to HSCT is high (Burt et al. 2012).

3.10 Future Studies and Prospects

Well-designed randomized controlled studies that systematically investigated the effect and safety of HSCT compared to other relevant treatments are needed in order to clarify treatment benefits. Two randomized controlled phase III trials are being conducted to compare AHSCT with FDA-approved DMDs (MIST Study [ClinicalTrials.gov identifier: NCT00273364 and NCT03133403]. iPSCs and transdifferentiated somatic cells have been proposed as an alternative source of HSCs for possible applications that include autologous, autologous and genetically modified, or allogeneic cells (van Bekkum and Mikkers 2012). Currently, transplantation of iPSC-derived hematopoietic cells is still limited to preclinical animal models. Low hematopoietic differentiation efficiency and tumor formation risk must be overcome for clinical application (Hwang et al. 2017). If pathogenic gene variants are detected in MS patients, iPSC or transdifferentiated cells derived from differentiated somatic

cells can be genetically corrected using the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 gene editing and converted into normal hematopoietic cells that can be used in AHSCT.

4 Mesenchymal Stem Cell Therapy

4.1 Biology of Mesenchymal Stem Cells

MSCs are self-replicating cells which were first described by Friedenstein et al. in 1968 (Friedenstein et al. 1974). They were first isolated from BM; besides BM a wide range of adult tissues including adipose tissue, umbilical cord blood, placenta, and dental pulp have been used as sources of MSCs (Gharibi et al. 2015; Giacoppo et al. 2017). There is no specific marker to discriminate MSCs from other cells. The minimum criteria for MSCs determined by the International Society for Cell and Gene Therapy are plastic adherence; presence of CD105, CD73, and CD90 expression; absence of hematopoietic surface markers (CD45, CD34, CD14, CD11b, CD79α, CD19, and HLA-D); and differentiation capacity to osteoblasts, adipocytes, and chondroblasts in vitro (Dominici et al. 2006; Sarkar et al. 2017).

4.2 In Vivo Studies

In vivo studies investigating the effect of MSCs on EAE have been started after demonstration of their suppressive effects on T cell proliferation in vitro and in vivo. MSCs derived from several different adult tissues were used in acute and chronic MS animal models via different routes (Giacoppo et al. 2017; Sarkar et al. 2017). An early EAE study showed that administration of murine BM-derived MSCs at the early stage of disease reduced clinical scores, demyelination, and inflammatory infiltrates in the CNS (Zappia et al. 2005). MSC transplantation also ameliorated proteolipid protein (PLP)-induced EAE symptoms in SJL/J mice through the inhibition of pathogenic T and B cell responses (Gerdoni et al. 2007). Human BM-derived MSCs promote clinical recovery in chronic and relapsing-remitting mouse models of MS, possibly via reduced Th1 and Th17 cells and increased IL-4-producing Th2 cells (Bai et al. 2009). Various studies reported therapeutic effects of adipose tissue-derived MSCs (AD-MSCs) in EAE (Giacoppo et al. 2017; Li et al. 2017; Scruggs et al. 2013; Semon et al. 2014; Strong et al. 2016). Intraperitoneal administration of AD-MSCs provides more Treg cells and IL-4 production than intravenous route (Yousefi et al. 2013). Age and body mass index of donors alter therapeutic effect of MSCs. MSCs derived from older donors are less effective than younger donors in myelin oligodendrocyte glycoprotein (MOG)-induced EAE (Scruggs et al. 2013). AD-MSCs from obese donors could not suppress clinical signs of EAE and inflammation in the brain (Strong et al. 2016). As autologous AD-MSCs had no therapeutic effect on the EAE progression (Semon et al. 2014), allogenic AD-MSC treatment may be preferred in clinical trials of MS. Fetal tissues including the placenta, amnion epithelial cells, umbilical cord, umbilical cord matrix, and decidua have been used for MSC generation. These fetal tissue-derived MSCs ameliorate EAE disease severity and inflammation of CNS (Giacoppo et al. 2017). Fetal tissues contain large number genetically stable stem cells, so they have become candidate alternative tissue sources for the MSCs. However, these favorable results of MSC transplantation in EAE may not necessarily indicate that MSC treatment will be effective in clinical studies. Because, animal models of EAE do not reflect all aspects of MS and human MSCs have special features that differ them from mouse stem cells.

4.3 Clinical Studies

In an initial pilot human study, autologous MSC treatment was carried out in ten PMS patients via intrathecal route (Mohyeddin Bonab et al. 2007). Mild improvement in clinical sign of MS was

observed in a 13–26-month follow-up period. Several studies in small patient groups have provided evidence on safety and efficacy of a single-dose MSC treatment (Table 1) (Bonab et al. 2012; Cohen et al. 2017; Connick et al. 2012; Karussis et al. 2010; Llufriu et al. 2014; Mohajeri et al. 2011; Mohyeddin Bonab et al. 2013; Yamout et al. 2010). Bonab et al. reported that improvement or stabilization of clinical or MRI findings was seen in 15 of 25 patients (Bonab et al. 2012; Mohyeddin Bonab et al. 2013). Their results support that MSC therapy will be effective in unresponsive MS cases. Intrathecal administration of MSCs did not alter cytokine expression in peripheral blood (Mohyeddin Bonab et al. 2013), but increased Treg cells and suppressed the proliferative responses of lymphocytes, and the expression of CD40+, CD83+, CD86+, and HLA-DR+ myeloid DCs (Karussis et al. 2010). Repeated intravenous infusions of autologous MSCs every month during 4–8 months resulted in clinical improvement in EDSS scores in six of eight patients (Odinak et al. 2011). BM-derived MSCs were converted to neural progenitor cells (NPCs) and administrated to eight PMS patients via intrathecal route. Two to five repetitive treatments of MSCs provided improvement of clinical sign in four of eight patients. Repeated intrathecal administrations of MSC-derived NSCs were well-tolerated by six PMS patients in both short-term and long-term periods (7.4 years) (Harris et al. 2016). No serious adverse events were reported during follow-up periods of clinical MSC transplantation studies. Mild self-limited adverse events, such as headache, fever, nausea, vomiting, and weakness in the lower limbs, have been observed.

