Biology of Blood and Marrow Transplantation 14:23-32 (2008)

1083-8791/08/1401-0001$32.00/0
doi:10.1016/j.bbmt.2007.10.008

The Contribution of Endothelial Activation and Injury to End-Organ Toxicity following Allogeneic Hematopoietic Stem Cell Transplantation

Kenneth R. Cooke,[1] *Anne Jannin*,[2] *Vincent Ho*[3]

[1] Blood and Marrow Transplantation Program, Case Comprehensive Cancer Center and Rainbow Babies and Children's Hospital, Case Western Reserve University, School of Medicine, Cleveland, Ohio; [2] Department of Pathology, Hospital Saint Louis, Université ParisVII, Paris, France; and [3] Center for Hematologic Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts

Correspondence and reprint requests: Kenneth R. Cooke, MD, Blood and Marrow Transplantation Program, Case Western Reserve University, School of Medicine, Department of Pediatrics, Wolstein Research Building, Room 6524, 2103 Cornell Road, Cleveland, OH 44106-7288 (e-mail: Kenneth.cooke@uhhospitals.org).

ABSTRACT

Over the last 25 years, allogeneic hematopoietic stem cell transplantation (HSCT) has been used increasingly as a curative treatment option for patients with hematologic and neoplastic diseases. Despite major advances in transplant immunology and improvements in supportive and critical care medicine, HSCT is still plagued by several life-threatening complications. As such, the establishment of effective therapeutic options for these complications will be crucial as increasing numbers of high-risk transplants are performed each year. This brief review will discuss the contribution of vascular endothelial cell activation and injury to inflammation and end-organ toxicity that occurs following allogeneic HSCT, and will highlight translational research efforts that have paved the way to the development of novel strategies to treat and prevent disease. Finally, we will discuss in detail the clinical manifestations and challenges encompassed by the syndrome of thrombotic microangiopathy following HSCT.

KEY WORDS

Endothelial cell • Graft-versus-host disease • Idiopathic pneumonia syndrome • Veno-occlusive disease • Thrombotic microangiopathy • Hematopoietic stem cell transplantation • Tumor necrosis factor-alpha • Cytokines • Inflammation • Microvasculature

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative therapeutic option for a number of malignant and nonmalignant disorders. Unfortunately, HSCT is associated with several life-threatening complications. Specifically, the development of graft-versus-host disease (GVHD), idiopathic pneumonia syndrome (IPS), veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS), and thrombotic microangiopathy (TMA) are associated with significant morbidity and mortality, and limit the ultimate success of HSCT, particularly when full-intensity conditioning is administered. The establishment of effective strategies to reduce toxicity after HSCT is critical as increasing numbers of high-risk transplants are performed each year.

The development of such approaches is fundamentally dependent upon a basic understanding of pathophysiologic mechanisms of disease. To this end, a significant body of experimental and clinical data has enhanced our knowledge as to how both cellular and soluble effectors contribute to the pro-inflammatory milieu that is associated with acute toxicity following allogeneic HSCT. Endothelial cell (EC) damage is observed in this setting, and has been implicated as a direct contributor to the development of several complications including GVHD, VOD, TMA, and endothelial leak syndrome (ELS) [1-3]. Support for this hypothesis has been provided by clinical studies that have measured various markers of endothelial damage such as soluble thrombomodulin, plasminogen-activator inhibitor, von Willebrand's factor, and soluble ICAM-1

after HSCT [1,4-6]. Several in vitro and in vivo systems have also demonstrated that EC respond to stimuli such as irradiation, lipopolysaccharide (LPS), and tumor necrosis factor-alpha (TNF-α) (all of which are operative early after HSCT), by either becoming activated or undergoing programmed cell death, or apoptosis [7,8].

Cellular and soluble inflammatory effectors are known to play a role in the major complications after allogeneic HSCT including GVHD and IPS, and may also contribute to others like VOD, but the molecular mechanisms by which inflammatory proteins and white blood cells (WBCs) gain access to "target" organs and cause damage have yet to be fully elucidated. Capillary permeability is a tightly regulated feature of the microcirculation in all organ beds and is fundamentally altered in inflammatory conditions, resulting in net extravasation of fluid out of the vascular space and into tissues. Endothelial barrier integrity is believed to result from a balance between contractile forces within ECs that create intercellular gaps and adhesive forces between ECs that restrict such gaps; an increase in contractile force within ECs is associated with increased permeability, whereas approaches that decrease this effect reduce capillary permeability. The passage of donor cells across the vascular endothelium and into inflamed tissues is also tightly controlled. This process is regulated in part by the expression of adhesion molecules on endothelial surfaces and ultimately by the integrity of the endothelial barrier itself. The recruitment of leukocytes from the vascular space and into target tissue can be divided into 4 steps: (1) weak adhesion of WBCs to the vascular endothelium, (2) firm adhesion of WBCs to endothelial cells, (3) transmigration of leukocytes through the vascular wall, and (4) migration of cells through the extracellular matrix along a chemotactic gradient. Thus, vascular ECs are the primary barrier separating donor-derived leukocytes and allogeneic target tissue. Injury to the vascular endothelium has been recently observed early in the development of experimental IPS [9] and identified as the initial target of an allogeneic GVH response elicited in SCID mice [2]. Taken together, it is possible that diffuse microvascular injury represents a common pathology underlying many transplant-related complications, and this possibility can help to explain the close clinical association observed between TMA/ transplant-associated microangiopathy (TAM) (discussed in detail later) and GVHD, VOD/SOS, and infection.

