37]. Nephrons are of mesenchymal origin and stroma cells are of crucial importance for signaling, leading to the differentiation of both nephrons and collecting ducts [38]. Bone marrow-derived mesangial cell progenitors may play a crucial role in the development and progression of extracellular matrix accumulation and mesangial cell proliferation in the db/db mouse. Future studies to clarify extra-cellular matrix accumulation will focus on the donor-derived BM cells in the kidneys of the recipient.

Type IV collagen is a major structural component of all basement membranes, including the glomerular basement membrane of the kidney in vertebrates and invertebrates [39, 40]. Our results show that the expression of type IV collagen in the glomerulus was attenuated after IBM-BMT+TT. BM-derived cells fuse with existing glomerular cells and thereby provide therapeutic benefit or, alternatively, transfer their nuclei to damaged podocytes and thereby enable repair. Bone marrow-derived stem cells repair basement membrane collagen defects and reverse genetic kidney disease [35].

Age-related hematologic changes are associated with a decline in BM cellularity and a decline in adaptive immunity [41, 42]. The thymus involutes steadily with increasing age, resulting in a decreased release of new naïve T cells to the periphery, thereby affecting adaptive immunity [43]. The thymus also undergoes age-related progressive involution with decreased thymic lymphopoiesis, reduced thymic size and disrupted thymic architecture. Our previous studies demonstrated that the thymus is significantly lighter in db/db mice than in age-matched lean mice. When thymi from newborn C57BL6 mice were transplanted into db/db mice, the percentage of double-positive, double-negative and CD4+ cells in the thymus was normalized, as was the CD4/CD8 ratio in the peripheral blood [7], and the total cell number of the thymus, suggesting that IBM-BMT+TT is capable of restoring the immune repertoire and overcoming the autoimmune response, which is considered to be partly responsible for the development of diabetes.

IBM-BMT appears to be the most appropriate strategy for allogenic BMT [4]. Moreover, allogenic IBM-BMT+TT normalizes T cell subsets, cytokine imbalance and insulin sensitivity in db/db mice [7]. Allogenic IBM-BMT+TT upregulates the expression of HO-1 in kidney. This is followed by the upregulation of peNOS and pAKT and a reduction in iNOS levels, resulting in an improvement in renal function. This therapeutic approach offers decided advantages in the treatment of both autoimmune and hematological diseases.

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Conflict of interest

The authors declare no conflict of interest.

Abbreviations

DN: diabetic nephropathy; GVHD: Graft versus host disease; HO: Heme oxygenase; IBM-BMT: Intra bone marrow-bone marrow transplantation; iNOS: inducible nitric oxide synthase; NO: Nitric oxide; OS: Oxidative stress; pAKT: phosphorylated AK transforming; peNOS: phosphorylated endothelial nitric oxide synthase; pLKB1: phosphorylated liver kinase B1; pAMPK: phosphorylated adenosine monophosphate-activated protein kinase; TT: thymus transplantation.

Competing Interests

The authors have declared that no competing interest exists.

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Review Article

Bone-Marrow-Derived Mesenchymal Stem Cells for Organ Repair

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Mesenchymal stem cells (MSCs) are prototypical adult stem cells with the capacity for self-renewal and differentiation with a broad tissue distribution. MSCs not only differentiate into types of cells of mesodermal lineage but also into endodermal and ectodermal lineages such as bone, fat, cartilage and cardiomyocytes, endothelial cells, lung epithelial cells, hepatocytes, neurons, and pancreatic islets. MSCs have been identified as an adherent, fibroblast-like population and can be isolated from different adult tissues, including bone marrow (BM), umbilical cord, skeletal muscle, and adipose tissue. MSCs secrete factors, including IL-6, M-CSF, IL-10, HGF, and PGE2, that promote tissue repair, stimulate proliferation and differentiation of endogenous tissue progenitors, and decrease inflammatory and immune reactions. In this paper, we focus on the role of BM-derived MSCs in organ repair.

1. Introduction

The shortage of donor organs and the need of lifelong immunosuppression for the thousands of patients suffering from end-stage diseases worldwide are problems that need to be resolved. The repair, replacement, and regeneration of organs can restore impaired functions and are regarded as a potential solution to allotransplantation [1]. The bone marrow (BM) is an invaluable source of adult pluripotent stem cells, including hematopoietic stem cells (HSCs), endothelial progenitor cells (EPCs), and mesenchymal stem cells (MSCs). MSCs are prototypical adult stem cells with the capacity for self-renewal and differentiation with a broad tissue distribution. MSCs have been identified as an adherent, fibroblast-like population, originally isolated from BM [2]. These multipotent cells can be differentiated *in vitro* and *in vivo* into various cell types of mesenchymal origin, such as osteoblasts, adipocytes, and chondrocytes [3, 4]. Recently, more reports have demonstrated that MSCs secrete a variety of factors that promote tissue repair, stimulate proliferation and differentiation of endogenous tissue progenitors, and decrease inflammatory and immune reactions [5–7]. Because MSCs do not evoke an immune response, they are useful for allogenic organ and tissue repair.

2. Source, Multilineage Potential and Definition of MSCs

MSCs were first isolated from BM and have since been isolated from different adult tissues, including skeletal muscle [8], adipose tissue [9], umbilical cord [10], synovium [11], the circulatory system [12], dental pulp [13], amniotic fluid [14], fetal blood [15], lung [16], liver, and BM [17]. Friedenstein and coworkers first reported the existence of adherent, fibroblast-like cells isolated from BM [2], and that these cells could differentiate into mesodermal lineage such as osteoblasts, adipocytes, and chondrocytes *in vitro* [18] and cardiomyocytes [19]. Also, MSCs have been reported to differentiate into types of cells of endodermal and ectodermal lineages, including lung [20], retinal pigment [21], skin [22], sebaceous duct cells [23], renal tubular cells [24], and neural cells [25, 26], hepatocytes [27], and pancreatic islets [28]. There has hitherto been no specific surface marker for the identification of MSCs. For the isolation of human MSCs, the International Society for Cell Therapy proposed criteria [18] that comprise (1) adherence to plastic in standard culture conditions; (2) expression of the surface molecules CD73, CD90, and CD105 in the absence of CD34, CD45, HLA-DR, CD14 or CD11b, CD79a, or CD19 surface molecules as

assessed by fluorescence-activated cell sorter analysis; (3) a capacity for differentiation to osteoblasts, adipocytes, and chondroblasts *in vitro*. Similarly, murine MSCs have been shown to differ from human MSCs in terms of marker expression and behavior and have been identified as an adherent, fibroblast-like population, negative for CD45, CD11b, and CD 31, and positive for Scal1 and CD106 [29].

3. MSCs and the Immune System

MSCs have the ability to modify and influence almost all the cells of the innate and adaptive immune systems, to interfere with and affect cellular proliferation, differentiation, maturation, and function to induce an anti-inflammatory phenotype, and to modulate the immune response mediated by MSC soluble factors, including IL-6, M-CSF, IL-10, TGFβ, HGF, and PGE2 [7, 30, 31]. The innate immune cells include neutrophils, dendritic cells (DCs), natural killer (NK) cells, eosinophils, mast cells, and macrophages. MSCs modulate DC function, indirectly regulate T and B cell activities, delay and prevent the development of acute graft versus host disease (GVHD) [32], and suppress DC function during allogeneic islet transplantation [33]. MSCs have been shown to suppress these inflammatory cells [34] and to alter NK cell phenotype and suppress proliferation, cytokine secretion, and cytotoxicity against HLA class I expressing targets [35]. MSCs mediated NK cell suppression via soluble factors such as indoleamine 2,3-dioxygenase, PGE2, and TGFβ [36]. The adaptive immune system, which is composed of T and B lymphocytes generates specific immune responses to pathogens with the production of memory cells. It has been reported that MSCs upregulate anti-inflammatory Th2 cytokines, including IL-3, -5, -10, and -13, and downregulate proinflammatory Th1 cytokines, including IL-1α and β, IFNγ, and TNFα [37]. MSCs induced an alteration of DC cytokine secretion, inducing a decreased secretion of pro-inflammatory cytokines such as TNFα, IFNγ, and IL-12, and increased IL-10, which is a suppressive cytokine and inducer of reg T cells [38]. MSCs exert an inhibitory effect on B cells, but MSCs have stimulatory effect in low doses [39]. Concerning the immunomodulatory properties of MSCs in a mouse model, one report [40] has suggested that allogeneic MSCs are not intrinsically immunoprivileged, and under appropriate conditions, allogeneic MSCs induce a memory T-cell response resulting in rejection of an allogeneic stem cell graft. Another report [41] has suggested that MSCs could potentially improve experimental autoimmune encephalomyelitis in mice.

4. Homing of MSCs

Intravenously injected MSCs can migrate to the BM [42, 43] in the steady state and home to the inflammation site by migrating across the endothelium and then entering the injured organ [20, 44–47]. The fact that MSCs confer protection cannot be entirely attributed to their ability to home and engraft to the site of damage, suggesting that they are also capable of mediating protection in an endocrine manner [1]. MSCs have many chemokine receptors that assist in their migration to inflammatory sites via the SDF1/CXCR4 pathway [48]. Moreover, studies have demonstrated that platelet-derived growth factor-AB, IGF-1, and CD44 are the most potent chemoattractants for MSCs [44, 49].

