1 Introduction

There has recently been a rapid increase in cancer, and inflammation has been shown to play an important role in its development (Ahmad et al., 2009). Inflammatory cells produce cytokines such as TNF IL and IL which promote tumor growth, and tumor cells also produce macrophage colony-stimulating factor (M-CSF), which accelerates the immune response (Marx, 2004; Borrello et al., 2008). Aging leads to a marked malfunction of multiple cellular and molecular events that ultimately get translated into various chronic ailments and diseases such as Type 2 diabetes mellitus (T2DM), Alzheimer’s disease (AD), and osteoporosis, Parkinson’s disease, atherosclerosis, and an increased risk of cancer (Caruso et al., 2004). Aging leads to decreases in humoral and cellular immune responses, aged dendritic cells (DCs) being less able to activate T and B cells, and aged-natural killer (NK) cells being less efficient at killing tumor cells (Frasca et al., 2008).

Based on histological type, cancer can be classified into five major categories: carcinoma, sarcoma, myeloma, lymphoma and mixed types. Treatment mainly includes surgery, radiotherapy, chemotherapy, immunotherapy, and stem cell transplantation. Radiotherapy and chemotherapy play a role in preventing cancer development, but cause adverse side effects. Immunotherapy is an effective method that aims to harness the body’s own immune system to fight the cancer. Using this approach, T cells are more able to recognize aberrant proteins from tumor cells, and either destroy the tumor cells or inhibit their growth (Schreiber et al., 2011; McNutt, 2013). The first to report a strong correlation between tumor-infiltrating T cells and patient survival in human colorectal cancer was Naito et al in 1998 (Naito et al., 1998). Moreover, it has been shown that increased CD8+ T cells infiltrate non-small-cell lung cancers, and may thus improve patient survival (Zhuang et al., 2010). In terms of immunotherapy, the most successful cancer vaccine has been described by Kenter et al, who suggest that complete responses are correlated with the induction of human papillomavirus type 16-specific immunity (Kenter et al., 2009). Recently, we have seen the development of new immunotherapies such as vaccination approaches and the use of monoclonal antibodies for tumors (Davies, 2014). For example, melanoma-associated antigen A3 (MAGE-A3) gene is expressed during embryogenesis and in a wide variety of tumors (Van den Eynde and van der Bruggen, 1997), and one report has shown that MAGE-A3 may facilitate antigen uptake by antigen-presenting cells, promoting T-cell responses, although it
did not directly kill the tumor cells (Moeller et al., 2012). The programmed death 1 (PD-1) receptor is a negative regulator of T-cells controlling immune responses to cancer, and anti-PD-L1 monoclonal antibodies have been shown to prevent tumor growth in patients with advanced melanoma (Hamid et al., 2013). DCs are antigen presenting cells that actively promote T-cell tolerance to self-tumor antigens in tumor-bearing hosts (Chaput et al., 2008). Thus, DC-based vaccines such as Sipuleucel-T have been used for patients with asymptomatic or minimally symptomatic metastatic hormone-refractory prostate cancer. Antibodies potentially exert antineoplastic effects, and have been used in cancer treatment in preclinical and clinical trials (Galluzzi et al., 2012; Vacchelli et al., 2013). For example, brentuximab vedotin, an anti-CD30 monomethyl auristatin E (MMAE) conjugate, has been approved to treat relapsed Hodgkin lymphoma (Younes et al., 2012). Denosumab, a receptor activator of NFκB ligand (RANKL)-targeting antibody has been used to treat osteoporosis, and also to increase bone mass in patients with breast cancer (Coleman, 2012).

There have been rapid developments into research in the clinical use of stem cells. Allogeneic stem cell transplantation (ASCT) has been proven to be a useful method of treating hematological malignancies via donor-derived lymphocytes, which are used to eradicate residual tumor cells (Parmar and Ritchie, 2014). ASCT has also been shown to be an effective immunotherapy for leukemia (Oliansky et al., 2007). Intra bone marrow-bone marrow transplantation has proven to be a valuable method for allogeneic BMT (ABMT), since intra bone marrow-bone marrow transplantation only requires low-dose irradiation as a preconditioning regimen and suppresses graft versus host disease (GVHD) (Kushida et al., 2000; Nishida et al., 2009). The thymus is a lymphoid organ in which T cells develop and then migrate to the peripheral blood. In this chapter, we focus on intra bone marrow-bone marrow transplantation + thymus transplantation (TT) to treat cancer in tumor-bearing mice.

