Check for updates

Altered B-cell homeostasis and excess BAFF in human chronic graft-versus-host disease

Stefanie Sarantopoulos,[1] Kristen E. Stevenson,[2] Haesook T. Kim,[2] Corey S. Cutler,[1] Nazmim S. Bhuiya,[1] Michael Schowalter,[1] Vincent T. Ho,[1] Edwin P. Alyea,[1] John Koreth,[1] Bruce R. Blazar,[3] Robert J. Soiffer,[1] Joseph H. Antin,[1] and Jerome Ritz[1,4,5]

[1]Division of Hematologic Malignancies and Department of Medical Oncology and [2]Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA; [3]Cancer Center and Department of Pediatrics, Division of Bone Marrow Transplantation, University of Minnesota, Minneapolis; [4]Cancer Vaccine Center, Dana-Farber Cancer Institute, Boston, MA; and [5]Harvard Stem Cell Institute, Harvard Medical School, Boston, MA

Downloaded from https://ashpublications.org/blood/article-pdf/113/16/3865/1307974/zh801609003865.pdf by NEW YORK UNIVERSITY user on 20 January 2020

Chronic graft-versus-host disease (cGVHD) causes significant morbidity and mortality in patients otherwise cured of malignancy after hematopoietic stem cell transplantation (HSCT). The presence of alloantibodies and high plasma B cell–activating factor (BAFF) levels in patients with cGVHD suggest that B cells play a role in disease pathogenesis. We performed detailed phenotypic and functional analyses of peripheral B cells in 82 patients after HSCT. Patients with cGVHD had significantly higher BAFF/B-cell ratios compared with patients without cGVHD or healthy donors. In cGVHD, increasing BAFF concentrations correlated with increased numbers of circulating pre–germinal center (GC) B cells and post-GC "plasmablast-like" cells, suggesting in vivo BAFF dependence of these 2 CD27^{+} B-cell subsets. Circulating CD27^{+} B cells in cGVHD comprised in vivo activated B cells capable of IgG production without requiring additional antigen stimulation. Serial studies revealed that patients who subsequently developed cGVHD had delayed reconstitution of naive B cells despite persistent BAFF elevation as well as proportional increase in CD27^{+} B cells in the first year after HSCT. These studies delineate specific abnormalities of B-cell homeostasis in patients with cGVHD and suggest that BAFF targeting agents may be useful in this disease. (Blood. 2009;113:3865-3874)

Introduction

The limited understanding of the immune mechanisms that result in chronic graft-versus-host disease (GVHD) hinders our ability to develop effective targeted therapies that would improve the survival of patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT).[1-3] In acute GVHD, tissue injury is mediated primarily by donor T cells that specifically target minor histocompatibility antigens (mHAs) in affected organs.[4,5] However, despite effective prevention of acute GVHD with agents that primarily inhibit T cells (calcineurin inhibitors, mTOR inhibitors, and purine analogues),[2,6] the incidence and severity of chronic GVHD (cGVHD) remain high.[1,2] This suggests that immune mechanisms of cGVHD are distinct from acute GVHD. Studies in mHA-mismatched murine transplantation models demonstrate involvement of B cells in the development of cGVHD.[7,8] After allogeneic HSCT in humans, alloantibodies to Y chromosome–encoded proteins are detectable in 50% of male recipients if they received hematopoietic stem cells from a female donor.[9] Such alloantibodies develop 4 to 9 months after HSCT, and the presence of alloantibodies correlated significantly with clinical cGVHD development.[10] HY antibodies were not associated with acute GVHD and did not develop when recipient and donor were sex matched. When studied in greater detail, patients with HY antibodies were also found to have coordinated T-cell responses to distinct epitopes in the same HY mHA (DBY).[11] These studies have suggested that donor B cells play a role in the development of cGVHD in humans, and several phase 1 or 2 trials of B cell–directed therapy with rituximab in steroid-refractory cGVHD have revealed clinical responses.[12-14] Although these observations provide compelling evidence that B cells play an important role in the immune pathology of human cGVHD, the mechanisms that promote and sustain B-cell involvement have not been elucidated.