5. BM-Derived MSCs (BMMSCs) and Organ Repair

Many reports have indicated that MSCs have the capacity to differentiate into endodermal, mesodermal, and ectodermal lineage cells. Recently, a report has indicated that the ability of MSCs to alter the tissue microenvironment via the secretion of soluble factors may contribute more significantly than their capacity for differentiation in tissue repair [50]. Adipose tissue and BM are the most readily available sources of MSCs because they are easy to harvest, and because of their relative abundance of progenitors and the lack of ethical concerns. Although adipose tissue-derived MSCs and BMMSCs show the same immunoregulatory and supporting hematopoiesis [51], BMMSCs have a higher degree of commitment to differentiate into chondrogenic and osteogenic lineages than adipose tissue-derived MSCs [52]. BMMSCs have been shown to ameliorate tissue damage and to improve function after lung injury [53–55], kidney disease [56, 57], diabetes [58, 59], myocardial infarction [60, 61], liver injury [62, 63], and neurological disorders [64].

5.1. BMMSCs and Lung. The lung is an organ that is highly susceptible to edema and endothelial permeability after traumatic injury. BMMSCs inhibit endothelial cell barrier permeability and preserve pulmonary endothelial cell integrity by preserving adherent junctions, tight junctions and decreasing inflammation. BMMSCs address both components of endothelial permeability and inflammation induced by hemorrhagic shock [54]. Interstitial lung diseases are characterized by epithelial injury, fibroblast proliferation, expansion of the lung matrix, and dyspnea. Of these diseases, idiopathic pulmonary fibrosis (IPF) is the most frequent and lethal. Proinflammatory cytokines IL-1 and TNF-α induce endothelial cells to express adhesion molecules and chemokines that attract other white cells from the blood to the site of injury [65]. IL-1 and TNF-α also stimulate proliferation of endothelial cells and fibroblasts that increase the blood supply at the site of injury and repair damage by the formation of scar tissue [66]. BMMSCs protect lung tissue from bleomycin-induced injury by blocking TNF-α and IL-1, two fundamental proinflammatory cytokines in the lung [53]. BMMSCs enhance the restoration of systemic oxygenation and lung compliance and decrease lung inflammation and histological lung injury. They also secrete cytokines, enhance lung repair, and attenuate the inflammatory response following ventilator-induced lung injury [55].

5.2. BMMSCs and Kidney. Acute and chronic kidney injuries after transplantation have a complex pathophysiology involving ischemic, inflammatory, and immunologic mechanisms, and adult stem cells have been used in the treatment of

these kidney diseases. Adult BM stem cells and the kidney precursors have been demonstrated to have an ability to differentiate into the kidney's specialized structures [67]. Nephrons are of mesenchymal origin, and stromal cells are of crucial importance for signaling, leading to the differentiation of both nephrons and collecting ducts [67]. Ischemic acute renal failure (ARF), characterized by a sharp decline in the glomerular filtration rate, is a very common complication in hospitalized patients and particularly in patients with multiorgan failure. When BMMSCs are injected after ARF, they can histologically become located in the kidney and significantly enhance the recovery of renal function by transdifferentiation into renal tubular or vascular endothelial cells [24, 68]. A single intrarenal administration of BMMSCs 7 days after ischemia-reperfusion significantly improved renal function and modified renal remodeling. The improvement of renal function was associated with a reduction in extracellular matrix accumulation. In addition, MSC administration also reduced tubular dilation, which is a classical feature of progressive renal failure in a renal ischemia rat model [57].

5.3. BMMSCs and Pancreas. Diabetes is caused by absolute insulin deficiency due to autoimmune destruction of insulin-secreting pancreatic β-cells (type 1 diabetes) or by relative insulin deficiency due to decreased insulin sensitivity, usually observed in overweight individuals (type 2 diabetes). In both types of the disease, an inadequate mass of functional β-cells is the major determinant for the onset of hyperglycemia and the development of overt disease. BM and BMMSCs induce the regeneration of recipient-derived pancreatic insulin-secreting cells, and MSCs inhibit T-cell-mediated immune responses against newly formed β-cells, which are able to survive in this altered immunological milieu [69].

Acute pancreatitis (AP) is characterized by a rapid onset and disease progression, with high fatality. Pancreatic acinar cells are the functional unit for the external secretion of the pancreas, which accounts for 80% of pancreatic tissue. During the process of severe AP, inflammatory mediators, metabolic products of arachidonic acid, and oxygen-derived free radicals enhance vascular permeability and cause tissue thrombosis and hemorrhage, thereby inducing necrosis of the pancreas [70]. BMMSCs can effectively relieve injury to pancreatic acinar cells and small intestinal epithelium, promote the proliferation of enteric epithelium and repair of the mucosa, and attenuate systemic inflammation in rats with severe acute peritonitis [71].

Human BM stem cells are able to differentiate into insulin-expressing cells *in vitro* by a mechanism involving several transcription factors of the β-cell developmental pathway when cultured in an appropriate microenvironment [72]. Human BMMSCs can be induced to express insulin in sufficient quantities to to reduce blood glucose in a diabetic mouse model [73] and to protect human islets from proinflammatory cytokines [74]. The use of human BMMSCs could be developed as a cell therapy for pancreatitis because of the ability, as shown in a rat model of acute pancreatitis, to reduce inflammation and damage to pancreatic tissue by reducing levels of cytokines and inducing Foxp3(+) regulatory T cells [75].

5.4. BMMSCs and Heart. Cardiovascular diseases are the first cause of death worldwide, and myocardial infarction (MI) is responsible for 12.8% of all deaths [76]. BMMSCs have been shown to differentiate into myogenic phenotype [77] and show a potent antifibrotic action, as their conditioned medium decreases cardiac fibroblast proliferation and the expression of collagen types I and III [78, 79] and increases the secretion of antifibrotic molecules such as matrix metalloproteinases 2, 9, and 14 [80]. BMMSCs exhibit the ability to differentiate into cardiomyocytes, smooth muscle cells, and endothelium in a swine model of chronic ischemic cardiomyopathy [81]. They have been shown to prolong survival compared with controls when hearts of Wistar rats were transplanted to Fisher 344 rats with intravenous MSC infusion [82]. Intravenous fusion of MSCs is the easiest and most practical method for delivery, though the MSCs must travel through the pulmonary circulation, where entrapment of cells is a concern [83]. Intracoronary infusion of stem cells is delivered with a standard over-the-wire balloon angioplasty catheter placed into the target coronary artery [84]. Injected BMMSCs improve cardiac function and reduce scar size in acute MI [85, 86]. Early-phase clinical trial data demonstrate that MSC therapy for post-MI is safe and has favorable effects on cardiac structure and function [87, 88].

5.5. BMMSCs and Liver. FGF-4 is one of the most important members of the fibroblast growth factor family; it can initiate the proliferation of mesodermal and endodermal cells and improve the development of fetal liver [89]. HGF is essential for the development of several epithelial organs and has been one of the most well-characterized cytokines for the stimulation of DNA synthesis in primary hepatocyte cultures and for liver development [90]. Oncostatin M is a member of the interleukin-6 family produced by hematopoietic cells and induces the differentiation of fetal hepatic cells, conferring various metabolic activities of adult liver [91]. These three factors participate in different developmental stages of the liver. FGF4, HGF, and oncostatin M have been shown to be key cytokines for hepatic differentiation from mouse BMMSCs [92]. Transplantation of BMMSCs alleviates GalN-induced acute liver injury in rats and stimulates the recovery systems, as evidenced by an earlier surge of cellular proliferation and differentiation into functional hepatocytes. IL-6 exerts hepatoprotective and mitogenic effects by stimulating the induction of acute-phase proteins as well as by suppressing apoptosis. Transplantation of BMMSCs could ameliorate acute liver injury. It promotes cell proliferation and organ repair, and the activation of the IL-6/gp130-mediated STAT3 signaling pathway via soluble IL-6 receptor is crucial in hepatic differentiation of BMMSCs [93].

