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Growth and Differentiation Advantages of $CD4^+OX40^+$ T Cells from Allogeneic Hematopoietic Stem Cell Transplantation Recipients

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ABSTRACT

OX40 (CD134), an activation-induced costimulatory molecule, is mainly expressed on $CD4^+$ T cells. Several reports, including previous reports from our laboratory, suggest that OX40-mediated signaling plays an important role in the development of graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (Allo HSCT). Here, we show that peripheral blood $CD4^+OX40^+$ T cells are a unique cell subset as they possess the homing receptors of lymph nodes, and some of them have an exceptional capacity to produce high levels of interleukin-2 (IL-2) upon the stimulation through T cell receptors. Stimulation with IL-7 acts selectively on $CD4^+OX40^+$ T cells not only to induce antigen-independent growth but also to increase the frequency of cells with IL-2-producing potential. Simultaneous, but not sequential, ligation of the T cell receptor and OX40 induces $CD4^+OX40^+$ T cells to produce far more IL-2, which causes them to proliferate abundantly and differentiate readily into Th1- or Th2-biased effector memory T cells, especially in Allo HSCT recipients. Although not all the $CD4^+OX40^+$ T cells had IL-2-producing capacity, Allo HSCT recipients with chronic GVHD (cGVHD) had a significantly higher frequency of IL-2-producing $OX40^+$ cells in their peripheral blood $CD4^+$ T cell subset than Allo HSCT recipients without cGVHD. Collectively, $CD4^+OX40^+$ T cells with IL-2-producing potential are expected to be privileged for growth and differentiation in lymph nodes upon antigen presentation, suggesting that they might be involved in the process of inducing or maintaining cGVHD.

KEY WORDS

OX40 • CD4 T cell • Allogeneic hematopoietic stem cell transplantation • Chronic graft-versus-host disease (GVHD)

INTRODUCTION

Chronic graft-versus-host disease (cGVHD) remains a serious complication that affects long-term survivors of allogeneic hematopoietic stem cell transplantation (Allo HSCT). It is not only the leading cause of nonrelapse mortality (NRM), it is also associated with decreased quality of life [1]. To prevent and treat GVHD, immunosuppressive agents such as calcineurin inhibitors are generally used, which increases the risk of developing opportunistic infections. It has been shown that GVHD is initiated by donor-derived $CD4^+$ and $CD8^+$ T cells that recognize a subset of host antigens [2,3]. Indeed, it has been shown that ex vivo depletion of the T cells in the graft effectively reduces the incidence and severity of acute GVHD (aGVHD) [4,5]. Unfortunately, this technique is also associated with increased incidences of graft rejection, relapses, and infectious complications, which prevents it from being widely used. Another technique to specifically deplete donor-derived alloreactive T cells that is currently being developed involves stimulating the graft with recipient cells in vitro and then depleting the activated T cells with monoclonal antibodies (mAb) [6-8]. However, such depletion-based techniques would probably fail to prevent cGVHD because the alloreactive T cells that cause cGVHD are believed to be derived from hematopoietic stem cells (HSC) in the graft rather than already being mature T cells [9-12]. A better way to prevent and treat

cGVHD would be to first identify which alloreactive cells are directly responsible for this disease; these cells could then be readily detected in the blood and specifically depleted within the host.

OX40 (CD134) is a member of the tumor necrosis factor (TNF) receptor superfamily [13], and is an activation-induced antigen that is predominantly expressed on CD4$^+$ T cells [14]. The ligand for OX40 (OX40L) is mainly expressed on activated antigen-presenting cells (APCs) such as dendritic cells and B cells [15-17]. OX40 signaling acts as an important costimulatory signal, as it augments interleukin 2 (IL-2) production [18,19], prolongs cell survival by upregulating Bcl-2 and Bcl-x_L expression [20], induces the clonal expansion of naïve CD4$^+$ T cells [19,21], and generates memory T cells by promoting the survival of effector T cells [19,22,23]. OX40-mediated signaling is also indispensable for expanding memory T cells in secondary immune responses and prolonging their survival [24]. A large body of evidence suggests that OX40-mediated signaling plays a pivotal role in the development of several immune-mediated conditions such as experimental autoimmune encephalomyelitis [16,25], collagen-induced arthritis [26], allergic lung inflammation [24,27], inflammatory bowel disease [28], and GVHD [29,30]. Because the in vivo blockade of OX40-mediated signals ameliorates these diseases in murine models, it is possible that targeting OX40 may also be useful for treating human diseases [14].

