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Effects of Aging on Bone Marrow Stem Cells and Thymus

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ABSTRACT

The immune system protects the body from bacteria, viruses and other harmful substances, but the functional decline in the immune system with aging leaves the aged susceptible to infections. Myeloid and lymphoid progenitors are derived from bone marrow stem cells, and the progenitor cells develop into immune cells such as macrophages, monocytes, T cells and B cells. T cell progenitors differentiate and proliferate in the thymus, and are selected by thymic epithelial cells, the mature T cells then entering the peripheral blood. Aging is reflected in a deterioration of the thymus and bone marrow stem cells, resulting in a lowered functioning of the immune system. In this chapter, we summarize recent reports on how aging affects bone marrow stem cells and the thymus.

Keywords: Aging; Immune System; Bone Marrow Stem Cell: Thymus; Thymic Epithelial Cell.

INTRODUCTION

The immune system prevents viruses and bacteria from entering the human body and also limits their growth so as to maintain health. The cells of the immune system include myeloid and lymphoid lineage cells, each of which play an important role in innate and adaptive immunity. Lymphoid cells include T and B cells, while myeloid cells mainly include megakaryocytes, erythrocytes, and monocytes. Myeloid and lymphoid progenitors are both derived from

Hematopoietic Stem Cells **(HSCs)** in the bone marrow. One report has indicated that the number of myeloid cells increases with age while that of lymphoid cells decreases [1]. The thymus is a lymphoid organ, and aging-related changes result in a reduction in thymic lymphopoiesis and a disruption of the thymic architecture [2]. T cell progenitors from bone marrow enter the thymus, differentiate and mature by negative and positive selection, and the mature T cells then enter the peripheral blood [3]. The thymus reaches its peak weight in adolescence, but starts to weigh significantly less and to produce significantly fewer thymocytes with aging. In this chapter, we focus on the effect of aging on bone marrow stem cells and the thymus.

EFFECT OF AGING ON BONE MARROW STEM CELLS

The immune system can be classified into the innate immune and adaptive immune systems. Disorders of the immune system induce autoimmune diseases, severe infections and cancer. The innate immune system provides immediate defense against infection, macrophages, Dentritic Cells **(DCs)** and neutrophils being major componnents of the innate immune responses [4]. Major Histocompatibility Complex II **(MHC II)** molecules are surface antigens, critical for the initiation of the antigen-specific immune response. MHC II expression is lower in the macrophages of aged individuals when compared with young individuals [5], and DCs show decreased numbers of co-stimulatory molecules, and decreased IL-12 production, in old animals [6]. In contrast, T and B cells are components of the adaptive immune response, the adaptive immune system of vertebrates playing an important role in enhancing responses to pathogens via immunological memory [7].

Aging affects the immune system as a result of thymic involution and a decrease in primary lymphopoiesis [8]. HSCs show impaired adherence to the Mesenchymal Stem Cells **(MSCs)** in the bone marrow with aging [9]. Moreover, the number of lymphoid progenitors has been shown to decrease and that of myeloid progenitors to increase in aged mice [10]. Alterations in the T cell and B cell compartments are related to a decline in immune functions, and the number of memory T cells has been shown to increase with aging [11]. Lymphoid progenitors differentiate into B and T cells. B cells mature in the bone marrow, but aging reduces the diversity of the B cell repertoire and B cell responses, including antibody production [12]. In contrast, T cell progenitors develop into CD4-CD8- double-negative cells, then CD4+CD8+double-positive cells, and finally into CD4+ or CD8+ single-positive cells in the thymus. T cell progenitors interact with thymic stroma cells, which include Medulla Epithelial Cells **(mTEC)** and Cortical Epithelial Cells **(cTEC)** [13]. Aging affects the functions of HSCs, T cells and B cells through oxidative stress and reduced telomerase activity, while ROS decreases activity resulting from decreased DNA repair gene expression [14]. The reduced secretion of TGFβ1 and IL-7 affect the bone marrow environment in the elderly, while the increase in memory T cells and decrease in IL-2 production affect the immune functions in aged individuals. The generation of naïve T cells is dependent on the functioning of the thymus [15], and the number of naïve T cells decreases with aging as a result of cellular senescence and

response to DNA damage [16]. Regulatory T cells (T regs), which primarily consist of thymically-derived T regs and peripherally-derived T regs, are suppressive T cells in the immune response, and express surface markers such as CD4, CD25, and Forkhead Box Protein 3 **(Foxp3)** [17]. Aging reduces the expression of multiple DNA repair protein, and elevates levels of genome instability in HSCs [18]. Furthermore, ROS alter the proliferation and differentiation of HSCs, and AKT, MAPK and ATM pathways with aging [19,20].

