Biol Blood Marrow Transplant 20 (2014) 1056–1073

Brief Articles

Relative Defects in Mucosal Immunity Predict Acute Graft-Versus-Host Disease

Keith J. August [1], K.-Y. Chiang [2], Muna Qayed [2], Ashley Dulson [2], Diana Worthington-White [2], Conrad R. Cole [3], John T. Horan [2,*]

[1] Division of Pediatric Hematology-Oncology, Children's Mercy Hospitals and Clinics, Kansas City, Missouri
[2] Division of Pediatric Hematology-Oncology, Aflac Cancer and Blood Disorders Center, Emory University/Children's Healthcare of Atlanta, Atlanta, Georgia
[3] Division of Pediatric Gastroenterology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

Article history:
Received 8 January 2014
Accepted 11 March 2014

Key Words:
Allogeneic hematopoietic cell transplantation
Graft-versus-host disease
Gut mucosal immunity
Fecal biomarkers

A B S T R A C T

Impairment of gut mucosal immunity by the transplant process could facilitate translocation of commensal bacteria and thereby augment the graft-versus-host response. To begin to assess the influence of gut mucosal immunity on the development of acute graft-versus-host disease (GVHD), we conducted a prospective study in 24 pediatric allogeneic hematopoietic cell transplant recipients, assessing 4 fecal markers of mucosal immunity: calprotectin, soluble CD8 (sCD8), soluble intracellular adhesion molecule 1, and β-defensin-2. Stool samples were collected prospectively on transplant days 0, +5, +10, and +15 and analyzed by ELISA. Lower levels on day +5 (calprotectin and β-defensin-2) and day +10 (calprotectin, β-defensin-2, and sCD8) were associated with subsequent acute GVHD. The most striking difference was with calprotectin on day +10. Patients with levels below 424 mg/kg had an incidence of 77.8%, whereas those with levels above this threshold had a cumulative incidence of 0% ($P = .002$). Relative defects in gut mucosal immunity may be important in the pathogenesis of acute GVHD.

INTRODUCTION

The gut and its flora are central to the pathogenesis of acute graft-versus-host disease (aGVHD) [1]. In the triphasic model of aGVHD conceptualized by Ferrara and Antin [2], the process is initiated (phase I) by damage to the mucosal barrier induced by conditioning, permitting translocation of commensal bacteria and stimulating, in turn, inflammation. This inflammation primes and amplifies the host-targeted response of donor T cells.

It is possible, however, that the bacterial translocation, which is central to this model, is not simply the result of the disruption of the physical barrier. The weakening of mucosal immunity by the transplant process could also be important. The gut's mucosal immunity is broadly compromised during hematopoietic stem cell transplantation (HSCT); host neutrophils and intraepithelial lymphocytes, important weapons against microbial invasion of the mucosa, are both lost through the effects of conditioning [3,4]; damage to the epithelial cells, which not only provide a physical barrier but also direct the transmigration of leukocytes to the luminal surface and secrete antimicrobial peptides, also occurs [5]. In this way, the pathogenesis of aGVHD could resemble the pathogenesis of Crohn's disease, where more limited defects in mucosal immunity appear to be important [6].

The role of defective mucosal immunity in the pathogenesis of aGVHD could be assessed in a preliminary and noninvasive way by using fecal biomarkers. It might also be possible to exploit any differences in fecal biomarker levels observed to be associated with the development of aGVHD to devise an approach for detecting aGVHD before clinical manifestations emerge. Fecal markers have been established as valuable tools in other settings. Clinically, they are used in the management of a variety of gastrointestinal illnesses, including inflammatory bowel disease [7] and pancreatic insufficiency [8]. They are also being used increasingly as research tools for a variety of diseases, including colon cancer [9]. The potential of fecal biomarkers in aGVHD is beginning to be assessed, with 3 groups recently reporting results of research using markers for diagnosing aGVHD and for predicting its response to therapy [10-12].

We conducted a prospective, longitudinal study in pediatric allogeneic HSCT recipients. We assessed 4 fecal markers: calprotectin, soluble CD8 (sCD8), soluble intracellular adhesion molecule 1 (sICAM-1), and β–defensin-2. Calprotectin, an antimicrobial protein released from neutrophils [13], has been extensively studied in inflammatory bowel disease and is now routinely used as a diagnostic test in this setting [14]. β-Defensin-2, an antimicrobial peptide secreted by gut mucosal epithelial cells [15], is now also being examined in inflammatory bowel disease [16]. CD8 is released from the surface of activated CD8 + T cells [17], which form the majority of intraepithelial lymphocytes [18]. Although not previously used as a stool marker before, we

Financial disclosure: See Acknowledgments on page 1059.

* Correspondence and reprint requests: John T. Horan, MD, MPH, Hematology, Oncology and Stem Cell Transplantation, Morgan Stanley Children's Hospital of New York-Presbyterian, Columbia University, 3959 Broadway, CHN 10-03, New York, NY 10032.
E-mail address: jth2148@columbia.edu (J.T. Horan).

1083-8791/$ – see front matter © 2014 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2014.03.012

have shown that early post-transplant elevations in plasma sCD8 level predict aGVHD [19]. sICAM-1 is released from the surface of epithelial cells (as well as endothelial cells) during inflammation [20]. ICAM-1 is expressed on the luminal surface of gut epithelium, where it assists in the transmigration of neutrophils [21]. Although it also has not previously been used as a stool marker, elevations in its plasma levels have been associated with aGVHD [22].