2 Physiology of 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 thymic epithelial cells (TECs), which consist of medullary TECs (mTECs) and cortical TECs (cTECs), thymocytes, and DCs. TECs shape T cell receptor (TCR) repertoire via negative and positive selection (Anderson et al., 2009). Autoimmune Regulator (Aire) plays an important role in the induction of central tolerance, and Aire*mTECs are important in elucidating the stages and the checking points that regulate the formation of the thymic microenvironment. Moreover, the receptor activator of NF-kB (RANK) expression on the mTECs has been shown to be directly responsible for Aire*mTEC differentiation (Rossi et al., 2007). The cytokine RANK ligand (RANKL) was produced by positively selected thymocytes and regulated the cellularity of mTECs by interacting with RANK and osteoprotegerin. Forced expression of RANKL restored thymic medulla in mice lacking positive selection, whereas RANKL perturbation impaired medulla formation, suggesting that RANKL is related to the establishment of central tolerance by promoting thymic medulla formation (Hikosaka et al., 2008). 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 (Hubert et al., 2011). Thymic involution is accompanied by a drop in the number of TECs, and specifically in the number of cTECs after adolescence (Gray et al., 2006), cTECs being responsible for supporting the early stage of T cell development and determining the overall lymphopoietic capacity of the thymus (Rode and Boehm, 2012). Wnt signaling controls TEC development and consequent T cell lymphopoiesis, and decreased Wnt expression leads to reduced naïve T cell output with aging (Balcıunaite et al., 2002; Varečzka et al., 2011). Mouse embryonic stem cell-derived thymic epithelial cell progenitors can enhance thymopoiesis and increase the number of peripheral T cells after BMT, and this approach could thus be used to reverse thymic involution with aging (Lai et al., 2011). Human ESCs have been shown to differentiate into epithelial progenitor-like cells, and to promote T cell generation in vivo (Sun et al., 2013).

Our previous report showed the existence of donor-type stromal cells in the thymus of mice when treated with ABMT + bone. The bone (femurs and tibias) was simultaneously engrafted subcutaneously and under the renal capsules when allogeneic BMT was carried out. These findings strongly suggest that stromal cells can migrate from the BM to the thymus, where they participate in the positive selection of thymocytes (Y. Li et al., 2000). Donor-derived TECs were found in both the medullary and the cortical areas of the thymus in MRL/lpr mice treated with allogeneic intra bone marrow-bone marrow transplantation. Furthermore, bone marrow (BM) cells contain the precursors of functional TECs, and they can differentiate into TECs, which results in the correction of thymic function (Takaki et al., 2008).

BM-derived common lymphoid progenitors enter the thymus and differentiate into T lymphocytes upon receiving various signals in the thymic microenvironments (Petrie and Zuniga-Pflucker, 2007). Thymus involution results in decreased migration of naïve T cells to the periphery, which is associated with lowered immune function, which in turn increases the susceptibility to infection and cancer (Lynch et al., 2009). One report has shown a difference between the lymphoid progenitors in aged BM and those in young BM, and that there is a loss of T cell function in aged BM progenitors (Zediak et al., 2007). Another report has indicated that thymic involution is also induced by enhancing the contribution of memory cells to the peripheral T cell pool (Hale et al., 2006). Moreover, the ratio of CD4/CD8 in recent thymic emigrants (RTEs) in mice also decreased with aging, and the RTEs of old mice secrete less IL-2 than young mice (Hale et al., 2006).

3 Tumors Accelerate Thymic Atrophy

Thymic atrophy can be induced by aging and tumor development, which induces impaired T cell development via the impaired maturation of thymocytes, increased apoptosis, and decreased proliferation. Vascular endothelial growth factor, hepatocyte growth factor, and IL-7 and IL-5 play important roles in thymic atrophy, and it has also been reported that increased leukemia inhibitory factor, IL-6 and stem cell factors, as well as decreased growth hormone and insulin-like growth factor induce thymic atrophy with aging (Sempowski et al., 2000; Chen et al., 2010). T cell maturation was blocked at the negative selection, and the percentage of CD4⁺ or CD8⁺ single positive cells within the CD3⁺ population increased, while CD4⁺CD8⁺ double-positive cells decreased in the tumor bearing mice. Moreover, apoptosis of thymocytes was also observed to increase (Carrio and Lopez, 2009b). And, furthermore, the number
of Treg increased as a result of the apoptosis of thymocytes in the thymus by inducing the production of TGFβ from TECs and macrophages (Konkel et al., 2014). Thymic atrophy associated with tumor development resulted from increased CD4+ and CD8+ single-positive cells and CD4-CD8- double-negative cells in tumor-bearing mice (Y. Fu et al., 1989a; Y. X. Fu et al., 1989b). IFN- modulates a variety of cellular responses, including cell growth and apoptosis, and the IFN- level was increased in tumor-bearing mice, suggesting that the IFN- level is related to thymocyte apoptosis. Furthermore, it has been shown that the regulation of T cell responses by cytokines is via Jak/Stats signaling pathways. Reduced expression of Jak/Stats-related mRNA has been reported in the thymus of tumor-bearing mice, suggesting that tumor development impaired the Jak/Stat signaling pathways, and then impaired the T cell response (Carrio et al., 2009a; Carrio et al., 2011).