HSCT conditioning depletes normal recipient B cells and the expansion of normal donor B cells is altered as reconstitution occurs in the setting of constant exposure to "foreign" minor histocompatibility antigens. Although genetic disparity between donor and recipient must exist for cGVHD to develop, sustainable autoreactivity occurs after alloreactivity in murine models. Along with a more prolonged humoral immune deficiency, a complex autoimmune disease–like phenotype is found in cGVHD.[15,16] The frequent production of autoantibodies in patients with cGVHD[17-19] suggests that disease pathogenesis reflects a critical breakdown in peripheral B-cell tolerance after allogeneic HSCT. Furthermore, a relative decrease in immature B-cell number in cGVHD compared with other post-HSCT patients[20] suggests that altered B-cell homeostasis is a component of this disease.

B cell–activating factor (BAFF) plays a critical role in B-cell reconstitution and homeostasis after myeloablation.[21] Studies of B-cell development in murine models have shown that survival of normal mature B cells depends on the relative balance of both B-cell receptor (BCR) and BAFF signaling.[22,23] In addition, maintenance of B-cell homeostasis in mice depends on in vivo soluble BAFF concentration.[24,25] Murine models demonstrate that a

Submitted September 30, 2008; accepted January 11, 2009. Prepublished online as *Blood* First Edition paper, January 23, 2009; DOI 10.1182/blood-2008-09-177840.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

© 2009 by The American Society of Hematology

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

diverse B-cell pool is required for antigen-induced anergy and exclusion of autoreactive B cells from follicular niches.[26,27] A set of elegant studies subsequently identified a BAFF-mediated B-cell tolerance checkpoint in which limiting amounts of BAFF are required for ongoing B-cell turnover and avoidance of B-cell autoreactivity.[28,29] When the pool of normal B cells is reduced, excess BAFF promotes survival of autoreactive B cells.[29] However, even in the presence of high BAFF, high numbers of nonautoreactive B cells outcompete autoreactive B cells for available soluble BAFF, causing autoreactive B cells to undergo apoptosis.[28] Thus, B-cell autoimmunity in transgenic mouse models is determined by both the level of soluble BAFF and the numbers of competing naive B cells.

Defective censoring of autoreactive B cells in patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis has been observed[30-32] and high BAFF has been found in patients with a variety of autoimmune diseases,[33-36] suggesting that excessive BAFF stimulation in humans contributes to development of autoimmunity. Since high BAFF levels are significantly associated with presence of active cGVHD more than 1.5 years after HSCT,[37,38] we hypothesized that relative B lymphopenia and high BAFF after HSCT could support potentially pathologic activated alloreactive and autoreactive B-cell populations in cGVHD patients. Examining peripheral blood after allogeneic HSCT, we found that patients with cGVHD had significantly higher "BAFF/B-cell ratio" compared with patients without cGVHD. Serial analysis of patients for 1 year after HSCT showed that, in patients without cGVHD, BAFF levels gradually normalized as B-cell numbers recovered, indicating a normal homeostatic response to B lymphopenia. In contrast, patients who subsequently developed cGVHD exhibited delayed reconstitution of naive B cells despite persistent BAFF elevation. Using $CD27^+$ as a marker of antigen experience and ex vivo IgG production as a measure of B-cell activation, we found that circulating B cells, including pre-GC B cells, were activated in cGVHD. These results suggest that altered B-cell homeostasis and excess BAFF contribute to promotion of activated B cells in patients with cGVHD.

Methods

Patient characteristics

All patient samples were collected after written informed consent was obtained in accordance with the Declaration of Helsinki and approved by the Human Subjects Protection Committee of the Dana-Farber/Harvard Cancer Center.

