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Spontaneous Autologous Graft-versus-Host Disease in Plasma Cell Myeloma Autograft Recipients: Flow Cytometric Analysis of Hematopoietic Progenitor Cell Grafts

Hillard M. Lazarus,[1] Scott R. Sommers,[1] Lisa M. Arfons,[2] Pingfu Fu,[3] S. A. Ataergin,[4] N. M. Kaye,[5] F. Liu,[5] Tamila L. Kindwall-Keller,[1] Brenda W. Cooper,[1] Mary J. Laughlin,[1,] Richard J. Creger,[1] Paul M. Barr,[1,†] Stanton L. Gerson,[1] David Kaplan[5,6]*

Nine plasma cell myeloma patients spontaneously developed histologically proven autologous graft-versus-host disease (GVHD) limited predominantly to the gastrointestinal tract within 1 month of initial autologous hematopoietic cell transplantation (AHCT) using high-dose melphalan conditioning. All recipients responded promptly to systemic and nonabsorbable oral corticosteroid therapy. All patients previously received systemic therapy with thalidomide, lenalidomide, or bortezomib before AHCT. Using enzymatic amplification staining-enhanced flow cytometry, we evaluated expression of selected transcription regulators, pathway molecules, and surface receptors on samples of the infused hematopoietic cell grafts. We demonstrated significantly enhanced expression of GATA-2, CD130, and CXCR4 on $CD34^+$ hematopoietic progenitor cells of affected patients compared with 42 unaffected AHCT controls. These 3 overexpressed markers have not been previously implicated in autologous GVHD. Although we did not specifically evaluate T cells, we postulate that exposure over time to the various immunomodulating therapies used for induction treatment affected not only the $CD34^+$ cells but also T cells or relevant T cell subpopulations capable of mediating GVHD. After infusion, the affected hematopoietic progenitor cells then encounter a host that has been further altered by the high-dose melphalan preparative regimen; such a situation leads to the syndrome. These surface markers could be used to develop a model to predict development of this syndrome. Autologous GVHD potentially is a serious complication of AHCT and should be considered in plasma cell myeloma patients with otherwise unexplained gastrointestinal symptoms in the immediate post-AHCT period. Prompt recognition of this condition and protracted treatment with nonabsorbable or systemic corticosteroids or the combination may lead to resolution.

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KEY WORDS: Autologous hematopoietic progenitor cell transplant, Autologous graft-versus-host disease, Plasma cell myeloma

INTRODUCTION

Autologous hematopoietic cell transplantation (AHCT) is an integral part of standard therapy for plasma cell myeloma patients, and this disorder is the most common indication for AHCT in North America [1-3]. Although allogeneic hematopoietic

From the [1]Department of Medicine, Division of Hematology/Oncology, Case Comprehensive Cancer Center, University Hospitals Case Medical Center, Cleveland, Ohio; [2]Department of Medicine, Division of Hematology/Oncology, Louis Stokes Cleveland VAMC, Cleveland, Ohio; [3]Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, Ohio; [4]Department of Medical Oncology and Bone Marrow Transplantation Unit, Gulhane (GATA) Faculty of Medicine, Ankara, Turkey; [5]Pathfinder Biotech, Cleveland, Ohio; and [6]Department of Pathology, University Hospitals Case Medical Center, Cleveland, Ohio.

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*Current address: Department of Medicine, Division of Hematology/Oncology, University of Virginia, Charlottesville, VA 29908.

†Current address: Department of Medicine, Division of Hematology/Oncology, University of Rochester, Rochester, NY 14642.

Correspondence and reprint requests: Hillard M. Lazarus, MD, FACP, Department of Medicine, University Hospitals Case Medical Center, 11100 Euclid Avenue, Cleveland, OH 44106 (e-mail: hillard.lazarus@case.edu).

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cell transplantation may result in a higher rate of molecular remission via an allogeneic effect, that is, graft-versus-myeloma eradication of the malignant clone by immunocompetent donor lymphocytes, graft-versus-host disease (GVHD), and high rates of treatment-related mortality plague, this modality and use remains controversial [4,5].

Relapse rates after AHCT are extremely high compared with allogeneic hematopoietic cell transplantation. These results reflect, in part, lack of a graft-versus-myeloma effect and reinfusion of autologous tumor cells [6]. A number of investigators have proposed that the induction of GVHD and attendant graft-versus-tumor effect may reduce relapse rates and improve overall patient outcome in a variety of malignancies [7-13].

