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Circulating Angiogenic Factors Associated with Response and Survival in Patients with Acute Graft-versus-Host Disease: Results from Blood and Marrow Transplant Clinical Trials Network 0302 and 0802

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A B S T R A C T

Circulating angiogenic factors (AF) reflect tissue healing capacity, although some AF can also contribute to inflammation and are indicative of endothelial dysfunction. The AF milieu in acute graft-versus-host disease (aGVHD) has not been broadly characterized. We hypothesized that patients with abundant AF involved in repair/regeneration versus those mediating damage/inflammation would have improved outcomes. Circulating AF known predominantly for repair/regeneration (epidermal growth factor [EGF], fibroblast growth factor-1 and -2, heparin binding–EGF–like growth factor, and vascular endothelial growth factor-A [VEGF-A], -C, and -D) and for damage/inflammation (angiopoietin-2, endothelin-1, soluble endoglin [sEng], follistatin [FS], leptin, and placental growth factor [PlGF]) were measured in a discovery set of hematopoietic cell recipients with grade III and IV aGVHD and compared with controls, then validated in 2 aGVHD cohorts enrolled in Blood and Marrow Transplant Clinical Trials Network (BMT CTN) trials 0302 (n = 105, serum) and 0802 (n = 158, plasma) versus controls without aGVHD (n = 53, serum). Levels of EGF and VEGF-A were lower than in controls at the onset of aGVHD in both trials and higher with complete response to first-line aGVHD therapy in CTN 0802. FS and PlGF were elevated in aGVHD measured in either serum or plasma. At day 28 after initial aGVHD therapy, elevated FS was an independent negative prognostic factor for survival in both cohorts (hazard ratio, 9.3 in CTN 0302; 2.8 in CTN 0802). These data suggest that circulating AF are associated with clinical outcomes after aGVHD and, thus, may contribute to both pathogenesis and recovery.

BACKGROUND

In the United States, nearly 7000 patients undergo allogeneic hematopoietic cell transplantation (HCT) annually in an effort to cure hematologic malignancies and other bone marrow disorders. Approximately 50% of these patients will experience acute graft-versus-host disease (aGVHD), a complication in which cells from the immunocompetent donor graft attack the recipient's organs and tissues [1]. Only one half of aGVHD patients achieve a durable response to first-line therapy with corticosteroids [2] and many others develop severe infections resulting from the immunocompromised state and impairment of skin and mucosal barrier integrity. As a result, many patients with severe aGVHD are at significant risk of death [3]. Identification of novel treatment approaches that can alleviate inflammation, spare infectious immunity, and promote healing is critical for improving patient outcomes.

A host vascular proliferative response accompanying aGVHD was first described by Brent and Medawar in the

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1960s [4], although it was not clear whether the angiogenic response in aGVHD could improve outcomes by facilitating healing or if it was detrimental to outcomes by contributing to inflammation. Recently, mechanisms underlying this critical observation have been suggested using rodent models. Specifically, vasculogenesis accompanies aGVHD with a concomitant increase in alpha-v integrin expression on endothelial cells in tissues targeted by aGVHD, a pathologic reaction that can be inhibited by a negative regulator of neovascularization, micro RNA-100, and by the alpha-v integrin inhibitor cilengitide [5,6]. Despite these advances, tools to therapeutically target neovascularization in human aGVHD are lacking. Recent literature suggests that angiogenic factors (AF), soluble mediators that support the development of new blood vessels, may contribute to favorable outcomes by providing trophic factors for wound healing after injury. This principle has been demonstrated in the autoimmune inflammatory bowel disease (IBD) setting with epidermal growth factor (EGF), an epithelial and endothelial mitogen that enhances angiogenic responses in tissues [7,8]. In IBD, circulating EGF levels have been shown to be low [9], and supplementation has induced remission in a randomized trial [10].

Studies of such AF capable of repairing host tissues and their associations with aGVHD outcomes are emerging. Patients with single nucleotide polymorphisms associated with increased production of vascular endothelial growth factor (VEGF) have a reduced incidence of grade II to IV aGVHD, including gastrointestinal aGVHD [11]. In addition, single nucleotide polymorphisms within the gene encoding thrombomodulin, a constitutively expressed endothelial factor that enhances angiogenesis [12], are associated with survival after onset of aGVHD [13]. These genetic studies suggest that variations in the patient's capacity to repair tissues after both the damage from the conditioning regimen and at the onset of aGVHD are clinically relevant. However, not all reports have consistently shown a beneficial role for AF in aGVHD. High circulating levels of VEGF have been shown to be protective in some aGVHD studies [14,15] but not in others [16,17]. It is possible that other factors contributing to the angiogenic milieu may be responsible for the discrepant results. For example, angiopoietin-2 (Ang2) is an AF that can have either proangiogenic or antiangiogenic function, depending upon the context [18]. In allogeneic HCT, elevated levels of Ang2 are reported to reflect endothelial cell activation/dysfunction and are associated with a poor prognosis in aGVHD [17,19,20].

