Brief report

Genetic variation in recipient B-cell activating factor modulates phenotype of GVHD

William B. Clark,[1] Kristin D. Brown-Gentry,[2] Dana C. Crawford,[2] Kang-Hsien Fan,[3] Jennifer Snavely,[1] Heidi Chen,[3] Bipin N. Savani,[1] Adetola Kassim,[1] John P. Greer,[1] Friedrich G. Schuening,[1] Brian G. Engelhardt,[1] and Madan H. Jagasia[1]

[1]Hematology and Stem Cell Transplantation Section, Division of Hematology/Oncology, Department of Medicine, [2]Center for Human Genetics Research, and [3]Department of Biostatistics, Vanderbilt University Medical Center, Nashville, TN

B-cell activating factor (BAFF) single nucleotide polymorphisms (SNPs) are associated with autoimmune diseases. Because patients with classic and overlap chronic GVHD (cGVHD) have features of autoimmune diseases, we studied the association of recipient and/or donor *BAFF* SNPs with the phenotype of GVHD after allogeneic stem cell transplantation. Twenty tagSNPs of the *BAFF* gene were genotyped in 164 recipient/donor pairs. GVHD after day 100 occurred in 124 (76%) patients: acute GVHD (aGVHD) subtypes (n = 23), overlap GVHD (n = 29), and classic cGVHD (n = 72). In SNP analyses, 9 of the 20 tag SNPs were significant comparing classic/overlap cGVHD versus aGVHD subtypes/no GVHD. In multivariate analyses, 4 recipient *BAFF* SNPs (rs16972217 [odds ratio = 2.72, *P* = .004], rs7993590 [odds ratio = 2.35, *P* = .011], rs12428930 [odds ratio2.53, *P* = .008], and rs2893321 [odds ratio = 2.48, *P* = .009]) were independent predictors of GVHD subtypes, adjusted for conventional predictors of cGVHD. This study shows that genetic variation of *BAFF* modulates GVHD phenotype after allogeneic stem cell transplantation. (*Blood*. 2011;118(4):1140-1144)

Introduction

Chronic GVHD (cGVHD) is a major cause of morbidity and mortality after allogeneic stem cell transplantation (allo-SCT).[1,2] The National Institutes of Health consensus criteria for classifying cGVHD have prognostic significance.[3-6] Retrospective studies have shown that patients with classic and overlap cGVHD have improved overall survival.[4,6] B-cell activating factor (BAFF) is a cytokine of the tumor necrosis factor family that plays an important role in both normal and post-SCT B-cell homeostasis.[7-9] Single nucleotide polymorphisms (SNPs) of *BAFF* have been associated with the development of autoimmune diseases.[10-15] Patients with classic and overlap cGVHD after allo-SCT present with phenotypes that mimic these naturally occurring autoimmune diseases.[1] Elevated BAFF to B-cell ratios at 6 months after allo-SCT can predict for the development of cGVHD, thus further linking B-cell homeostasis to GVHD phenotype.[16,17] B cells have further been implicated in the pathogenesis of GVHD, as patients who develop antibodies to minor antigens have a higher risk of cGVHD.[18] This study hypothesizes that recipient and/or donor *BAFF* SNPs would be associated with the GVHD phenotype after allo-SCT.

Methods

Adult patients undergoing matched related or unrelated allo-SCT at Vanderbilt University Medical Center from January 1999 to September 2008 with availability of pretransplantation germline recipient and donor DNA samples and surviving until day 120 after allo-SCT (to enrich for patients with cGVHD) were eligible. Mismatched allo-SCT, age < 18 years, and cord blood transplant were excluded. The final cohort consisted of 164 recipient-donor pairs. As previously described, cGVHD classification and severity were determined as per National Institutes of Health criteria, and records were abstracted for transplant characteristics (Table 1).[3,5] Seventeen patients experienced either cGVHD or acute GVHD (aGVHD) subtypes between day 100 and day 120. All patients were transplanted on Institutional Review Board-approved or standard of care protocols.