Bone marrow stem cells mainly include HSCs and MSCs. MSCs are essential for supporting hematopoiesis, and MSCs differentiate into osteoblasts and adipocytes in the bone marrow [21]. A number of signaling pathways regulate the inverse balance between osteogenesis and adipogenesis [22]. Human MSCs differentiate into adipocytes more often than osteocytes in the aged bone marrow, and adipocytes in the bone marrow have been shown to negatively regulate the bone marrow microenvironment [23,24].

EFFECT OF AGING ON THE THYMUS

The thymus develops from both ectoderm and endoderm, reaches its peak weight in adolescence, and starts to atrophy with aging, starting to weigh significantly less and to produce significantly fewer thymocytes. The thymus mainly includes TECs, thymocytes, and DCs. TECs shape the T cell receptor repertoire via negative and positive selection [25]. The thymus is a lymphoid organ, and aging-related changes result in a reduction in thymic lymphopoiesis, and a disruption of the thymic architecture [2].

One report has shown that Aire controls thymic negative selection, and mediates tolerance by direct presentation of Aire-regulated antigens to both CD4+ and CD8+ T cells. In contrast, cTECs are required for positive selection of CD4+ and CD8+ T cells [26]. Thymic involution is accompanied by a drop in the number of TECs, and specifically in the number of cTECs after adolescence [27]. cTECs support the early stages of T cell development and determine the overall lymphopoietic capacity of the thymus, cTECs being required for positive selection of CD4+ and CD8+ T cells [26,28]. Wnt signaling is important for TEC development and T cell lymphopoiesis, but aging reduces naive T cell output due to decreased Wnt expression [29,30]. The cytokine RANK Ligand **(RANKL)** regulates the cellularity of mTECs by interacting with RANK and osteoprotegerin. RANKL is related to the establishment of central tolerance by promoting thymic medulla formation [31]. TECs provide signals during thymopoiesis, and are thus important for thymus involution, but one report indicates that adipocytes infiltrate the aged thymus, affecting the TECs [32]. Moreover, accelerated thymic aging is primarily a function of stromal cells, and stromal gene expression changes in the aged thymus [33]. Thus, preventing aging-related changes in the immune system and any loss of function in the thymus should help ameliorate aging-related diseases.

Bone marrow-derived common lymphoid progenitors enter the thymus and differentiate into T lymphocytes upon receiving various signals in the thymic microenvironments [34]. Thymus involution results in decreased migration of naive T cells to the periphery, which is associated

with lowered immune function, which in turn increases the susceptibility to infection and cancer [8]. One report has shown a difference between the lymphoid progenitors in aged bone marrow and those in young bone marrow, and that there is a loss of T cell function in aged bone marrow progenitors [35]. Another report has indicated that thymic involution is also induced by enhancing the contribution of memory cells to the peripheral T cell pool [36]. Moreover, the ratio of CD4/CD8 in recent thymic emigrants in mice also decreases with aging, and the recent thymic emigrants of old mice secrete less IL-2 than young mice [36]. Transcription factors such as Foxn1 play an important role in the development of TEC, but their expression decreases with aging [37].

STEM CELL THERAPY FOR TREATING CANCER

Allogeneic stem cell transplantation is a general therapy for cancer and immunodeficiency disorders, and works by reconstituting the immune system. Human Leukocyte Antigen **(HLA),** which is the human version of MHC, helps the immune system distinguish between self-and non-self-derived proteins or cells. Specifically, T cell reconstitution is affected by HLA, which is mismatched after allogeneic stem cell transplantation [38]. Fetal thymus-derived CD4+ cells produce higher levels of IFN-γ and IL-4 than adult-derived cells, and show different responses to antigen stimulation [39]. Transplanted thymus may regulate the homeostasis of T cells, and thymus transplantation is thus a simple and effective method of supplying T cells that are differentiated and regulated to treat tumors without acute rejection or infection [40]. Moreover, renal allografts were accepted without immune supressants when renal allografts and lobes of thymus were transplanted simultaneously, suggesting that thymus transplantation across a fully MHC-mismatched barrier induces tolerance in a large-animal model. Thymus transplantation is thus a potential strategy for tolerance induction in clinical transplantation [41].