Herein we report 9 cases of spontaneous autologous GVHD in plasma cell myeloma patients undergoing initial AHCT. We compared 6 of these 9 patients with 42 concurrently treated patients who did not develop this condition with respect to age, gender, stage at diagnosis, treatment type, $CD34^+$ cell dose, presence of diarrheal illness during the peritransplant period, and time to engraftment. Subsequently, we retrospectively analyzed all 9 patients' hematopoietic progenitor cell grafts using enzymatic amplification staining (EAS) enhanced flow cytometry to evaluate expression of various transcription regulators, pathway molecules, and surface receptors compared with 42 unaffected controls.

PATIENTS AND METHODS

Between March 2006 to September 2009, 48 patients underwent AHCT for plasma cell myeloma at University Hospitals Case Medical Center, Cleveland, Ohio. The institutional review board for human investigation granted approval for this retrospective study. Three additional patients with the clinical syndrome were added after the aforementioned time period for flow cytometric analysis, but were not included in the case-control analysis. All data were obtained via retrospective chart reviews using the electronic medical record and paper charts. Myeloma staging was reported using the Durie-Salmon [14] system. Blood cells were mobilized from patients who were treated uniformly with cyclophosphamide 4 g/m^2 intravenous, filgrastim (Amgen, Thousand Oaks, CA) 10 μg/kg once or twice daily subcutaneous (determined by resting-state the blood $CD34^+$ cell concentration), and prednisone 2 mg/kg/day by mouth for 4 days. Apheresis was undertaken when the blood $CD34^+$ cell count exceeded 10/μL. Filgrastim was continued until the last day of apheresis. A multilumen central venous apheresis catheter was placed either in the internal jugular or subclavian vein for blood cell mobilization and subsequent transplantation. The date of transplant was defined as day T = 0, and all days were calculated from this metric. The conditioning regimen consisted of amifostine 740 mg/m^2 i.v. on days T−2 and T−1 and melphalan 200 mg/m^2 i.v. on day T−1. All patients received filgastrim support from day T+1 until 3 consecutive days after blood neutrophil count >500/μL. Quantification of amounts and days of diarrhea could not be obtained in all patients because of inadequacies of documentation. Autologous GVHD was treated at the discretion of the attending physician using: corticosteroids (methylprednisolone or prednisone 1-2 mg/kg/day); budesonide 3 mg by mouth 3 times daily; or the combination. Subsequent weaning was undertaken at the discretion of the treating physician without use of a predetermined protocol.

Statistical Plan and Analysis

After recognizing 6 cases with autologous GVHD, we conducted a case-control study using 42 plasma cell myeloma patients who underwent an AHCT identically managed, yet who did not develop autologous GVHD during the same period (controls). The comparison between cases and controls was performed using the chi-square test or Fisher exact test for categoric variables and using the Student *t* test for comparing continuous measurements. The association between 2 continuous variables was estimated using the Pearson correlation coefficient. The cumulative neutrophil recovery rate was estimated using the Kaplan-Meier method, and the difference between cases and controls was tested using the log-rank test [15]. All tests were 2 sided and $P < .05$ was considered significant.

Flow Cytometric Analysis

Aliquots of peripheral blood mononuclear cells, cryopreserved at the time of initial hematopoietic cell mobilization, were available for the 9 affected patients and 42 controls. Cryopreserved samples were thawed and analyzed via EAS, which provides 10- to 100-fold greater sensitivity than standard staining procedures. This technology has been demonstrated to be precise, reproducible, quantitative, and stable upon freezing/thawing [16-20]. EAS obtains significant amplification by catalyzing the deposition of tyramide on the analyte-expressing cell. $CD34^+$ cells in the samples were assessed for expression of (1) pathway molecules (PTEN, pAkt[308], pAkt [473], β-catenin, GAB2); (2) transcription regulators (HoxB4, BMI-1, GATA-2, c-MYC, E47, RUNX1); and (3) surface receptors (IL-23R, IL-3R, CXCR4, CD117, CD130). The peripheral blood mononuclear cells were stained by Pathfinder Biotech (Cleveland, OH). Briefly, antibodies specific for CD34 and conjugated with AlexaFluor 647 were used to identify hematopoietic progenitor cells. The expression levels