IPS

Pulmonary complications occur in 25% to 55% of transplanted patients, and account for approximately 40% of treatment-related mortality (TRM). In approximately 50% of these cases no infectious etiology is identified, and this form of pulmonary dysfunction has been defined as IPS [10]. IPS remains a frequently fatal complication after allogeneic HSCT. Although a recent study showed a lower incidence and earlier onset of IPS than previously reported, the typical clinical course involving the rapid onset of respiratory failure leading to death remained unchanged, underscoring the critical nature of this transplant-related problem [11]. Mortality rates therefore remain unacceptably high, and the median time from diagnosis to death is a sobering 14 days [11,12]. Potential etiologies for IPS include direct toxic effects of HSCT conditioning regimens, occult pulmonary infections, and immunologic mechanisms as suggested by the association of IPS with GVHD [10,13,14]. Clinical and experimental data have shown that the intensity of HSCT conditioning [15,16], along with inflammatory cytokine secretion and the influx of donor derived cellular effectors, may all contribute to the development of IPS [17,18], but the mechanisms regulating leukocyte recruitment to the lung in this context are not completely understood.

Clinical and experimental IPS are associated with evidence for pulmonary vascular injury and leak as demonstrated by pulmonary edema, enhanced total protein levels in bronchoalveolar lavage fluid and increased wet-to-dry lung weight ratios [18,19]. Excess extravascular fluid in the lung impairs gas exchange across the alveolar membrane and contributes to reductions in lung compliance. However, the relationship between vascular EC damage and lung injury after HSCT has only recently been explored. Using a well-described murine system [9], we recently demonstrated that experimental IPS is associated with EC injury and activation. Significant EC apoptosis accompanies pulmonary toxicity 6 weeks after allogeneic HSCT when the extent of injury directly correlates with the severity of lung histopathology. At this point, pulmonary toxicity is robust, and approximately 40% of all vessels in the lung are injured, and microscopic inspection of these vessels reveal that most are surrounded by a dense mononuclear cell infiltrate. Detection of apoptotic ECs was confirmed by cytologic appearance, presence of activated caspase 3 in the cytoplasm, and TUNEL positivity of nuclei in allogeneic tissue samples. Importantly, we also found that damage to the endothelium occurs before maximal lung histopathology is present; apoptotic endothelia are readily apparent in the lung early after HSCT even when leukocyte infiltration is at a very early stage.

In addition EC apoptosis is associated with evidence for EC activation as measured by enhanced mRNA expression of adhesion molecules, and with elevated BAL TNF-α levels. Finally, a causal relationship between TNF-α and endothelial damage was ultimately established; neutralization of TNF-α using a soluble TNF-α binding protein (rhTNFR:Fc) from

week 4 to week 6 after allogeneic HSCT significantly reduces EC apoptosis, and the progression of lung histopathology observed during this interval. We have recently demonstrated that TNF-α production by both donor T cells and accessory cells (monocytes and macrophages) significantly contributes to the development of IPS [19], but it remains to be determined whether a subset of these infiltrating cells is also involved with direct cell-mediated killing of ECs. Indeed, we have shown that cytotoxicity via the Fas-FasL pathway contributes to the development of experimental IPS [20], but we have yet to extend these observations to EC injury that occurs in this setting. Collectively, our results demonstrate a role for vascular EC damage in leukocyte recruitment during IPS and confirm a significant contribution of TNF-α to each of these processes.

Our experimental findings are in accord with clinical reports showing an association between microangiopathy and diffuse alveolar hemorrhage after allogeneic HSCT; endothelial damage preceded pulmonary toxicity in 3 of 4 patients [21]. Disruption of pulmonary endothelial function is also observed during sepsis-associated acute respiratory distress syndrome (ARDS), and a recent study explored the mechanisms responsible for EC damage in this context [22]. Angiopoietin-1 (Ang-1) and Ang-2 are peptide ligands that bind the Tie-2 receptor tyrosine kinase found that is primarily found on ECs. The 2 proteins have been identified as an agonist/antagonist pair that regulates early vascular development and endothelial barrier integrity during physiologic homeostasis and disease. Ang-2 is a known antagonist of the Tie-2 that competitively binds the receptor and interferes with agonistic Ang-1:Tie-2 receptor:ligand functions. Moreover, Ang-2 release can be driven by inflammatory stimuli such as TNF-α. By contrast, Ang-1 activates Tie-2, resulting in receptor phosphorylation and subsequent signal transduction that promotes EC survival and vessel stability. Ang-1 may also have an anti-inflammatory action by signaling the downregulation of surface-adhesion molecules such as VCAM-1 and E-selectin.

Parikh and colleagues [22] tested the hypothesis that Ang-2 contributes to the disruption of the pulmonary vascular endothelial barrier during sepsis-associated lung injury. They found that circulating Ang-2 is significantly elevated in humans with ARDS from sepsis, and sera from these patients disrupt endothelial architecture ex vivo. This disruptive effect correlated with measured Ang-2 levels, wanes when sera collected during clinical improvement is tested, and is reversed by Ang-1. In addition, Ang-2 alone can reproduce endothelial damage ex vivo, and its administration to healthy mice results in pulmonary capillary leak and edema. These findings suggest that an imbalance in Tie-2 signaling could also arise in the inflammatory milieu that characterizes the immediate post-HSCT period. Because the expression of Tie-2 in quiescent adult tissues is highest in the lung, it is possible that such an imbalance may preferentially affect the lung in this context. Studies to explore this possibility in the setting of IPS are currently ongoing.

Finally, laboratory insights regarding the role of TNF-α in the development of IPS are being translated back to the clinic. Recent work from the University of Michigan and the Dana Farber Cancer Institute suggests that etanercept (Enbrel, Amgen Corp, Thousand Oaks, CA) may be a useful therapeutic option for IPS. Etanercept was administered in combination with systemic steroids to a total of 18 patients with IPS [23]. Etanercept was given subcutaneously at a dose of 0.4 mg/kg twice weekly for a maximum of 8 doses. Therapy was well tolerated overall. Thirteen of 18 patients were able to completely withdraw from supplemental oxygen support within 28 days of therapy. Survival at day 28 and day 56 (from the first etanercept dose) was 73% and 60%, respectively. Based upon these encouraging results, larger phase II (pediatric) and phase III (adult) trials are currently ongoing through the Children's Oncology Group (COG) and the BMT clinical trials network (CTN), respectively.