SNP selection and genotyping

A total of 24 tagSNPS were targeted for genotyping, of which 20 passed quality control. TagSNPs were identified using linkage disequilibrium select via the Genome Variation Server (Build 5.09) on the SeattleSNPs Web site (www.gvs.gs.washington.edu/GVS) using a combination of Seattle SNPs and HapMap with the following parameters: (1) minor allele frequency of > 10%, (2) r^2 threshold > 0.8, and (3) reference sequence encompassing the 2-kb 5′ and 3′ flanking region. Whole genome amplification was performed using the REPLI-g UltraFast Mini Kit (QIAGEN). Whole genome amplification was verified by RNASE-P analysis on the TaqMan 7200. Genotyping of amplified DNA was performed with a custom GoldenGate (Illumina) combined with VeraCode technology on the BeadXpress reader system according to the manufacturer's protocol (Illumina).

Statistical analysis

The primary endpoint for the study was to test for associations between BAFF SNPs and GVHD subtypes (classic/overlap GVHD compared with aGVHD subtypes/no GVHD). Classic and overlap were combined as one category as both of these groups have features of cGVHD. All SNPs analyzed passed quality control (test of Hardy-Weinberg equilibrium $P > .001$, minor allele frequency > 0.10, SNP call rate > 0.95, and pair-wise r^2 value < 0.8), and linkage disequilibrium was calculated for donors and recipients. All single SNP tests of association were performed using PLINK (www.pngu.mgh.harvard.edu/~purcell/plink) and included all European-American persons (153 donors and

Table 1. Patient characteristics and survival of all patients (N = 164)

Variable	Total cohort (N = 164), N (%)	Classic plus overlap cGVHD (N = 101), N (%)	aGVHD subtypes plus no GVHD (N = 63), N (%)
Pretransplantation characteristics			
Median age, y (range)	47 (18-69)	49 (21-67)	46 (18-69)
Sex			
Male (R)	80 (49)	49 (49)	31 (49)
Female (R)	84 (51)	52 (51)	32 (51)
Female D to male R	31 (19)	21 (21)	10 (19)
Race			
White (R/D)	156 (95)/153 (93)	94 (93)/92 (91)	62 (98)/61 (97)
Diagnoses			
Acute leukemia	86 (52)	49 (49)	37 (59)
Chronic leukemia	19 (12)	11 (11)	8 (13)
Lymphoma	54 (33)	38 (37)	16 (25)
Other	5 (3)	3 (3)	2 (3)
Disease risk*			
Low	65 (40)	38 (38)	27 (43)
Intermediate	30 (18)	21 (20)	9 (14)
High	40 (24)	25 (25)	15 (24)
Missing	29 (18)	17 (17)	12 (19)
Stem cell source			
Marrow	44 (27)	23 (22)	21 (33)
Peripheral blood stem cells	120 (73)	78 (78)	42 (67)
Regimen intensity			
Myeloablative	111 (68)	69 (68)	42 (67)
Other	53 (32)	32 (32)	21 (33)
Donor type			
Related	109 (67)	69 (68)	40 (63)
Unrelated	55 (33)	32 (32)	23 (37)
HLA match			
HLA identical sibling	108 (66)	68 (67)	40 (63)
HLA matched unrelated	55 (33)	32 (32)	23 (37)
Other	1 (1)	1 (1)	0
GVHD prophylaxis			
CSA/MTX	112 (68)	72 (71)	40 (63)
CSA/MMF	50 (31)	28 (28)	22 (36)
Other	2 (1)	1 (1)	1 (1)
TBI			
Yes	79 (48)	52 (51)	27 (43)
No	85 (52)	49 (49)	36 (57)
CMV			
R/D (+/+)	63 (38)	35 (34)	28 (44)
R/D (+/−)	19 (12)	12 (12)	7 (11)
R/D (−/+)	47 (29)	29 (29)	18 (29)
R/D (−/−)	35 (21)	25 (25)	10 (16)
Posttransplantation characteristics			
aGVHD†			
Grade 0 to 2 vs 3 to 4	129 (78) vs 32 (20)	78 (77) vs 21 (21)	51 (81) vs 11 (17)
Grade 0 to 1 vs 2 to 4	39 (24) vs 122 (74)	39 (24) vs 75 (74)	15 (24) vs 47 (75)
Platelets, × 10⁹/L at day 100, median (range)	110 (10-356)	105 (17-244)	112 (10-356)
Total bilirubin, mg/dL at day 100, median (range)	0.8 (0-3.6)	0.8 (0-2.4)	0.9 (0.2-3.6)

