Published in final edited form as:
Biol Blood Marrow Transplant. 2010 February ; 16(2): 287–291. doi:10.1016/j.bbmt.2009.10.008.

Monitoring and preemptive rituximab therapy for Epstein-Barr virus reactivation after anti-thymocyte globulin containing nonmyeloablative conditioning for umbilical cord blood transplantation

Anne H. Blaes, M.D.[1,2], **Qing Cao, M.S.**[1], **John E. Wagner, M.D.**[1,3], **Jo-Anne H. Young, M.D.**[1,4], **Daniel J. Weisdorf, M.D.**[1,2], and **Claudio G. Brunstein, M.D., Ph.D.**[1,2]

[1]University of Minnesota Blood and Marrow Transplant Program

[2]Division of Medical

[3]Division of Pediatric, Hematology, Oncology and Transplantation

[4]Division of Infectious Diseases

Abstract

Epstein Barr viremia (EBV) and posttransplantation lymphoproliferative disorder (PTLD) are complications of hematopoietic stem cell transplantation (HSCT). The use of antithymocyte globulin in recipients of umbilical cord HSCT is a known risk factor for the development of PTLD. In this high risk population, we implemented an EBV monitoring program with preemptive therapy with rituximab (375 mg/m2 IV) for EBV viremia (> 1,000 copies/mL). 8 of 35 patients treated with an UCB HSCT between 2007 and 2009 developed EBV viremia. 2 of 7 developed PTLD (with 1 of the 2 dying of PTLD), despite prophylactic rituximab use. When compared with our previously described cohort[6] where 6 of 30 developed EBV viremia and 5 of 6 patients developed PTLD (with 2 of 5 dying of PTLD), the incidence of PTLD appears to be less when prophylactic rituximab is administered. Despite small numbers, our observations suggest that in this high risk population, EBV monitoring accompanied by preemptive therapy may reduce the risk of progression to life-threatening PTLD; further follow-up of this cohort and a larger multi-institutional prospective study of this preemptive strategy is warranted.

Introduction

Epstein Barr virus (EBV) reactivation and posttransplantation lymphoproliferative disorder (PTLD) are complications of allogeneic hematopoietic stem cell transplantation (HSCT).[1–6] In HSCT recipients, the reported incidence can vary from 0.4% to 24%.[7, 8] The biggest risk factors for PTLD after HSCT are T-cell depletion, HLA disparity, use of antithymocyte globulin (ATG), and grade III/IV graft versus host disease (GVHD). There have also been

Address correspondence to: Dr. Anne H. Blaes, Department of Medicine, Mayo Mail Code 480, 420 Delaware Street, S.E., Minneapolis, MN, 55455, USA. Ph: 612 625-3918, Fax: 612 625-6919, blaes004@umn.edu.

Potential Conflicts of Interest and Financial Disclosures: J.H.Y. has received clinical trials funding from ViroPharma for evaluation of maribavir.

reports of an increased incidence of PTLD in subsets of patients who have received an umbilical cord blood (UCB) transplantation.[6, 9–11] In these reports, however, most of these patients had received ATG as part of their preparatory regimen.

High titer EBV viremia detected by quantitative polymerase chain reaction (qPCR) may be predictive of the development of PTLD.[12,13] EBV viral load monitoring and preemptive therapy with anti-CD20 antibodies may be one approach in reducing the incidence of PTLD.[14–20] We previously reported a high incidence of EBV viremia for recipients of an ATG-containing nonmyeloablative UCB.[6] Here we report the incidence and outcomes of EBV-related complications since we implemented an EBV monitoring program with preemptive therapy for this patient subset.