Apart from these published studies, several clinical trials for MSC treatment in MS are ongoing. A phase II open-label clinical trial for BM-derived MSC in PMS will include 80 patients, whose results are awaited (ACTiMuS) (ClinicalTrials.gov (NCT01815632)). A phase III MSC transplantation study was terminated due to limited number of participation (CMM-EM) (ClinicalTrials.gov (NCT01228266)). There are two AD-MSC transplantation clinical trials in MS listed in ClinicalTrials.gov (NCT01056471 and NCT02326935). A phase I/II randomized placebo-controlled study evaluating safety and feasibility of therapy of autologous MSCs in patients with SPMS was completed (NCT01056471). The other phase I multicenter study is currently recruiting patients (NCT02326935). The results of these two clinical trials are expected.

4.4 Mechanisms of Action

MSCs have immunomodulatory, immunosuppressive, neurotrophic, and repair functions (Giacoppo et al. 2017; (Sarkar et al. 2017). They inhibit both innate and adaptive immune responses. Decreased proliferation and immune responses of T cells, B cells, NK cells, and APC are observed (Gharibi et al. 2015). BM-derived MSCs have transdifferentiation capacity into neuron-like cells in vitro under certain conditions (Uccelli et al. 2011), but cell replacement effect of MSCs was not observed in EAE and clinical studies. Soluble factors secreted by MSCs and cell-to-cell contact have been the implicated mechanisms of MSCs' effects. Inoleamine-2,3-dioxygenase, transforming growth factor-β, hepatocyte growth factor (HGF), nitric oxide, and soluble HLA-G are soluble factors which mediate MSCs therapeutic effects (Bai et al. 2012; Mahfouz et al. 2017; Matysiak et al. 2008).

4.5 Challenges and Future Studies

There are several safety issues with MSC transplantation including infusion-related toxicity, infection, malignancy development, and disease activation. Standard procedures about dose, route of administration, repetition time, and culture conditions for MSC treatment in MS should be developed. The unsolved problems for MSC transplantation in MS are to provide migration of cells to lesion site and homing of cells into donor tissue. To avoid side effects of cell therapy, administration of MSC-derived exosomes could be a noncellular alternative therapy option to stem cell transplantation (SCT) in MS (Jarmalaviciute and Pivoriunas 2016). Recently, good

Table 1 Clinical studies with MSCs in multiple sclerosis

Author	Trial	Folow-up	MS type	N (F/M)	Age	EDSS	Transplanted cell	Route of administration	Single/ repeated	Outcomes	Side effects
Bonab 2007		13–26 months	PMS	10		3.5–6	Autologous BM-MSCs	IT	Single	EDSS of one patient improved from 5 to 2.5; four patients no change; five patients increased from 0.5 to 2.5 MRI: seven patients with no difference, two extra plaque, and one patient decrease in the number of plaques	
Karussis 2010	I/II	6–25 months	N/A	15 (8/7)	35.3	6.7 (4–8)	Autologous BM-MSCs	IT and IV	Single	EDSS score improved from 6.7 (1.0) to 5.9 (1.6) No new or Gd + lesions An increase in the proportion of Treg cells, decrease in proliferative responses of lymphocytes, and expression of CD40+, CD83+, CD86+, and HLADR on myeloid DCs	Mild self-limited febrile reaction, headache
Yamout 2010	Pilot	12 months	SP RR	7 (4/3)	42	6.42 (4–7.5)	Autologous BM-MSCs	IT	Single	EDSS improvement in 5/7, stabilization in 1/7, and worsening in 1/7 patients. MRI: a new or enlarging lesion in 5/7 and Gd + lesions in 3/7 patients. Vision and low-contrast sensitivity testing: improvement in 5/6	Transient encephalopathy with seizure in one patients
Mohajeri 2011		6 months	RR	7 (6/1)	35.5	N/A	Autologous BM-MSCs	IT	Single	FOXP3 mRNA increased	

