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Biology

Immune Cell Subset Counts Associated with Graft-versus-Host Disease

Peter J. Podgorny*, Yiping Liu, Poonam Dharmani-Khan, Laura M. Pratt, Kareem Jamani, Joanne Luider, Iwona Auer-Grzesiak, Adnan Mansoor, Tyler S. Williamson, Alejandra Ugarte-Torres, Mette Hoegh-Petersen, Douglas A. Stewart, Andrew Daly, Faisal M. Khan, James A. Russell, Jan Storek

Department of Medicine, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada

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Graft-versus-host disease (GVHD) is a major transplantation complication. The purpose of this study was to measure immune cell subsets by flow cytometry early after transplantation (before median day of GVHD onset) to identify subsets that may play a role in GVHD pathogenesis. We also measured the subsets later after transplantation to determine which subsets may be influenced by GVHD or its treatment. We studied 219 patients. We found that acute GVHD (aGVHD) was preceded by high counts of CD4 T cells and CD8 T cells. It was followed by low counts of total and naive B cells, total and cytolytic NK cells, and myeloid and plasmacytoid dendritic cells. Chronic GVHD (cGVHD) was preceded by low counts of memory B cells. In conclusion, both CD4 and CD8 T cells appear to play a role in the pathogenesis of aGVHD. Generation of B cells, NK cells, and dendritic cells may be hampered by aGVHD and/or its treatment. Memory B cells may inhibit the development of cGVHD.

INTRODUCTION

Graft-versus-host disease (GVHD) is the most important complication of allogeneic hematopoietic cell transplantation (HCT), leading to substantial mortality and poor quality of life. Multiple immune cell subsets, including subsets of T cells, B cells, NK cells, monocytes/macrophages, and dendritic cells, have been implicated in the pathogenesis of GVHD in animal models [1,2]. However, information is limited on whether these subsets play a role in the pathogenesis of GVHD in humans. Human studies depleting certain immune cell subsets from the graft or correlating numbers of immune cell subsets in the graft with the likelihood of developing GVHD have suggested a pro-GVHD role of T cells and B cells and anti-GVHD role of monocytes or invariant NKT cells [3-9]. These studies would be complemented by studies correlating numbers of immune cell subsets in the HCT recipient after transplantation, before the development of GVHD. However, only a few such studies have been reported [4,10-13], all using small numbers of patients, which precluded discovery-to-validation study design; thus, spurious associations may have been reported.

Here, we enumerated multiple immune cell subsets in the grafts and in the blood at predefined time points after transplantation in a large number of patients. Our main objective was to identify associations between *early* post-transplantation immune cell subset counts (before GVHD onset) and GVHD. We reasoned that such associations would suggest that these cells influence GVHD development. Our secondary objective was to identify associations between GVHD and immune cell subset counts at *later* time points (after GVHD onset). Such associations would suggest subsets influenced by GVHD and/or its treatment.

As we focused on clinically significant GVHD, we primarily compared the subset counts in patients with grade 0 to 1 versus 2 to 4 (significant) acute GVHD (aGVHD), and in patients with none or insignificant (not treated with systemic immunosuppressive therapy) versus significant (treated with systemic immunosuppressive therapy) chronic GVHD (cGVHD). First, in a discovery cohort, we determined for each subset whether there is an association with significant aGVHD or significant cGVHD. Second, all statistically significant associations from the discovery cohort analysis were subjected to validation in a validation cohort of patients of similar demographic and clinical characteristics. Third, we compared the subset counts in patients with grade 0 versus grade 2 to 4 aGVHD and in patients with no cGVHD versus significant cGVHD. This was done using only 1 combined discovery and validation cohort, as statistical power would be very limited if we used the discovery-to-validation design for this question.

As immune cells are suspected to play a role in not only GVHD but also in graft-versus-leukemia reaction, we also determined for each subset whether there is an association with relapse. As GVHD and relapse are the 2 most important causes of post-transplantation mortality, we also determined for each subset whether there is an association with death.

METHODS

Patients and Transplantation

For the discovery cohort, we studied 133 consecutive patients who underwent first allogeneic marrow or filgrastim-mobilized blood stem cell

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* Correspondence and reprint requests: Peter J. Podgorny c/o Storek Lab, Department of Medicine, 3330 Hospital Drive NW, Calgary, Canada T2N 4N1.