of PTEN, phospho-Akt (thr308), phospho-Akt (ser473), β-catenin, HoxB4, RUNX1, Bmi-1, GATA-2, E47, and GAB2 were assessed after fixation and permeabilization by processing the signals in the fluorescein channel with EAS. The expression levels of IL-23R, CD123, CD117, CD130, and CXCR4 were assessed on live cells by processing the signals in the fluorescein channel with EAS. Stained samples were analyzed on a FACSCalibur flow cytometer and CELLQuest software. Isotype/subtype matched immunoglobulins were used to control the expression levels of the various analytes. The median fluorescence ratio was obtained from the median fluorescence intensities for the specific antibodies versus matched control immunoglobulin.

Antibodies

Rabbit antibodies specific for PTEN, phospho-Akt (thr308), phospho-Akt (ser473), and β-catenin were purchased from Cell Signaling Technology (Danvers, MA). Rabbit antibodies specific for HoxB4 and RUNX1 were purchased from Epitomics (Burlingame, CA). Antibodies specific for Bmi-1, GATA-2, IL-23R, and CXCR4 were purchased from R&D Systems (Minneapolis, MN). Antibodies specific for E47 and CD123 were purchased from BD Biosciences (San Jose, CA). Antibodies specific for GAB2 and CD130 were purchased from abcam (Cambridge, MA). Antibodies specific for CD117 and CD34 were purchased from BioLegend (San Diego, CA). Antibody specific for c-MYC was purchased from Invitrogen (Seattle, WA).

RESULTS

Table 1 shows the patient-, disease-, treatment-, and symptom-related information for the 5 women and 4 men median (range) age 62 (38-68) years. Autologous GVHD was confirmed by histologic examination in all 9 patients. This phenomenon occurred at a median (range) time to onset of symptoms at 8 (2-27) days after initial AHCT. Biopsies were obtained at a median (range) of 14 (11-45) days after transplant. All symptoms responded promptly to therapy with no long-term sequelae. Three of the 9 patients have relapsed or died.

Case-Control Analysis

Six patients affected in the predetermined time period were included in a case-control analysis; the 3 additional affected patients were not included in this analysis. During the same time period, 42 plasma cell myeloma patients (controls) underwent an AHCT and were compared with the 6 affected patients (Table 2). Eleven of the patients (26%) in the unaffected group reportedly had a diarrheal illness compared with 100%

Table 1. Nine Patients Affected with Autologous GVHD

Age/Gender	Initial Stage*/Type	Prior Therapies	$CD34^+ \times 10^6$/kg Cell Dose	Symptom Onset after HCT (Days)	Time to Biopsy after HCT (Days)	Sites/Stage aGVHD	GVHD Therapy	Time To Neutrophil Recovery (Days)
55 years/M	IIIB IgG-κ	Bort/Dex; Cy	16.4	7	14	GI 3	Methylpred 1 mg/kg and Bud	8
62 years/F	IIIA IgG-κ	Thal/Dex;Bort/Dex;Mel/Pred	8.1	27	45	GI 4	Methylpred 1 mg/kg and Bud	11
53 years/F	IIIB IgG-κ	Thal/Dex	5.2	12	19	GI 3/liver 2	Methylpred 2 mg/kg	12
38 years/F	IIA IgG-κ	Thal/Dex/Bort	5.3	9	14	GI 3/liver 2	Pred 2 mg/kg	12
67 years/F	IIIA IgA-λ	Len/Dex	15.7	8	21	GI 3	Pred 1 mg/kg	10
56 years/M	IIIA IgA-κ	Len/Dex	15.0	8	11	GI 3/skin 2	Pred 2 mg/kg and Bud	10
64 years/F†	IIIB Free-κ	CyVD;Bort/Dox;Len/Dex	6.3	2	15	GI 3	Methylpred 1 mg/kg	11
66 years/M†	IA IgA-κ	Len/Dex;Bort/Dox	23.3	10	12	GI 3	Methylpred 2 mg/kg	10
68 years/M†	IIIA IgG-κ	Vel/Len/Dex	8.7	3	12	GI 2	Pred 1 mg/kg	10

HCT indicates hematopoietic cell transplantation; aGVHD, acute graft-versus-host disease; GVHD, graft-versus-host disease; Bort, Bortezomib; Dex, Dexamethasone; Cy, Cyclophosphamide; Methylpred, Methylprednisolone; Bud, Budesonide; Thal, Thalidomide; Pred, Prednisone; Len, Lenolidamide; Dox, liposomal doxorubicin.
*Stages reported as per Durie-Salmon [14].
†These 3 patients were added to the original 6 affected patients and were not included in the case-control analysis (Table 2). Their hematopoietic progenitor cells were analyzed in the flow cytometric analysis.