ACUTE GVHD (aGVHD)

GVHD is the major cause of morbidity and mortality following allogeneic HSCT. The onset of aGVHD is usually within the first several weeks following the infusion of stem cells, and classically involves immune-mediated damage to the skin, liver, and gastrointestinal tract. Recently, clinical and experimental data suggest that the lung may also be a target organ, with disease manifesting itself as IPS (described above). The pathophysiology of aGVHD is complex, and is now known to involve aspects of both the adaptive and innate immune responses. Advances in basic transplant immunology have demonstrated how interactions between cells of the lymphoid and myeloid lineage are governed by cytokines, and much recent research has focused on the role of these cellular and soluble effectors in the pathogenesis of aGVHD. The pathologic findings of aGVHD characteristically include epithelial damage that is usually apoptotic in nature. Although vascular endothelia represent the primary barrier between donor-derived cells, their secretory products, and allogeneic target tissue, the role of ECs in the development of GVHD has yet to be fully appreciated.

When microvascular damage occurs in patients after HSCT, it is difficult to recognize what is linked to radiotherapy, to chemotherapy, or to immune damage. Several lines of investigation have added to our understanding of how ECs regulate immune-mediated responses during both allograft and xenograft rejection [9]. As noted, vascular endothelial cells are the primary barrier separating the engrafting, donor-derived,

cellular immune system and GVHD target tissue, and as such, represent the first allogeneic cells encountered by primed T cell effectors. Microvascular ECs can express MHC Class I, MHC Class II, and minor histocompatibility (H) antigens, and the expression of these molecules is enhanced by TNF-α and interferon-gamma (IFN-γ). It is conceivable, therefore, that ECs can serve as targets for direct cell-mediated damage. When cocultured with recipient type endothelium in vitro, activated allogeneic T cell effectors enhance mRNA expression of TNF-α, adhesion molecules like ICAM-1, and cause EC injury [24].

Using an unirradiated animal model of splenocyte transfer in immunodeficient mice wherein characteristic epithelial damage in the GVHD target organs is consistently reproduced, Dr. Janin and colleagues [2] were able to assess EC damage induced solely by allogeneic reactions. Janin's group of investigators found that ECs were targets of the allogeneic reaction, because no damage was found in mice following splenocyte transfer from syngeneic donors. In addition, the severity of endothelial injury was linked to the amount of allogeneic splenocytes injected, and the most severe damage led to rupture of the microvessel wall and pericapillary hemorrhage, but not to thrombosis. The team also determined that ECs underwent apoptosis through the Fas/FasL pathway; splenocytes from FasL-deficient mice did not induce injury, and the development of EC lesions was blocked by an inhibitor of caspase (zVAD) and by injections of anti-Fas antibodies. Importantly, endothelial damage occurred before epithelial damage in the targets organs, thereby identifying disseminated EC apoptosis as the earliest target of an allogeneic GVH response in several tissues [2].

Systematic analyses of microvessels in human biopsies also reveal that EC damage is present in the gut and skin. EC apoptosis in the digestive tract of patients with aGVHD and the number of apoptotic cells was linked to the severity of GVHD, leading to transmural microvascular lesions and pericapillary hemorrhage in the more severe cases. In patients with chronic GVHD (cGVHD), another European team performed a quantitative study of endothelial cell surface and found a reduction of endothelial areas in patients with cGVHD. Taken together, these clinical and experimental data suggest that endothelial cell damage in GVHD could lead to a "disappearing microvessel syndrome," as bile duct epithelial damage leads to a "disappearing bile duct syndrome" common to cGVHD, chronic liver allograft rejection, and primary biliary cirrhosis.

Evidence for EC damage in the context of GVHD has also been provided by other clinical studies. Endothelial microparticles (EMP) are vesicles shed from the membrane of ECs upon activation of apoptosis, and are thus good markers for endothelial stress [25]. EMPs are also believed to promote disseminated platelet aggregation and microangiopathy and to have pro-inflammatory effects because they can bind and activate monocytes, thereby stimulating release of cytokines that may further damage the endothelium [25]. It is interesting to note that in a recent study where EMP levels were analyzed in 19 patients after HSCT, EMP levels were significantly higher in patients who had aGVHD compared to those without aGVHD [26]. Whether these endothelial microparticles play a significant role in the pathogenesis of other complications after HSCT remains to be elucidated.

Vascular endothelial growth factor (VEGF) is a member of a family of 6 structurally related proteins that regulate the growth and differentiation of multiple components of the vascular system, especially blood and lymph vessels. VEGF (specifically VEGF-A) stimulates vascular EC growth, survival, and proliferation, and is important for endothelial integrity and tissue repair. Nachbaur and colleagues [27] recently retrospectively measured endogenous VEGF serum levels in seventy allogeneic HSCT recipients. VEGF levels were significantly decreased within the first 2 weeks after the HSCT, but returned toward pretransplant levels by day +15. At this time, higher levels (as determined using the median value as a cutoff) were associated with less TRM, faster neutrophil recovery, a lower incidence of severe aGVHD, and significantly improved overall survival (OS). These findings are in accord with a similar study that showed that low VEGF levels after allogeneic HSCT are associated with non-relapse mortality (NRM) with increased incidence and severity of aGVHD [28]. Taken together, experimental and clinical data suggest that EC damage and levels of proteins that are critical to vascular endothelial integrity and tissue repair may participate in the development of aGVHD.

