Biol Blood Marrow Transplant 22 (2016) 418–422

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

ASBMT American Society for Blood and Marrow Transplantation

Biology: Biomarkers

A Genetic Modifier of the Gut Microbiome Influences the Risk of Graft-versus-Host Disease and Bacteremia After Hematopoietic Stem Cell Transplantation

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Article history:
Received 26 May 2015
Accepted 21 November 2015

Key words:
Fucosyltransferase 2
FUT2
Microbiome
Graft-versus-host disease
Bacteremia
Hematopoietic stem cell transplantation

A B S T R A C T

The human gut microbiome is involved in vital biological functions, such as maintenance of immune homeostasis and modulation of intestinal development and enhanced metabolic capabilities. Disturbances of the intestinal microbiota have been associated with development and progression of inflammatory conditions, including graft-versus-host disease (GVHD). The *fucosyltransferase 2 (FUT2)* gene produces an enzyme that is responsible for the synthesis of the H antigen in body fluids and on the intestinal mucosa. *FUT2* genotype has been shown to modify the gut microbiome. We hypothesized that *FUT2* genotype influences risk of GVHD and bacterial translocation after allogeneic hematopoietic stem cell transplantation (HSCT). *FUT2* genotype was determined in 150 consecutive patients receiving allogeneic HSCT at our center. We abstracted clinical characteristics and outcomes from the transplantation database. Cumulative risk of any acute GVHD varied by *FUT2* genotype, with decreased risk in those with A/A genotype and increased risk in those with G/G genotype. In contrast, the cumulative incidence of bacteremia was increased in those with A/A genotype. We conclude that the *FUT2* genotype influences risk of acute GVHD and bacteremia after HSCT. We hypothesize that the mechanisms involve altered intestinal surface glycosylation and microbial composition but this requires additional study.

INTRODUCTION

Recent studies have shown that intestinal inflammation secondary to graft-versus-host disease (GVHD) is associated with major shifts in the composition of the intestinal microbiota [1]. We hypothesized that *fucosyltransferase 2 (FUT2)* genotype, a genetic modifier of the gut microbiome, influences the risk of GVHD and bacterial translocation after allogeneic hematopoietic stem cell transplantation (HSCT).

The mucin layer overlying the intestinal epithelium has an important role as the first line of host defense and enables contact between the intestinal microbiota and the host [2-4]. In a healthy microbial community, there is an optimal balance of organisms that provide signals to the developing immune system, leading to a balance of Treg and TH17 cell activities [5]. The *FUT2* gene regulates expression of ABH blood group antigens in the mucin layer [6]. ABH mucosal antigens are carbohydrates that serve as a bacterial energy source [7] and as adhesion receptors for many microbes [8], and they play an important role in shaping the microbiota of the host [9-11]. A common nonsense mutation (428G > A) in the *FUT2* gene means that about 25% of Caucasians who have an AA genotype do not express ABH antigens in the mucin layer; these people are considered *nonsecretors* [12,13]. The potential importance of FUT2 genotype and disease is illustrated by large GWAS (genome-wide association study) that reported a significant association between risk of Crohn's disease, differences in the gut microbiome, and FUT2 nonsecretor status [14].

Our first hypothesis was that FUT2 genotype would modify risk of GVHD. GVHD is initiated by translocation of lipopolysaccharide (LPS) across the gastrointestinal lining disrupted by chemotherapy, radiation, and or infection [15]. Systemic LPS triggers secretion of proinflammatory cytokines, causing T cell attack and tissue damage typical of

Financial disclosure: See Acknowledgments on page 422.
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http://dx.doi.org/10.1016/j.bbmt.2015.11.017
1083-8791/

GVHD of the skin, gut, and liver. Recent murine data have demonstrated differential response to systemic LPS in secretor and nonsecretor mice, with reduced ability to recover from a systemic LPS challenge in the nonsecretor animals [16], leading us to expect that risk of GVHD would be increased in nonsecretor transplant recipients.

Our second hypothesis was that FUT2 genotype would modify risk of bacteremia. Many bacteria and fungi initiate infection by noncovalent binding to mucosal cell surface carbohydrate-binding proteins expressed by FUT2. Nonsecretor status has been associated with increased risk of a number of infections, including recurrent bacterial (pneumococcus, meningococcus, and haemophilus influenza) and fungal infections, including oral infections, urinary tract infections, and gastroduodenal disease [17-21]. In addition, in murine studies, LPS challenge led to significantly increased expression of microbial virulence genes in FUT2-negative but not FUT2-sufficient mice [16]. These data led us to hypothesize that the frequency of invasive bacterial infections would be increased in nonsecretor transplant recipients.