Table 2. Comparison of Clinical Factors

Variable	Unaffected (n = 42)	Autologous GVHD (n = 6)	*P* value
Median (range) age (years)	55 (41-69)	55.5 (38-67)	.954
Gender (male/female)	27/15	2/4	.197
Stage (Durie-Salmon)			.191
I/II	22 (52%)	1 (17%)	
III	20 (48%)	5 (83%)	
$CD34^+$ cell dose (10^6/kg): mean (range)	8.82 (3.51-17.5)	10.9 (5.2-16.4)	.269
Pretransplant therapies			
Thalidomide	25/42 (60%)	3/6 (50%)	.683
Melphalan	2/42 (5%)	1/6 (17%)	.336
Lenalidomide	20/42 (48%)	2/6 (33%)	.674
Bortezomib	10/42 (24%)	3/6 (50%)	.323
Doxorubicin	7/42 (17%)	0/6 (0%)	.573
Cyclophosphamide	1/42 (2%)	1/6 (17%)	.237
Multiple lines therapy	12/42 (29%)	2/6 (33%)	.99
Neutrophil recovery (days): median (range)	11 (9-26)	10.5 (8-12)	.357
Tandem autologous transplant	11/42 (26%)	0/6 (0%)	.313
Discharge summary reported diarrheal illness	11/42 (26%)	6/6 (100%)	.001

GVHD indicates graft-versus-host disease.

for the affected cases ($P = .001$). Sigmoidoscopy or biopsies were not obtained in these unaffected patients because of other clinical diagnoses. Indeed, 2 evaluations revealed *Clostridium difficile* enterocolitis; the other patients were suspected of having antibiotic-associated diarrhea or melphalan-induced mucositis that resolved without sequelae or extended duration. Age, $CD34^+$ cell dose infused, time to neutrophil recovery, and number of regimens and types of therapy were similar for both groups. With a limited number of cases, there were trends but not of statistical significance: Patients affected with autologous GVHD were more likely to be female (67% vs. 36%, $P = .197$) and more had advanced-staged disease (83% vs. 48%, $P = .191$).

Flow Cytometric Analysis

Coincident with this investigation, we were independently investigating molecular expression levels in $CD34^+$ hematopoietic progenitor cells from the same patients. Because the onset of autologous GVHD was early after transplantation, we considered the possibility that there are differences in the cellular inocula between samples that resulted in autologous GVHD and those that did not. Consequently, we analyzed the molecular expression results from the 9 clinically affected patients compared with controls, with the idea that molecular expression levels in the $CD34^+$ cells may relate to molecular expression levels in T cells or relevant T cell subpopulations (Table 3). Three targets, GATA-2, CD130, and CXCR4, had statistically significant increased expression ($P < .05$) compared with controls. A fourth target (c-MYC) was marginally significant ($P = .073$). Representative results are shown (Figure 1). There was no correlation between expression of CXCR4 and CD130, CXCR4 and c-MYC, CXCR4 and GATA-2, CD130 and c-MYC, or CD130 and GATA-2. GATA-2 and c-MYC positively correlated in $CD34^+$ cells from patients affected with autologous GVHD, with $r = .67$ ($P = .048$) compared with control patients (Figure 2).

DISCUSSION

More than 20 years ago, Hood and colleagues [21] described an autoimmune syndrome similar to acute GVHD after AHCT termed "autoaggression" syndrome, or autologous GVHD. This condition tended to be milder than classic GVHD after allogeneic transplantation, most commonly involved the skin (rarely the gastrointestinal tract or liver), infrequently required therapy, and often was self-limited [7,21-24]. Subsequently, autologous GVHD uncommonly has been reported to occur spontaneously and can be induced by design after administration of posttransplant immune modulation with cyclosporine A, interferon-γ, or the combination, in an attempt to stimulate a graft-versus-tumor response [8-13,21,24].