Stem cell transplantation +adult thymus transplantation prevented tumor development with mild graft-versus-host reaction resulting from the induction of high thymopoiesis and a strong graft-versus-tumor effect in tumor-bearing mice [42]. The number of T cell receptor rearrangement excision circles was higher, while the number of CD4+ FoxP3+ (T regs) cells was lower, in the Meth A sarcoma-bearing mice treated with stem cell transplantation +adult thymus transplantation than in those treated with stem cell transplantation alone. Furthermore, the number of CD8+ cells infiltrating the tumor and the levels of IFN-γ were higher in the mice treated with stem cell transplantation+adult thymus transplantation than in those treated with stem cell transplantation alone [43]. Although T regs have been reported to suppress the graft-versus-host reaction induced by CD4+T cells, they did not reduce the graft-versus-tumor induced by CD8+ T cells [44].

Leukemias are hematologic malignancies, and include acute and chronic myeloid leukemia. Allogenic stem cell transplantation is an effective immunotherapy for acute leukemia in children [45], and we can maximize the graft-versus-leukemic effect with minimal GVHD by our basic studies. We performed experiments to treat leukemia by allogenic stem cell transplantation using leukemia-

bearing mice induced by EL-4 cells. We compared the effects of stem cell transplantation +donor lymphocyte infusion with stem cell transplantation +adult thymus transplantation in leukemia-bearing mice. Donor lymphocyte infusion is sometimes used to treat leukemia, but it also induces a risk of GVHD in the recipients [46]. Our results showed that stem cell transplantation +adult thymus transplantation prevented the growth of leukemia by improving mitogen responses to both T and B cells, and significantly increased IL-2 production, IL-2 having been reported to protect against allogeneic bone marrow transplantation-induced GVHD [47,48]. Moreover, the number of CD62L-CD44+ effector memory T cells was higher in mice treated with stem cell transplantation + thymus transplantation than in those treated with stem cell transplantation + donor lymphocyte infusion between the two groups. These results showed that stem cell transplantation+adult thymus transplantation induces strong graft-versus-leukemic effects with mild GVHD, suggesting that thymus transplantation is useful for treating leukemia.

Our previous report showed that newborn liver cell transplantation with newborn thymus transplantation can ameliorate intestinal injury following irradiation in supralethally irradiated mice by increasing the number of CD4+ T cells and B cells when compared with newborn liver cells transplantation alone. The production of IL-7 and keratinocyte growth factor play an important role in protection against radiation injury in the intestine, and their levels were higher in newborn thymus than fetal or adult thymus [49]. We therefore compared the results of bone marrow transplantation+adult thymus transplantation, newborn liver cell transplantation+newborn thymus transplantation, or fetal liver cells transplantation+fetal thymus transplantation on tumor suppression. Our results showed that tumors were suppressed to a greater extent as a result of the increased CD4+ and CD8+ T cells and decreased number of Gr-1+/CD11b+ myeloid suppressor cells and Foxp3+/CD4+ T regs in Meth A sarcoma-bearing mice treated with stem cell transplantation+thymus transplantation than in those treated with stem cell transplantation alone. Furthermore, the tumors were suppressed in mouse Meth A sarcoma-bearing mice treated with newborn liver cell transplantation+newborn thymus transplantation or fetal liver cell transplantation+fetal thymus transplantation. Moreover, the production of CD62L-CD44+ effector memory T cells and IFN-γ were also higher in these two groups than in the stem cell transplantation+thymus transplantation group [50]. Our results showed that fetal thymus transplantation grafts showed greater growth than newborn thymus transplantation or adult thymus transplantation, and some atrophic features were observed in adult thymus transplantation grafts, suggesting aging-related changes in the thymus. These results suggested that fetal liver cell transplantation+fetal thymus transplantation is an effective method of treating tumors without GVHD.

In conclusion, aging affects functions of bone marrow stem cells and thymus, and induces immune dysfunction in experimental animals. Stem cell transplantation has been shown to improve aging-related diseases due to an improvement in immune functions. Thus, prevention of the aging process in bone marrow stem cells and thymus, would help ameliorate aging-related diseases.

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