Patient group 1

Blood samples for analysis of B-cell reconstitution were obtained from 57 patients who had undergone allogeneic HSCT between 1994 and 2005 and were more than 12 months after HSCT. Within this group, BAFF levels from 28 samples have been reported previously.[37] Clinical characteristics of the 57 patients are summarized in Table 1. The study included patients who received nonmyeloablative or myeloablative conditioning regimens and bone marrow or mobilized peripheral blood stem cell grafts. Patients whose primary disease relapsed within 1 year of transplantation and patients receiving high-dose steroids (> 0.5 mg/kg prednisone) at the time of sample collection were excluded. Chronic GVHD status at the time of sample collection was categorized according to documented clinical examination and laboratory studies using both Seattle criteria and National Institutes of Health (NIH) cGVHD consensus criteria. Twenty-two patients target organs affected per modified Seattle criteria.[39] Twenty-three patients had "inactive cGVHD" at the time of sample collection. Inactive cGVHD was used to describe patients with cGVHD who had achieved a complete response to immune-suppressive therapy at the time of analysis. Patients who never developed cGVHD after HSCT were designated as having "no cGVHD." Unlike patients with inactive or active cGVHD, patients with no cGVHD were receiving no immune-suppressive agents, except one patient who received low-dose steroids for a non-GVHD indication (Table 1). Median time of analysis was 20.9 months after transplantation for patients with active cGVHD compared with 26.6 months after transplantation for patients with no cGVHD ($P = .10$). Patients with inactive cGVHD were significantly later in their post-HSCT course (30.9 months) compared with patients with active cGVHD (20.9 months, $P = .007$). All other clinical characteristics including age, sex, type of transplant, and underlying hematologic malignancy were similar in patients with active and inactive cGVHD. We also studied 33 healthy controls.

Patient group 2

We prospectively followed an additional group of 25 patients from day of stem cell transplantation (day 0), with samples obtained every 3 months over a 19-month period. These patients received either myeloablative or nonmyeloablative conditioning regimens and tacrolimus and/or sirolimus as GVHD prophylaxis.[6,40] Within this cohort, 8 patients did not develop cGVHD by 12 months after HSCT and 17 patients developed cGVHD between 3 and 12 months after HSCT. All patients in group 2 were analyzed, regardless of prednisone dose or treatment with other immunosuppressive agents. Six of the 17 patients who developed cGVHD had prior grades 2 to 4 acute GVHD. None of the patients without cGVHD were taking immunosuppressive agents after the 6-month time point. Control blood samples were obtained from 8 patients with multiple myeloma after the second of a planned tandem autologous transplantation.

Processing of patient plasma and peripheral blood cells

Whole blood was drawn into standard EDTA-containing collection tubes. Plasma was separated from whole blood cells by centrifugation at 600*g*. Plasma was stored in aliquots at −80°C and used after first thaw for BAFF measurements. Whole blood for flow cytometry studies was also collected on day of use in EDTA-containing tubes. Peripheral blood leukapheresis products were obtained from 2 cGVHD patients. Discarded leukocyte filters from anonymous healthy platelet donors were obtained from the Kraft Blood Donor Center at Dana-Farber Cancer Institute (DFCI).

BAFF enzyme-linked immunosorbent assay

Soluble BAFF in patient plasma samples was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) and the manufacturer's recommended procedures (R&D Systems, Minneapolis, MN).

Flow cytometric analysis of peripheral B cells

Antibodies used for flow cytometry were as follows: CD19 ECD or CD19 PC7 (both clone J4.119), CD20 ECD (clone B9E9), CD38 PE (clone LS198), CD27 PCD5 (clone 1A4), and IgD FITC (clone IA6-2) from BD Biosciences Pharmingen (San Diego, CA); BR3-FITC (eBioscience, San Diego, CA); and TACI-PE (R&D Systems). Whole blood was processed for flow cytometry using the Prep Plus 2 system (Beckman Coulter, Fullerton, CA). The lymphocyte gate was established using forward and side scatter. A minimum of 50 000 lymphocytes were collected for all samples to ensure adequate subset analysis. Gates positive for CD19, IgD, CD38, and CD27 were first set according to isotype controls. Only $CD19^+$ cells were analyzed to ensure that $CD3^+$ cells were not included in the analysis of CD38 or CD27 expression. Anti-CD19 PC7 (Beckman Coulter) was used per the manufacturer's instructions. Red blood cells (RBCs) were lysed and leukocytes were fixed using the Beckman Coulter TQPrep system before analysis. Cells were analyzed using a Cytomics FC 500 instrument and