We report herein 9 plasma cell myeloma patients who appeared to develop the syndrome of autologous GVHD spontaneously at a median (range) time to onset of symptoms at 8 (2-27) days after initial AHCT. Forty-two similarly treated patients during this 2-year period did not exhibit symptoms to warrant clinical evaluation, and we found no clinical differences to be able prospectively to identify patients at highest risk. In contrast to previous autologous GVHD reports, only 1 patient had clinical skin involvement, whereas in all others the gastrointestinal (GI) tract findings predominated, and 2 subjects had liver dysfunction as well. Ideally, it would have been useful to compare diarrheal output and incidence between affected and unaffected patients to identify this syndrome on clinical grounds; the retrospective nature of this analysis, however, precluded this undertaking.

Table 3. Correlations between Autologous GVHD and Molecular Expression in Hematopoietic Progenitor Cells

	Autologous GVHD	Mean (Standard Deviation)	N	*P*
PTEN	−	13.0 (8.8)	38	.518
	+	15.2 (2.5)	8	
Phospho-Akt (thr308)	−	8.8 (1.0)	32	.329
	+	11.1 (1.2)	6	
Phospho-Akt (ser473)	−	3.0 (0.3)	36	.285
	+	2.5 (0.3)	9	
β-catenin	−	6.8 (0.6)	34	.402
	+	5.7 (0.9)	9	
GAB2	−	2.6 (0.3)	20	.579
			8	
	+	3.1 (087)		
HoxB4	−	6.0 (0.7)	38	.744
	+	5.6 (0.6)	9	
Bmi-1	−	14.7 (2.0)	38	.377
	+	16.4 (2.2)	9	
GATA-2	−	37.5 (4.9)	38	.005
	+	69.1 (6.7)	9	
c-MYC	−	50.9 (7.1)	30	.073
	+	80.0 (16.5)	9	
E47	−	27.9 (3.0)	26	.609
	+	31.3 (7.2)	9	
RUNX1	−	9.8 (0.8)	20	.412
	+	8.5 (1.4)	9	
IL-23R	−	33.9 (4.7)	27	.654
	+	38.4 (10.2)	9	
IL-3R	−	12.0 (1.8)	38	.166
	+	8.5 (1.6)	9	
CXCR4	−	5.4 (0.7)	38	<.0001
	+	1.9 (0.3)	9	
CD117 (c-Kit)	−	34.9 (5.4)	37	.853
	+	32.8 (6.5)	9	
CD130	−	6.2 (0.7)	36	.02
	+	9.8 (1.5)	9	

GVHD indicates graft-versus-host disease.

These features are most consistent with the autologous GVHD syndrome; engraftment syndrome is an unlikely possibility [25-27]. Signs and symptoms in our patients were confined mainly to the GI tract. Only 1 patient had a rash and 3 had a fever at time of biopsy, features considered the most salient diagnostic aspects of engraftment syndrome. Additionally, affected patients did not exhibit the findings of capillary leak, marked weight gain, noncardiogenic pulmonary edema, and multiple organ dysfunction syndrome.

Further, we examined a number of molecular targets in the infused hematopoietic progenitor graft. We identified CD130 [28,29], GATA-2 [30-32], CXCR4 [33-35], and c-MYC [36,37], molecules well-described as integral in hematopoietic cell development, as having increased expression in $CD34^+$ hematopoietic progenitor cells in those patients affected with autologous GVHD.

Our patients were treated with bortezomib. CD130 [38,39] signaling is perturbed under the influence of bortezomib. Those patients unaffected by autologous GVHD (controls) did not exhibit these findings in their autologous grafts. Overexpression of these 4 markers has not been implicated previously in autologous GVHD. Although we did not specifically evaluate T cells, we postulate that exposure over time to the various immunomodulating therapies used for induction treatment affected not only the $CD34^+$ cells but also T cells or relevant T cell subpopulations capable of mediating GVHD. After infusion, these affected hematopoietic progenitor cells then encounter a host that has been further altered by the high-dose melphalan preparative regimen; such a situation leads to autologous GVHD. Further prospective study is needed to validate these findings.