VENO-OCCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME (VOD/SOS)

Recently, a role for endothelial activation and injury has been implicated in the pathogenesis of VOD/SOS. Markers of endothelial damage including plasma thrombomodulin (TM), selectins, tissue factor pathway inhibitor (TFPI), soluble tissue factor (sTF), and plasminogen activator inhibitor (PAI-1) are elevated in patients with VOD/SOS [29]. Thrombocytopenia with platelet refractoriness is common in patients with established VOD/SOS, and although this may be because of splenic sequestration from portal hypertension, it may also reflect increased platelet consumption through EC injury. Not surprisingly, thrombopoietin and von Willebrand's factor multimers are often elevated, whereas factor VII levels are generally depressed, suggesting activation of the coagulation cascade with ongoing EC injury. In concordance with this, investigators have demonstrated

lower levels of anticoagulants, either at baseline or shortly after high-dose cytoreductive therapy, in patients who subsequently develop VOD/SOS, whereas there appears to be fluctuation in levels of procoagulant proteins, including serum proteases and fibrinogen, and fibrin breakdown products, such as D-dimer at similar time points.

Based upon the histopathologic findings of microthrombosis and fibrin deposition that are present during VOD/SOS, and the mechanisms of injury that may be operative in the development of disease, several therapeutic approaches have been explored. The majority of treatment strategies have focused upon fibrinolytic therapy with or without anticoagulation. Unfortunately, approaches utilizing heparin, antithrombin III (AT III), prostaglandin E1 (PGEI), and activated protein C have been associated with limited response rates and significant bleeding complications. Defibrotide (DF), a polydisperse oligonucleotide with a molecular weight of 23 kD [30], is an agent that modulates EC injury without enhancing bleeding or compromising the antitumor effects of cytotoxic therapy [31]. DF binds specifically to the vascular endothelium via adenosine receptors A1 and A2, which are part of the growing family of nucleotide receptors involved in EC regulation adhesion and response to injury. DF has antithrombotic, anti-ischemic, anti-inflammatory, antiadhesive, and thrombolytic properties, without significant, anticoagulant activity. Recent studies of human-derived, LPS-exposed microvascular and macrovascular endothelium have shown preferential protective effects of DF toward microvascular injury [31].

Nonrandomized clinical trials using DF for the treatment of severe VOD/SOS and multi-organ failure (MOF) following HSCT have been associated with minimal toxicity, resolution of hyperbilirubinemia, and MOF and a corresponding improvement in day 100 survival in a significant number of patients [32]. In the largest experience reported to date, where 88 HSCT patients with severe VOD/SOS and MOF were treated with DF, complete response was 36% and OS at day +100 was 35% (versus <10% expected) [32]. Predictors of survival included younger age, autologous HSCT, and abnormal portal flow, whereas busulfan-based conditioning and encephalopathy predicted worse outcome. Decreases in mean creatinine and PAI-1 levels during DF therapy also predicted better survival, suggesting that certain features associated with successful outcome could correlate with DF-related treatment effects. DF also appears to be effective in VOD/SOS following Mylotarg therapy. Prospective, multicenter trials of DF in the prevention and treatment of severe VOD/SOS are now underway in the United States and Europe, and preliminary results have been encouraging.

HSCT-ASSOCIATED THROMBOTIC MICROANGIOPATHY

Clinical syndromes presenting with microangiopathic hemolytic anemia, consumptive thrombocytopenia, often with renal insufficiency and encephalopathy, have long been recognized as dangerous complication following autologous and allogeneic HSCT. In the literature, the terms used to describe these microangiopathic processes have included hemolytic uremic syndrome (HUS), microangiopathic hemolytic anemia (MAHA), and thrombotic thombocytopenic purpura (TTP). The BMT Clinical Trials Network (CTN) has proposed the term TMA associated with transplantation to encompass these HSCT-related HUS/TTP syndromes. Similarly, the International Working Group from the EBMT recommended TAM as an umbrella term to cover these microangiopathic processes. Although the proposed definitions for "TMA" and "TAM" vary to a certain extent, such efforts from recognized groups like the CTN and IWG are essential, and should provide a framework for future clinical and laboratory research into this important, yet poorly understood complication of transplantation. In deference to both definitions, the combined terminology "TMA/TAM" will be used in this review.

Clinical Features of TMA/TAM

The classic presentation of TMA/TAM includes the relatively acute onset of anemia and thrombocytopenia with evidence of RBC fragmentation on the peripheral blood smear. This is invariably associated with a rapid increase in the serum lactate dehydrogenase (LDH) level. Almost all patients with TMA/TAM will be thrombocytopenic, and many patients who develop TMA/TAM early after HSCT will present with a failure of the platelet count to rise appropriately [33]. Concomitant acute renal dysfunction, often associated with proteinuria and hypertension, occurs in a majority of patients. Neurologic deficits, including, but not restricted to, confusion and seizures, occur in up to half the cases. The onset of TMA/TAM usually occurs within the first 100 days after HSCT, and the median time of onset ranged from 44 to 67 days in 2 large retrospective reports [34,35]. It is worth noting, however, that there is a distinct subset of TMA/TAM that occurs later and is more exclusively associated with renal impairment. These later occurring TMA/TAM syndromes, commonly referred to in the literature as HSCT nephropathy, radiation nephritis, or conditioning associated HUS, can develop several months to years after HSCT [36].

Recent reports have also shown that TMA/TAM can affect the intestines and present with abdominal pain and bloody diarrhea, thereby imitating enteric GVHD or infectious colitis [37]. Diagnosis of intestinal TMA relies primarily on histopathologic evaluation demonstrating hyaline thrombi in capillaries of

intestinal biopsies, or presence of thrombonecrotic arteriolar lesions in the intestine on autopsy [37]. Many of these intestinal TMA cases were, in fact, detected by intestinal biopsy when patients developed increasing bloody diarrhea despite resolving skin GVHD after therapy with corticosteroids.