Downloaded from https://ashpublications.org/blood/article-pdf/113/16/3865/1307974/zh801609003865.pdf by NEW YORK UNIVERSITY user on 20 January 2020

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

Table 1. Clinical characteristics of 57 patients who underwent allogeneic HSCT

Characteristic	Chronic GVHD: Active	Chronic GVHD: Inactive	Chronic GVHD: No	*P*
No. (%)	22 (45)	23 (36)	12 (18)	
Median age, y (range)	46 (24-64)	47 (19-66)	44 (25-58)	.65
Female sex (%)	10 (45)	11 (48)	4 (33)	.73
Conditioning regimen (%)				
Myeloablative	14 (64)	17 (74)	10 (83)	.52
Nonmyeloablative	8 (36)	6 (26)	2 (17)	
Cell source (%)				
Peripheral blood stem cells	19 (86)	15 (65)	9 (75)	.26
Bone marrow	3 (14)	8 (35)	3 (25)	
HLA matching (%)				
Matched, unrelated	10 (45)	13 (57)	2 (17)	.11
Matched, related	11 (50)	9 (39)	10 (83)	
Mismatched	1 (5)	1 (4)	0 (0)	
Prednisone dose (%)				
0 mg/day	9 (30)	10 (42)	11 (92)	
0-30 mg/day	13 (59)	13 (57)	1 (8)	
Cellcept (%)	7 (32)	5 (21)	0 (0)	
Tacrolimus (%)	8 (36)	3 (13)	0 (0)	
Rapamycin (%)	8 (36)	3 (13)	0 (0)	
Months after transplantation, median (range)	20.9 (13.6-79.2)	30.9 (16.7-134.6)	26.6 (18.7-93.2)	.01*
BAFF level, ng/mL, median (range)	7.0 (1.6-24.5)	5.5 (1.5-24.3)	3.0 (1.4-4.9)	.001
Grades II-IV aGVHD (%)	2 (9)	5 (22)	2 (17)	.52
Disease (%)				
AML/AML from MDS	8 (36)	5 (22)	2 (17)	
ALL	2 (9)	1 (4)	3 (25)	
CML	0 (0)	9 (39)	3 (25)	
CLL	3 (14)	0 (0)	0 (0)	
MDS	4 (18)	4 (17)	0 (0)	
NHL	2 (9)	1 (4)	4 (33)	
HL	1 (5)	0 (0)	0 (0)	
MM	0 (0)	1 (4)	0 (0)	
Other	2 (9)	2 (9)	0 (0)	

AML indicates acute myeloid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; CLL, chronic lymphoblastic leukemia; NHL, non-Hodgkin leukemia; HL, Hodgkin lymphoma; and MM, multiple myeloma.

*$P = .101$ for active group versus none; $P = .007$ for active versus inactive group.

leukapheresis samples (Miltenyi Biotec, Auburn, CA) were stained with anti–BAFF-R, BR3 PE (clone 8A7; eBioscience), or biotinylated BMCA or TACI (R&D Systems) followed by streptavidin-APC antibody (Invitrogen, Carlsbad, CA).