Our data confirm an increased risk of bacteremia in nonsecretor recipients, but in contrast to our expectations, risk of GVHD was reduced in nonsecretors.

METHODS

Patients and Transplantation Procedure

One hundred fifty consecutive patients receiving allogeneic HSCT at our center between 2010 and 2014 were included in this study. The study was approved by the institutional review board and all participants gave written informed consent. We abstracted clinical characteristics and outcomes from the transplantation database.

Study Population

Patient characteristics are summarized in Table 1. All patients received piperacillin/tazobactam as empiric antibiotic therapy with episodes of fever, and vancomycin was added with deterioration of clinical status.

Donor Selection

Donor characteristics are summarized in Table 1. High-resolution typing was performed for all donor-recipient pairs [22]. Among donors, 29% were matched sibling donors (n = 43), 49% matched unrelated or other family member donors (n = 74) and 22% were mismatched donors (n = 33).

Genotyping

Peripheral blood neutrophils were obtained before transplantation. Using the single nucleotide polymorphism rs601338 (428G > A), secretor status was genotyped. DNA was extracted from patient neutrophils with a Qiagen Gentra Puregene Blood Kit (Qiagen, Hilden, Germany), and the quantity and quality were analyzed on a NanoDrop 2000 Spectrophotometer. The DNA was amplified by PCR in a total reaction volume of 20 μL containing Taqman 2x PCR Master Mix (4304437; Applied Biosystems,

Table 1
Characteristics of Patients who Underwent Allogeneic HSCT

Characteristic	A/A	A/G	G/G	*P* Value
No. of patients	34 (23%)	78 (52%)	38 (25%)	
Age at transplantation, median (range), yr	5.27 (.3-29.78)	6.55 (.41-28.67)	6.61 (.72-32.79)	.54
Male sex	24 (70%)	49 (63%)	24 (63%)	.77
Race				N/A*
Caucasian	32 (94%)	69 (88%)	29 (76%)	
African American	2 (6%)	7 (9%)	6 (16%)	
Mixed	0	2 (3%)	1 (2.6%)	
Asian	0	0	1 (2.6%)	
Pacific Islander	0	0	1 (2.6%)	
Ethnicity				.38
Non-Hispanic	33 (97%)	75 (96%)	34 (89%)	
Hispanic	1 (3%)	3 (4%)	4 (11%)	
Diagnosis				N/A*
Hematologic malignancies	10 (29%)	20 (26%)	15 (40%)	
Immune deficiencies	11 (32%)	24 (30%)	8 (21%)	
BM failure syndromes	9 (26%)	17 (22%)	6 (16%)	
Hemophagocytic lymphohistiocytosis	2 (6%)	12 (15%)	4 (10%)	
Metabolic diseases	1 (3%)	2 (3%)	4 (10%)	
Hemoglobinopathies	0	3 (4%)	1 (3%)	
Evans syndrome	1 (3%)	0	0	
Conditioning regimen				.05
Myeloablative	16 (47%)	38 (49%)	27 (71%)	
Reduced intensity	18 (53%)	40 (51%)	11 (29%)	
Graft source				.36
BM	30 (88%)	63 (81%)	29 (76%)	
PBSC	3 (9%)	10 (13%)	3 (8%)	
Cord blood	1 (3%)	4 (5%)	6 (16%)	
Cord blood and BM	0	1 (1%)	0	
HLA match				.65
Matched sibling donor	11 (32%)	19 (24%)	13 (34%)	
Matched family or unrelated donor	15 (44%)	43 (55%)	16 (42%)	
Mismatched donor	8 (24%)	16 (21%)	9 (24%)	
Use of T cell antibodies	16 (47%)	36 (46%)	10 (26%)	.09
Sex mismatch	14 (41%)	36 (46%)	21 (55%)	.46
CMV status (donor/recipient)				.44
Neg/neg	3 (9%)	13 (17%)	9 (24%)	
Neg/pos	14 (41%)	23 (29%)	15 (39%)	
Pos/neg	2 (6%)	9 (17%)	2 (5%)	
Pos/pos	15 (44%)	33 (42%)	12 (32%)	
Malignant disease status				.35
CR1 or CR2	4 (40%)	12 (60%)	9 (60%)	
Other	6 (60%)	8 (40%)	6 (40%)	

Data presented are n (%), unless otherwise indicated.
NA indicates not available; BM, bone marrow; PBSC, peripheral blood stem cells; CMV, cytomegalovirus; neg, negative; pos, positive; CR, complete remission.
* Not applicable, small sample size per cell precludes computing of meaningful *P* value.