4 Intra Bone Marrow-bone Marrow Transplantation + TT Prevents Tumor Growth

Hematopoietic stem cell transplantation (HSCT) is a useful therapy for hematological malignancies, but this intervention also induces GVHD, which injures the thymus, including inducing the apoptosis of TECs and delaying T cell recovery, and thereby injuring the immune system (Toubert et al., 2012). Allogeneic HSCT is a general therapy for cancer and immunodeficiency disorders that works by reconstituting the immune system. Human leukocyte antigen (HLA), which is the human version of major histocompatibility complex (MHC), helps the immune system distinguish between self- and non-self-derived proteins or cells. Specifically, T cell reconstitution is affected not only by aging but also by HLA, which is mismatched after allogeneic HSCT (Seggewiss and Einsele, 2010). Fetal thymus-derived CD4+ cells produced higher levels of IFN-γ and IL-4 than adult-derived cells, and showed different responses to antigen stimulation (Adkins, 2003). Neonatal T cells respond more rapidly to antigen stimulation than adult-derived T cells, and the activation of T cells results in cellular proliferation (Adkins et al., 2003). Transplanted thymus may regulate the homeostasis of T cells, and TT is thus a simple and effective method of supplying T cells that are differentiated and regulated to treat tumors without acute rejection or infection (Ikehara, 2011). TT has also been used to treat DiGeorge syndrome and immune deficiency (Chinn and Markert, 2011). Moreover, renal allografts were accepted without immunosuppressants when renal allografts and lobes of thymus were transplanted simultaneously, suggesting that TT across a fully MHC-mismatched barrier induces tolerance in a large-animal model. TT is thus a potential strategy for tolerance induction in clinical transplantation (Kamano et al., 2004). Our previous studies showed that intra bone marrow-bone marrow transplantation + TT improved hyperglycemia in type 2 diabetes model mice by increasing heme oxygenase-1 expression and insulin receptor activity (M. Li et al., 2010).

Intra bone marrow-bone marrow transplantation has been shown to suppress GVHD even when pretreatment consisted only of low-dose irradiation (Nakamura et al., 2004). ABMT + TT can improve autoimmune diseases in chimeric-resistant MRL/lpr mice without GVHD (Hosaka et al., 2007; Ryu et al., 2008). Intra bone marrow-bone marrow transplantation + adult TT (ATT) prevented tumor development with mild graft-versus-host reaction (GVHR) resulting from the induction of high thymopoiesis and a strong graft-versus-tumor (GVT) ef-
fect in tumor-bearing mice. Meth A sarcoma cells were subcutaneously inoculated into mice, and intra bone marrow-bone marrow transplantation + ATT was then used to treat these mice when the tumor had grown to 5mm. The number of T cell receptor rearrangement excision circles (TRECs) was higher, and the number of CD4+ FoxP3+ cells was lower, in the mice treated with intra bone marrow-bone marrow transplantation + ATT than in those treated with intra bone marrow-bone marrow transplantation alone. Furthermore, the number of CD8+ cells infiltrating the tumor and the levels of IFN-γ were higher in the mice treated with intra bone marrow-bone marrow transplantation + ATT than in those treated with intra bone marrow-bone marrow transplantation alone (Miyake et al., 2009). Although Tregs have been reported to suppress the GVHR induced by CD4+T cells, they did not reduce the GVT induced by CD8+ T cells (Edinger et al., 2003).