Liver fibrosis is the excessive accumulation of extracellular matrix proteins, including collagen, that occurs in most types of chronic liver disease. Advanced liver fibrosis results in cirrhosis, liver failure, and portal hypertension, and often requires liver transplantation [94]. Although liver

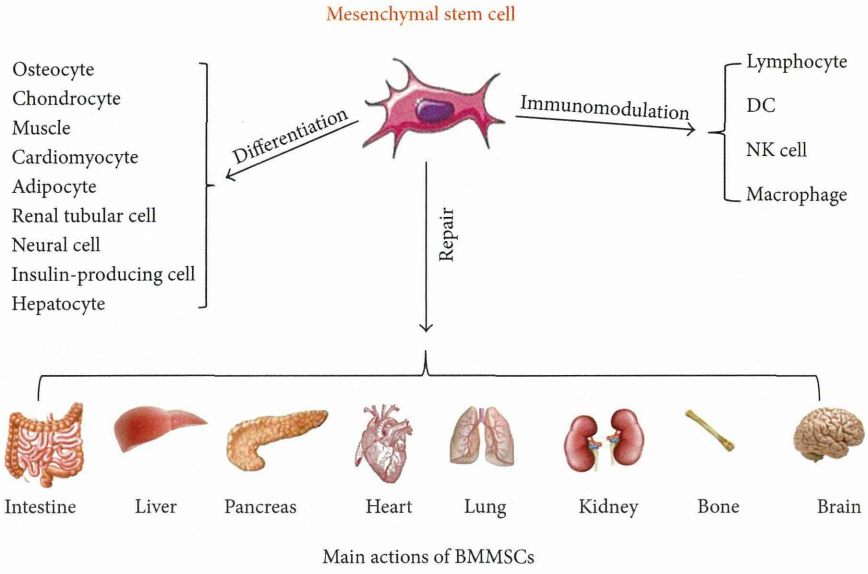

FIGURE 1: Main actions of BMMSCs.

transplantation is by far the most effective treatment for liver cirrhosis, extensive clinical application of the technique is limited by the lack of donor organ availability [95]. Cell-based hepatocyte transplantation, a potential interventional procedure, provides an effective strategy and holds great promise for the treatment of impaired livers. BMMSCs can protect against experimental liver fibrosis through promotion of IL-10 expression in CCl4- or dimethylnitrosamine-induced rats [63, 96].

5.6. BMMSCs and Brain. The development of effective treatments for human brain and spinal cord injury remains a serious challenge. In this regard, the transplantation of stem cells may help repair injured nerve tissue through the replacement of damaged cells, neuroprotection, or the creation of an environment conductive to regeneration by endogenous cells [97]. BMMSCs have been shown to promote cell proliferation and neurotrophic function of Schwann cells *in vitro* and *in vivo* [98]. Transplantation of BMMSCs can significantly reduce the behavioral abnormalities of these animals during the six weeks after engraftment [64]. Intravenously transplanted MSCs are capable of improving functional recovery and restoring neurological deficits in experimental intracerebral hemorrhage. The mechanisms are associated with enhanced survival and differentiation of neural cells and increased expression of antiapoptotic proteins and atrophic factors [99]. Human BMMSCs can improve neurological functional recovery in mice with experimental autoimmune encephalitis, possibly via a reduction of inflammatory infiltrates and areas of demyelination, stimulation of oligodendrogenesis, and by elevating brain-derived neurotrophic factor (BDNF) expression [41, 100]. Human BMMSCs transfected with the BDNF gene also showed improved functional recovery and reduced infarct size through a reduction in apoptosis [101]. Patients with Parkinson's disease transplanted with BMMSCs in the early stages of the disease (less than 5 years) showed greater improvement than in the later stages (11–15 years) [102].

5.7. BMMSCs and Intestine. Inflammatory bowel disease comprises a spectrum of chronic and relapsing diseases, including Crohn's disease (CD) and ulcerative colitis [103]. CD is characterized by a background of mucosal T-cell dysfunction, inflammatory cell infiltration, and abnormal cytokine production leading to uncontrolled and persistent intestinal transmural inflammation. Intraperitoneally injected cryopreserved BMMSCs home to and engraft into the inflamed colon and ameliorate trinitrobenzene sulfonic acid-induced colitis in rats [104]. Similarly, the injection of adipose-derived MSCs facilitated colonic mucosal repair and reduced the infiltration of inflammatory cells in the experimental colitis model [105].

Small intestinal permeability and villi injuries were significantly reduced in an MSC-administered group compared with the control group. MSC administration accelerated the recovery of the intestinal barrier dysfunction in a rat model of ischemia/reperfusion injury [106].

5.8. BMMSCs and Bone. Bone is regarded as an organ, and small bone damage can repair spontaneously without intervention. However, bone transplantation and surgery are required when there is extensive bone damage. As adult stem cells, BMMSCs possess a number of characteristics that make them appropriate for use in promoting bone regeneration [107]. BMMSCs may differentiate into tissue cells in order to restore lost morphology as well as function and to secrete a wide spectrum of bioactive factors that help to create a repair environment through their antiapoptotic effects, immunoregulatory function, and the stimulation of endothelial progenitor cell proliferation [108]. One report shows that

BMMSCs stimulate growth with osteogenesis imperfecta when children received allogeneic BMMSCs [109].

6. Conclusion

Figure 1 summarizes the main actions of BMMSCs. The original use of BMMSCs was to accelerate hematopoiesis, since they have the potential to differentiate into various cells, and to secrete cytokines and growth factors. BMMSCs have immunomodulatory properties through paracrine and endocrine mechanisms to repair damaged tissue. Homing and immunomodulation are important aspects of MSC functioning and their clinical effects. It has been proposed that the anti-inflammatory and antiapoptotic effects of MSCs may promote tissue regeneration. The use of allogenic nonimmunogenic BMMSCs would be a more acceptable strategy clinically. The potential role of BMMSCs to promote engraftment of organs and prevent rejection may be multifactorial and might be dependent on secretion of soluble growth factors, increasing angiogenesis, suppressing alloreactive T cells, and interacting with several arms of the immune system. However, the long-term safety of transplanted BMMSCs for organ repair needs to be proven prior to their clinical application.

Conflict of Interests

None of the authors has conflict of interests to declare.

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$CD4^+$ T Cell–Depleted Lymphocyte Infusion Impairs Neither the Recovery of Recipient Thymus nor the Development of Transplanted Thymus

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Thymus transplantation, in conjunction with bone marrow transplantation (BMT), has been attracting attention for the treatment of various diseases. Recently, donor lymphocyte infusion (DLI) has been used as a helpful tool for establishing donor chimerism and preventing a relapse of leukemia/lymphoma. However, the effects of DLI on transplanted and recipient thymuses have not been explored. We therefore performed DLI in the intrabone marrow–BMT + thymus transplantation setting. We have found that DLI leads to derangements in both recipient thymuses and transplanted thymuses; by 2 wk after BMT, we saw a decrease in total cell number, a lower percentage of $CD4^+CD8^+$ cells, and the obliteration of the thymic corticomedullary junction. Four weeks later, the thymic impairment became more serious. However, when we depleted the $CD4^+$ T cells ($CD4^-$-DLI), the recipient thymic recovery and transplanted thymic development were significantly restored by the treatment. In addition, there were much greater levels of TNF-α and Fas ligand, and a lower percentage of regulatory T cells in the DLI group than in the $CD4^-$-DLI group. These findings indicate that inflammation induced by DLI, especially by $CD4^+$ T cells, plays a crucial role in the thymic impairment. ***The Journal of Immunology*****, 2013, 190: 2976–2983.**

Allogeneic bone marrow transplantation (allo-BMT) is a potentially curative therapy for certain diseases of the hematopoietic system, immunodeficiencies, autoimmune diseases, solid malignant tumors, and so on (1–6). We have developed a new and powerful bone marrow transplantation (BMT) method: intrabone marrow–BMT (IBM-BMT) (7), in which donor bone marrow cells (BMCs) are directly injected into the recipient's bone marrow cavity. Therefore, a much greater number of donor hematopoietic stem cells and mesenchymal stromal cells (including mesenchymal stem cells) can be inoculated into the recipient bone marrow by IBM-BMT than by conventional i.v. BMT. This results in the rapid reconstitution of donor hematopoietic cells and permits a reduction in the doses of irradiation used as a conditioning regimen (8–10).

The thymus is an organ for inducing T cells and maintaining homeostasis. However, thymic functions are impaired by the conditioning regimen and the acute graft-versus-host disease (GvHD) that occurs after allo-BMT, resulting in deficient cell immunity (11, 12). In addition, there is a strong association between posttransplant autoimmune disease and the thymic dysfunction caused by chronic GvHD (13). Thymus transplantation (TT), an attractive method for improving T cell functions, has been applied clinically for patients with DiGeorge syndrome or HIV infection, which elicits the hypoplasia of the thymus (14). However, in mice, although T cell functions were restored or enhanced by TT, no concomitant GvHD was observed after TT in conjunction with allo-BMT (15). Therefore, TT can be used to treat autoimmune diseases in chimeric-resistant MRL/lpr mice and type 2 diabetes mellitus, and to suppress tumor growth (16–18).

Donor lymphocyte infusion (DLI) is often used after allo-BMT to prevent disease relapse in the setting of T cell–depleted BMT or nonmyeloablative conditioning regimens. It is also a combined method to convert from mixed chimerism to full donor chimerism (19, 20). However, DLI-induced GvHD is always associated with an increase in therapy-related morbidity because of its uncontrollable and fatal characteristics (21). It has been reported that many factors are involved in the damage to the recipient thymus after DLI (22, 23), whereas the effects of DLI on the transplanted thymus have hitherto remained unexplored.