R indicates recipient; D, donor; HLA, human leukocyte antigen; CSA, cyclosporine; MTX, methotrexate; MMF, mycophenolate mofetil; TBI, total body irradiation; and CMV, cytomegalovirus.

*Disease risk based on Committee of International Bone Marrow Transplant Registry risk criteria.

†Three cases were missing.

156 recipients). Univariate logistic regression was performed to test for association between GVHD subtype and each SNP using an unadjusted, additive genetic model. P values were adjusted using false discovery rate,[19] and SNPs retaining significance were used in multivariate models. In multivariate analyses, logistic regression was used to test for association of SNPs with GVHD subtypes while adjusting potential confounding variables, such as age at transplantation (continuous variable), donor type (related or unrelated), and source of stem cell (marrow or peripheral blood). As we included patients with a minimum survival of 120 days after allo-SCT, multivariate analyses were done for the entire cohort and also for the subset that experienced GVHD only after day 120 (N = 147). Descriptive statistics, including median and interquartile range for continuous variables as well as percentages and frequencies for categorical variables, were
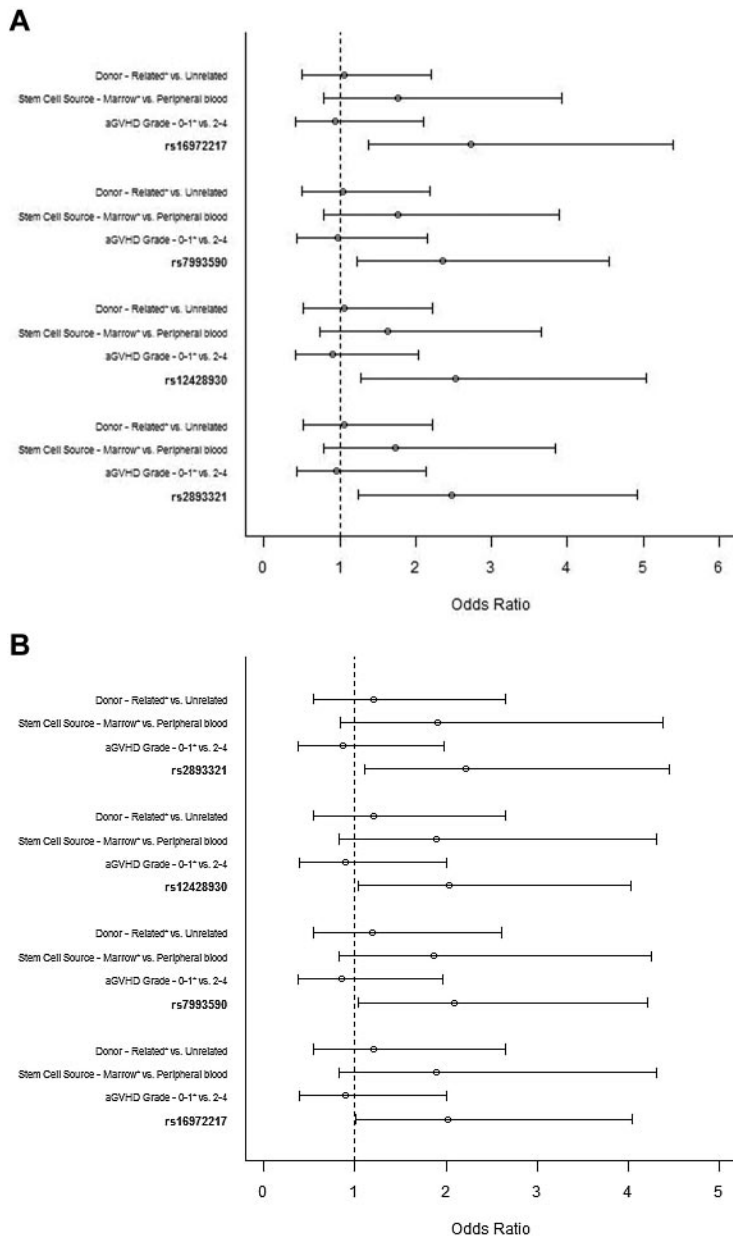
A