Connick 2012	IIA	6 months	SP	10 (3/7)	48.8	N/A (2.0–6.5)	Autologous BM-MSCs	IV	Single	Improvement after treatment in visual acuity and visual evoked response latency, increase in optic nerve area	No serious adverse events
Bonab 2012, 2013		12 months	PP SP	25 (19/6)	34.7 (23–50)	5.5–7	Autologous	IT	Single	MRI (3 T) and EDSS scores improved or remained unchanged in 15 (68.18%) patients, whereas 7 (31.81%) patients showed new T2 or Gd + lesions or increased EDSS. Three (13.63%) patients refused to undergo further follow-up after 6 months	Transient low-grade fever, nausea/vomiting, weakness in the lower limbs, and headache
							BM-MSCs			No changes in gene expression and serum level of cytokines	
Odinak 2012		4–12 months	RR PPSP	8 (3/5)	37.5 (24–47)	5.56 (3.5–6.5)	Autologous BM-MSCs	IV	Repeated per month, 4–8 months	Improvements on the EDSS by 0.5–1 point were seen in six of the eight patients, with stabilization in one and progression in another patient	No serious adverse events. General weakness
Lilufru 2014	II	6 months	RR	9 (7/2)	36.8	3.5 (3.0–6.0)	5 patient BM-MSCs; 4 patient placebo	IV	single	No clinical significant change, nonsignificant reduction in Gd + MRI lesions and Th1 cells	No serious adverse events. Facial flushing, herpes labialis
Harris 2016	Pilot study	7.4 years	SP PP	6(4/2)	43	(6.5–9)	Autologous BM-MSCs-NPCs	IT	Repeated (2–5)	Four of the six patients showed measureable clinical improvement	No serious adverse events.
Cohen 2017	I	6 months	RR SP	24 (16/8)	46.5	6 (3.0–6.5)	Autologous BM-MSCs	IV	Single	Cell infusion was tolerated well without treatment-related severe or serious adverse events or evidence of disease activation	No serious adverse events

manufacturing practices (GMP)-grade standard protocol for hMSC-derived exosomes have been developed (Pachler et al. 2017).

5 Neural Stem Cell Therapy

5.1 Biology of Neural Stem Cells

Neural stem cells are self-renewing multipotent cells located in the subventricular zone (SVZ) and the subgranular zone of the dentate gyrus (Xiao et al. 2017). NSCs can differentiate into both neurons and glia. Either embryonic or adult brain tissue can be used as source of NSCs. They can also be generated from ESCs, MSCs, and iPSCs (Volpe et al. 2016). Due to differentiation capacity of NSCs to OPCs and oligodendrocytes, it is conceivable that NSCs can be used for cell replacement in demyelinating diseases such as MS.

5.2 In Vivo Studies

The therapeutic effect of NSCs in animal models of MS has been shown in several preclinical studies (Table 2). Immunization of SJL/J mice with PLP causes relapsing-remitting-type animal model of MS. But, protein immunization with MOG leads to chronic form of EAE in C57/BL6 mice. In addition to mice, NSC transplantation in EAE was performed in other species including Lewis rat, SD rat, and common marmoset (Ben-Hur et al. 2003; Einstein et al. 2003; Lee et al. 2015; Pluchino et al. 2009). Mostly embryonic or fetal cells have been used as the source of NSCs; however adult NSCs were also transplanted into mice (Pluchino et al. 2005; Wu et al. 2013). NSC transplantation was performed at different time points from the first day of immunization to 35 days after. NSC transplantation at 10 days of post immunization (dpi) delayed disease onset in addition to suppressing clinical findings of EAE (Yang et al. 2009). NSC transplantation in EAE was performed via different routes such as intravenous, subcutaneous, intracerebroventricular, intraspinal, intrathecal, intracerebral, and intranasal. Direct route via intracerebral or intraspinal seems more effective, while peripheral route also suppresses clinical signs of EAE by reducing peripheral immune responses (Einstein et al. 2007). More safe and effective route for administration of NSCs, such as intranasal route, may also be used in MS (Wu et al. 2013).

5.3 Mechanisms of Actions

NSCs exert therapeutic effects by several mechanisms including cell replacement, immunomodulation, trophic support to endogenous repair mechanisms, and stimulation of progenitor cell differentiation (Volpe et al. 2016; Xiao et al. 2017). The cell replacement effect of transplanted NSCs has been reported in limited EAE studies (Ben-Hur et al. 2003). Several studies reported that transplanted NSCs reduce clinical signs and inflammatory findings of EAE even though they persist in perivascular area and they do not migrate toward the lesion site (Ben-Hur et al. 2003; Einstein et al. 2003, 2006). These findings suggest that NSCs exert beneficial effects via other mechanisms such as immune modulation. Local and peripheral immune modulatory effects of these cells were supported by several EAE studies (Einstein et al. 2006, 2003, 2007; Pluchino et al. 2009; Yang et al. 2009). Transplantation of NSCs reduces perivascular infiltrates, CD3+ cells, and ICAM-1 and LFA-1 expression and increases Treg cells in the brain and spinal cord (Einstein et al. 2003, 2006). IL-10 overexpressing adult NSCs significantly suppress CD45+ cells, CD4+ T cells, CD68+ macrophages/microglia, and CD8+ T cells in the spinal cord (Yang et al. 2009). Peripheral immunosuppressive effects of NSCs also contribute to attenuation of clinical findings. NSC transplantation via intravenous route decreased the number of CD3+ T cells and Mac3+ macrophages infiltrating the spinal cord (Einstein et al. 2007). Subcutaneous injection of NSCs inhibits generation of effector T cells, DC maturation, and cytokine production (Pluchino et al. 2009).