In this study, we investigate the influence of DLI on both recipient and transplanted thymuses in the IBM-BMT + TT setting. Because we have found that TT using newborn thymus is most effective in tumor suppression (18), we used newborn thymus in this study. We show in this article that $CD4^+$ T cell–depleted lymphocyte infusion ($CD4^-$-DLI) impairs neither the recovery of recipient thymus nor the development of transplanted thymus.

Materials and Methods

Mice

C57BL/6 (B6), enhanced GFP (eGFP) transgenic (tg) B6, and BALB/c mice were purchased from Shimizu Laboratory Supplies (Shizuoka, Japan). Eight- to 12-wk-old male mice were used for BMT and DLI. For TT, 1 d after birth, B6 mice were sacrificed to obtain newborn thymuses. All the mice were maintained in a specific pathogen-free room.

Experimental protocol

As shown in Fig. 1, BALB/c mice were lethally irradiated with 7 Gy using the Gammacell 40 Exactor (MDS Nordion, Kanata, ON, Canada) with two

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Abbreviations used in this article: allo-BMT, allogeneic bone marrow transplantation; B6, C57BL/6; BMC, bone marrow cell; BMT, bone marrow transplantation; DLI, donor lymphocyte infusion; eGFP, enhanced GFP; FasL, Fas ligand; GvHD, graft-versus-host disease; IBM-BMT, intrabone marrow–BMT; tg, transgenic; Treg, regulatory T cell; TT, thymus transplantation; WSP, whole spleen.

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[^{137}Cs] sources, and the next day, these mice received IBM-BMT from B6 mice (group I). Some mice additionally received TT from B6 mice (group II). On the same day, some mice also received whole spleen (WSP-), CD4$^-$-, or CD8$^-$-DLI from B6 mice: WSP-DLI (group III), CD4$^-$-DLI (group IV), and CD8$^-$-DLI (group V). The treated mice were sacrificed 5 d, 2 wk, or 4 wk after the treatments.

Reagents and flow cytometric analysis

The Abs used in this study were as follows: purified rat anti-mouse CD4 and CD8 Ab (eBioscience, San Diego, CA); FITC-conjugated anti-mouse CD4 and H-2K^b Ab; PE-conjugated anti-mouse H-2K^d, CD4, CD8, and B220 Ab; and PerCP-Cy5.5–conjugated anti-mouse CD45 Ab (BD Pharmingen, San Diego, CA). For the thymus and peripheral blood analysis, leukocytes were first gated by CD45$^+$ cells, which were estimated as nuclear cells. To analyze the percentage of regulatory T cells (Tregs) in the spleen, we performed intracytoplasmic FoxP3 staining using an eBioscience FITC–anti-mouse/rat FoxP3 staining set in accordance with the manufacturer's instructions. Samples were analyzed using an FACSCalibur flow cytometer (BD Biosciences).

IBM-BMT and TT

One day after irradiation, the BMCs were prepared by flushing them from the medullary cavities of the femurs and tibias of B6 mice with PBS. The BMCs (1×10^7) were then injected directly into the tibial cavity of the recipient mice via the IBM route, as previously described (24). One whole newborn thymus from a B6 mouse was transplanted under the renal capsule of each recipient of IBM-BMT.

Cell preparation and DLI

Whole splenocytes from donor B6 mice were injected via the tail vein in the WSP-DLI group. CD4$^+$ or CD8$^+$ T cells were depleted from the splenocytes using purified CD4 or CD8 Ab with Dynabeads anti-rat IgG (Invitrogen Dynal AS, Oslo, Norway) for the CD4$^-$- or the CD8$^-$-DLI group, respectively. A total of 1×10^7 splenocytes were injected in the WSP-, CD4$^-$-, and CD8$^-$-DLI groups.

Assessment of GvHD

Survival was monitored daily. The severity of GvHD was assessed as previously described (25). In brief, the recipients were scored every 4 or 5 d for six clinical parameters: weight loss, posture, activity, fur texture, skin integrity, and diarrhea. A severity scale of 0–2 was used for each parameter, with a maximum score of 12. Histopathological analysis was performed by scoring the changes in the skin, intestine, and liver specimens. A severity scale from 0–4 was used, with a maximum score of 12.

ELISA

Peripheral blood was collected from the recipient mice by EDTA tube (BD, Franklin Lakes, NJ) and centrifuged to get the mouse plasma within 30 min. The plasma was stored at −20°C until assay. TNF-α and Fas ligand (FasL) were measured using the commercial kit (R&D Systems, Minneapolis, MN). Samples and standards were run in duplicate.

Blockade of inflammatory cytokine

For cytokine blockade, purified rat anti-mouse TNF-α Ab and FasL Ab were purchased from BioXCell and BioLegend, respectively. After WSP-DLI, some mice were injected i.p. with 1 mg anti–TNF-α Ab and 2 mg anti-FasL Ab (WSP + Abs group) on days 0, 4, 8, and 12 as described previously (26).

Statistical analysis

The results are represented as means ± SD. The Student t test was used to determine any statistical significance. A p value <0.05 was considered to be a significant difference.

Results

In the IBM+TT setting, WSP-DLI induces serious GvHD but CD4$^-$ -DLI does not

We carried out IBM-BMT, TT, and DLI from B6 to BALB/c mice. The protocol is described in detail in Fig. 1 and in *Materials and Methods*.

The TT group (group II) showed similar results to group I (Fig. 2), although TT provides an abundance of thymocytes (immature T cells), whereas DLI contains mature T cells and attacks the target organs after priming. Serious GvHD was induced not only according to the clinical scores (Fig. 2A), but also according to the histopathological evaluation (Fig. 2B) in the WSP-DLI group (group III). However, when CD4$^+$ T cells were depleted, the severity of the GvHD decreased dramatically (group IV), whereas CD8$^+$ T cell–depleted DLI (CD8$^-$-DLI: group V) induced GvHD, as seen in the WSP-DLI group (group III). Therefore, we omitted group V from the subsequent experiments because in vivo experiments require a large number of mice. Thus, the mice with CD4$^-$-DLI exhibited only a slight and transient loss of body weight, but no serious GvHD (Fig. 2).

The recovery of recipient thymus is impaired by WSP-DLI but not CD4$^-$-DLI

The cytoreductive conditioning regimens in the context of BMT, especially irradiation, result in severe recipient thymic atrophy and defects in the immune system (13). Moreover, after BMT, the transplanted bone marrow–derived thymic progenitors migrate to the recipient's thymus where they support thymopoiesis. We examined the effects of DLI on the recovery of the recipient thymus. The recipient mice were sacrificed and analyzed 5 d, 2 wk, and 4 wk after BMT. Five days after BMT, the recipient thymuses in all groups showed severe atrophy (Fig. 3A) and the total cell number decreased. The cell numbers in the WSP- and CD4$^-$-DLI groups were much lower than in the TT group (Fig. 3B). The size and weight of the recipient thymus in the TT group gradually increased (Fig. 3A, 3C, 3D). Histologically, the recipient thymus already displayed well-defined cortical and medullary areas 2 wk after BMT (Fig. 3E). In addition, the percentage of CD4$^+$CD8$^+$ cells had also increased to the normal level (Fig. 3F).

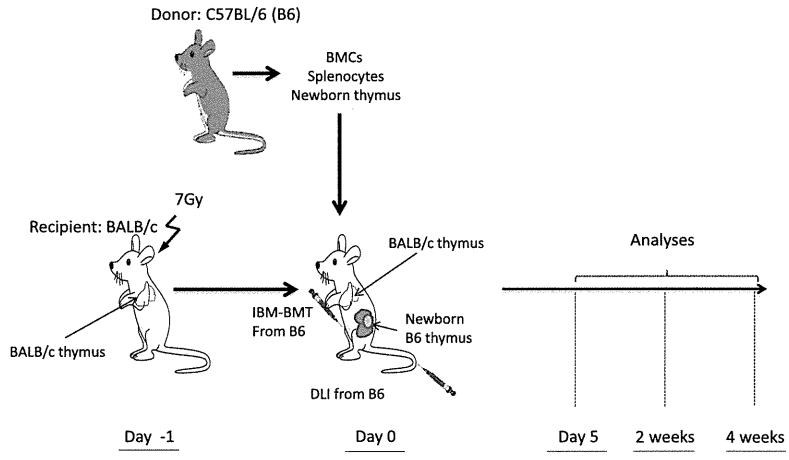

FIGURE 1. Experimental protocol. BALB/c mice were lethally irradiated (7 Gy from [^{137}Cs]). The next day, all the mice received IBM-BMT from B6 mice (group I). Some mice additionally received newborn TT from B6 mice (group II). On the same day, some mice also received WSP-, CD4$^-$-, or CD8$^-$-DLI from B6 mice: WSP-DLI (group III), CD4$^-$-DLI (group IV), and CD8$^-$-DLI (group V). The treated mice were sacrificed 5 d, 2 wk, or 4 wk after the treatments.