Figure 1. Multivariate analysis of factors associated with GVHD phenotype. (A) Entire cohort. Odds ratio ≥ 1 is associated with increased odds of developing aGVHD subtypes or no GVHD after day 100 from allo-SCT. *Reference group. The tested alleles were as follows: rs16972217, A; rs7993590, T; rs12428930, C; and rs2893321, G. (B) Subset of patients with GVHD after day 120. Odds ratio ≥ 1 is associated with increased odds of developing aGVHD subtypes or no GVHD after day 100 from allo-SCT. *Reference group. The tested alleles were as follows: rs16972217, A; rs7993590, T; rs12428930, C; and rs2893321, G.

B

calculated. To test for differences between the 2 groups, χ^2 test was used for the categorical variables and Wilcoxon rank-sum test for the continuous variables. All statistical analyses were performed using PLINK and R 2.10.1 (www.R-project.org).

Results and discussion

Patients

Patient characteristics are summarized in Table 1. GVHD after day 100 occurred in 124 (76%) patients: recurrent/persistent/delayed aGVHD (N = 23), overlap cGVHD (N = 29), and classic cGVHD (N = 72). Among patients with overlap and classic cGVHD (N = 101), mild, moderate, and severe GVHD were seen in 13 (13%), 22 (22%), and 66 (65%) persons, respectively.

Univariate analysis

Because of shared clinical phenotype, overlap and classic cGVHD were combined into a single outcome variable and were compared with patients with aGVHD subtypes and no GVHD after day 100. There were no significant differences in clinical characteristics between patients with or without classic plus overlap cGVHD (Table 1). In single SNP analysis, 9 (2 donors, 7 recipients) of the 20 tagSNPs had significant *P* values of < .05 when comparing classic plus overlap with aGVHD plus no GVHD (supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Because the majority of significant SNPs identified were recipient derived, the remainder of the analysis was focused on recipient SNPs. Among patients with classic or overlap cGVHD, there was no significant association between *BAFF* SNPs with organ involvement or global

severity of GVHD (mild/moderate vs severe or mild vs moderate/severe), overall survival, or relapse-free survival (data not shown).

Multivariate analysis

Although no clinical variable was associated with cGVHD (classic plus overlap), multivariate analyses was adjusted for conventional predictors of cGVHD (donor status, stem cell source, aGVHD [before day 100]). After adjusting for false discovery rate, 4 intronic recipient *BAFF* SNPs remained significant and were used in multivariate analyses. All 4 BAFF SNPs (rs16972217 [odds ratio = 2.72; 95% confidence interval, 1.38-5.39, P = .004]; rs7993590 [odds ratio = 2.35; 95% confidence interval, 1.22-4.55, P = .011]; rs12428930 [odds ratio = 2.53; 95% confidence interval, 1.27-5.04, P = .008]; and rs2893321 [odds ratio = 2.48; 95% confidence interval, 1.25-4.92, P = .009]) were independent predictors of GVHD phenotype (Figure 1A). The same SNPs retained statistical significance in multivariate analyses done on the subset patients experiencing GVHD after day 120 from allo-SCT (Figure 1B). The other known predictors of cGVHD were not significant.