Table 2 Neural stem cell transplantation studies in EAE

Organism	Model	Transplanted cells	Genetic modification of cells	Transplantation time	Route of administration	Outcome	References
Lewis rat	SC and CFA	Rat- neonatal striatal spheres		Disease peak	ICV or IT	Cells migrated into the brain or spinal cord (inflamed white matter). Cell differentiation (neuronal and glial)	Ben-Hur et al. (2003)
Lewis rat	SC and CFA	Rat- neonatal striatal spheres		On day immunization	ICV	Decreased clinical severity of EAE and the brain inflammation (reduction in perivascular infiltrates and decreased expression of ICAM-1 and LFA-1)	Einstein et al. (2003)
C57BL/6mice	MOG	NPCs		On day 10,15, and 22 dpi	ICV or IV	Reduction of astrogliosis and marked decrease in the extent of demyelination and axonal loss	Pluchino et al. (2003)
C57B/6 mice	MOG	Neurospheres		On day 6 dpi	ICV	Downregulated inflammatory process, reducing demyelinating process and axonal injury	Einstein et al. (2006)
C57B/6 mice	MOG	hESC-derived NPCs		On day 7 dpi	ICV	Reduction in clinical signs, axonal damage and demyelination, suppression of encephalitogenic T cells	Aharonowiz et al. (2008)
C57BL/6 mice	MOG	ESC-derived NPCs		On the day of immunization or on day 10 dpi	IV	Substantial delay of the disease onset, marked reduction in EAE severity, decreased inflammation and demyelination	Cao et al. (2011)
C57BL/6 mice	MOG	mMSC-NSC		On day 21, 28, and 35 dpi	IT repeated	Reduced immune cell infiltration, area of demyelination, increased number of endogenous nestin+ progenitor cells	Harris et al. (2012)
C57BL/6 mice	MOG	aNSCs		On days 14 and 21 dpi	Intranasal, IV	Reduction in clinical sign, no peripheral immune responses, decreased spinal inflammation	Wu et al. (2013)
C57BL/6 mice	JHMV	hESCs-derived NSC transcriptomic signature-based selection		On day 14 dpi	Intraspinal	Significant neurological recovery, reduced neuroinflammation, decreased demyelination, and enhanced remyelination. Decreased accumulation of CD4+ T cells, increase in Tregs	Chen et al. (2014)
C57BL/6 mice	JHMV	Human EB-derived NSC		On day 14 dpi	Intraspinal	Decreased accumulation of CD4 + Tcells in the CNS, reduced demyelination at the site of injection, transient increase in Tregs	Plaisted et al. (2016)
C57B/6 mice SJL/J mice	MOG, adoptive transfer (PLP)	Mouse neurospheres		On day 8 dpi	IV	Suppression of encephalitogenic T cells, reduced demyelinating process and axonal injury	Einstein et al. (2007)

(continued)

Table 2 (continued)

Organism	Model	Transplanted cells	Genetic modification of cells	Transplantation time	Route of administration	Outcome	References
SJL mice	PLP139–151	Mice SVZ aNPCs		First disease episode or first clinical relapse	IV	Promoted brain repair, induced apoptosis of CNS-infiltrating encephalitogenic T cells	Pluchino et al. (2005)
SJL mice	PLP139–151	Mouse NPCs		On day 3 and 10 dpi or 10 dpi only	SC	Clinical improvement, inhibition of the generation of encephalitogenic T cells, BMP-4-dependent impairment of the DC maturation	Pluchino et al. (2009) b
Common marmoset	MOG 1–125	Human NPCs		Disease onset	IT or IV	Increase of the CCT velocities at the lower limb, decrease in the number of inflammatory infiltrates	Pluchino et al. (2009)a
C57BL/6 mice	MOG	Mouse aNSCs	IL-10-transduced	On day 10, 22, or 30 dpi	IV or ICV	Suppression of clinical sign, enhanced anti-inflammatory effect, induction of T cell apoptosis, promotion of remyelination	Yang et al. (2009)
C57BL/6 mice	MOG, adoptive transfer EAE	Mouse BM-derived NPCs	CCR5-transduced	On day 22 dpi (peak)	IV	Suppressed CNS inflammatory infiltration, myelin damage, and clinical sign of EAE	Yang et al. (2012)
FVB mice, Biozzi ABH mice	MOG	Mouse NPCs	Olig2-transduced	Disease onset or first relapse	ICV	Reduced the clinical signs of acute and relapsing disease	Sher et al. (2012)
C57BL/6 mice	MOG	NSPCs	IL-10 producing	On day 7 dpi or on first sign of disease	ICV, IV	Suppress clinical signs, inhibit T-cell activation, proliferation, and cytokine production	Klose et al. (2013)
SD rats	MOG	Rat/fetal NSCs	IDO	On day 5 dpi	IV	Attenuated clinical scores and faster remission in local immune suppression in the cervical lymph nodes and CNS, reduction in the number of activated T lymphocytes and an increase in Treg	Lee et al. (2015)
C57BL/6 mice	MOG	Mice/BM-derived NSCs	Overexpression IL-10, NT-3, and LINGO-1-Fc	At the onset (day 10 dpi) or chronic stage (day 60 dpi) of disease	IV	Cocktail-NSCs suppress acute and chronic stage of EAE, inducing M2 macrophages/microglia, reducing astrogliosis, and promoting axonal integrity, remyelination, and endogenous oligodendrocyte/neuron differentiation	Li et al. (2016)

Human NSCs (hNSCs) inhibit allogeneic immune cell responses when cocultured with T cells or DCs. They suppress T cell proliferation, decrease DC differentiation from myeloid precursors and maturation, suppress antigen-presenting capacity of DCs, and inhibit costimulatory molecule (CD80, CD86, and MHC-II) expression (Pluchino et al. 2009).