This phenomenon in our case series appears to be more common and with features different than most previously reported cases of spontaneously occurring autologous GVHD. Only 1 of our patients appeared to have skin involvement, although we did not perform random cutaneous biopsies in unaffected skin and cannot exclude subclinical manifestations. Further, hepatic evidence of autologous GVHD, an extremely uncommon finding, apparently affected 2 AHCT recipients. Onset in our patient population was much earlier than in any of the other series.

Kline and colleagues [40] reviewed reports of spontaneously occurring autologous GVHD. This syndrome developed in a variety of disorders after AHCT, but the incidence was uncommon. Most affected patients had skin involvement, but GVHD

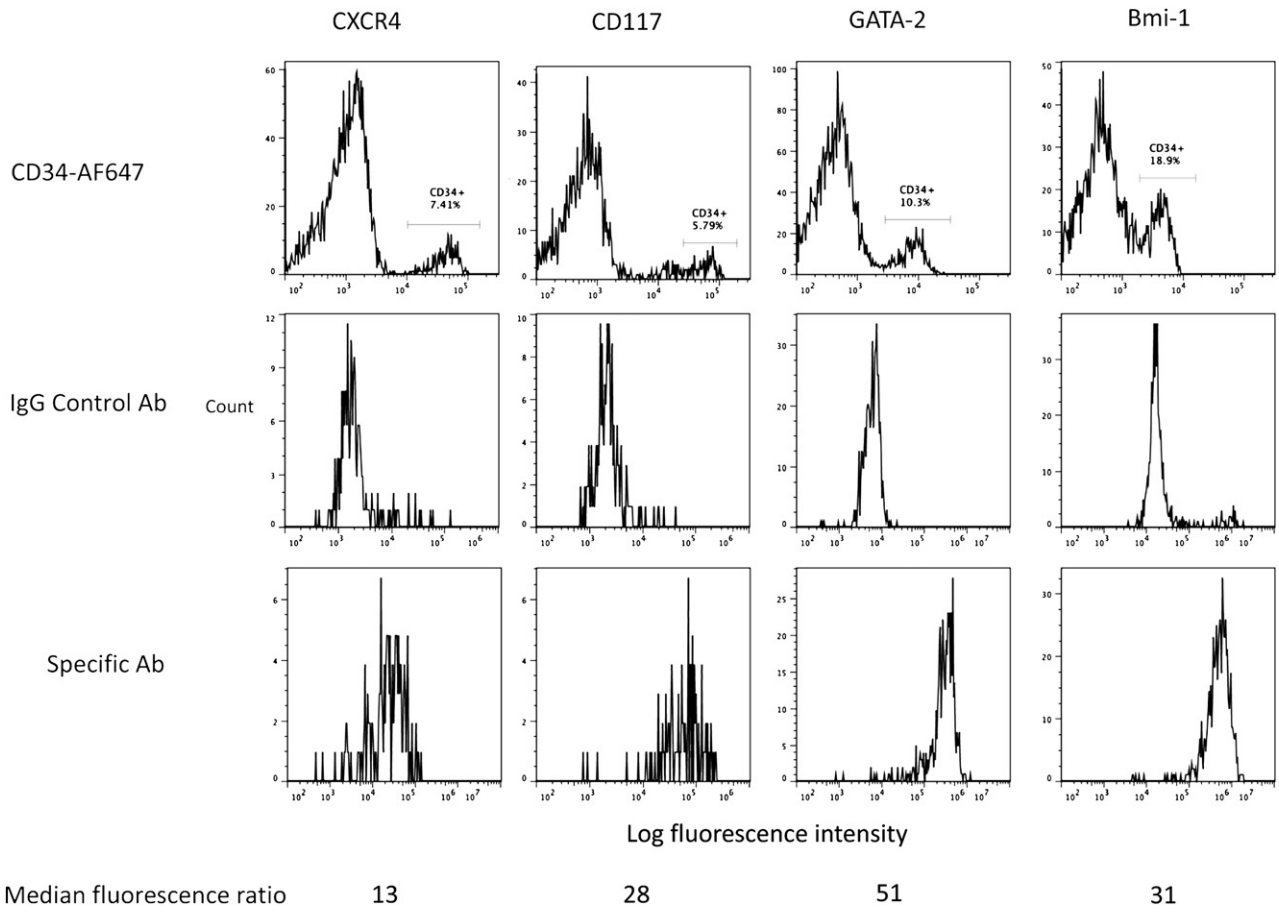

Figure 1. Representative flow cytometric analysis of $CD34^+$ cells in mobilized blood samples. Mononuclear cells from 4 different mobilized blood samples were stained for CD34 expression (upper row). The cells were also stained with control immunoglobulin (middle row) or with specific antibodies (lower row) and processed by EAS for high-resolution immunophenotyping. The specific antibodies shown are CXCR4 (lower left), CD117 (lower middle left), GATA-2 (lower middle right), and Bmi1 (lower right). The stains for CXCR4 and CD117 are surface stains without fixation and permeabilization. The stains for GATA-2 and Bmi-1 are intracellular stains with fixation and permeabilization. The amplified signals (middle and lower rows) are shown only for the $CD34^+$ gates (upper row). Representative results are shown from 4 different donors in order to demonstrate the consistency in $CD34^+$ cell delineation.