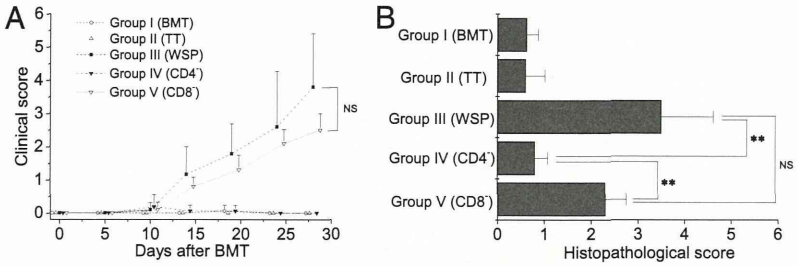

FIGURE 2. Clinical and histopathological scores of different groups. Irradiated BALB/c mice received IBM-BMT alone (BMT) or with newborn TT. Whole, $CD4^+$, or $CD8^+$ T cell–depleted splenocytes were injected in the WSP-, $CD4^-$- and $CD8^-$-DLI groups, respectively. Donor BMCs, thymuses, and splenocytes were all from B6 mice. After BMT, the body weight of the recipients was recorded and clinical signs of GvHD were assessed every 4 or 5 d (**A**). Autopsies were performed on mice that had been sacrificed 4 wk after BMT. Tissues from GvHD target organs (liver, skin, and intestine) were prepared for histopathological scoring (**B**). n = 4–8/group; experiments were performed three times. $^{**}p < 0.01$.

However, when the whole B6 splenocytes were injected, the process of recovery was disturbed: 1) the recipient thymus remained atrophic (Fig. 3A, 3C, 3D); 2) it was structurally damaged and, histologically, lost the corticomedullary junction (Fig. 3E); and 3) the percentage of $CD4^+CD8^+$ cells decreased seriously (Fig. 3F, 3G).

When $CD4^+$ T cells were depleted, the effects of DLI on the impairment of the recipient thymus appeared to be ameliorated at 2 wk after the BMT (Fig. 3C, 3E, 3F). Four weeks after the BMT, there was no significant difference in the structure or percentage of $CD4^+CD8^+$ cells between the TT and $CD4^-$-DLI groups (Fig. 3E, 3G). These findings suggested that DLI, especially with $CD4^+$ T cells, impaired the recovery of the recipient thymus.

The development of transplanted thymus is also impaired by WSP-DLI but not $CD4^-$-DLI

The transplanted thymus was also examined at the time points described earlier. In the TT group (group II), macroscopic findings indicated that the transplanted thymus developed well in all the recipient mice (Fig. 4A). Two weeks after the BMT, the transplanted thymus showed normal architecture and percentage of $CD4^+CD8^+$ cells (Fig. 4C, 4D). The thymus had also grown large by 4 wk after BMT (Fig. 4A, 4C). In the WSP-DLI group, the total cell number of the transplanted thymus was lower than that of the TT group on day 5 after BMT, but there was no significant difference between the TT and $CD4^-$-DLI groups (Fig. 4B). In the WSP-DLI group, only 37.5% of transplanted thymuses could be observed at 2 wk after the BMT, and those thymuses were smaller, had lost the thymic corticomedullary junction, and had a decreased percentage of $CD4^+CD8^+$ cells (Fig. 4A, 4C, 4D). The impairment in the transplanted thymus had become even more serious by the later time point, at which time only 16.7% of the transplanted thymuses could be observed (Fig. 4A, 4C, 4E). In contrast, 2 wk after BMT, the transplanted thymus in the $CD4^-$-DLI group was much larger than that in the WSP-DLI group. In addition, both the thymic structure and the percentage of $CD4^+CD8^+$ cells were similar to those in the TT group. Four weeks after BMT, there were no significant differences in any of the thymic parameters between the TT and $CD4^-$-DLI groups (Fig. 4A, 4C–E). These results indicated that the WSP-DLI had a remarkable impact on the development of the transplanted thymus, and that the $CD4^+$ T cells played a crucial role in the impairment.

DLI-derived $CD4^+$ T cells induce thymic impairment

Because both the recovery of the recipient's thymus and the development of the transplanted thymus had already been disturbed in the WSP-DLI group by 2 wk after BMT, we investigated the origin of the population that played a role in the impairment at an earlier time point. It has been reported that TT can also significantly increase the percentage of $CD4^+$ T cells (but not $CD8^+$ T cells) compared with BMT alone (27). Because no serious GvHD or thymus impairment was induced by TT, we hypothesized that the derivation of $CD4^+$ T cells could be the key point. We used splenocytes of eGFP tg B6 mice to distinguish the derivation of the T cells and confirm the role of the $CD4^+$ T cells. In this model, BMCs and newborn thymus were from B6 mice, whereas splenocytes from eGFP tg mice were injected for DLI. The percentages of non-DLI (donor BMCs or TT) and DLI-derived $CD4^+$ and $CD8^+$ T cells were analyzed 5 d after the BMT. The results showed that the percentage of DLI-derived alloreactive $CD4^+$ T cells was much higher in the WSP-DLI group than in the $CD4^-$-DLI group, suggesting that the DLI-derived $CD4^+$ T cells induced the impairment of both the recipient and transplanted thymus (Fig. 5A). Although the percentage of $CD8^+$ T cells in both the WSP- and $CD4^-$-DLI groups was higher than in the TT group, there was no significant difference between the WSP- and $CD4^-$-DLI groups, indicating that the $CD8^+$ T cells were likely not responsible for the thymus impairment (Fig. 5B). Moreover, there were no significant differences in the percentages of non–DLI-derived $CD4^+$ and $CD8^+$ T cells between the WSP- and $CD4^-$-DLI groups (Fig. 5A, 5B). These data showed that mature $CD4^+$ T cells from DLI played an important role in the impairment of the recovery of the recipient thymus and the development of the transplanted thymus. In addition, lymphopenia, especially B lymphopenia, was seen in the WSP-DLI group, whereas normal reconstitution was observed in the TT and $CD4^-$-DLI groups at 4 wk after BMT (Table I), proving that donor $CD4^+$ (not $CD8^+$) T cells were responsible for the induction of hematopoietic niche injury (28). The recipients in all groups showed full donor chimerism (>98%, data not shown).

Mechanism for $CD4^+$ T cell–induced thymic impairment

It has been shown that alloreactive $CD4^+$ T cells induce epithelial and organ damage in recipients, otherwise known as acute GvHD; many kinds of soluble inflammatory agents, such as TNF-α, IFN-γ, IL-1, and NO, are involved (29). The target organs in acute GvHD include the skin, liver, intestine, and thymus. Teshima and colleagues (30) have shown that the damage does not require alloantigen expression on the target epithelium, and that the neutralization of TNF-α prevents the damage. In addition, FasL-dependent donor T cell–mediated cytotoxicity has been implicated in the development of the thymus and bone marrow GvHD (22, 28). Therefore, we hypothesized that the inflammatory cytokines of TNF-α and FasL should induce the impairment of both recipient and transplanted thymuses. We thus examined the plasma levels of TNF-α and FasL 5 d after BMT, because we know that, at that