Ex vivo purification of B-cell subsets

For purification of $CD19^+CD27^+$ and $CD19^+CD27^-$ B cells, whole EDTA anticoagulated blood (10-12 mL) was obtained from post-HSCT patients or healthy individuals. For purification of IgD versus CD38 B-cell subsets (Table 2), large volume leukapheresis products from 2 cGVHD patients or leukocyte filters from 3 healthy donors were obtained. Lymphocytes were isolated in a sterile fashion using Ficoll-Paque PLUS (GE Healthcare, Uppsala, Sweden). $CD27^+$ or $CD27^-$ B cells were sorted by flow cytometry to more than 98% purity (BD FACSAria Special Order Cell-Sorting System; Becton Dickinson, San Jose, CA). Purified $CD27^+$ or $CD27^-$ B cells (> 95%) were also obtained using the indirect magnetic labeling system B Cell Isolation Kit II, human (Miltenyi Biotec). In brief, non-B cells were retained on magnetic-activated cell sorting (MACS) columns after staining with a cocktail of biotin-conjugated antibodies against CD2, CD14, CD16, CD36, and CD43 and binding to streptavidin magnetic microbeads. B cells passed through the column and were positively selected with CD27 magnetic beads and enriched to more than 95% $CD20^+CD27^+$. IgD versus CD38 B-cell subsets were isolated using a BD FACSAria cell sorter (BD Biosciences, San Jose, CA).

IgG measurement

B cells purified to more than 98% purity as assessed by CD20 staining were incubated in vitro in complete RPMI medium with 100 ng/mL IL-10 (R&D Systems); 250 U IL-2 (BD Biosciences); and 0, 25, 250, or 2500 ng/mL BAFF (Axxora LLC, San Diego, CA). IgG was measured using the Human IgG Elisa Quantitation Kit (Bethyl Laboratories, Montgomery, TX).

Table 2. "Antigen-naive" (naive and transitional) and $CD27^+$ "antigen-experienced" B cells in patients after HSCT

B-cell subset	Active, n = 22	Inactive, n = 23	No, n = 12	Healthy, n = 33
Total naive B cells ×1000/L	79.8	99.1	260.5	89.5
P vs none	.001	.001		.001
Total transitional B cells ×1000/L	8.1	8.5	28.7	14.4
P vs none	.04	.04		.02
Total $CD27^+CD19^+$ B cells ×1000/L	17.5	25.3	24.9	68.1
P vs none	.14	.37		.01

Downloaded from https://ashpublications.org/blood/article-pdf/113/16/3865/1307974/zh801609003865.pdf by NEW YORK UNIVERSITY user on 20 January 2020

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

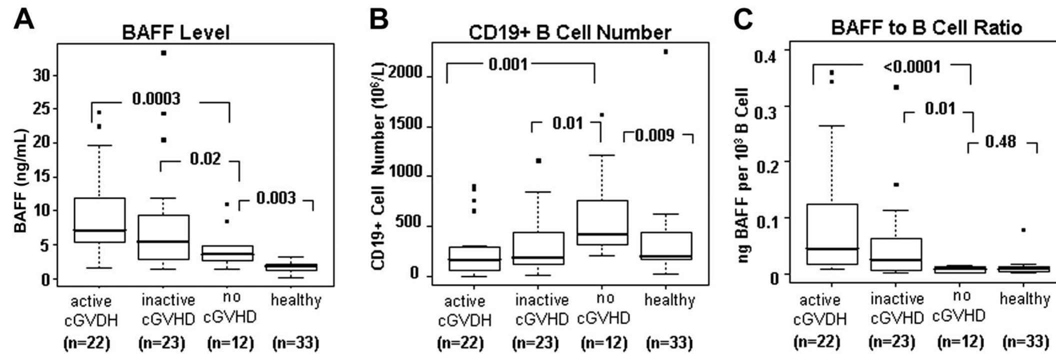

Figure 1. High BAFF levels and low B-cell numbers result in high BAFF/B-cell ratios in patients with cGVHD. Plasma BAFF concentrations and numbers of CD19$^+$ B cells were measured in 3 groups after allogeneic HSCT: 22 patients with active cGVHD, 23 with inactive cGVHD, and 12 who did not develop cGVHD. Results were compared with 33 healthy donors. (A) Plasma BAFF concentrations in each patient group after HSCT and healthy donors. (B) Total number of CD19$^+$ B cells in each patient group and healthy donors. (C) Median BAFF/B-cell ratio for each patient group and healthy donors. The BAFF/B-cell ratio was defined as nanograms of BAFF per 10^3 CD19$^+$ B cells. Box plots in each figure depict 75th percentile; median and 25th percentile values and whiskers represent maximum and minimum values.