Incidence of TMA/TAM

TMA/TMA after HSCT may be part of a spectrum of transplant-related toxicities, including VOD/SOS, GVHD, and IPS which, as discussed above, are associated with injury to the vascular endothelium. Research efforts in TMA/TAM have been hampered by the absence of a unified set of diagnostic criteria. Reflective of this problem, the reported incidence of this complication in the HSCT literature has varied from 0.5% to 76% [33-35]. TMA/TAM occurs less frequently after autologous HSCT (up to 2.6%) compared to the allogeneic setting where the incidence is generally closer to 10% [34]. Indicative of the lack of a unified definition for this condition, a recent review found 28 different sets of diagnostic criteria used in 35 publications. The most frequently cited criteria required for diagnosis were: red cell fragmentation (31 of 35), elevated LDH (25 of 35), platelet decline (18 of 35), renal insufficiency (17 of 35), and neurologic dysfunction (8 of 35).

Diagnostic Criteria for TMA/TAM

Recognizing the need to standardize definitions for toxicity reporting in multicenter clinical trials and facilitate future clinical investigative efforts, the BMT CTN and EBMT IWG have recently proposed their own sets of clinical criteria for reporting microangiopathy associated with transplantation (Table 1) [35,38]. The BMT CTN definition of TMA places focus on the presence of red cell fragmentation (schistocytosis) and elevation of serum LDH, in conjunction with end-organ dysfunction manifested as either acute renal or central nervous system (CNS) compromise [38]. The IWG definition for TAM does not require the presence of renal/CNS dysfunction, but requires the presence of RBC fragmentation, reduced haptoglobin, and increased LDH with prolonged/progressive thrombocytopenia. By limiting the diagnosis to patients with microangiopathy and concurrent renal/CNS dysfunction, the CTN definition captures only more severe cases of TMA/TAM (with organ damage) compared to the broader IWG definition [35]. The IWG placed particular focus on de novo or prolonged thrombocytopenia as a sine qua non for the diagnosis of TAM, which concurs with the finding in the review by George et al. [34], where low platelets was the third most commonly cited criteria in the literature. Because the CTN and IWG definitions of TMA/TAM were conceived with distinct purposes in mind, they are expectedly different, although not mutually exclusive, of each other. Both of these sets of criteria deserve further testing and validation in large clinical trials to assess their utility as a diagnostic and prognostic tool.

Table I. *CTN and IWG Consensus Criteria for Diagnosis of TMA/TAM*

BMT CTN Toxicity Committee Consensus Definition for TMA
1. RBC fragmentation, ≥2+ schistocytes per high-power field on peripheral smear
2. Concurrent elevated serum LDH above institutional baseline
3. Concurrent renal* and/or neurologic dysfunction without other explanations
4. Negative direct and indirect Coombs tests
International Working Group (IWG) definition for TAM
All the following are present:
1. Increased percentage (>4%) of schistocytes in the blood
2. De novo, prolonged or progressive thrombocytopenia (platelet $<5 \times 10^9$/l or ≥50% decrease from previous counts)
3. Sudden and persistent increase in LDH
4. Decrease in hemoglobin concentration or increased red blood cell transfusion requirement
5. Decrease in serum haptoglobin concentration

*Doubling of serum creatinine from baseline (baseline = creatinine before hydration and conditioning), or 50% decrease in creatinine clearance from baseline.

Endothelial Cell Injury and TMA/TAM

The primary inciting events leading to the development of TMA/TAM remain poorly understood, but evidence suggests that damage to the vascular endothelium is central to its pathogenesis. Direct support of this concept has come from scanning electron microscopy showing EC damage in patients with TMA/TAM [39]. As noted above, increased levels of markers of EC injury/activation, including tissue factor, von Willebrand's factor antigen, thrombomodulin, and PAI-1, have also been associated with patients developing TMA/TAM, as well as other transplant complications including VOD/SOS, GVHD, and sepsis after allogeneic HSCT. Elevated levels of inflammatory cytokines, including IL-1, TNF-α, IFN-γ, and IL-8 in patients with VOD/SOS, GVHD, IPS, and sepsis may further contribute to EC damage. These findings point to diffuse microvascular injury as a common pathology underlying all of these transplant complications, and may explain the close clinical association observed between TMA/TAM and GVHD, VOD/SOS, and infection [34]. Proposed mechanisms leading to EC injury and TMA/TAM posttransplant include direct toxicity from high-dose conditioning, infection, GVHD or a variety of medications, especially those used as GVHD prophylaxis or treatment.

Calcineurin inhibitors (CIs), including cyclosporine and tacrolimus, are directly toxic to endothelial cells. CIs can also damage EC via secondary mechanisms by triggering microvascular thrombosis. Sirolimus, a macrocyclic lactone antibiotic with immune suppressive properties, has been used increasingly following allogeneic HSCT as a therapeutic and prophylactic agent against GVHD. Although Sirolimus is in itself not associated with the occurrence of TMA/TAM, its use in conjunction with calcineurin inhibitors does potentiate the deleterious effects of CI on the endothelium [40].

Risk Factors, Prognostic Factors, and Staging for TMA/TAM

TMA/TAM has been associated with advanced recipient age, female sex, unrelated or HLA-mismatched donor grafts, aGVHD, viral or fungal infections, and the use of CI, or Sirolimus in conjunction with a CI [33,34,41]. Unfortunately, interpretation of the literature is challenging, because most reports include small numbers of cases and use varying definitions for the diagnosis of TMA/TAM. In the large recent retrospective series involving 1219 allogeneic transplants and 66 cases of TMA/TAM, significant risk factors in multivariate analysis included female gender, lymphoid malignancy, unrelated donors, and aGVHD [41].

The clinical spectrum of TMA/TAM is highly variable. According to the NCI Common Terminology Criteria for Adverse Events (CTC AE) scoring system (Table 2), clinical severity may range from grade 1 "evidence of RBC destruction (schistocytosis) without clinical consequence," to a moderately severe problem with concomitant renal insufficiency (grade 3), to a life-threatening hemolytic anemia/thrombocytopenia with renal failure and/or devastating neurologic complications (grade 4). In the review of the literature review published by George et al. [34], the overall mortality in 379 cases of TMA/TAM after allogeneic SCT reported in 31 articles was 61%. A majority (81%) of patients died within 3 months of the diagnosis of TMA/TAM. Death may result directly from the sequelae of TMA/TAM such as acute renal failure, cerebral ischemia, and hemorrhage, or indirectly from commonly associated disorders such as GVHD, infection, and VOD/SOS.