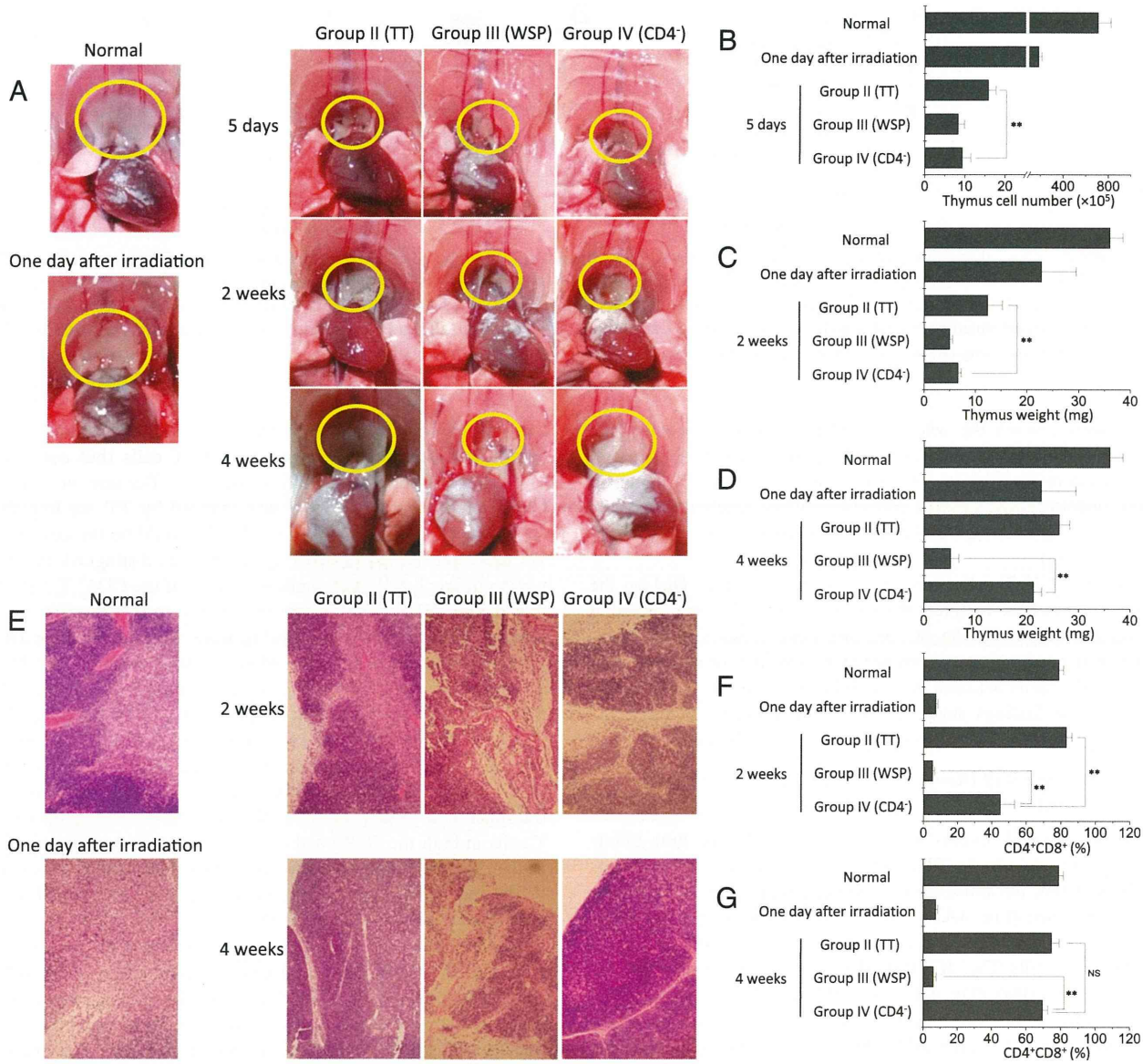

FIGURE 3. WSP-DLI, but not $CD4^-$-DLI, induces recipient thymic impairment. Recipient mice were sacrificed for analysis 5 d, 2 wk, or 4 wk after BMT. Thymuses from normal B6 mice and B6 mice 1 d after irradiation (7 Gy, no BMT) were used for control. (**A**) Representative macroscopic findings. Yellow circles indicate location of recipient thymus. (**B**) Total cell number of recipient thymus on day 5. (**C–G**) Weight, representative histological findings, and $CD4^+CD8^+$ cell percentage of recipient thymus are shown for later time points [(C, F): 2 wk; (D, G): 4 wk]. (E) H&E staining (original magnification ×100). n = 4–8/group; experiments were performed three times. $^{**}p < 0.01$.

time point, the processes of recipient thymic recovery and transplanted thymic development were disturbed by WSP-DLI. The levels of TNF-α and FasL were, indeed, significantly higher in the WSP-DLI group than in the TT and $CD4^-$-DLI groups on day 5 after BMT (Fig. 6A). To determine whether the production of inflammatory cytokines was causally related to the thymic impairment, we blocked TNF-α and FasL after WSP-DLI by Abs as described previously (26). The mice injected with Abs were sacrificed 2 or 4 wk after BMT. Both recipient and transplanted thymuses in the WSP + Abs group showed similar results to those in the $CD4^-$-DLI group 2 wk after BMT, including macroscopic (Supplemental Fig. 1A) and histological (Supplemental Fig. 1B) observations, weight of recipient thymus (Supplemental Fig. 1C), and percentages of $CD4^+CD8^+$ cells in the recipient (Supplemental Fig. 1D) and transplanted thymuses (Supplemental Fig. 1E). Blockade of TNF-α and FasL also prevented thymic impairment, according to observations made 4 wk after BMT (data not shown). These results proved that the inflammatory cytokines of TNF-α and FasL mediated the impairment of both recipient and transplanted thymuses.

We also compared the percentage of Tregs in the recipient spleen, which reflects the suppressive capability of inflammation 4 wk after BMT at the time lymphopenia occurs. The percentage of Tregs in the WSP-DLI group was much lower than in the TT and $CD4^-$-DLI groups (Fig. 6B). These data suggested that inflammation induced by DLI-derived $CD4^+$ T cells resulted in the impairment of recipient thymic recovery and transplanted thymic development.

Discussion

In this study, we examined the effects of $CD4^-$-DLI on both recipient and transplanted thymuses after allo-IBM-BMT + TT. In

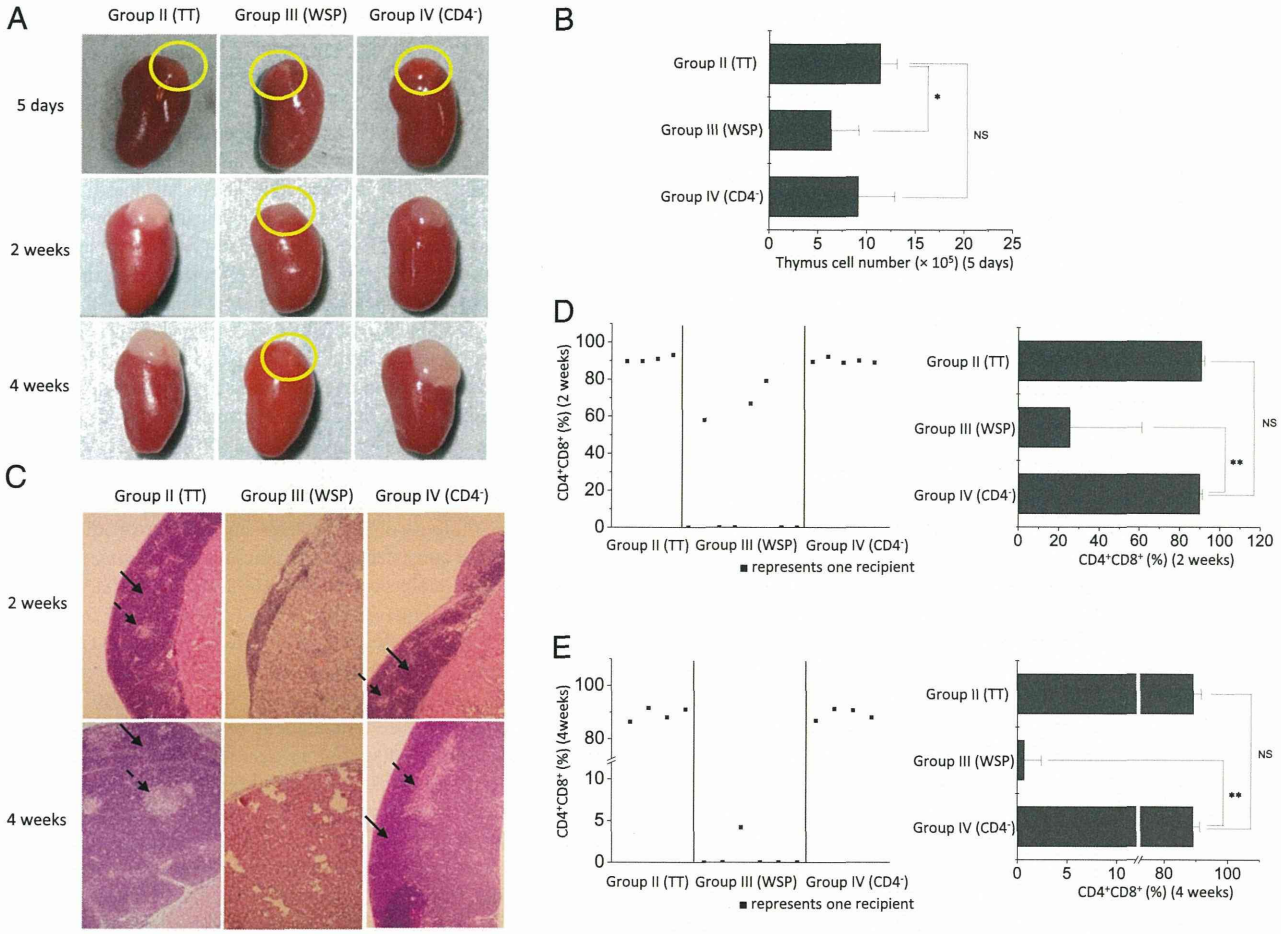

FIGURE 4. The development of transplanted thymus is impaired by WSP-DLI, but not $CD4^-$-DLI. Analysis of transplanted thymus was also performed at the same time as that in Fig. 3. (**A**) Representative macroscopic findings. Yellow circles indicate location of transplanted thymus. (**B**) Total cell number on day 5. (**C**) Representative histological findings. H&E staining (original magnification ×100). Plain arrows indicate cortex; dotted arrows indicate medulla. (**D** and **E**) Percentage of $CD4^+CD8^+$ cells is shown 2 or 4 wk after BMT. Each small square (■) represents the percentage for each recipient mouse in the *left panels*. Values of small squares on the x-axis are 0 and mean that no transplanted thymus could be observed or obtained for analysis. The *right panels* show the percentage by mean ± SD. $*p < 0.05$, $**p < 0.01$.