Conclusion

This study shows that recipient *BAFF* SNPs modulate GVHD phenotype. Multiple observations suggest that B cells play an important role in the pathogenesis of cGVHD.[16-18,20-23] The finding of the association of *BAFF* SNPs with GVHD allows potentially for identification of a subset of patients who may benefit from intervention that modulates the BAFF pathway.

This study is the first to report the association of recipient *BAFF* SNPs and GVHD phenotype. None of the known clinical predictors of limited/extensive cGVHD was associated with classic or overlap cGVHD. The impact of recipient *BAFF* SNPs predominated. BAFF production from residual host-derived macrophages,[24] dendritic cells, and salivary gland epithelial cells[25] possibly explains the association of recipient *BAFF* SNPs with GVHD subtypes. Of the 3 SNPs previously reported to be associated with increased BAFF levels and risk for non-Hodgkin lymphoma,[26] one SNP (rs12428930) was also an independent predictor of GVHD phenotype.

Our study is limited as we included only patients with a minimum survival of 120 days after allo-SCT. These findings need to be confirmed in a larger cohort along with functional correlation of *BAFF* SNPs with BAFF levels. Rituximab, starting at 3 months after transplantation, has been used for prophylaxis of cGVHD.[27] The association of recipient *BAFF* SNPs with GVHD phenotype may allow for an individualized approach in the management of GVHD as a predictor of response with rituximab therapy, using rituximab for cGVHD prophylaxis in high-risk patients, and by developing new strategies that target BAFF or alter B-cell homeostasis.

Authorship

Contribution: W.B.C. and M.H.J. designed the research, analyzed data, and wrote the manuscript; W.B.C. and J.S. performed experiments; K.D.B.-G. and D.C.C. advised SNP assay design and analyzed SNP data with PLINK; H.C. and K.-H.F. analyze data and performed statistical analysis; B.G.E. and B.N.S. reviewed and provided critical input in revision of the manuscript; and A.K., J.P.G., and F.G.S. provided critical review for the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Madan H. Jagasia, Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, 2665 Vanderbilt Clinic, Nashville, TN 37232-5505; e-mail: madan.jagasia@vanderbilt.edu.

References

1. Lee SJ, Vogelsang G, Flowers ME. Chronic graft-versus-host disease. *Biol Blood Marrow Transplant.* 2003;9(4):215-233.

2. Shlomchik WD, Lee SJ, Couriel D, Pavletic SZ. Transplantation's greatest challenges: advances in chronic graft-versus-host disease. *Biol Blood Marrow Transplant.* 2007;13(1 suppl 1):2-10.

3. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant.* 2005;11(12):945-956.

4. Arora M, Nagaraj S, Witte J, et al. New classification of chronic GVHD: added clarity from the consensus diagnoses. *Bone Marrow Transplant.* 2009;43(2):149-153.

5. Jagasia M, Giglia J, Chinratanalab W, et al. Incidence and outcome of chronic graft-versus-host disease using National Institutes of Health consensus criteria. *Biol Blood Marrow Transplant.* 2007;13(10):1207-1215.

6. Jagasia MH, Savani BN, Stricklin G, et al. Classic and overlap chronic graft-versus-host disease (cGVHD) is associated with superior outcome after extracorporeal photopheresis (ECP). *Biol Blood Marrow Transplant.* 2009;15(10):1288-1295.

7. Moore PA, Belvedere O, Orr A, et al. BLyS: member of the tumor necrosis factor family and B lymphocyte stimulator. *Science.* 1999;285(5425):260-263.

8. Gorelik L, Gilbride K, Dobles M, et al. Normal B cell homeostasis requires B cell activation factor production by radiation-resistant cells. *J Exp Med.* 2003;1986(6):937-945.

9. Sasaki Y, Casola S, Kutok JL, Rajewsky K, Schmidt-Supprian M. TNF family member B cell-activating factor (BAFF) receptor-dependent and -independent roles for BAFF in B cell physiology. *J Immunol.* 2004;173(4):2245-2252.

10. Matsushita T, Hasegawa M, Yanaba K, et al. Elevated serum BAFF levels in patients with systemic sclerosis: enhanced BAFF signaling in systemic sclerosis B lymphocytes. *Arthritis Rheum.* 2006;54(1):192-201.