affecting the GI tract was rare. Time to clinical onset in several series ranged from 11 to 37 days after AHCT. Holmberg and colleagues [22] noted that 90 of 681 (13%) AHCT recipients, many of whom received the GI-toxic regimen "BUMELT" (busulfan/melphalan/thioTEPA), developed autologous GVHD. Findings included persistent nausea, vomiting, and diarrhea, mucosal abnormalities at endoscopy, and histology showing apoptotic crypt cells with or without lymphoid infiltrates. They surmised that the resultant GI mucosal damage increases the risk of autologous GI GVHD as activated cytotoxic T cells "home" to and cause further GI mucosal damage. Women, especially those with the diagnosis of breast cancer, had a 3-fold higher rate of developing this syndrome compared with men. Mean time to establishing a histologically proven diagnosis and starting prednisone treatment was 42 and 45 days, respectively, after AHCT. Most patients responded promptly to corticosteroid therapy. It should be noted that 20% of affected patients were treated with interleukin-2.

Recently, Drobyski and colleagues [41] described 5 cases of severe GI tract autologous GVHD in a population of 250 plasma cell myeloma AHCT recipients. The syndrome was corticosteroid-refractory in all 5 and was fatal in 4 of the affected patients. In contrast to our patients, autologous GVHD tended to develop after the second (tandem) autograft and was much later in onset. Only 2 of the 5 affected patients received therapy with thalidomide, lenalidomide, or bortezomib; the remaining patients received vincristine, adriamycin, and dexamethasone. The T and B cell subsets in the hematopoietic cell grafts did not differ between affected and the unaffected patients. They speculated that the second AHCT might compromise endogenous peripheral regulatory mechanisms and predispose these patients to autoimmunity. Certainly, in comparison to Drobyski and colleagues' [41] report, our population lacked the severe morbidity and mortality.

We hypothesize several reasons for these findings including perturbation of the immune system