the BMT + TT setting, DLI induced serious damage, as evidenced in the classical GvHD target organs such as the skin, liver, and intestine. In addition, recovery of the recipient thymus was impaired, as was the development of the transplanted thymus. DLI led to decreased total cell number, lower percentages of $CD4^+$ $CD8^+$ cells, and loss of the thymic corticomedullary junction in

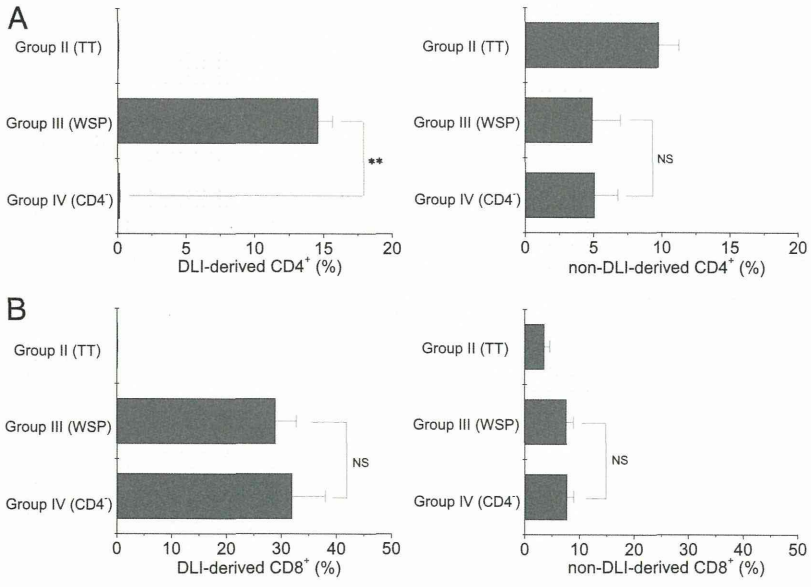

FIGURE 5. Percentages of DLI- and non–DLI-derived $CD4^+$ and $CD8^+$ T cells 5 d after BMT. BMCs and newborn thymus from B6 mice were used for BMT and TT, whereas splenocytes from eGFP tg mice were injected for DLI. Peripheral blood was analyzed 5 d after BMT. The percentages of DLI- ($eGFP^+$) and non–DLI-derived ($eGFP^-$) $CD4^+$ (**A**) and $CD8^+$ (**B**) T cells in the leukocytes are shown. $n = 3$–4/group; experiments were performed three times. $**p < 0.01$.

Table I. Reconstitution of donor-derived hematopoietic cells 4 wk after BMT

Groups	Percentage of Donor Cells		
	CD4	CD8	B220
Group II (TT)	11.4 ± 2.7	7.7 ± 0.9	44.7 ± 1.0
Group III (WSP)	2.4 ± 1.1	4.7 ± 1.4	2.0 ± 1.2
Group IV ($CD4^-$)	10.7 ± 2.4**	7.0 ± 1.3*	45.5 ± 4.0**

Four weeks after BMT, the reconstitution was analyzed using the peripheral blood by flow cytometer. Full donor chimerism was observed in all the recipient mice (>98%, checked by H-$2K^b$ and H-$2K^d$ staining). The percentages of H-$2K^{b+}CD4^+$, H-$2K^{b+}CD8^+$, and H-$2K^{b+}B220^+$ cells in the peripheral blood are shown. n = 4–6/group, experiments were performed three times.

*$p < 0.05$, **$p < 0.01$, $CD4^-$-DLI group versus WSP-DLI group. There were no significant differences in the percentages of donor-derived CD4, CD8, and B220 cells between the TT and $CD4^-$-DLI groups.

both the recipient and transplanted thymuses. However, the thymic impairment was significantly ameliorated after $CD4^+$ T cell depletion, indicating that $CD4^+$ T cells play a crucial role in the impairment.

Complete reconstitution of the recipient's immune system is imperative for there to be a favorable outcome from allo-BMT. The recovery of peripheral T cells occurs via both thymus-independent and thymus-dependent pathways (31), and it seems likely that mature T cells injected by DLI expand rapidly in response to recipient APCs and provide protective immunity after allo-BMT via the thymus-independent pathway. However, they induce uncontrollable and fatal GvHD.

Donor hematopoietic progenitors enter and restore the thymus, and differentiate into T cells via the thymus-dependent pathway after BMT. Because the recipient thymus is impaired by the conditioning regimen (especially irradiation), the thymic reconstitution takes ~10 wk; T cells are the last hematopoietic lineage cells to recover after BMT in mice and humans (32). To improve this situation, we combined TT with BMT to provide a new environment for T cell induction and differentiation. TT was able to significantly enhance not only the percentage, but also the number of $CD4^+$ T cells, which helped restore early T cell functions whereas avoiding any concomitant GvHD (14, 27).

In this study, we combined IBM-BMT with TT and DLI, because IBM-BMT can efficiently recruit donor-derived stromal cells (including mesenchymal stem cells), support the donor-derived hematopoietic stem cells, and help to suppress GvHD. As shown in Fig. 2, GvHD was induced after WSP-DLI but was preventable with $CD4^-$-DLI. The process of recipient thymic recovery was disturbed, and the recipient thymus showed decreased total cell number, a lower percentage of $CD4^+CD8^+$ cells, and obliteration of the thymic corticomedullary junction at 2 or 4 wk after BMT (Fig. 3C–G). Because GvHD is considered to be systemic, the recipient thymus can also be considered to be a target for GvHD. Lower doses of DLI resulted in thymic damage without any obvious clinical signs of GvHD, suggesting that the thymus is probably exquisitely sensitive to GvHD (22, 31).

Effector mechanisms of acute GvHD are thought to include both CTL-mediated and cytokine-mediated cytotoxicity. In our model, recipient thymic impairment was significantly ameliorated in the $CD4^-$-DLI group, indicating that mature $CD4^+$ T cells play a central role in the impaired recovery of the recipient thymus. We also excluded the effects of TT-derived $CD4^+$ T cells and DLI-derived $CD8^+$ T cells by using splenocytes from eGFP tg B6 mice (Fig. 5). Our findings of the relative importance of allo-reactive $CD4^+$ T cells are consistent with recent reports showing that $CD4^+$ T cells are much more powerful (>100 times) than $CD8^+$ T cells in destroying the hematopoietic niche (28), although it has been reported that $CD8^+$ T cells play a key role in mediating GvHD (33, 34). The mature T cells from DLI are activated and proliferate in the secondary lymphoid tissues under the stimulation of APCs. The activated $CD4^+$ T cell–dependent GvHD damage is mediated primarily by soluble inflammatory agents such as TNF-α, IFN-γ, IL-1, and NO. The attack on the epithelium, induced by the soluble inflammatory agents, has been proved to not require Ag expression (30). This novel evidence explains the impairment of not only the recovery of the recipient thymus, but also the development of the transplanted thymus (Fig. 4B–E). Methods to inhibit the activated $CD4^+$ T cell–induced inflammation, such as neutralization of TNF-α and the administration of anti-CD4 Ab, ameliorated the effects of inflammatory impairment on the recipient epithelium or bone marrow niche (28, 30). Recent reports have proved that FasL, which is a T cell cytolytic molecule, is required for the thymus and bone marrow GvHD (22, 28). The results from one clinical study show a clear correlation between the level of serum-soluble FasL and liver damage during GvHD (35). Based on these findings, we examined the concentration of TNF-α and soluble FasL in the plasma, and compared the levels in

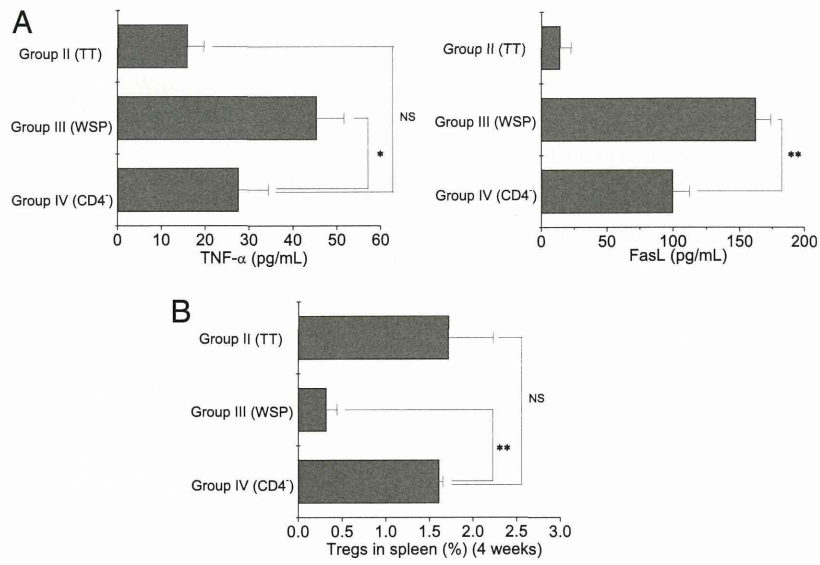

FIGURE 6. The levels of TNF-α and FasL and percentage of Tregs. (**A**) The levels of TNF-α and FasL in the recipient plasma were measured 5 d after BMT. (**B**) Percentage of Tregs in the recipient splenocytes was analyzed 4 wk after BMT. The Tregs were of donor origin as they showed H-$2K^{b+}$. n = 3–4/group; experiments were performed three times. *$p < 0.05$, **$p < 0.01$.

the TT, WSP-DLI, and CD4$^-$-DLI groups. The results showed that the TNF-α and FasL levels in the plasma in the WSP-DLI group were much higher than in the other two groups. Blockade of TNF-α and FasL by Abs prevented inflammatory cytokine-mediated impairment of both recipient and transplanted thymuses, confirming the role of TNF-α and FasL in thymic impairment. In addition, the percentage of Tregs that could suppress the inflammatory reaction was also decreased dramatically after WSP-DLI, but not CD4$^-$-DLI. It thus appears that the inflammation induced by the allo-CD4$^+$ T cells mediates the unselective impairment of both the recovery of the recipient's thymus and the development of the transplanted thymus.

