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Brief communication

Frequent expression of C4d in hepatic graft-versus-host disease: Potential clue for diagnosis and distinguishing acute and chronic form

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ABSTRACT

Background: Graft-versus-host disease (GVHD), a common complication of hematopoietic stem cell transplant, is generally regarded to develop through cell-mediated immune response following activation of helper T cells. Since production of antibodies is also mediated by helper T cells, the role of humoral immunity in GVHD is questioned and has not yet been explored in clinical practice. We conducted a pilot study to evaluate the role of antibody production in hepatic H-GVHD and whether it can distinguish acute and chronic forms.

Results: C4d expression was increased in portal vessels and hepatic sinusoids of patients with histological proven evidence of GVHD 11/16 ($P=0.007$). Patients classified as chronic GVHD were statistically more likely to have C4d expression in the portal vasculature and liver sinusoids ($P=0.011$).

Conclusion: Humoral activation seems to play a role in pathophysiology of hepatic, especially chronic GVHD.

1. Introduction

Graft-versus-host disease (GVHD) remains the most series complication after allogeneic hematopoietic stem cell transplantation (1,2). Despite HLA identity between donors and recipients, approximately 40–80% of patients still develop GVHD even though, acute and chronic forms of GVHD appear to involve different immune cell subsets, produce different cytokine profiles, affect different targets, and respond differently to treatment. The presentations sometimes overlap, which makes the distinction hard and somewhat arbitrary based on the time of onset.

In the liver, the hallmarks of hepatic (H-) GVHD are T cell-mediated bile duct damage (BDD) along with cholestasis. In acute form, more inflammatory cells and bile duct epithelial damage are present. While, chronic form is more of an absence of bile ducts (ductopenia) and fibroses of portal tract (3). Nonetheless, the distinction between the two forms continue to be a major challenge and often impossible on a given biopsy. One area of particular interest to the author has been B-cell activation with presence of autoantiboides. In this study we attempt to investigate the rule of antibody production in a group of post-allogeneic hematopoietic stem cell transplant (HSCT) who were diagnosed and followed up in our institution. Our objective is to first determine if there is a role of humoral activity in GVHD and second if this activity can be used in the clinical practice to distinguish one form of the disease from other.

2. Background on C4d

Complement split product 4d (C4d) is a degradation product of complement factor C4, a component of the classical complement cascade, which is typically initiated by binding of antibodies to specific target molecule e.g. HLA antigens. Following activation of C4 molecule, thio-ester groups are exposed which allow transient and covalent binding of the degradation product C4d to the endothelial cell surfaces, intracytoplasmic and to the extracellular matrix of vascular basement membranes. This binding renders C4d a stable molecule that can be detected by immunohistochemial technique (4). Nowadays C4d expression is regarded as an indirect 'footprint' sign and solid diagnostic marker of antibody mediated rejection in solid organ transplant e.g. renal and cardiac. In allogeneic HSCT, differences in MHC are a pre-requisite for GVHD to occur, which in turn may trigger anti-host antibody production and activates classic complement pathway. This antibody response theoretically can be detected by using C4d immunohistochemical antibodies. Such an example; C4d expression was noted in colonic biopsies of a small number of patients with chronic gut GVHD (5).

In liver, C4d detection has been tried to distinguish acute cellular rejection from recurrent hepatitis C in orthotropic liver transplanted individuals. Results had shown that C4d positive

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Table 1
Patients data ± Myeloablative patients; † HBV + recipient; Dix = primary bone marrow disease; Days BMT-Bx = days from the bone marrow transplantation to the day of the liver biopsy; H-GVHD = histological GVHD grade (scale 0–3); Fe++ = iron grading in liver biopsy; HLA = number of HLA antigens mismatch; Liver GVHD = clinical liver GVHD status; other GVHD and Max-GVHD = clinical acute grade/chronic grade; survival = as of May 2009; COD = cause of death; MOF = multiorgan failure; Bu = busulfan; CY = cyclophosphamide; TBI = total body irradiation; HPC-A Allo = peripheral blood stem cell, related allogeneic donor; HPC-M URD = bone marrow, unrelated donor; HPC-C URD = cord blood, unrelated donor; HPC-M Allo = bone marrow, related donor; ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; CLL = chronic lymphocytic leukemia; NHL = non-Hodgkin's lymphoma; HL = Hodgkin lymphoma; MDS = myelodysplastic syndrome; AA = aplastic anemia; MPD = myeloproliferative disorder.