IL-10 overexpressing adult NSCs lead to reduced numbers of CD68+ and CD4+ cells and CD8+ cells, inhibit production of inflammatory cytokines IFNγ and IL-17, and induce apoptosis of encephalitogenic T cells (Yang et al. 2009). Reduced proliferation capacity of autoreactive T cells in draining lymph nodes was observed in EAE mice treated with IL-10-producing NSCs (Klose et al. 2013). Additionally, they inhibit proliferation and cytokine production of T cells. There are some evidence supporting the notion that soluble factors can mediate the immunosuppressive effects of NSCs. NSC supernatant suppresses differentiation of Th17 cells in vitro and in Th17 cell-driven EAE model with IFNγ−/− mice, in vivo (Cao et al. 2011). After testing various cytokines and neurotrophins, leukemia inhibitory factor (LIF) was found as a soluble factor that is responsible from immunosuppressive effects of NSCs (Cao et al. 2011).

5.4 Limitations and Solutions

NSC-based treatment in MS still needs improvement. Transplanted cells usually remain in the perivascular area and do not migrate to the lesion site. To increase the migration capacity, genetically modified NSCs with CCR5 transduction were generated and used in EAE models (Yang et al. 2012). CCR5 transduction accelerated migration of NSCs toward lesion site, even when given intravenously. Several genetic modifications were performed to increase therapeutic effects of NSCs such as Olig2, IL-10, and indoleamine 2,3-dioxygenase (IDO) transduction (Burt et al. 2015; Klose et al. 2013; Nam et al. 2015; Sher et al. 2012; Yang et al. 2009). Finally, cocktail transduction with IL-10, NT-3, and Lingo-1 Fc-engineered cells was used to enhance immunosuppressive and cell protective effect of NSCs (Zhang et al. 2016).

Another trouble in NSC therapy in MS is inflammatory microenvironment at lesion sites during demyelination. Several soluble factors and microglial cells affect transplanted NSCs' viability, differentiation, and migration capacity to the lesion site. Nonpermissive microenvironment conditions can be reversed by different modulations of NSCs. Preconditioning of NSCs with minocycline enhances survival of grafted cells, increases proliferation of NSCs, and induces release of cytoprotective factors such as Nrf2 (Sakata et al. 2012). Treatment of NSCs with IL-10 and IL-4 increases expression of adhesion molecules LFA-1 and chemokine receptors CXCR4 and CCR5 and enhances their migration capacity (Guan et al. 2008).

Due to the differentiation capacity to all neuronal cell lineages and the presence of anti-inflammatory and trophic effects, NSCs are ready for clinical applications. A phase 1 NSC transplantation study was performed in patients with Pelizaeus–Merzbacher disease (PMD), which is a leukodystrophy characterized by hypomyelination (Gupta et al. 2012). Results demonstrated that allogeneic NSCs transplantation is safe and effective in PMD. Transplanted cells engrafted to recipient tissue and produced myelin.

6 Induced Pluripotent Stem Cells

6.1 Generation of Induced Pluripotent Stem Cell

Induced pluripotent stem cells (iPSCs) are pluripotent stem cells produced from adult somatic cells by reprogramming process using particular transcription factors. They were first generated from mouse embryonic and adult fibroblasts by Shinya Yamanaka and his colleagues at Kyoto University. Yamanaka's group used retroviral vectors Octamer 3/4 (OCT3/4), SRY-box-containing gene 2 (SOX2), cytoplasmic Myc protein (c-MYC), and Krueppel-like factor 4 (KLF4) for reprogramming both mouse and human

fibroblasts, whereas the group of James Thomson used lentiviral vectors encoding OCT4, SOX2, NANOG, and Lin28 for reprogramming human fibroblasts. These iPSCs exhibited similar characteristics with ESCs as they can self-renew and differentiate into all cell types in the body. Several factors affect reprogramming efficiency including initial cell type, reprogramming factors, delivery method, and culture conditions. Apart from fibroblasts, several types of somatic cells have been used for iPSC generation, but fibroblast is still the favorable cell type for iPSC generation. Classical reprogramming factors have tumor-forming capacities. Therefore, new efficient reprogramming methods by using chemicals and microRNAs were developed. Retroviral and lentiviral vectors have been mainly used to deliver these factors into somatic cells. The major disadvantage of the original delivery method is that viral vectors integrate into genome of iPSCs and may cause tumor development. New approaches such as the use of nonviral delivery methods or omitting the oncogenic factors c-MYC and KLF4 could prevent tumorigenicity (Durnaoglu et al. 2011; Harding and Mirochnitchenko 2014).

6.2 In Vivo Studies

The recent successful improvements in generation of iPSCs and their differentiation into neural precursor cells (NPCs) and oligodendrocyte precursor cells (OPCs) initiated autologous iPSC therapy studies in MS. First, in vivo therapeutic effect of iPSCs was evaluated in EAE, chemically induced demyelination, and genetic hypomyelination models (Table 3). Mouse iPSC-derived NPCs (miPSC-NPCs) were transplanted to C57Bl/6 mice with MOG-induced EAE intrathecally after disease onset (Laterza et al. 2013). miPSC-NPC treatment reduced clinical scores of EAE and decreased demyelinated areas and axonal damage in the spinal cord. Transplanted miPSC-NPCs did not differentiate to neither neuron nor oligodendrocyte and did not migrate from perivascular space to lesion site. Neuroprotective effects of miPSC-NPCs were partly through the secretion of LIF that support resident oligodendrocyte survival and differentiation. Microarray analysis revealed that miPSC-NPCs counterbalanced the EAE-associated transcriptional changes in the spinal cord. These cells also limit BBB damage and decrease CNS-infiltrating inflammatory cells (Laterza et al. 2013). Intraventricular transplantation of miPSCs also improved the functional recovery of EAE mice and reduced T cell infiltration and white matter damage (Zhang et al. 2016). The effect of hiPSC-derived embryoid body intermediate-stage NPCs (EB-NPCs) was examined in neurotropic JHM strain of mouse hepatitis virus (JHMV)-induced EAE (Plaisted et al. 2016). Significant clinical recovery was not observed in EB-NPCs transplanted mice. EB-NPCs were rapidly eliminated, but they decreased accumulation of CD4+ T cells in the CNS, reduced demyelination at the site of injection, and increased the number of Treg cells (Plaisted et al. 2016). While healthy hiPSC-derived NPCs decreased inflammation, PPMS patient-derived NPCs failed to provide any profit in demyelination process (Nicaise et al. 2017).

