

Review Article

Bone Marrow Stem Cell as a Potential Treatment for Diabetes

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Diabetes mellitus (DM) is a group of metabolic diseases in which a person has high blood glucose levels resulting from defects in insulin secretion and insulin action. The chronic hyperglycemia damages the eyes, kidneys, nerves, heart, and blood vessels. Curative therapies mainly include diet, insulin, and oral hypoglycemic agents. However, these therapies fail to maintain blood glucose levels in the normal range all the time. Although pancreas or islet-cell transplantation achieves better glucose control, a major obstacle is the shortage of donor organs. Recently, research has focused on stem cells which can be classified into embryonic stem cells (ESCs) and tissue stem cells (TSCs) to generate functional β cells. TSCs include the bone-marrow-, liver-, and pancreas-derived stem cells. In this review, we focus on treatment using bone marrow stem cells for type 1 and 2 DM.

1. Introduction

Diabetes mellitus (DM) is a devastating disease [1] that includes 2 main types: type 1 and type 2 DM. DM therapies mainly include diet, insulin, oral hypoglycemic agents, and pancreas or islet-cell transplantation. Exogenous insulin replacement has been the primary therapeutic technique for controlling plasma glucose levels. However, because of the shortage of donor organs, recent research has focused on stem cells, including pancreatic stem cells, hepatic stem cells, bone marrow stem cells (BMSCs), induced pluripotent stem cells (iPS), and embryonic stem cells (ESCs) to generate β cells for DM treatment [2]. BM is an invaluable source of adult pluripotent stem cells, and this review focuses on the treatment of DM using BMSCs.

2. Pathophysiology of Type 1 DM

Type 1 DM is a T-cell-mediated autoimmune disease accompanying lymphocytic infiltration of the pancreatic islets. The nonobese diabetic (NOD) mouse is a spontaneous mouse model of type 1 DM and involves many of the same autoantigens targeted by human T cells [3, 4]. BM-derived T-cell precursors go to the thymus to differentiate into mature T cells via positive and negative selection. Thymocytes expressing TCRs recognize self-antigen-MHC complexes with high affinity/avidity undergoing central deletion. In contrast, thymocytes expressing low-affinity TCRs for the same complexes differentiate into mature T cells and populate

the peripheral lymphoid organs where they become available for recognizing foreign antigens [5]. Autoreactive T cells, with their relatively high avidity and pathogenic potential, can escape thymocyte negative selection and elicit autoimmunity in the absence of adequate peripheral regulation [6]. Approximately 20% of individuals with spontaneous mutation of autoimmune gene Aire develop type 1 DM with other autoimmune diseases, which is thought to reflect their inability to select against islet antigen reactivity during T-cell development [7]. Insulin is an Aire-regulated islet protein ectopically expressed in the thymic medullary epithelial cells. Insulin was detected in all thymus tissues examined and class III VNTR alleles were associated with a 2-to-3-fold higher INS mRNA level than class I. It has been proposed that higher levels of thymic INS expression, which facilitates the induction of immune tolerance, are a mechanism for the dominant protective effect of class III alleles [8].

In the NOD mouse model, mice that are genetically deficient in B cells from birth develop a very low incidence of diabetes [9, 10]. One report has suggested that depleting B cells delayed and/or reduced the onset of diabetes and reversed diabetes in over one-third of mice and that B cells are among the regulatory populations [11].

3. BMSC Therapies for Type 1 DM

Insulin injection is the standard therapy for type 1 DM. Unfortunately, however, exogenous insulin injection cannot