Buenafe et al [31] reported that the antigen-specific T cells in the spinal cord of Lewis rats displaying experimental autoimmune encephalomyelitis are frequently CD4$^+$OX40$^+$ T cells. Tittle et al [32] showed that CD4$^+$OX40$^+$ T cells are the alloreactive T cells in a murine GVHD model. In addition, we previously showed that the occurrence of cGVHD correlates positively with the frequency of peripheral blood CD4$^+$OX40$^+$ T cells [33]. Consequently, we speculated that the circulating CD4$^+$OX40$^+$ T cell subset of Allo HSCT recipients contains alloreactive T cells that are involved in the process of inducing and maintaining cGVHD. To further understand the role CD4$^+$OX40$^+$ T cells play in the development of cGVHD, we here isolated the CD4$^+$OX40$^+$ T cells from Allo HSCT recipients and healthy volunteers (HVs) and assessed their characteristics.

SUBJECTS, MATERIALS, AND METHODS

Subjects

Peripheral blood samples were obtained from 13 HVs and 43 Allo HSCT recipients who had undergone transplantation at least 100 days previously. Each subject gave written informed consent. Allo HSCT recipients were required to be in complete donor chimerism as well as in complete remission at the time of sampling. The clinical characteristics of the Allo HSCT recipients are summarized in Table 1. Standard conditioning for patients with hematologic malignancies consisted of 12 Gy total-body irradiation (TBI) and cyclophosphamide (Cy; 120 mg/kg), 12 Gy TBI and melphalan (Mel; 140 mg/m^2), or busulfan (Bu/Cy; 16 mg/kg) and Cy (120 mg/kg). Patients with aplastic anemia (AA) received 200 mg/kg Cy and antithymocyte-globulin (ATG), and a patient with adrenoleukodystrophy was treated with Bu (8 mg/kg), Cy (120 mg/kg), and 7.5Gy total lymphoid irradiation [34]. Reduced-intensity conditioning (RIC) was performed using 2-4 Gy TBI, fludarabine (Flu; 125 mg/m^2), and either Bu (8 mg/kg) or Mel (80-140 mg/m^2). The presence of cGVHD in Allo HSCT recipients was defined as the presence of active symptoms, for which immunosuppressive therapy was required [1,35]. In other words, patients defined as positive for cGVHD included patients with extensive cGVHD and patients with limited cGVHD, which manifests itself as significant hepatic dysfunction (value of Alkaline Phosphatase greater than twice the normal upper limit). All studies involving these blood samples were approved by the institutional review board of Kyoto University.

Table 1. *Patient Characteristics*

Characteristics	Data
No. male/no. female	20/23
Median age, years (range)	52 (25-74)
Diagnosis, no. (%)	
Acute lymphoblastic leukemia	2 (5)
Acute myelogenous leukemia	12 (28)
Myelodysplastic syndrome	7 (16)
CML/MPD	7 (16)
Adult T cell Leukemia	1 (2)
Lymphoma	8 (19)
Myeloma	3 (7)
Aplastic anemia	2 (5)
Adreno-leukodystrophy	1 (2)
Donor type, no. (%)	
Matched related	19 (44)
Matched unrelated	16 (37)
Mismatched related	5 (12)
Mismatched unrelated	3 (7)
Conditioning regimen, no. (%)	
Standard	24 (56)
Reduced intensity	20 (44)
Stem cell source, no. (%)	
Bone marrow	27 (62)
Peripheral blood	14 (33)
Cord blood	2 (5)
cGVHD, no. (%)	
Yes	23 (53)
No	20 (47)
Immunosuppression at the time of analysis, no. (%)	29 (67)
Median time after Allo HSCT for analysis, mo (range)	12 (4-149)

CML/MPD indicates chronic myelogenous leukemia/myeloproliferative disorder; cGVHD, chronic graft-versus-host disease; Allo HSCT, allogeneic hematopoietic stem cell transplantation.