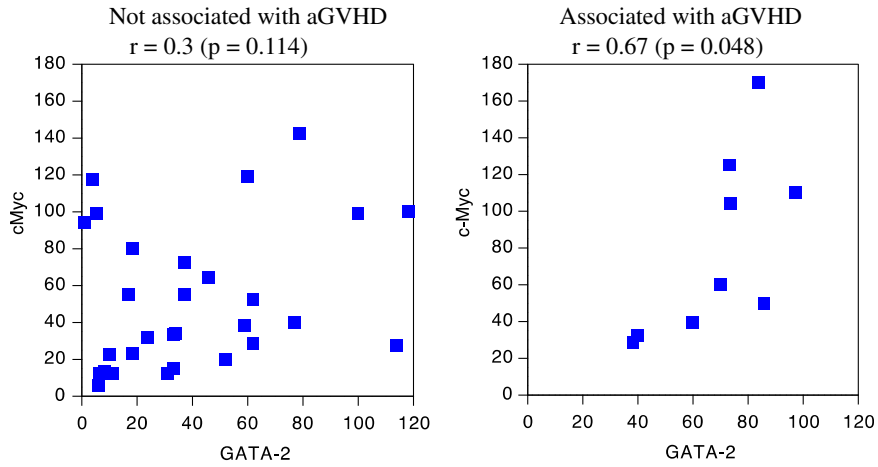

Figure 2. Correlation of cMyc and GATA-2 expression levels in $CD34^+$ cells. $CD34^+$ cells from various samples of mobilized blood from patients with multiple myeloma were stained for the expression of c-MYC and separately for the expression of GATA-2. The cells were processed by EAS for high-resolution immunophenotyping. Samples not associated with the subsequent development of autologous GVHD in the transplanted patients (left panel) and samples that were associated with the subsequent development of autologous GVHD in the transplanted patients (right panel) are shown. The distributions of median fluorescence ratios are shown for the various samples. Coefficients of correlation and *P* values are shown. Abbreviations: aGVHD indicates acute graft-versus-host disease.

composition because of the underlying disease, the use of new immunomodulating medications, and the conditioning regimen for the AHCT. Frassanito and colleagues [42] demonstrated that $CD8^+/CD57^+$ lymphocytes contribute to the immune dysregulation in plasma cell myeloma. Many investigators have described the perturbations in the immune system that occur in response to myeloma therapies, especially the new immunomodulating therapies. The antitumor properties of thalidomide and its analogues are incompletely understood but likely include antiangiogenic, antiproliferative, and anti-inflammatory effects, as well as immunomodulation of T cells and NK cells. Galustian and colleagues [43] demonstrated that lenalidomide and pomalidomide inhibit T regulatory cell expression and suppressor activity against self-reactive cells in vitro. Also, thymic function in adults receiving high-dose cytotoxic therapy is poor, and patients are susceptible to attack by autoreactive immune cells. Two groups [44,45] have demonstrated in separate reports that high-dose melphalan significantly suppresses the overall number and function of T cells.

Melphalan is an agent used commonly in conditioning plasma cell myeloma patients for AHCT that frequently leads to profound mucositis and symptoms akin to GVHD of the GI tract. This overlap could lead to delay in diagnosing autologous GVHD and instituting appropriate therapy. We believe it important to follow myeloma patients who develop autologous GVHD to know if they experienced differences in relapse and survival rates compared with patients who did not develop autologous GVHD. To date, autologous GVHD has not been associated with an enhanced graft-versus-tumor effect [40]. In contrast to chronic GVHD, autologous GVHD in the allogeneic transplant setting is not reportedly associated with a decreased relapse rate. Indeed, in our initial cohort, 3 of 9 patients had disease progression.

This uncommon complication of autologous GVHD in plasma cell myeloma AHCT recipients needs to be recognized early, as it appears to be treatable. This syndrome may be underreported as 1 of the many possible causes of diarrhea in this population. These findings easily can be mistaken for melphalan-induced mucositis or antibiotic-associated colitis. Therefore, patients who develop intractable symptoms and signs of GI tract injury, with or without hepatic dysfunction, should undergo appropriate investigations that may include endoscopy and possibly mucosal biopsy in order to start GVHD therapy promptly. Expression of CD130, GATA-2, and CXCR4 is altered in the hematopoietic progenitor cells of patients affected with autologous GHVD. We hypothesize that the differences we observed in the hematopoietic progenitor cells are associated with differential molecular expression levels in T cells or in relevant T cell subpopulations. Further prospective study is needed to validate these markers to see if a model could be developed to predict this syndrome.

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AUTHORSHIP STATEMENT

H.M. Lazarus designed research, performed research, collected data, analyzed and interpreted data,

and wrote the manuscript. S.R. Sommers performed research, collected data, analyzed and interpreted data, and wrote the manuscript. L.M. Arfons analyzed and interpreted data. P. Fu analyzed and interpreted data, and performed statistical analysis. S.A. Ataergin collected, analyzed and interpreted data, and wrote the manuscript. N.M. Kaye contributed vital new reagents or analytical tools. F. Liu contributed vital new reagents or analytical tools. T.L. Kindwall-Keller analyzed and interpreted data. B.W. Cooper analyzed and interpreted data. M.J. Laughlin analyzed and interpreted data. R.J. Creger analyzed and interpreted data. P.M. Barr analyzed and interpreted data. S.L. Gerson analyzed and interpreted data. D. Kaplan designed research, performed research, analyzed and interpreted data, wrote the manuscript, and contributed vital new reagents or analytical tools.

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