OPCs derived from iPSCs were also transplanted into animal models of MS. Intracerebrally transplanted OPCs derived from miPSCs and hiPSCs survive and differentiate to MBP-expressing oligodendrocytes in both cuprizone- and lysolecithin-induced models (Czepiel et al. 2011; Nicaise et al. 2017). The recovery effect of OPC transplantation on demyelination was also confirmed in congenital hypomyelination model (Douvaras et al. 2014; Terzic et al. 2016; Wang et al. 2013). Transplanted OPCs differentiate into MBP-expressing oligodendrocytes (Terzic et al. 2016) and contribute to myelination (Douvaras et al. 2014; Terzic et al. 2016; Wang et al. 2013). The effect of iPSC-derived OPC transplantation was also evaluated in EAE model (Thiruvalluvan et al. 2016). Transplanted OPCs reduced EAE scores, cell infiltration, and demyelination in the cerebellum. Histological analysis revealed that transplanted OPCs remained within the ventricles; therefore their effect on clinical and histological features of EAE occurs most

Table 3 Induced pluripotent stem cells derived cell therapies in demyelinated animal models

Organism	Model	Original cell	Intermediate cell	Transplanted cell	Route of administration	Outcome	References
C57Bl/6 mice	Cuprizone induced	Mouse embryonic fibroblast	miPSCs	OPCs	IC (corpus callosum)	Differentiate to MBP-expressing oligodendrocytes, contributed to the remyelination	Czepiel et al. (2011)
C57Bl/6 mice	Cuprizone induced	Human blood cells with PPMS	hiPSCs	NPCs	IV	NPCs from PPMS patients failed to provide any benefit in preserving compact CNS myelination during active demyelination	Nicaise et al. (2017)
Sprague Dawley rats	Lysolecithininduced	Human fibroblast	hiPSCs	OPCs	IC (optic chiasm)	Recovery from symptoms (measured with visual evoked potential), transplanted cells survived and integrated within the chiasm, differentiated to PLP and/or MBP expressing oligodendrocytes, contributed to the remyelination	Pouya et al. (2011)
C57Bl/6 mice	MOG 35–55 induced EAE	Mouse embryonic fibroblasts	miPSCs	NPCs	IT	Decreased EAE score, reduction of demyelinated areas and axonal damage, transplanted miPSC-NPCs counterbalanced the EAE-associated transcriptional changes	Laterza et al. (2013)
C57BL/6 mice	MOG 35–55 induced EAE	miPSCs	miPSCs	NPCs	Intraventricular	Reduced T cell infiltration, ameliorated WM damage	Zhang et al. (2016)
C57BL/6 mice/ marmoset	Human MOG 34–56 induced EAE	Human fibroblast	hiPSCs	OPCs	ICV (cisterna magna), IC	Significant reduction of subsequent EAE scores and cell infiltration; transplanted hiPSCs in ventricule, migration and differentiation to MBP producing cells in marmoset model	Thiruvalluvan et al. (2016)
C57BL/6 mice	Intracranially JHMV	Primary fetal human fibroblasts	hiPSCs	NPCs	Intraspinal	EB-NPCs were rapidly rejected, decreased accumulation of CD4+ T cells in the CNS, reduced demyelination at the site of injection, modest pathological improvements, no significant clinical recovery	Plaisted et al. (2016)
Shiverer mice	Congenital hypomyelination	Mouse embryonic fibroblast	miPSCs	OPCs	IC (corpus callosum and striatum)	Transplanted cells expressing MBP	Terzic et al. (2016)
Shiverer mice	Congenital hypomyelination	Human fibroblast, keratinocyte	hiPSCs	OPCs	IC (5-site forebrain and brainstem)	Increased the survival, myelination of the brain, brainstem, and cerebellum	Wang et al. (2013)
Shiverer mice	Congenital hypomyelination	Human fibroblast with PPMS	hiPSCs	OPCs	IC (forebrain)	Host mouse axons were ensheathed, mature compact myelin observed with electron microscope	Douvaras et al. (2014)

(continued)

Table 3 (continued)

