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Role of soluble tumor necrosis factor-related apoptosis-inducing ligand concentrations after stem cell transplantation

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Abstract

Although stem cell transplantation (SCT) is being used for hematopoietic reconstitution following high-dose chemotherapy for malignancy, it involves certain serious transplant-related complications such as graft-versus-host disease (GVHD). Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) plays important roles in regulating cell death, immune response, and inflammation. However, the role of soluble TRAIL (sTRAIL) after SCT is poorly understood. In this study, 42 patients underwent SCT; 22 patients received allogeneic SCT, while the remaining 20 received autologous SCT. In these patients, levels of sTRAIL, cytokines, and soluble factors were measured by enzyme-linked immunosorbent assay (ELISA). In addition, a basic study of the generation of endothelial cell-derived microparticle (EDMP) by TNF-α and soluble Fas ligand (sFasL) was conducted. sFasL and EDMP exhibited significant elevation in the early phase (2–3 weeks) after SCT. In addition, the elevation of IL-6, TNF-α, and sIL-2R after allogeneic SCT was observed. EDMP also exhibited changes similar to sFasL. The patients with high sTRAIL exhibited significant decrease of sFasL and EDMP as compared with those without high sTRAIL. TNF-α and sFasL induced an increase in procoagulant and apoptotic markers in endothelial cells, and EDMP shedding was observed. Furthermore, sTRAIL inhibited the EDMP elevation caused by TNF-α and sFasL. The apoptotic markers such as sFasL and sTRAIL exhibited particular changes after SCT. Our results suggest that sTRAIL generation after allogeneic SCT relates to the prevention of GVHD.

Keywords: TRAIL; GVHD; Stem cell transplantation; sE-selectin; EDMP

1. Introduction

Stem cell transplantation (SCT) involves some serious transplant-related complications [1,2], such as graft-versus-host disease (GVHD), and vascular disorders, such as veno-occlusive disease (VOD), pulmonary vasculopathy, thrombotic microangiopathy (TMA), and capillary leak syndrome [3–5]. Although the complex pathophysiology of acute GVHD involves the conditioning regimen, cytokines, nitric oxide, and non-T effector cells, the cytolytic activity of donor T-cells is essential for the development of GVHD activity [6,7]. The cytolytic activity of cytotoxic T-lymphocytes (CTLs) is primarily mediated through certain effector mechanisms such as the Fas/FasL and perforin/granzyme pathways [8,9]. Interaction of FasL, expressed on the CTL cell surface, with the Fas receptor on the target cell membrane results in the initiation of the Fas cell death pathway [10]. Recent accumulating evidence indicates that the Fas/FasL system is implicated in the pathogenesis of acute GVHD [7,11–13].

Cellular microparticles are fragments that shed almost spontaneously from the plasma membrane blebs of virtually all cell types when subjected to a number of stress conditions [14,15]. In addition, these microparticles have more recently been shown to reflect in vitro cell stimulation, and testify to cellular activation and/or tissue degeneration occurring in vivo under various pathophysiologic conditions [14,15]. Thus, there is a

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possibility that the cellular microparticles exhibit a dynamic change after SCT [16]. In contrast, diagnosing vascular complications in patients undergoing SCT is challenging, and damage to endothelial cells is regarded as the common feature of these complications [17,18]. Furthermore, endothelial damage, perpetuated by $CD8^+$CTL, has been linked to GVHD which is described in the skin and gut [18–22].

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)/Apo-2L is a member of the TNF family of cytokines, which are structurally related proteins playing important roles in regulating cell death, immune response, and inflammation [23]. TRAIL is a type II membrane protein, which can be proteolytically cleaved to a soluble form [24], as previously shown also for TNF-α and CD95 (Apo-1/Fas). The unique feature of TRAIL, as compared with other members of the TNF family, is its ability to induce apoptosis in a variety of malignant cells both in vitro and in vivo, displaying minimal toxicity on normal cells and tissues [25,26]. TRAIL interacts with four cellular receptors that form a distinct subgroup within the TNF receptor superfamily. TRAIL receptor 1 (TRAIL-R1 or DR4) and TRAIL receptor 2 (TRAIL-R2 or DR5) have cytoplasmic death domains and signal for apoptosis and NF-κB [27–29]. Two additional receptors, TRAIL receptor 3 (TRAIL-R3 or DcR1) and TRAIL receptor 4 (TRAIL-R4 or DcR2), are homologous to DR4 and DR5 in their cysteine-rich extracellular domain, but they lack intracellular death domains and apoptosis-inducing capability [30,31]. It has been shown that endothelial cells express TRAIL-R3 and TRAIL-R4 [30,32], further suggesting the relationship between TRAIL and endothelial function. Furthermore, there are several indications that TRAIL could be involved in the pathophysiology of autoimmune diseases [33–35]. However, the role of soluble TRAIL (sTRAIL) after SCT is poorly understood.

We measured and compared levels of sTRAIL, cytokines, and soluble factors in patients undergoing SCT. The results suggested that sTRAIL plays a unique role after SCT.