Clinical parameters associated with poor survival in TMA/TAM include: age ≥18, unrelated or haploidentical donor, high LDH, high schistocyte count (>5-10/hpf), and presence of nephropathy. Although the literature suggests a number of factors that predict worse survival in patients with TMA/TAM, there is insufficient data to support any staging system for this condition at this time. Until such a system is in place, adverse vent reporting of TMA/TAM in HSCT clinical trials should follow the guidelines specific to the clinical protocol, or default to the NCI Common Terminology Criteria for Adverse Events v3.0 (Table 2) for determination of severity.

Table 2. *NCI Common Terminology Criteria for Adverse Events (CTC AE) for TMA*

Thrombotic Microangiopathy*	Version 2.0	Version 3.0
Grade 1	Evidence of RBC destruction (schistocytosis) without clinical consequences	Evidence of RBC destruction (schistocytosis) without clinical consequences
Grade 2	Evidence of RBC destruction with elevated creatinine ≤3× upper limit of normal (ULN)	—
Grade 3	Evidence of RBC destruction with creatinine >3× ULN not requiring dialysis	Laboratory findings present with clinical consequences (eg, renal insufficiency, petechiae)
Grade 4	Evidence of RBC destruction with renal failure requiring dialysis, and/or encephalopathy	Laboratory findings and life-threatening or disabling consequences (eg, CNS hemorrhage/ bleeding or thrombosis/ embolism or renal failure)

*Must have microangiopathic changes on blood smear (eg, schistocytes, helmet cells, red cell fragments).

Management of TMA/TAM

There is currently no standard treatment for TMA/TAM following HSCT. There is, however, a general consensus that once a diagnosis of TMA/TAM is established, withdrawal of potential offending drugs such as calcineurin inhibitors or Sirolimus should be the primary intervention. In patients who have or are at risk for GVHD, replacement of these drugs with alternative immune suppressive agents are necessary to mitigate GVHD exacerbation and further endothelial damage from the GVH reaction. Aggressive management of concurrent GVHD and infections is crucial because these are common causes of mortality in patients with TMA/TAM. Despite its relative ineffectiveness, many centers continue to include plasma exchange (PE) in their management algorithm for patients with TMA/TAM, perhaps as a testament to the

lack of other viable treatments available. The fact that TMA/TAM results from direct injury to ECs, and not from circulating antibody, may explain the low response rates to PE in this disease compared to classic TTP; response rates for HSCT recipients with TMA/TAM are generally less than 50%, and mortality rates among patients treated with PE remain unacceptably high [38].

As mentioned above, DF, a polydisperse oligonucleotide that has protective effects on vascular endothelium, has shown great promise in clinical trials as a treatment for patients with hepatic VOD/SOS. Limited data exist currently for the use of DF in patients with TMA/TAM after HSCT. Corti and colleagues [42] have reported a series of 12 allogeneic HSCT patients treated with DF after the diagnosis of TMA/TAM. Five patients had a complete response and 3 had a partial response. Complete responders included 3 of 10 patients who had "severe" disease, based on the presence of neuropathy, nephropathy, gastrointestinal hemorrhage, or multiorgan failure. Further investigation into the use of DF for patients with TMA/TAM is indicated. The humanized monoclonal antibody directed against CD20, rituximab, has been reported in several small case series to have activity in patients with relapsing or refractory TTP and HUS [43-45], and Au and colleagues [46] recently reported on 5 patients with TMA/TAM refractory to plasma exchange and steroids who were treated with rituximab (375 mg/m^2 twice a week $\times$ 4). Four of 5 patients achieved a CR, and 3 remained in remission with extended follow-up.

SUMMARY

TMA/TAM is an underrecognized, but often devastating complication of HSCT especially in the allogeneic setting because of its close association with calcineurin inhibitors, GVHD, and infections. Diagnosis of TMA/TAM is based on clinical criteria. The recently proposed IWG and CTN definitions need further validation, but should improve uniformity in diagnosis and reporting of TMA/TAM across transplant centers, and should facilitate future clinical trials. Withdrawal of offending agents such as calcineurin inhibitors, supportive care, and aggressive management of concurrent GVHD or infections, remain the standard treatment for patients with TMA/TAM. Plasma exchange has demonstrated limited efficacy, and has not been endorsed as a standard treatment. Measurement of ADAMTS13 activity or testing for ADMATS13 inhibitors is not warranted during TMA/TAM following HSCT because these levels do not correlate with onset, severity, or response of therapy in this disease. Based on its protective activity on microvascular endothelium and efficacy in hepatic VOD/SOS, DF should be a promising agent to investigate both as treatment and prophylaxis.

CONCLUSIONS

Over the last 25 years, the use of HSCT as a curative treatment option for patients with hematologic and neoplastic diseases has steadily increased. Despite major advances in transplant immunology and improvements in supportive and critical care medicine, HSCT is still plagued by several life-threatening complications. Specifically, the development of GVHD, IPS, VOD/SOS, and TMA/TAM significantly limit the ultimate success of HSCT, particularly in the allogeneic setting when full-intensity conditioning is administered. The establishment of effective therapeutic options for these complications will be crucial as increasing numbers of high-risk transplants are performed every year. Further research into mechanisms of injury incurred following HSCT should improve our understanding of these disease processes.

To this end, clinical and preclinical data suggest that injury to the vascular endothelium may contribute to both the initiation and propagation of transplant-related complications, and demonstrate that EC apoptosis mediated by TNF-α and Fas-FasL is linked to the pathogenesis of IPS and GVHD in experimental models. Whether EC damage is a prerequisite for GVHD, IPS, VOD/SOS, and TMA or develops as a consequence of the cellular and inflammatory response underlying these disorders remains to be fully elucidated. It is likely, however, that EC damage contributes to progressive disease in each setting, and therefore conceivable that strategies that maintain EC integrity may be effective at preventing or treating each form of toxicity.