Organism	Model	Original cell	Intermediate cell	Transplanted cell	Route of administration	Outcome	References
Shiverer mice	Congenital hypomyelination	Mouse embryonic fibroblast	iNSCs	iNSCs	IC	MPB+ myelin sheets were detected in white matter tracts of the cerebellum	Lujan et al. (2012)
Shiverer mice	Congenital hypomyelination	Mouse embryonic fibroblast	iOPCs	iOPCs	The dorsal region of the spinal cord	Transplanted cells colonized the dorsal column white matter, generated compact myelin sheaths around dorsal column axons	Najm et al. (2013)
Shiverer mice	Congenital hypomyelination	Rat fibroblast	iOPCs	iOPCs	IC (corpus callosum and cerebellum)	Oligodendroglial ensheathment of host axons by the transplanted iOPCs, myelin formation	Yang et al. (2013)

likely through secreted factors. In contrast to this, intracerebrally transplanted hiPSC-derived OPCs migrated toward the lesion and differentiated to MBP producing mature oligodendrocytes in marmoset model of EAE, suggesting that differences between species or route of administration are important (Thiruvalluvan et al. 2016). Genetic modification of transplanted cells can be used to enhance their targeted migration. Polysialylating enzyme sialyltransferase X (STX) overexpression in iPSC-derived OPCs increased their migration along the axons in cuprizone-induced model (Czepiel et al. 2014).

After the successful reprogramming of somatic cells to iPSCs, more direct neural lineage conversion methods have been developed. Functional neurons were obtained by transdifferentiation of fibroblasts using defined factors. Afterward, similar methods were developed for the generation of iNSCs and iOPCs using neural lineage-specific sets of TFs. iNSC and iOPC transplantations were performed in dysmyelinated *Shiverer* mice (Lujan et al. 2012; Najm et al. 2013; Yang et al. 2013). Transplanted iNSCs differentiated into oligodendrocytes capable of integration into dysmyelinated *Shiverer* brain (Lujan et al. 2012). iOPCs when given into the spinal cord, corpus callosum, and cerebellum survived, ensheathed host axons, and produced myelin (Najm et al. 2013; Yang et al. 2013).

6.3 Mechanisms

Because of inadequate endogenous remyelination, cellular therapy is moving forward in MS treatment. Therapeutic effects of iPSCs are not limited with cell replacement. iPSCs also exhibit immunosuppressive effect and provide trophic support on endogenous repair mechanisms. NPC transplantation decreases T cell infiltration (Plaisted et al. 2016; Zhang et al. 2016). Secreted LIF from transplanted NPCs exerts trophic action on endogenous oligodendrocytes (Laterza et al. 2013).

Apart from cell replacement, iPSCs have been used in vitro to model diseases in order to understand the underlying mechanisms and for screening drugs that modify the disease process. MS patient-specific iPSCs were first generated in 2011 by using fibroblasts from a 35-year-old patient with RRMS (Song et al. 2012). Patient-derived iPSCs were successfully differentiated to neural progenitors and mature neurons. Subsequently, patient iPSCs were also generated from PPMS patients (Douvaras et al. 2014; Nicaise et al. 2017). In the study by Douvaras et al., four iPSC lines were converted to NSCs and OPCs which carry normal karyotypes. Transplanted OPCs provided myelination in *Shiverer* mice. Similar studies should be continued especially in MS patients with high genetic load for disease modeling.

6.4 Practical Considerations

There are still some concerns about iPSC-based treatment in MS. Firstly, iPSC generation methods still need to be improved. The other concern is preference of initial cell type for transplantation. OPCs are superior for cell replacement, but anti-inflammatory and trophic effects of these cells have not been demonstrated yet. NSCs can differentiate into all neuronal cell lineages and also have anti-inflammatory and trophic effects, but they may differentiate to astrocytes as well and therefore lead to unwanted astrogliosis in MS. Also, they have tumorigenicity potential. Another major concern is the route of administration of cells. Direct intracerebral or intraspinal injection seems more effective, but they are not practical in clinical setting. Intranasal route may also be used effectively, and it is a less invasive route for administration of iPSCs in MS (Wu et al. 2013). Finally, use of allogeneic or autologous iPSCs should be considered. Autologous iPSCs are preferable, but they may be ineffective due to intrinsic disease factors (Nicaise et al. 2017). To avoid immunogenicity, healthy

allogeneic iPSC therapy needs immunosuppressive treatment that may cause MS relapses.

7 Challenges and Future Perspectives of Stem Cell Transplantation for MS

Stem cell transplantation can be regarded as a potential source of treatment for MS. Nevertheless, before introducing stem cell treatment wholesale into clinics, methodological, ethical, and clinical challenges must be overcome in stem cell therapy studies.

7.1 Sources

HSCs and MSCs have been used in clinical MS stem cell trials. In spite of the better availability of HSCs and MSCs compared to NSCs, remyelination capacity of NSCs makes them the preferred cell type for MS stem cell clinical trials particularly in the progressive stages of MS (Mariano et al. 2015; Sarkar et al. 2017). Although iPSC-derived stem cells have not been used in clinical trials of MS, they are suitable candidates for individualized cell replacement therapy due to their advantage of being easily obtained from the patient's own tissue.

The autologous and allogeneic stem cells contain different advantages and disadvantages in MS treatment (Cohen 2013). Autologous stem cell is less immunogenic, but generation from PSCs takes a longer time period, which makes it disadvantageous particularly in the acute phase of MS because of the necessity of immediate SCT. Additionally, autologous stem cells may be ineffective due to intrinsic disease factors (Nicaise et al. 2017). However, genetic defects can be corrected with several gene-editing methods including zinc finger nucleases, transcription activator-like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 systems (Maeder and Gersbach 2016), which means that autologous stem cells could be used in MS therapy upon genome editing, if necessary. The use of allogeneic stem cells has advantages such as avoiding the risk by genetic susceptibility of the recipient to develop MS. Since these cells are readily available from biobanks (Natalwala and Kunath 2017), they may be useful in the acute and progressive phase of MS.