Given these disparate associations of AF with outcomes in aGVHD, we sought to more broadly characterize the angiogenic milieu in aGVHD and determine the association of individual factors with clinical outcomes. Furthermore, we compared serum versus plasma levels of AF, because of the potential for variability contributed by factor release by activated platelets, a nontrivial matter for future studies of AF in human disease. Thirteen AF were first tested in a pilot study, followed by the validation of 6 AF using samples from 2 multicenter aGVHD treatment trials, Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 0302 [21], a randomized 4-arm phase II clinical trial for patients with newly diagnosed aGVHD where serum was collected, and BMT CTN 0802 [22], a randomized phase III study of the addition of mycophenolate mofetil versus placebo to corticosteroids in patients with newly diagnosed aGVHD, where plasma was collected. Overall, we hypothesized that patients with abundant circulating AF involved in repair/regeneration would have improved outcomes compared with those with higher levels of AF involved in damage/inflammation.

PATIENTS AND METHODS

Discovery Cohort

We measured circulating levels of EGF, fibroblast growth factor (FGF)-1, FGF-2, heparin binding–EGF–like growth factor, VEGF-A, -C, and -D, Ang2, endothelin-1, endoglin (sEng), follistatin (FS), leptin, and placental growth factor (PlGF) in HCT recipients at onset of grade III and IV aGVHD (patient discovery set, n = 17) and compared them with recipients without aGVHD at 3 months after HCT (n = 17) and healthy stem cell donors (n = 16). Therefore, samples from both HCT patients without aGVHD and healthy donors were tested as controls. Samples were analyzed by MILLIPLEX (Millipore, Billerica, MA) magnetic bead array and performed in duplicate. For the pilot study, plasma samples were collected in sodium heparin tubes, except for 13 of the aGVHD specimens, which were serum. The mean coefficient of variation for the analytes ranged from 1.9% to 7.5%.

Validation Cohorts

AF demonstrating statistically significant <.5-fold or >1.5-fold differences from controls in the pilot study were selected for validation in samples from 2 multicenter treatment trials for initial therapy of aGVHD: BMT CTN 0302 (n = 105) and BMT CTN 0802 (n = 158). Serum (0302) or plasma (0802) samples obtained at aGVHD onset and at day 28 after GVHD treatment were selected for study. All patients with available aGVHD onset and day 28 post-aGVHD therapy samples were included in this study. Samples from aGVHD onset in these 2 trials were further compared with serum samples from a control cohort of HCT patients without acute or chronic GVHD (n = 53). These control samples were collected 3 months after HCT in University of Minnesota allogeneic HCT recipients, a time point consistent with the pilot study. Samples were acquired after obtaining informed consent and approval from the University of Minnesota institutional review board and in accordance with the Declaration of Helsinki.

Statistical Analysis

Statistical comparisons across categorical variables were completed using chi-square tests. Differences in continuous variables across categories were completed using Kruskal-Wallis tests for nonparametric data [23]. Differences between AF levels in onset and day 28 samples were determined using Wilcoxon signed rank tests [24]. Kaplan-Meier estimates were used to determine the probability of 2-year survival, with differences between curves determined using log-rank tests [25]. Cox regression was used to determine the independent effects of clinical factors and angiogenic biomarkers on 6-month and overall survival [26]. Correlations between continuous variables were determined by Spearman's rank correlation [27]. Receiver operating characteristic (ROC) curves were generated to determine the value of the tested AF in discriminating aGVHD from no aGVHD. Statistical analyses were performed using JMP 10.0.0 (SAS Institute, Cary, NC).

RESULTS

Discovery Set

In the discovery cohort, levels of multiple AF differed at aGVHD onset compared with levels for controls (Figure 1). Specifically, among the repair and regeneration factors, the median EGF level was 10-fold lower in aGVHD patients compared with those of allogeneic HCT patients without aGVHD. Similar to EGF, the median VEGF-A level was 5-fold lower in patients with aGVHD compared with that of control allogeneic HCT patients and 1.5-fold higher than normal donors (overall *P* value of .07). Based on these data and its importance in angiogenesis, VEGF-A was retained for validation studies. Four AF associated with damage/inflammation were significantly elevated in aGVHD patients compared with the levels in healthy donors: Ang2, sEng, FS, and PlGF. Too few patients had detectable levels of endothelin-1, FGF-1, and FGF-2 for statistical comparison (not shown). Using the pilot study data, 6 AF were selected for validation: FS, EGF, VEGF-A, Ang2, sEng, and PlGF.

Validation Cohorts

Characteristics of the patients in the validation cohorts are detailed in Table 1. The median age was 6 to 8 years