Combinatorial approaches aimed at identifying patients at risk, minimizing conditioning-related injury, and reducing the toxicity of HSCT without compromising graft-versus-leukemia activity or further suppressing donor immunologic reconstitution, hold promise in making allogeneic HSCT a more safe and feasible option for a greater number of patients. Success in these endeavors will require collaborative efforts between basic laboratory programs and large transplant centers to conduct robust prospective studies, with collection of tissue and blood samples for translational research to identify the mechanisms of endothelial injury crucial in the pathogenesis of these feared transplant complications. Fortunately for our HSCT patients, such trials are currently underway!

ACKNOWLEDGMENTS

Dr. Cooke is an Amy Strelzer-Manasevit Scholar of the National Marrow Donor Program, a Leukemia and Lymphoma Society Scholar in Clinical Research, and the recipient of a Clinical Scientist Award in

Translational Research from the Burroughs Wellcome Fund.

REFERENCES

1. Richard S, Seigneur M, Blann A, et al. Vascular endothelial lesions in patients undergoing bone marrow transplantation. *Bone Marrow Transplant*. 1996;18:955-959.
2. Janin A, Deschaumes C, Daneshpouy M, et al. CD95 engagement induces disseminated endothelial cell apoptosis in vivo: immunopathologic implications. *Blood*. 2002;99:2940-2947.
3. Salat C, Holler E, Kolb HJ, Pihusch B, Reinhardt B, Hiller E. Endothelial cell markers in bone marrow transplant recipients with and without acute graft-versus-host disease. *Bone Marrow Transplant*. 1997;19:909-914.
4. Testa S, Manna A, Porcellini A, et al. Increased plasma level of vascular endothelial glycoprotein thrombomodulin as an early indicator of endothelial damage in bone marrow transplantation. *Bone Marrow Transplant*. 1996;18:383.
5. Salat C, Holler E, Kolb HJ, Reinhardt B, Pihusch R. Plasminogen activator inhibitor-1 confirms the diagnosis of hepatic venoocclusive disease in patients with hyperbilirubinemia after bone marrow transplantation. *Blood*. 1997;89:2184-2188.
6. Holler E, Kolb HJ, Hiller E, et al. Microangiopathy in patients on cyclosporine prophylaxis who developed acute graft-versus-host disease after HLA-identical bone marrow transplantation. *Blood*. 1989;73:2018-2024.
7. Eissner G, Lindner H, Behrends U, et al. Influence of bacterial endotoxin on radiation-induced activation of human endothelial cells in vitro and in vivo. Protective role of IL-10. *Transplantation*. 1996;62:819-827.
8. Robaye B, Mosselmans R, Fiers W, Dumont J, Galand P. Tumor necrosis factor induces apoptosis (programmed cell death) in normal endothelial cells in vitro. *Am J Pathol*. 1991;138:447.
9. Gerbitz A, Nickoloff BJ, Olkiewicz K, et al. A role for tumor necrosis factor-alpha-mediated endothelial apoptosis in the development of experimental idiopathic pneumonia syndrome. *Transplantation*. 2004;78:494-502.
10. Clark J, Hansen J, Hertz M, Parkman R, Jensen L, Peavy H. Idiopathic pneumonia syndrome after bone marrow transplantation. *Am Rev Respir Dis*. 1993;147:1601-1606.
11. Kantrow SP, Hackman RC, Boeckh M, Myerson D, Crawford SW. Idiopathic pneumonia syndrome: changing spectrum of lung injury after marrow transplantation. *Transplantation*. 1997;63:1079-1086.
12. Yanik G, Hellerstedt B, Custer J, et al. Etanercept (Enbrel) administration for idiopathic pneumonia syndrome after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2002;8:395-400.
13. Afessa B, Litzow MR, Tefferi A. Bronchiolitis obliterans and other late onset non-infectious pulmonary complications in hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2001;28:425-434.
14. Crawford S, Longton G, Storb R. Acute graft versus host disease and the risks for idiopathic pneumonia after marrow transplantation for severe aplastic anemia. *Bone Marrow Transplant*. 1993; 12:225.
15. Fukuda T, Hackman RC, Guthrie KA, et al. Risks and outcomes of idiopathic pneumonia syndrome after nonmyeloablative and conventional conditioning regimens for allogeneic hematopoietic stem cell transplantation. *Blood*. 2003;102:2777-2785.
16. Shankar G, Cohen DA. Idiopathic pneumonia syndrome after bone marrow transplantation: the role of pre-transplant radiation conditioning and local cytokine dysregulation in promoting lung inflammation and fibrosis. *Int J Exp Pathol*. 2001;82: 101-113.
17. Cooke KR, Kobzik L, Martin TR, et al. An experimental model of idiopathic pneumonia syndrome after bone marrow transplantation. I. The roles of minor H antigens and endotoxin. *Blood*. 1996;88:3230-3239.
18. Panoskaltsis-Mortari A, Taylor PA, Yaegar TM, et al. The critical early proinflammatory events associated with idiopathic pneumonia syndrome in irradiated murine allogenic recipients are due to donor T cell infusion and potentiated by cyclophosphamide. *J Clinl Invest*. 1997;100:1015-1027.
19. Hildebrandt GC, Olkiewicz KM, Corrion LA, et al. Donor-derived TNF-alpha regulates pulmonary chemokine expression and the development of idiopathic pneumonia syndrome after allogeneic bone marrow transplantation. *Blood*. 2004;104: 586-593.
20. Cooke KR, Kobzik L, Teshima T, Lowler K, Clouthier S, Ferrara JLM. A role for Fas-Fas ligand but not perforin mediated cytolysis in the development of experimental idiopathic pneumonia syndrome. *Blood*. 2000;96(Suppl 1):768a.
21. Srivastava A, Gottlieb D, Bradstock K. Diffuse alveolar hemorrhage associated with microangiopathy after allogeneic bone marrow transplantation. *Bone Marrow Transplant*. 1995;15:863.
22. Parikh SM, Mammoto T, Schultz A, et al. Excess circulating angiopoietin-2 may contribute to pulmonary vascular leak in sepsis in humans. *PLoS Med*. 2006;3:e46.
23. Yanik GA, Levine JE, Ferrara JLM, et al. Survival following etanercept therapy for the treatment of idiopathic pneumonia syndrome following allogeneic stem cell transplantation. *Blood*. 2004;104:104a.
24. Joucher F, Mazmanian GM, German-Fattal M. Endothelial cell early activation induced by allogeneic lymphocytes in isolated perfused mouse lung. *Transplantation*. 2002;74:1461-1469.
25. Goon PK, Lip GY, Boos CJ, Stonelake PS, Blann AD. Circulating endothelial cells, endothelial progenitor cells, and endothelial microparticles in cancer. *Neoplasia*. 2006;8:79-88.
26. Pihusch V, Rank A, Steber R, et al. Endothelial cell-derived microparticles in allogeneic hematopoietic stem cell recipients. *Transplantation*. 2006;81:1405-1409.
27. Nachbaur D, Schumacher P, Auberger J, Clausen J, Kircher B. Vascular endothelial growth factor and activin-a serum levels following allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2007;13:942-947.
28. Min CK, Kim SY, Lee MJ, et al. Vascular endothelial growth factor (VEGF) is associated with reduced severity of acute graft-versus-host disease and nonrelapse mortality after allogeneic stem cell transplantation. *Bone Marrow Transplant*. 2006; 38:149-156.
29. Richardson P, Krishnan A, Wheeler C, et al. Elevation of plasminogen activator inhibitor-1 [PAI-1] levels in BMT-associated veno-occlusive disease [VOD] and changes seen with the use of defibrotide [DF]. *Blood*. 1996;88:1821.
30. Bianchi G, Barone D, Lanzarotti E, et al. Defibrotide, a single-stranded polydeoxyribonucleotide acting as an adenosine receptor agonist. *Eur J Pharmacol*. 1993;238:327-334.
31. Eissner G, Multhoff G, Gerbitz A, et al. Fludarabine induces apoptosis, activation, and allogenicity in human endothelial and epithelial cells: protective effect of defibrotide. *Blood*. 2002; 100:334-340.