N	Age	Sex	Dix	Days BMT-Bx	H-GVHD	C4d	Fe++	Fibro.	Tx. Type	HLA	Reg./myelo.	Liver - GVHD	Other GVHD	Max GVHD	Survival	COD
1	47	M	NHL	210	0	0	3	0	HPC-A Allo	0	CBV±	0	Skin-2/Lmt	2/Lmt	1842	
2	58	F	AML	200	2	0	4	1	HPC-A URD	0	Bu/Cy±	2	Skin-2	2	415	CGVHD/Infection
3	27	M	AA	-182	0	3+	0	0	HPC-C URD	-1	Bu/Cy/Thio±	0	0	0	12	Fungal infection
4	59	F	CLL	393	2	0	3	0	HPC-A Allo	0	None	1	Skin-1/Gut-4	3	610	Infection/ Disease progression
5	43	M	MDS	53	1	2+	1	0	HPC-A URD	-1	Bu/Cy±	0	0	0	63	Disease progression
6	26	F	HL	230	0	0	2	0	HPC-C URD	-1	Bu/Cy/Thio±	0	Skin-3/Ext	2/Ext	1560	
7	31	M	MDS	844	1	0	1	0	HPC-A URD	0	Flu/Cy	3	Gut-4	4	260	GVHD/ Infection
8	62	M	AML	174	1	0	1	0	HPC-A Allo	0	Flu/Bu±	0	Skin-2	1	1272	Pulm hem/ Disease progression
9†	56	M	AML	178	1	0	3	0	HPC-A Allo	0	Flu/Bu±	1	Skin-2	1	388	MOF /Hep B reactivity
10	63	F	RCC	31	0	0	1	0	HPC-A URD	0	Flu/Cy±	3	Gut-4	4	38	MOF
11	61	F	NHL	24	0	0	1	1	HPC-A Allo	0	BEAM±	0	0	0	32	Disease progression
12	24	F	ALL	36	3	2+	4	1	HPC-A URD	0	Cy/TBI±	2	Skin-1	2	148	Disease progression
13	30	M	ALL	48	2	1+	3	0	HPC-A URD	0	Cy/TBI±	2	Skin-2	3	86	Disease progression/ GVHD
14	37	F	AML	84	1	2+	3	0	HPC-A Allo	0	Bu/Cy±	3	0	2	283	Disease progression
15	33	F	AA	30	2	1+	3	0	HPC-A Allo	0	Flu/Cy±	4	Skin-3	4	204	GVHD/ Disease progression
16	59	F	CML	155	3	1+	1	0	HPC-A Allo	0	Bu/Cy±	4	Skin-3/Gut-4	4	191	Infection/ GVHD
17	41	F	CML	399	2	1+	1	1	HPC-A URD	0	Bu/Cy±	2/Lmt	Skin-1/Lmt Gut-4/ Lmt	4/Lmt	742	
18	55	M	AML	146	3	1+	4	1	HPC-A Allo	-1	Bu/Cy±	Ext	Skin-Lmt/ Gut-Ext	Ext	393	ARDS/ Infection/MOF
19	37	M	AML	-133	0	0	3	0	HPC-C URD	0	Bu/Cy/Thio±	0	0	0	3191	
20	47	F	MPS	140	2	1+	2	0	HPC-M Allo	0	Bu/Cy±	Lmt	Skin - Lmt	Lmt	315	
21	55	M	MDS	225	1	1+	3	0	HPC-A Allo	0	Bu/Cy±	Lmt	Skin-Lmt/ Gut-Ext	Ext	245	Infection/ MDS/GVHD
22	61	F	MDS	223*	3	3+	3	0	HPC-A Allo	0	Bu/Cy±	Ext	Skin-Lmt/ Gut-Lmt	Ext	238	

staining is identified in 33–69.2% of biopsies of patient with acute cellular rejection, (6,7) and to a lesser degree, 0–33.3%, in patients with recurrent viral hepatitis (7–9). In control group, C4d was identified in 6.9% (9).

3. Materials and methods

3.1. Patients

After obtaining the approval to perform the study from the Institutional Review Board, the bone marrow transplant data-base was searched for allogenic bone marrow transplanted patients with additional liver biopsy between January 2004 and March 2009. A total of 23 allogenic HSCT patients were identified. One patient was eliminated because of the liver biopsy was intended for a mass lesion and had no reliable liver parenchymal tissue to evaluate. Two patients were found to have their liver biopsies before the transplantation which we included in the histological and immunohistochemical review for comparison. A total of 20 biopsies from 20 post allogenic HSCT patients were chosen for the study and statistical analysis. Patients demographics in summary were 10 men; twelve women, with an average of 43 (range 24–62) years. Nine patients had acute leukemia, two patients with chronic lymphocytic leukemia, two patients with non-Hodgkin lymphoma, four patients with myelodysplastic syndrome, two patients with aplastic anemia, one patient each with myeloproliferative, metastatic carcinoma and Hodgkin lymphoma. Twenty patients received peripheral stem cell transplantation (11 related; 8 unrelated; 1 cord blood). Only four patients had single HLA mismatched antigen. All patients were serologically negative for HIV, hepatitis C; while one patient was positive for HBV.

Table 2
Statistical analysis for the 20 patients.

Histologic-grading	C4d expression score				Total ($n=20$)			
H-GVHD	0	1+	2+	3+				
0	4	0	0	0	4 (20%)			*P*-value = 0.007
								r-value = 0.58
1	3	1	2	0	6 (30%)			
2	2	4	0	0	6 (30%)			
3	0	2	1	1	4 (20%)			
						Mean	SD	*P*-value = 0.011
Chronic	0	2	3	0	5 (25%)	1.6	0.55	
Acute	5	5	0	1	11 (55%)	0.73	0.90	
0	4	0	0	0	4 (20%)	0	0	
	After omitting the "0" category							*P*-value = 0.04
Chronic	0	2	3	0	5 (31%)	1.6	0.55	
Acute	5	5	0	1	11 (69%)	0.73	0.90	

3.2. GVHD assessment

GVHD was assessed in two ways. The first way which is the focus of this article, was to assess the occurrence of GVHD before immune manipulation. In this case, we re-evaluated GVHD retrospectively in respect to the clinical presentations and time of disease onset. Liver biopsy was performed when liver dysfunction started. Interpretations of biopsy results of H-GVHD, however, were regarded to the overall clinical impression of GVHD. Acute GVHD was evaluated within 100 days after HSCT and graded on a 5-point scale from 0 to IV(10). While, diagnosis of chronic GVHD was based on clinical manifestations; gut, skin and liver abnormalities which were considered part of chronic GVHD if they occurred beyond 100 days of transplantation. Chronic GVHD was graded as limited and extensive (11).

Second we determine the incidence of the maximum GVHD grade throughout the patient course of treatment (i.e., maximum acute alone, maximum chronic grade alone and maximum combined acute and chronic grade) (Table 1). Mortality was considered if the patient died of GVHD related complications. Follow up of patients were available till last hospital visit/admission or till the time of this report, May 2009 or time of death.