Statistical analyses

For 2-sample comparison of continuous variables, a Wilcoxon rank sum test was performed. The Fisher exact test was used to compare categoric variables and the Spearman rank test was used for correlation analysis. All tests performed were 2-sided and considered significant at the .05 level.

Results

Relative B lymphopenia and high BAFF/B-cell ratio are associated with cGVHD

A detailed analysis of plasma BAFF levels and B-cell phenotype was carried out in 57 patients who underwent allogeneic HSCT more than 12 months ago (Table 1). As shown in Figure 1A, all patients after HSCT had significantly higher BAFF levels compared with healthy individuals. Patients with active cGVHD had BAFF levels that were significantly higher than patients without cGVHD (no cGVHD; $P < .001$) or healthy donors ($P < .001$). Patients without cGVHD also had higher than normal BAFF levels (3.0 ng/mL vs 1.9 ng/mL, $P = .003$). Consistent with a previously described “surge” in B-cell number after HSCT,[20] B-cell numbers in patients without cGVHD were significantly higher than normal (Figure 1B). In contrast, patients with active or inactive cGVHD had significantly lower numbers of total CD19$^+$ B cells compared with patients without cGVHD ($P = .001$ and $P = .01$, respectively). Since BAFF level per B cell has been determined in murine models to be a critical determinant of autoreactive B-cell survival,[28,29] we calculated BAFF/B-cell ratios (level of BAFF per 10^3 B cells) for each patient in each post-HSCT group. Figure 1C shows that despite higher than normal BAFF levels, supranormal B-cell numbers in patients without cGVHD resulted in BAFF/B-cell ratios that were similar to those found in healthy individuals. In contrast, patients with cGVHD had significantly higher BAFF/B-cell ratios compared with other post-HSCT patients and healthy individuals ($P < .001$ each). As shown in Figure 1A and B, high BAFF/B-cell ratios in patients with cGVHD were due to both high BAFF levels and B lymphopenia.

Chronic GVHD is associated with decreased numbers of naive B cells

In murine models, high BAFF/B-cell ratios promote survival of therefore examined the relative numbers of antigen-naive B cells and antigen-experienced B cells in post-HSCT patients (Table 1) and in healthy individuals. In this analysis, antigen-naive B cells were defined as naive (CD19$^+$IgDCD38^{Lo}CD27$^-$) B cells and transitional (CD19$^+$IgD$^+$CD38^{Hi}CD27$^-$) B cells; antigen-experienced B cells were defined as CD19$^+$CD27$^+$ B cells. As shown in Table 2, patients with active cGVHD had low numbers of naive B cells compared with patients with no cGVHD (median time after HSCT, 21 months vs 27 months, respectively, $P = .01$). Patients without cGVHD had supranormal numbers of both naive and transitional B cells (260.5×10^6/L vs 89.5×10^6/L [$P = .001$] and 28.7×10^6/L in cGVHD vs 14.4×10^6/L [$P = .02$], respectively). High total numbers of circulating recent bone marrow emigrants or transitional cells and naive B cells in patients greater than 1 year after HSCT without cGVHD suggests increased bone marrow output and prolonged survival of naive B cells compared with healthy individuals and with patients with cGVHD. In contrast to naive B cells, numbers and frequencies of CD27$^+$ antigen-experienced B cells were significantly decreased compared with healthy donors but these cells were not different among post-HSCT groups (Table 2). In the setting of relative naive B lymphopenia, active cGVHD patients tended to have higher frequencies of CD27$^+$CD19$^+$ B cells (19.0% in active cGVHD vs 6.1% in patients without cGVHD), but this difference did not reach significance ($P = .06$).