32. Richardson PG, Soiffer R, Antin JH, et al. Defibrotide (DF) appears effective and safe in a phase II, randomized study of patients (pts) with severe veno-occlusive disease (VOD) and multi-system organ failure (MOF) post stem cell transplantation (SCT). *Blood*. 2002;100:112A.
33. Fuge R, Bird JM, Fraser A, et al. The clinical features, risk factors and outcome of thrombotic thrombocytopenic purpura occurring after bone marrow transplantation. *Br J Haematol*. 2001; 113:58-64.
34. George JN, Li X, McMinn JR, Terrell DR, Vesely SK, Selby GB. Thrombotic thrombocytopenic purpura-hemolytic uremic syndrome following allogeneic HPC transplantation: a diagnostic dilemma. *Transfusion*. 2004;44:294-304.
35. Ruutu T, Barosi G, Benjamin RJ, et al. Diagnostic criteria for hematopoietic stem cell transplant-associated microangiopathy: results of a consensus process by an International Working Group. *Haematologica*. 2007;92:95-100.
36. Kersting S, Verdonck LF. Stem cell transplantation nephropathy: a report of six cases. *Biol Blood Marrow Transplant*. 2007; 13:638-643.
37. Nishida T, Hamaguchi M, Hirabayashi N, et al. Intestinal thrombotic microangiopathy after allogeneic bone marrow transplantation: a clinical imitator of acute enteric graft-versus-host disease. *Bone Marrow Transplant*. 2004;33:1143-1150.
38. Ho VT, Cutler C, Carter S, et al. Blood and marrow transplant clinical trials network toxicity committee consensus summary: thrombotic microangiopathy after hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2005;11:571-575.
39. Cohen H, Bull HA, Seddon A, et al. Vascular endothelial cell function and ultrastructure in thrombotic microangiopathy following allogeneic bone marrow transplantation. *Eur J Haematol*. 1989;43:207-214.
40. Cutler C, Henry NL, Magee C, et al. Sirolimus and thrombotic microangiopathy after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2005;11: 551-557.
41. Oran B, Donato M, Aleman A, et al. Transplant-associated microangiopathy in patients receiving tacrolimus following allogeneic stem cell transplantation: risk factors and response to treatment. *Biol Blood Marrow Transplant*. 2007;13:469-477.
42. Corti P, Uderzo C, Tagliabue A, et al. Defibrotide as a promising treatment for thrombotic thrombocytopenic purpura in patients undergoing bone marrow transplantation. *Bone Marrow Transplant*. 2002;29:542-543.
43. Kameda T, Dobashi H, Kittaka K, et al. Two cases of refractory thrombotic thrombocytopenic purpura associated with collagen vascular disease were significantly improved by rituximab treatment. *Clin Rheumatol*. 2007 (in press).
44. Heidel F, Lipka DB, von Auer C, Huber C, Scharrer I, Hess G. Addition of rituximab to standard therapy improves response rate and progression-free survival in relapsed or refractory thrombotic thrombocytopenic purpura and autoimmune haemolytic anaemia. *Thromb Haemost*. 2007;97:228-233.
45. Patino W, Sarode R. Successful repeat therapy with rituximab for relapsed thrombotic thrombocytopenic purpura. *J Clin Apher*. 2007;22:17-20.
46. Au WY, Ma ES, Lee TL, et al. Successful treatment of thrombotic microangiopathy after haematopoietic stem cell transplantation with rituximab. *Br J Haematol*. 2007;137: 475-478.