7.2 In Vitro Cell Expansion and Manipulation

Numerous technical factors affect the yield, viability, function, and efficacy of SCT (Bang et al. 2016). The use of fetal bovine serum in culture medium raises safety concerns, including possible transmission of zoonoses and infusion-related allergic reactions (Cohen 2013). Stem cell production for clinical trials should be done under current GMP standards (Galvez-Martin et al. 2016).

Various approaches for in vitro cell expansion increase the stem cell proliferation, survival, and trophic support and reduce senescence of stem cells (Bang et al. 2016). Preclinical studies showed that ex vivo treatment of stem cells with trophic factors or chemical agents enhanced the migration of stem cells and trophic support in the brain. Lastly, genetic modification of stem cells, such as overexpressing chemokine receptors or IL-10, increased their efficacies and migration capacities in animal models of MS (Klose et al. 2013; Phillips and Tang 2008; Yang et al. 2012).

7.3 Practical Considerations

7.3.1 Storage

The ability to freeze with xeno-free freezing media and storing them in proper conditions are essential steps for clinical use of stem cells (Sarkar et al. 2017). Automated methods of thawing cells may increase viability of cells (Nishiyama et al. 2016). Other considerations

for storage conditions are cell number, cell density, vial size, and total volume.

7.3.2 Passage Number

The passage number of transplanted stem cells affects their proliferation, differentiation, and therapeutic capacities (Sisakhtnezhad et al. 2017). For example, lower than five passages of MSCs appear to be optimal for transplantation (Liu et al. 2016).

7.3.3 Route

The routes of cell administration vary among studies. Direct intracerebroventricular or intrathecal injections seem more effective, but IV route could be superior for peripheral immunosuppressive effect (Cohen 2013). However, entry to CNS is very low, and most of the injected cells are trapped by the lung, liver, spleen, and lymph nodes. Effective and less invasive routes for administration of stem cells such as intranasal route may also be used in MS (Li et al. 2015).

7.3.4 Dose

As the differences in the number of doses could be an important factor to the observed variation in responses, standardization is required for optimal doses for each transplanted cell type (Cohen 2013). Additionally, repeated administrations should be used to increase clinical benefit especially in progressive MS (Harris et al. 2016).

7.4 Tracking of Transplanted Cells

Noninvasive cell-tracking methods allow real-time monitoring of survival, migration, and homing of administered stem cells. MRI, magnetic particle imaging, positron emission tomography, single-photon emission computed tomography, and optical imaging methods can be used for in vivo tracking of stem cells (Filippi et al. 2017a; Ngen and Artemov 2017). To date, an optimal technique for in vivo cell tracking does not yet exist in the clinical setting.

7.5 Risks

In general, HSCT and MSC transplantation in MS patients have been well-tolerated, but several potential acute and chronic adverse effects should be considered. Infusion-related toxicity and infection are acute risks of stem cell therapy, and additive immunosuppressive treatment increases the infection risk following transplantation (Sarkar et al. 2017). Ectopic tissue formation and malignant transformation of stem cells are theoretical concern, although it has not been reported in MSC therapy studies (Cohen 2013). iPSCs have more tumorigenicity potential depending on pluripotency induction method. The use of iOPCs or iNSCs obtained by direct reprogramming without pluripotent stage can avoid tumor development risk (Xie et al. 2016). The other main risk in stem cell treatment is allogeneic immune rejection that is primarily mediated by T cell-dependent immune responses and needs lifelong immunosuppressive treatment. Previous studies supported that SCTs may also lead to disease activation in MS, presumably by leading to fever (Cohen 2013).

7.6 Cost-Effectiveness

The initial costs of stem cell therapies are extremely high, and cost-effective stem cell treatments must be developed (Sarkar et al. 2017). To assess the risk-benefit ratio of stem cell therapies, randomized studies should be performed comparing the efficacy of stem cells against other conventional therapies.

Stem cell tourism is a ridiculous term used to describe traveling abroad to undergo medical stem cell treatments that are not approved or available in patients' home country. Anecdotal evidence suggests that stem cell tourism leads to physical and financial risk to patients (Marks et al. 2017). Clinicians and regulators should work together to prevent deregulated cell-based therapies.

8 Conclusion

Stem cell-based therapies are attractive alternatives for the treatment of MS. There are still some issues which need to be resolved such as low efficacy of iPSCs, lack of proper differentiation protocols, epigenetic alternation in donor cells, and heterogeneity of transplanted cells. Improvement of stem cell technology will contribute to overcome these problems. For example, screening and selection of viable, genetically stable, and desired stem cells can prevent tumorigenicity and immunogenicity side effects of SCT.

Apart from these technical problems, several clinical difficulties should be considered. One of main concerns in SCT is to make a choice between autologous versus allogeneic stem cells for MS treatment. In older MS patients, HLA-matched young donor cells for transplantation may be more appropriate (Phanthong et al. 2013).

Combination therapy with anti-inflammatory and remyelinating agents (Anti-Lingo1 ab) and also other stem cells should be considered to increase the efficacy of stem cell therapy (Harlow et al. 2015). Emerging, innovative treatment approaches such as using cell-free stem cell products (conditioned media, exosome) should be investigated in animal models of MS (Derkus et al. 2017; Harlow et al. 2015).

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