Identification of circulating pre-GC (IgD$^+$CD38^{Hi}CD27$^+$) B cells in cGVHD

In humans, CD27 expression identifies antigen-experienced B cells committed to plasma cell differentiation.[41] CD27$^+$ B-cell subsets include IgD^{Lo} post–germinal center (GC) memory and plasmablast-like (PB)/plasma cell–like (PC) populations. CD27$^+$ IgD$^+$ B-cell populations include “IgD$^+$ memory” V region–mutated memory B cells.[42] In addition, the presence of CD27 on IgD$^+$ CD38^{Hi}–expressing B cells allows distinction of human peripheral transitional B cells from pre-GC B cells.[43] Pre–germinal center cells (GCs), also called BM2′, GC founder, or preplasmablast cells, are IgD$^+$CD38^{Hi}CD27$^+$. These cells are typically found in human tonsils and have also been described in peripheral blood in SLE patients.[44,45] Table 3 summarizes peripheral CD27$^+$ B-cell subset phenotypes and their corresponding functional characteristics. We used multiparameter flow cytometry to determine whether any of these distinct circulating CD27$^+$ B-cell subsets were more preva-

Downloaded from https://ashpublications.org/blood/article-pdf/113/16/3865/1307974/zh801609003865.pdf by NEW YORK UNIVERSITY user on 20 January 2020

DOCKET ALARM Find authenticated court documents without watermarks at docketalarm.com.

Table 3. Peripheral blood B-cell subsets analyzed in this study

Peripheral blood B-cell subset	Cell-surface phenotype
CD27⁻ subsets	
Naive B cells	IgD⁺CD38ᴸᵒCD27⁻
Transitional B cells[42,43]	IgD⁺CD38ᴴⁱCD27⁻
CD27⁺ subsets	
IgD⁺ memory B[42,45]	IgD⁺CD38ᴸᵒCD27⁺
Pre-GC (or "GC founder")[42]	IgD⁺CD38ᴴⁱCD27⁺
Post-GC memory B	IgDᴸᵒ/⁻CD38ᴸᵒCD27⁺
Plasmablast/plasma cell (PB/PC)	IgDᴸᵒ/⁻CD38ᴴⁱCD27⁺

Table 4. Proportions of CD27⁺ B-cell subsets in patients after HSCT

CD19⁺ subset	Active, n = 22	Inactive, n = 23	No, n = 12	Healthy, n = 33
IgD⁺ memory	8.4	2.8	2.3	4.1
P vs none	.04	.45		.11
Post-GC memory, %	20.4	18.4	10.8	32.8
P vs none	.06	.06		< .001
Pre-GC, %	41.3	9.7	2.6	4.7
P vs none	.06	.42		.65
PB/PC, %	86.9	66.4	36.8	40.7
P vs none	.08	.23		.80

and examined relative levels of surface IgD, CD38, and CD27⁺ on these cells. Figure 2 shows representative examples of flow cytometric profiles of CD27⁺ B-cell subgroups identified in patients after HSCT and in healthy individuals. Table 4 compares the median frequency of each CD27⁺ B-cell subset in patients with and without cGVHD and in healthy individuals. Consistent with previous reports, all post-HSCT patients had fewer post-GC (IgDᴸᵒ).[46] We found that the post-GC memory (CD27⁺IgDᴸᵒCD38ᴸᵒ) B-cell subgroup was less frequent and lower in number in each post-HSCT group compared with healthy individuals, whereas all other CD27⁺ subsets were not proportionally or numerically different from normal (Table 4 and data not shown). As shown in Table 4, median IgD⁺ naive (CD38ᴸᵒ) or pre-GC (CD38ᴴⁱ) percentages in all post-HSCT groups were the same, but these populations tended to have higher CD27⁺ expression in cGVHD patients compared with patients without cGVHD (although differences did not reach significance).

Overall increased CD27⁺ expression on B-cell subsets in the active cGVHD group could not be attributed to time after HSCT, since the active cGVHD group was closest in time to HSCT (median, 20.9 months) compared with the patients with inactive or without cGVHD, and therefore would have been expected to have