11. Nossent JC, Lester S, Zahra D, Mackay CR, Rischmueller M. Polymorphism in the 5′ regulatory region of the B-lymphocyte activating factor gene is associated with the Ro/La autoantibody response and serum BAFF levels in primary Sjogren's syndrome. *Rheumatology (Oxford).* 2008;47(9):1311-1316.

12. Mariette X, Roux S, Zhang J, et al. The level of BLyS (BAFF) correlates with the titre of autoantibodies in human Sjogren's syndrome. *Ann Rheum Dis.* 2003;62(2):168-171.

13. Groom J, Kalled SL, Cutler AH, et al. Association of BAFF/BLyS overexpression and altered B cell differentiation with Sjogren's syndrome. *J Clin Invest.* 2002;109(1):59-68.

14. Kawasaki A, Tsuchiya N, Fukazawa T, Hashimoto H, Tokunaga K. Analysis on the association of human BLYS (BAFF, TNFSF13B) polymorphisms with systemic lupus erythematosus and rheumatoid arthritis. *Genes Immun.* 2002;3(7):424-429.

15. Zhang J, Roschke V, Baker KP, et al. Cutting edge: a role for B lymphocyte stimulator in systemic lupus erythematosus. *J Immunol.* 2001;166(1):6-10.

16. Sarantopoulos S, Stevenson KE, Kim HT, et al. High levels of B-cell activating factor in patients with active chronic graft-versus-host disease. *Clin Cancer Res.* 2007;13(20):6107-6114.

17. Sarantopoulos S, Stevenson KE, Kim HT, et al.

Altered B-cell homeostasis and excess BAFF in human chronic graft-versus-host disease. *Blood.* 2009;113(16):3865-3874.

18. Miklos DB, Kim HT, Miller KH, et al. Antibody responses to H-Y minor histocompatibility antigens correlate with chronic graft-versus-host disease and disease remission. *Blood.* 2005;105(7):2973-2978.

19. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B.* 1995;57(1):289-300.

20. Cutler C, Miklos D, Kim HT, et al. Rituximab for steroid-refractory chronic graft-versus-host disease. *Blood.* 2006;108(2):756-762.

21. Ratanatharathorn V, Ayash L, Reynolds C, et al. Treatment of chronic graft-versus-host disease with anti-CD20 chimeric monoclonal antibody. *Biol Blood Marrow Transplant.* 2003;9(8):505-511.

22. Ratanatharathorn V, Logan B, Wang D, et al. Prior rituximab correlates with less acute graft-versus-host disease and better survival in B-cell lymphoma patients who received allogeneic peripheral blood stem cell transplantation. *Br J Haematol.* 2009;145(6):816-824.

23. Shimabukuro-Vornhagen A, Hallek MJ, Storb RF, von Bergwelt-Baildon MS. The role of B cells in the pathogenesis of graft-versus-host disease. *Blood.* 2009;114(24):4919-4927.

24. Polverino F, Baraldo S, Bazzan E, et al. A novel insight into adaptive immunity in COPD: B cell activating factor belonging to the TNF family (BAFF). *Am J Respir Crit Care Med.* 2010;182(8):1011-1019.

25. Ittah M, Miceli-Richard C, Eric GJ, et al. B cell-activating factor of the tumor necrosis factor family (BAFF) is expressed under stimulation by interferon in salivary gland epithelial cells in primary Sjogren's syndrome. *Arthritis Res Ther.* 2006;8(2):R51.

26. Novak AJ, Slager SL, Fredericksen ZS, et al. Genetic variation in B-cell-activating factor is associated with an increased risk of developing B-cell non-Hodgkin lymphoma. *Cancer Res.* 2009;69(10):4217-4224.

27. Cutler C, Lixian S, Kim H, et al. Prophylactic rituximab after allogeneic stem cell transplantation prevents steroid requiring chronic graft-versus-host disease [abstract]. *Blood.* 2011;116(21):214.