METHODS

Subjects

The institutional review boards of Emory University School of Medicine and Children's Healthcare of Atlanta approved the study protocol. Patients under age 21 years undergoing an allogeneic HSCT at Children's Healthcare of Atlanta for a malignant or nonmalignant disease were eligible. Patients with a condition deemed to have the potential to raise marker levels, such as inflammatory bowel disease or other pre-existing gastrointestinal disease, active infection, treatment-unresponsive hemophagocytic lymphohistiocytosis, treatment-unresponsive hematologic malignancy, or high risk for graft rejection, were ineligible. Midway through the study, in an effort to capture a sufficient number of patients developing aGVHD, enrollment was restricted to those receiving myeloablative conditioning and alternative donor transplants. Patients were followed for aGVHD [23] and infection through day +100.

Biomarkers

Stool samples were collected prospectively on transplant days 0, +5, +10, and +15. The stool samples were analyzed using commercially available ELISA kits for calprotectin (Buhlmann Laboratories, Schönenbuch, Switzerland), β-defensin-2 (Immundiagnostik, Bensheim, Germany), sCD8 (Bender MedSystems, Vienna, Austria), and sICAM-1 (R&D Systems, Minneapolis, MN). Solution was extracted from the stool samples, using a commercial kit (Buhlmann Laboratories), and then aliquoted and stored at −80°C. All testing was performed according to manufacturers' specifications.

Statistical Analysis

Cut-offs in biomarker levels were determined using recursive partitioning. The cumulative incidences of aGVHD for patients with levels above and below the thresholds were compared using the log rank test. The potential screening test characteristics of the markers were assessed using a receiver-operating characteristic curve analysis. An area under a receiver-operating characteristic curve was considered statistically significant if the confidence intervals around the estimate did not include .5, the value expected for a nondiscriminating test. Statistical analyses were performed using SPSS version 18 (SPSS Inc., Chicago, IL).

RESULTS

Twenty-four patients were enrolled between November 2008 and October 2010 (Table 1). All patients survived through at least day +30 and could be assessed for aGVHD. The median patient age was 10 years (range, 1 to 23 years), with 16 boys and 8 girls. Seventeen patients received an unrelated or mismatched related transplant, and 20 patients were transplanted for an acute leukemia or myelodysplastic syndrome. The rest had nonmalignant diseases. Thirteen patients received marrow grafts, and the rest received single or double cord blood grafts. All but 1 patient received myeloablative conditioning. All patients received T cell–replete grafts with calcineurin inhibitor–based GVHD prophylaxis.

aGVHD of any grade was diagnosed in 10 patients. The median day of diagnosis was day +25 (range, 19 to 49) in 10 patients. All but 1 patient had biopsies to confirm their diagnoses. The maximal grade of aGVHD attained by day +100 was grade I in 1 patient, grade II in 5 patients, grade III in 2 patients, and grade IV in 2 patients. Two patients had disease that was initially steroid-refractory and a third had a steroid-refractory recurrence. All 3 of these patients died. Seven patients had gastrointestinal involvement, and all but 1 had involvement of the lower tract. One patient with steroid-refractory disease was diagnosed with cytomegalovirus enteritis by endoscopic biopsy at day +72. None of the other patients was diagnosed with gastrointestinal infections.

Table 1
Patient Characteristics (N = 24)

Characteristic	Value
Median age, yr	10
Sex	
Female	8 (33.3%)
Male	16
Disease	
Acute lymphoblastic leukemia	7 (29.2%)
Acute myeloid leukemia/MDS	11 (45.8%)
Mixed phenotype acute leukemia	2 (8.3%)
Nonmalignant	4 (16.7%)
Conditioning	
Myeloablative	23 (95.8%)
Reduced intensity	1
Transplant	
HLA matched related marrow	7 (29.2%)
Mismatched related marrow	1 (4.2%)
Unrelated marrow	6 (25%)
Unrelated cord blood, single	6 (25%)
Unrelated cord blood, double	4 (16.7%)
aGVHD	10 (40%)
Grade I	1
Grade II	5
Grade III	2
Grade IV	2
Gastrointestinal involvement	7

MDS indicates myelodysplastic syndrome.

For calprotectin and β-defensin-2, levels below the threshold on day +5 and day +10 were associated with a higher cumulative incidence of aGVHD (Table 2). For sCD8, levels above the threshold on day 0 and below the threshold day +10 were associated with aGVHD. For sICAM-1, none of the results was statistically significant. There was, however, a trend on day 0 with levels below the threshold associated with aGVHD. The most striking difference was with calprotectin on day +10. Patients with levels below 424 mg/kg had an incidence of 77.8%, whereas those with levels above this threshold had a cumulative incidence of 0% ($P = .002$) (Figure 1). The median calprotectin levels at this juncture were 188 mg/kg (range, 64 to 294 mg/kg) in patients who went on to develop GVHD and 655 mg/kg (range, 147 to 1953 mg/kg) in those who did not.

The receiver-operating characteristic curve analysis yielded statistically significant results for calprotectin and β-defensin-2 (Table 2). The highest area under the curve was obtained for calprotectin on day +10 (.89, 95% confidence interval, .72 to 1). The corresponding sensitivity, specificity, positive predictive value, and negative predictive value were 1, .8, .78 and 1, respectively.

Although we did not consistently collect samples beyond day +15, we obtained samples in 6 patients at the time of diagnosis. We obtained a second sample in 1 of these 6, who initially had a complete response to steroids, during a severe and steroid-refractory recurrence. Finally, we obtained a sample in a seventh patient with steroid-refractory disease 10 days into treatment. These 8 calprotectin levels ranged from 102 to 4312 mg/kg (median, 568 mg/kg). The 3 highest levels, 2077, 2681, and 4312 mg/kg, were from the 3 samples obtained during steroid-refractory disease. The highest level obtained from a sample during steroid-responsive disease was 745 mg/kg. No such pattern was noted for the 3 other markers (data not shown).