mimic insulin secretion from normal β cells when blood glucose changes all the time. Thus, how to generate new β cells is an important approach in the treatment of type 1 DM. BM-, liver-, and pancreas-derived stem cells, iPS cells, and ESCs can differentiate into β cells. Hepatic stem cells are excellent candidates for generating β -cell surrogates because the liver and pancreas are both differentiated from endoderm during development, and liver stem cells expressing duodenum homeobox protein-1 long-term exhibited similar profiles for the expression of genes related to pancreatic development and β -cell function and reversed hyperglycemia in diabetic mice [12]. The acinar cells in the pancreas produce digestive enzymes and duct cells that form the afferent system to the duodenum. Adult mouse pancreas contains islet cell progenitors, which differentiate into β cells after partial duct ligation. This cell population represents an obvious target for therapeutic regeneration of β cells in DM [13]. iPS cell transplantation corrected hyperglycemia in two mouse models of types 1 and 2 DM [14]. Although ESCs are pluripotent stem cells and can generate insulin-positive cells *in vitro* [15], their differentiation *in vitro* cannot be controlled [16].

BM is the flexible tissue found in the interior of bones, and includes hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), and endothelial progenitor cells (EPCs). HSCs give rise to red blood cells, platelets, monocytes, granulocytes, and lymphocytes; MSCs can differentiate into myogenic, osteogenic, chondrogenic, and adipogenic lineages [17, 18]. EPCs are a population of rare cells that circulate in the blood with the ability to differentiate into the endothelial cells that make up the lining of blood vessels [19]. BM cells (BMCs) have the ability to differentiate *in vivo* into functionally competent β cells [20].

NOD mice that received transplanted BALB/c nu/nu bone marrow cells displayed normal T- and B-cell functions, and newly developed T cells in the allogeneic bone marrow recipients were tolerant to cells with both donor- and host-type major histocompatibility complex determinants. These results suggest that allogeneic bone marrow transplantation (ABMT) might contribute to the prevention of islet destruction and to the restoration of self-tolerance [21]. However, ABMT alone could not be used to treat overt diabetes in NOD mice whose islets had been completely destroyed. One report has demonstrated that BMT promotes β -cell regeneration after acute injury through BM mobilization [22]. NOD mice showed a reduction of the glycosuria and a normal response in the glucose-tolerance test after the transplantation of bone marrow plus newborn pancreas [23, 24]. The transplantation of pancreatic islets from two MHC-disparate donors was achieved in combination with IBM-BMT, resulting in the improvement of blood glucose levels and the amelioration of streptozotocin-induced DM in rats [25]. SDF-1 could potentially be used to improve the homing of stem cells and β -cell regeneration, and it improves glycemia and insulin production in diabetic mice [26]. The transplantation of insulin-producing cells from adult hBMSCs into nude diabetic mice resulted in the control of their diabetic status for 3 months [27]. Recent reports have demonstrated that human bone marrow MSCs (hBMSCs) improved blood glucose control and ameliorated diabetes in animal models [28–31]. One

report has described the possible benefit of hBMSCs for the treatment of insulin-dependent diabetes and gives new insight into the mechanism of β -cell recovery after injury mediated by hBMSC therapy [32].

Hyperglycemia affects EPC function by generating diffused endothelial damage, microvascular remodeling, and a reduction in the number of c-kit+Scal-1+ cells [33]. Reports have shown that adverse metabolic stress factors in type 1 DM are associated with reduced EPC numbers and angiogenicity [34]. Type 2 DM may alter EPC biology in processes critical for new blood vessel growth and may identify a population at high risk for morbidity and mortality after vascular occlusive events [35]. EPCs-mediated neovascularization of the pancreas could in principle be exploited to facilitate the recovery of nonterminally injured β -cells or to improve the survival and/or function of islet allografts [36].

BMSCs can not only promote endogenous angiogenesis [37] but also directly differentiate into smooth muscle [38] and endothelial cell [39] phenotypes *in vitro* and into functional vascular structures [40]. They contribute to myocardial recovery after injury *in vivo* [41]. MSCs have been shown to significantly suppress β cell-specific T-cell proliferation in the pancreas. Moreover, the increase in β -cell regeneration may be partly attributable to the protective qualities of MSCs, which shield newly formed β cells from destruction by T cells and thereby overcoming the inherent autoimmune pathology associated with type 1 DM [29]. BMCs have already been used in Phases I to III of clinical trials (NCT00821899, NCT01143168, NCT01157403, and NCT00465478) for the treatment of type 1 DM, as has been listed in the Clinical Trails.gov registry (update to August 2012).