Leukemias are hematologic malignancies, and include acute and chronic myeloid leukemia. Some leukemia cells express CD80, CD86, and some may express MHC class I and II, which can be recognized and eradicated by T cells (Whiteway et al., 2003). ABMT is an effective immunotherapy for acute leukemia in children (Oliansky et al., 2007) and, from our experience, we can maximize the graft-versus-leukemic (GVL) effect with minimal GVHD. We performed experiments to treat leukemia by ABMT using leukemia-bearing mice induced by EL-4 cells. EL-4 cells are derived from the thymoma of mice, and can induce the mimicking of leukemia in mice. We compared the effects of intra bone marrow-bone marrow transplantation + donor lymphocyte infusion (DLI) with intra bone marrow-bone marrow transplantation + ATT in leukemia-bearing mice. DLI is sometimes used to treat leukemia, but there is also a risk of GVHD in the recipients (Deol and Lum, 2010). Our results showed that intra bone marrow-bone marrow transplantation + ATT 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 ABMT-induced GVHD (Zhang et al., 2012; Satake et al., 2014). Moreover, the number of CD62L+CD44+ effector memory T cells was higher in mice treated with intra bone marrow-bone marrow transplantation + TT than in those treated with intra bone marrow-bone marrow transplantation + DLI, although there was no difference in the number of CD62L+CD44+ central memory or CD62L−CD44- naive T cells between the two groups. These results showed that intra bone marrow-bone marrow transplantation + ATT induces strong GVL effects with mild GVHD, suggested that TT is useful for treating leukemia.

Our previous report showed that newborn liver cell transplantation (NLT) with newborn TT (NTT) can ameliorate intestinal injury following irradiation in supralethally irradiated mice, by increasing the number of CD4+ T cells and B cells when compared with NLC transplantation alone. The production of interleukin (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 (Ryu et al., 2008). We therefore compared the results of BMT+ATT, NLT+NTT, or fetal LT (FLT) + fetal TT (FTT) 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+ Tregs in Meth A sarcoma-bearing mice treated with HSCT + TT than in those treated with HSCT alone. Furthermore, the tumors were suppressed in mouse Meth A sarcoma-bearing mice treated with NLT+NTT or FLT+FTT. Moreover, the production of CD62L+CD44+ effector memory T cells and IFN-γ were also higher in these two
groups than in the HSCT+TT group (Zhang et al., 2011). Our results showed that FTT grafts showed greater growth than NTT or ATT, and some atrophic features were observed in ATT grafts, suggesting aging-related changes in the thymus. These results suggested that FLT+FTT is an effective method of treating tumors without GVHD.

5 Conclusions and Future Directions

Our previous studies showed that HSCT+TT could be a useful intervention for treating tumors. Specifically, FLT+FTT was shown to be superior for tumor therapy (Figure 1), although it may be difficult to obtain both HSCs and fetal human thymus from the same donor.

Donor thymus may be obtained from donors who donate the heart or other organs for transplantation or from aborted fetuses. Moreover, thymus can be generated from induced pluripotent stem cells (Inami et al., 2011). We have, however, shown that triple chimeric mice survived for a long period with sufficient T-cell functions, and almost all the hematolymphoid cells were derived from donor bone marrow cells that showed tolerance to all three types of MHC determinants with donor-derived thymic dendritic cells, suggesting third-party fetal thymus can be used to induce tolerance in elderly recipients with low thymic function (Cui et al., 2008). Future studies will further clarify the mechanism of TT in cancer therapy in tumor-bearing mice.
Tracking the fate of transplanted stem cells is important in order to analyze the molecular pathways and control the stem cell migration. Molecular imaging is a noninvasive method of tracking stem cell migration, survival, and differentiation, and for monitoring the transplanted cells in the live recipient (Gu et al., 2012). Molecular imaging may allow treatments to be modified to maximize their efficacy for brain tumors. Moreover, stem cells are of great interest as they show a tropism to tumor cells and will even migrate long distances to track down single tumor cells (Aboody et al., 2000). The identification of genetic and biochemical mechanisms underlying tumor growth and progression, along with the unraveling of the human genome, has provided a plethora of new targets for cancer detection, treatment and monitoring. The biological processes and target expression can be visualized by positron emission tomography or single photon emission computed tomography. Molecular imaging may help us understand the mechanisms by which stem cells migrate toward invasive tumors and to identify appropriate tissue sources when stem cells are used to treat brain tumors (Sandu et al., 2012a; Sandu and Schaller, 2012b; Spiriev et al., 2013). Similarly, molecular imaging will likely play an important role in tracking stem cell migration and in continuously observing the effects of stem cells when used in other cancer therapies.

Acknowledgments

We would like to thank Mr. Hilary Eastwick-Field and Ms. Keiko Ando for their help in the preparation of the manuscript.

References