There are thymic epithelial cells, endothelial cells, and fibroblasts in the thymic stroma. The destruction on the thymic stroma impairs the thymic cellularity and function. Recipient thymic GvHD is largely due to the impairment of the stromal cells of the thymus induced by allo-activated T cells. We have further proved a causative link between the activated CD4$^+$ T cells (but not CD8$^+$ T cells) and the impairment of the thymic stromal cells using an in vitro system (data not shown).

Newborn TT increased the percentage of CD4$^+$ T cells significantly in the peripheral blood after BMT (27). This population did not induce any serious recipient organ injury, suggesting that these cells experienced reconstitution via a thymus-dependent pathway. We have obtained encouraging results using thymus-independent CD8$^+$ T cells in treating solid tumors (36). This infers that the graft-versus-tumor effects of TT and CD4$^-$-DLI can be combined for tumor suppression without severe thymic impairment or GvHD.

In this study, we have shown that DLI leads to derangements in both recipient and transplanted thymuses in the IBM-BMT + TT setting. The inflammation mediated by allo-CD4$^+$ T cells plays a central role in the impairment of the recipient thymic recovery and the transplanted thymic development. The impairment would be significantly ameliorated by the depletion of CD4$^+$ T cells.

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Disclosures

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REVIEW

Novel conditioning regimens for bone marrow transplantation

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Abstract: Bone marrow transplantation (BMT) has evolved into an effective strategy for the treatment of hematological and oncological disorders. Radiotherapy and chemotherapy are used as conditioning regimens prior to BMT to suppress host immunity and reduce tumor burden. High doses of total body irradiation are conventionally administered along with alkylating agents, ie, the myeloablative regimen, to help ensure rapid engraftment of donor cells and to prevent relapse. However, the toxicity of the myeloablative conditioning regimen and unacceptable nonrelapse mortality rules out this approach for older patients by whom less intense preparative regimens are likely to be better tolerated. The reduced-intensity and nonmyeloablative conditioning regimens have been demonstrated by many investigators to be novel approaches resulting in a lower nonrelapse mortality rate and lower incidence of severe acute graft versus host disease. Here, we review the conditioning regimens employed as a pretreatment for BMT, and focus on the novel conditioning regimens and cutting edge developments.

Keywords: myeloablative conditioning regimen, reduced-intensity conditioning, nonmyeloablative conditioning regimen, relapse, nonrelapse mortality, graft versus host disease

Introduction

Bone marrow transplantation (BMT) was originally developed to treat congenital immunodeficiencies and hematologic disorders.[1,2] BMT has also become a powerful strategy for treating autoimmune and metabolic diseases.[3–7] Diseases frequently encountered in BMT are listed in Table 1. Radiotherapy and/or chemotherapy are prerequisites for recipients of BMT, because these conditioning regimens are essential for successful transplantation. Because the majority of BMT procedures are performed for the treatment of malignant disease, the conditioning regimens could be used to provide tumor cytoreduction and ideally disease eradication. The therapeutic effects of BMT on malignancies are also mediated via induction of the graft versus tumor effect by immunocompetent cells in the graft. Conditioning regimens that can minimize graft versus host disease without jeopardizing engraftment and graft versus tumor effects are being explored.[8]

The intensity of the conditioning regimens varies significantly. Based on the expected duration and reversibility of cytopenia after BMT, Bacigalupo et al classified the conditioning regimens into three categories, ie, myeloablative, reduced-intensity, and nonmyeloablative conditioning regimens.[9] Myeloablative conditioning regimens result in irreversible cytopenia, and stem cell support is required after BMT. The high-dose radiotherapy and chemotherapy used in the myeloablative conditioning regimens

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Table 1 Diseases frequently encountered in bone marrow transplantation

Autologous BMT	Allogeneic BMT
Multiple myeloma	Acute myeloid leukemia
Non-Hodgkin lymphoma	Acute lymphoblastic leukemia
Hodgkin disease	Chronic myeloid leukemia
Acute myeloid leukemia	Chronic lymphocytic leukemia
Neuroblastoma	Myeloproliferative disorders
Germ cell tumors	Myelodysplastic syndromes
Autoimmune disorders	Multiple myeloma
	Non-Hodgkin lymphoma
	Hodgkin disease
	Aplastic anemia

Note: Multiple myeloma continues to be the most common indication for autotransplantation and acute myeloid leukemia for allogeneic transplantation.

reduce immunocompetent cells in the recipient, permitting rapid engraftment of even unrelated, mismatched donor bone marrow cells. However, the myeloablative conditioning regimens are associated with considerable morbidity and mortality.[10] Therefore, these approaches have been restricted to young patients without comorbidities, and 50 years is considered an upper age limit.

In contrast with the consensus on a definition of myeloablative conditioning regimens, there are different opinions about reduced-intensity and nonmyeloablative conditioning regimens. Researchers sometimes refer to some of the conventional nonmyeloablative regimens as "reduced-intensity" conditioning regimens.[11–13] "Reduced-intensity conditioning" has also been used instead of "nonmyeloablative" directly.[14] Even though these regimens have been variably named nonmyeloablative conditioning or reduced-intensity conditioning regimens, they share one important characteristic, ie, both result in reversible myelosuppression (usually within 28 days) when given without stem cell support.[12] Above all, these methods use lower doses of cytoreductive treatments and result in low nonhematological toxicity. Some researchers use terms such as "intermediate-intensity" conditioning and semi-intensive conditioning rather than reduced-intensity conditioning.[15,16] Therefore, we have put reduced-intensity and nonmyeloablative conditioning regimens together in this review when discussing those regimens that are not myeloablative and are less toxic.[17–19]

Here, we review the conditioning regimens that are performed as pretreatments for BMT, and focus on some novel conditioning methods (reduced-intensity and nonmyeloablative conditioning regimens) with lower intensity that have expanded the use of BMT to older patients and to those with comorbidities.

Myeloablative conditioning regimens

Total body irradiation was the first conditioning method developed from research of radiation exposure and has been widely used in the conditioning regimens for its powerful immunosuppressive effects and activity against a variety of malignancies. Early myeloablative total body irradiation regimens were carried out using single large fractions of 8–10 Gy. However, such treatment was not tolerated well and was associated with serious toxicity, which resulted in interstitial pneumonitis, and severe nausea/vomiting.[20,21] To reduce the side effects of these high doses of total body irradiation while maintaining or improving efficacy, both fractionation and reductions in dose rates were developed.[22,23]

Host bone marrow can also be ablated with chemotherapy. Furthermore, chemotherapy can reduce or eradicate the tumor burden, while reducing the long-term sequelae of total body irradiation, including cataracts, sterility, and secondary malignancies. The combination of busulfan and cyclophosphamide is currently the most widely used myeloablative conditioning regimen without incorporating total body irradiation to treat malignant and nonmalignant hematological disorders with allogeneic BMT.[24–26] Yet, specific metabolites of cyclophosphamide are known to be associated with increased transplantation-induced mortality after conditioning, especially with busulfan. Therefore, fludarabine, a purine analog, has been used in an attempt to replace the cyclophosphamide in the busulfan and cyclophosphamide combination for myeloablative allogeneic BMT as well as nonmyeloablative transplantation. Fludarabine has considerable efficacy in both immunosuppression and tumor cell killing with minimal extramedullary toxicity. The regimen of busulfan and fludarabine has exhibited lower nonrelapse mortality and higher overall survival in patients with low-risk disease than busulfan and cyclophosphamide.[25,27]

Other possible alkylating agents, such as nitrosoureas (eg, carmustine), melphalan, thiotepa, and etoposide, have been included in the conditioning regimens in some trials. For example, the combination of carmustine, etoposide, cytarabine, and melphalan (BEAM) was designed to provide antilymphoma activity without the toxicity of total body irradiation. BEAM has proven to be an effective preparative regimen for its feasibility and tolerability in patients with lymphoma treated by both autologous and allogeneic BMT.[28,29]

Total body irradiation is often combined with chemotherapy in conditioning regimens and appears to provide benefit over conditioning with chemotherapy alone in many settings.[30–32] The combination of cyclophosphamide and total body irradiation (CyTBI) is considered to be one of the