4. Pathophysiology of Type 2 DM

Metabolic and immune systems are among the most fundamental requirements for survival. Obesity, insulin resistance, and type 2 DM are closely associated with chronic inflammation [42]. Genetic and environmental factors are involved in the development of type 2 DM, which shows relative insulin deficiency because of insulin resistance [43]. Adipocytes regulate and mediate inflammatory cytokines such as tumor necrosis factor- α (TNF α) and IL-6, which, respectively, inhibit or enhance each other, contributing to insulin resistance [44]. The basic mechanism of type 2 DM is that β cells fail to compensate for the effect of insulin resistance [45, 46].

Some youths with a clinical diagnosis of type 2 DM show evidence of islet-cell autoimmunity, with autoantibodies including islet-cell antibodies (ICA), glutamic acid decarboxylase (GAD), islet-autoantibodies-(IA-) 2, and insulin antibodies [47, 48]. Obese youths with a clinical diagnosis of type 2 DM may show evidence of islet autoimmunity contributing to insulin deficiency [49].

5. BMSC Therapies for Type 2 DM

We previously showed that KK-Ay mice, a type 2 DM model reconstituted with KK-Ay bone marrow cells, showed glycosuria, hyperinsulinemia, and hyperlipidemia. However,

KK-Ay mice showed improved serum insulin and lipid levels 4 months after BMT from normal BALB/c mice [50]. A previous report suggested that the transplantation of BMMSCs via intra-bone-marrow-BMT (IBM-BMT) in conjunction with the induction of HO-1 could eradicate type 2 DM. The beneficial effect of HO-1 induction further suggests that the abnormality in endothelial progenitor cells is due to a MSC-stromal cell disorder exacerbated by oxidative stress and decreases in adiponectin [51]. Autologous BM-derived rat MSCs promote PDX-1 and insulin expression in the islets and alter T-cell cytokine patterns [52]. Combined therapy of intrapancreatic autologous stem cell infusion and hyperbaric oxygen treatment can improve metabolic control and reduce insulin requirements in patients with type 2 DM [53].

The leptin receptor-deficient db/db mouse, a type 2 DM mouse model, exhibits severe hereditary obesity [54] and displays hormonal imbalances and hematolymphoid defects [55, 56]. Db/db mice exhibit a marked reduction in the size and cellularity of the thymus [57, 58]. We have reported that, in db/db mice, increased insulin sensitivity and decreased blood glucose levels result from normalizing any imbalance in lymphocyte subsets by IBM-BMT+ thymus transplantation (TT). The novel effects of IBM-BMT+TT are that this combination induces adiponectin secretion, followed by enhanced pLKB1-AKT-AMPK crosstalk, signaling pathway, insulin phosphorylation, and also HO-1 [59]. Moreover, IBM-BMT+TT has been proved to upregulate the expression of HO-1, peNOS, and pAKT, while decreasing iNOS levels in the kidney of db/db mice [60]. BMCs have already been used in Phases I to III of clinical trials (NCT00465478, NCT00644241, NCT01142050, NCT01677013, and NCT01759823) for the treatment of type 2 DM, as has been listed in the Clinical Trails.gov registry (update to August 2012). Combined therapy of intrapancreatic BMT and hyperbaric oxygen treatment can improve glucose control and reduce the dose of insulin and/or oral hypoglycemic drugs in type 2 DM patients, even if only to improve pancreatic β -cell function transiently [61].

6. Conclusion

There are problems which have a tendency to form teratomas and the ethical debate revolving around their derivation on the use of ESCs. iPS cells have been shown to have an embryonic-like pluripotent state that enables the development of an unlimited source of any type of human cell. However, the safety of using iPSCs needs to be proven in clinical trials. In contrast, BMSCs are an invaluable source of adult pluripotent stem cells and have already been used in Phases I to IV of clinical trials for the treatment of type 1 and 2 DM. BMSCs are thus a potential agent for the treatment of DM.

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