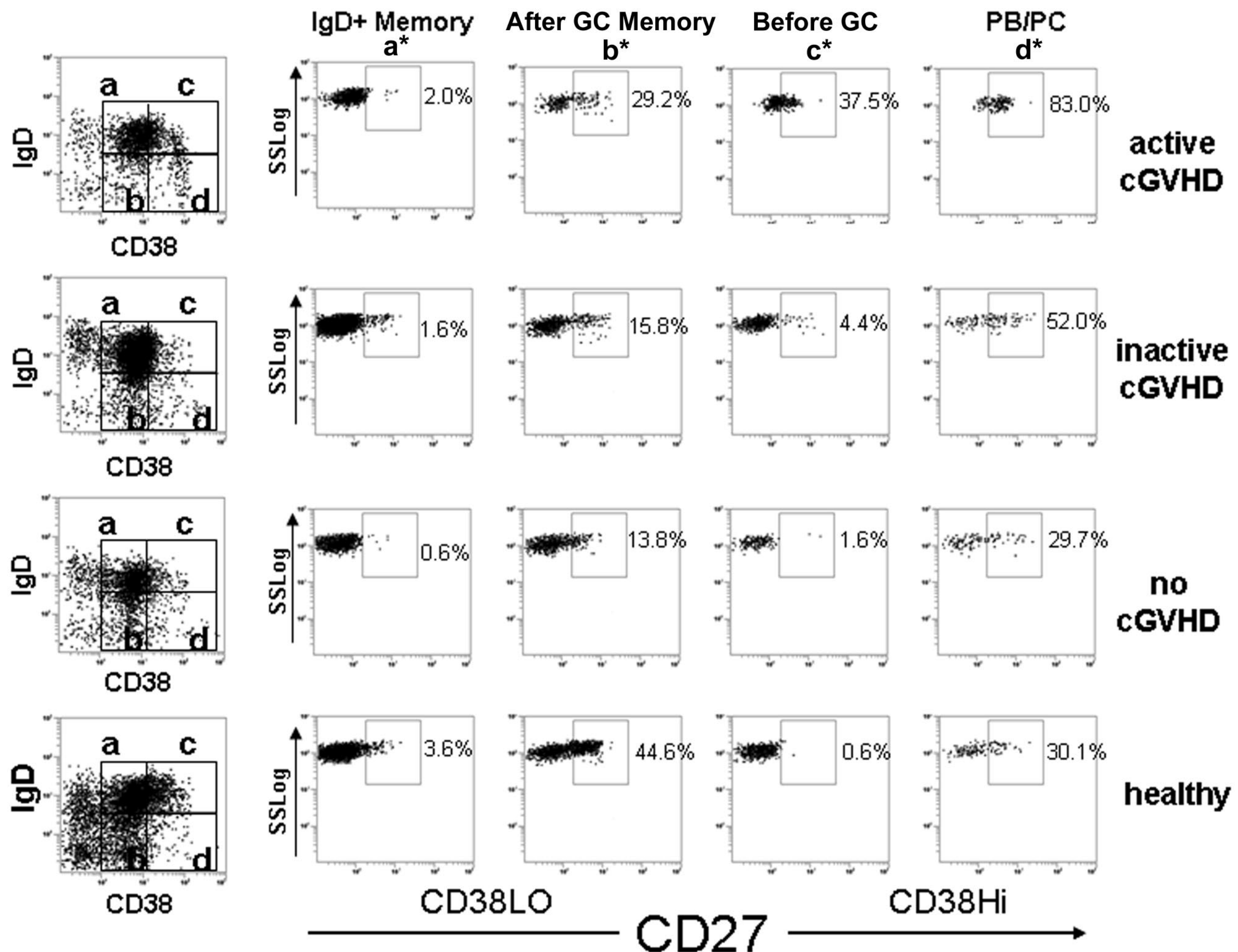

Figure 2. Flow cytometric gating algorithm demonstrating circulating CD27⁺ B-cell subsets in patients after HSCT. Gated CD19⁺ B cells were first examined for IgD

Downloaded from https://ashpublications.org/blood/article-pdf/113/16/3865/1307974/zh801609003865.pdf by NEW YORK UNIVERSITY user on 20 January 2020

DOCKET ALARM
Find authenticated court documents without watermarks at docketalarm.com.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.

WHAT WILL YOU BUILD? | sales@docketalarm.com | 1-866-77-FASTCASE

fastcase®
Smarter legal research.