E-mail address: pjpodgor@ucalgary.ca (P.J. Podgorny).

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Table 1
Patient Characteristics

Characteristic	Discovery Cohort	Validation Cohort	Sig. of Difference*
No. of patients	133	86	
Patient age, median (range), yr	47 (19-66)	50 (19-66)	.164
Donor age, median (range), yr	35 (15-67)	37 (12-68)	.786
Patient sex	79 M, 54 F (59% M, 41% F)	50 M, 36 F (58% M, 42% F)	.853
Donor sex	89 M, 44 F (67% M, 33% F)	48 M, 38 F (56% M, 44% F)	.097
Diagnosis/disease stage at transplantation†			
Poor risk	58 (44%)	38 (44%)	.933
Good risk	75 (56%)	48 (56%)	
Diagnosis			
AML in first remission	42 (32%)	27 (31%)	.007
AML beyond first remission‡	16 (12%)	10 (12%)	
ALL in first remission	10 (8%)	12 (14%)	
ALL beyond first remission	5 (4%)	5 (6%)	
CML in first chronic/accelerated phase	8 (6%)	0	
CML in blast or second chronic/accelerated phase	2 (~1%)	0	
CMML	5 (4%)	0	
CLL	2 (~1%)	7 (8%)	
Non-Hodgkin's lymphoma	21 (16%)	7 (8%)	
Hodgkin's lymphoma	2 (~1%)	0	
Myelodysplasia/myelofibrosis	14 (11%)	17 (20%)	
Aplastic anemia	4 (3%)	0	
Other§	2 (~1%)	1 (1%)	
Stem cell source			
Bone marrow	8 (6%)	0	.020
Blood stem cells	125 (94%)	86 (100%)	
Donor/recipient CMV serostatus at HCT			
Positive/positive	37 (28%)	28 (33%)	.687
Positive/negative	11 (8%)	7 (8%)	
Negative/positive	30 (23%)	19 (22%)	
Negative/negative	54 (41%)	27 (31%)	
Unknown or indeterminate	1 (~1%)	5 (6%)	
Donor/recipient EBV serostatus at HCT			
Positive/positive	117 (88%)	65 (76%)	.106
Positive/negative	4 (3%)	7 (8%)	
Negative/positive	9 (7%)	9 (10%)	
Negative/negative	0	1 (1%)	
Unknown or indeterminate	3 (2%)	4 (5%)	
Conditioning (in addition to ATG)			
Fludarabine + busulfan	44 (33%)	27 (31%)	.058
Fludarabine + busulfan + TBI	81 (61%)	59 (69%)	
Other‖	8 (6%)	0	
Donor type			
HLA-matched sibling	64 (48%)	37 (43%)	.460
Other¶	69 (52%)	49 (57%)	
Acute GVHD by grade			
None	59 (44%)	33 (38%)	.138
Grade 1	34 (26%)	32 (37%)	
Grade 2	27 (20%)	12 (14%)	
Grade 3	12 (9%)	5 (6%)	
Grade 4	1 (~1%)	3 (3%)	
Indeterminate	0	1 (1%)	
Acute GVHD grade 2-4 developing before the day 28 blood draw	16 (12%)	3 (3%)	.044
Chronic GVHD			
None	72 (54%)	44 (51%)	.091
Not needing systemic therapy	13 (10%)	15 (17%)	
Needing systemic therapy	48 (36%)	27 (32%)	
Chronic GVHD needing systemic therapy developing before the day 84 blood draw	3 (2%)	0	.141
Relapse	26 (20%)	18 (21%)	.868
Second malignancy#	11 (8%)	5 (6%)	.495
Death	41 (31%)	27 (31%)	.929
Follow-up (days after transplantation), median (range)			
For GVHD or relapse**	795 (38-1657)	512 (34-1027)	<.001
For GVHD or relapse among patients without relapse/second malignancy/death	1034 (181-1532)	641 (68-1027)	<.001
For death	835 (59-1657)	546 (44-1027)	<.001
For death among surviving patients	1018 (181-1532)	650 (68-1027)	<.001
Actual blood draw days after transplantation, median (range)			
Projected day 28	28 (23-36)	27 (23-34)	.025
Projected day 56	56 (50-68)	55 (51-64)	.172
Projected day 84	84 (77-105)	83 (75-86)	.028

(*Continued*)