2. Materials and methods

2.1. Subjects

The subjects were 42 patients who underwent SCT between June 2001 and May 2006 at the institution of residence. In all, 22 patients received allogeneic SCT, while the remaining 20 received autologous SCT (Table 1). The 10 male and 12 female allogeneic SCT patients ranged in age from 6 to 68 years (median: 31 years), and the 12 male and 8 female autologous SCT patients ranged in age from 36 to 67 years (median: 51 years). Patient diagnoses consisted of 4 acute myeloid leukemia, 5 acute lymphoblastic leukemia, 2 chronic myeloid leukemia, 3 acute promyelocytic leukemia, 14 non-Hodgkin's lymphoma, 6 multiple myeloma, and 8 others. Conditioning applied was: total body irradiation for 13 and non-total body irradiation for 29. For allogeneic SCT, prophylaxis included cyclosporine for 19 patients with GVHD. The donor sources were 6 bone marrow transplantations, 10 peripheral blood stem cell transplantations, and 6 cord blood transplantations. Twenty-four patients received filgrastim and 18 received lenograstim. Written informed consent was obtained from all the patients.

2.2. Cytokine evaluation

Blood samples from each patient were collected into plastic tubes and immediately centrifuged to obtain serum. The serum was divided into aliquots and frozen at −30 °C until use. As a positive control, recombinant products were used in each assay, as well as standard solutions provided with the commercial kits. Human TNF-α, IFNγ, IL-4, and IL-6 ELISA kits were purchased from BioSource International, Inc. (Camarillo, California, USA). Serum levels of cytokines were measured according to the 'manufacturers' instructions. Normal ranges were as follows: TNF-α: 5–20 pg/ml, IFNγ: 0–12.5 pg/ml, IL-4: 0–3.5 pg/ml, and IL-6: 0.2–4.5 pg/ml.

Table 1
Clinical profiles of SCT patients

Characteristics	Allogeneic SCT	Autologous SCT
Gender		
Male/Female	10/12	12/8
Age (years) median (range)	31 (6–68)	51 (36–67)
Diagnosis		
Leukemia	AML: 4 APL: 3 ALL: 5 CML: 2	
Malignant lymphoma	DLBC: 1	DLBC: 8 FCL: 5
Others	AA: 1 MDS: 5 Renal cancer: 1	MM: 6 Lung cancer: 1
Conditioning		
TBI	CY: 5	L-MAP: 3 Flu/L-PAM: 5
Non-TBI	Flu: 3 Flu, Bu: 5 Flu, L-PAM: 4	VP-16, CY: 4 MCNU, IFO, CBDCA, VP-16: 8 MCNU, L-PAM, Ara C, VP-16: 5
Donor source	BMT: 6 PBSCT: 10 CBT: 6	
G-CSF		
Filgrastim	14	10
Lenograstim	8	10

AML: acute myeloblastic leukemia; APL: acute promyeloblastic leukemia; ALL: acute lymphoblastic leukemia; CML: chronic myeloblastic leukemia; DLBC: diffuse large B cell lymphoma; FCL: Follicular cell lymphoma; AA: aplastic anemia; MDS: myelodysplasia syndrome; MM: multiple myeloma; TBI: total body irradiation; CY: cyclophosphamide; Flu: fludarabine; L-PAM:melphalan; VP-16: etoposide; IFO: ifosfamide; CBDCA: carboplatin; BMT: bone marrow transplantation; PBSCT: peripheral blood stem cell transplantation; CBT: cord blood transplantation.

2.3. Measurement of sFasL, sTRAIL, sIL-2R, sVCAM-1 and sE-selectin

sFasL, sTRAIL, sIL-2R, sVCAM-1, and sE-selectin ELISA kits were purchased from BioSource International Inc. For measurement of sFasL, sTRAIL, sIL-2R, sVCAM-1 and sE-selectin in serum, all the kits were used according to the manufacturers' instructions. Normal ranges were as follows: sFasL: 0.02–0.14 ng/ml, sTRAIL: 100–500 pg/ml, sIL-2R: 150–450 IU/ml, sVCAM-1: 395–714 ng/ml, and sE-selectin: 23.0–79.2 ng/ml.

2.4. Assessment of endothelial cell-derived microparticle (EDMP)

EDMPs were detected using a previously reported method with some modifications [36]. A 10-μl aliquot of washed intact platelets (3×10^8/ml) was added to the plasma, and the mixture was incubated for 30 min in dark at room temperature, with FITC-labeled Annexin V (FITC-Ann V) and phycoerythrin (PE)-labeled CD51 (αvβ3) to detect EDMP. The samples were diluted 1:10 with HEPES-Tyrodes buffer containing 5 mmol/l EGTA and analyzed using the Ortho Cytoron Absolute Analyzer (Ortho Diagnostic Systems, Inc., Tokyo, Japan), set to detect only the particles bound to FITC-labeled Annexin V and PE-labeled CD51. This method was designed to ensure the detection of only procoagulant EDMP. The concentrations of these microparticles were then calculated per μl of the whole blood.

2.5. Activation of endothelial cells

Endothelial cells isolated from freshly obtained human umbilical cord veins were cultured according to the method of Jaffe et al. [37]. Second-passage cells