Patients and Methods

In January 2007, we instituted a monitoring protocol for all patients at our institution receiving an ATG-containing nonmyeloablative UCB HSCT. The preparatory regimen consisted of a single dose of cyclophosphamide (CY) 50 mg/kg intravenously (IV) on day −6, fludarabine (FLU) 40 mg/m^2 IV daily on days −6 to −2, a single fraction of total body irradiation (TBI) 200 cGy without shielding on day −1, and equine anti-thymocyte globulin (ATG, ATGAM, Pharmacia, Kalamazoo, MI) at 15 mg/kg IV every 12 hours on days −6 to −4. Graft selection, posttransplantation immunosuppression, GVHD therapy, and antiviral prophylaxis/supportive care after UCB have been previously described.[19, 20]

EBV Monitoring and Complication Definitions

Patients were monitored with on site serial qEBV PCR testing on whole blood using real-time TaqMan PCR every-two-weeks between days +30 and +180 post UCB transplantation. The amplicon was a 71-bp portion of the Ebna1 gene. qEBV results were reported as viral copies per milliliter (ml). The limit of detection of the assay was 10 viral copies/reaction.[21] EBV viremia was defined as more than 1000 copies of EBV DNA per ml of whole blood on one occasion. EBV PTLD was defined as biopsy proven PTLD with positivity for in situ hybridization by Epstein Barr early response (EBER) staining, regardless of the presence of viremia.

Preemptive Therapy Strategy

In the event of EBV viremia, patients underwent CT scan imaging to determine whether adenopathy was present; palpable or radiographic adenopathy led to a biopsy whenever possible. At the time of EBV viremia, patients received one dose of rituximab (RTX) 375 mg/m^2 IV (Genentech, Inc., South San Francisco, CA). All patients had hepatitis B serologies tested before HSCT, and no patient had an active hepatitis B viremia at the time RTX was started. Patients with EBV-viremia were monitored weekly in order to follow response to RTX. For patients with a decrease in EBV viremia titer following one dose of rituximab, viral loads were monitored weekly in order to follow response to RTX. For patients with persistent viremia or pathologic evidence of PTLD, additional weekly doses of RTX were administered until resolution of the viremia. Whenever possible, immunosuppression was tapered.

All HSCT protocols and this analysis were approved by the University of Minnesota Institutional Review Board and registered at www.clinicaltrials.gov (NCT00365287, closed and NCT00305682). All patients or their legal guardians provided written informed consent according to the Declaration of Helsinki.

Results

Thirty-five consecutive patients who received an UCB transplant with this uniform ATG containing nonmyeloablative regimen at the University of Minnesota between January 2007 and 2009 were included in this analysis. Eight of 35 patients (23%) developed EBV viremia, in 2 cases associated with PTLD. The demographic characteristics of the current cohort were compared to the high risk group of recipients previously reported[6] who also received a nonmyeloablative ATG containing UCB transplant. The results are summarized in Table 1.

The characteristics of our current cohort that developed EBV-related complications are described in Table 2. In our current cohort, the patients had a median age 60 years (range, 36–68 years), median weight 84 kg (range, 69–101 kg), and median follow-up of 349 days (range, 31–403 days). All received two unit UCB grafts. Their median infused total nucleated cell dose was 0.35×10^8/kg (range, 0.29–0.40) with a median CD34 cell dose of 0.37×10^6/kg(0.14–1.32). The median time from transplantation to EBV viremia was 83 days (range, 71–342). Median peak EBV viremia was 31,500 copies (range, 3,100–311,300). Patient 4 had a qPCR titer of 3,100 copies 243 days posttransplantation, yet EBV viremia resolved without treatment. At the time of initial reactivation at 1,300 copies, the patient's immunosuppression was decreased. The patient's viremia initially became undetectable and then increased to 3,100 copies. Given the low titers for EBV reactivation and the interval from transplantation the treating physician felt it was not clinically necessary to treat with preemptive rituximab. The EBV viremia subsequently resolved, and the patient remains alive over 300 days posttransplant.

Seven patients were preemptively treated with RTX for a median of three weekly doses (Table 2). Two of these patients (6%) had EBV-viremia associated with PTLD. Patient 1 had PTLD on his left tonsil, and underwent a tonsillectomy and received 4 weekly doses of RTX; he remains in complete remission now one year post-UCB HSCT. Patient 7 developed EBV-viremia and biopsy-proven PTLD of the lung. Despite 7 weekly doses of RTX and a decrease in immunosuppression, the patient with PTLD died of progressive disease.

Discussion

Epstein Barr virus (EBV) reactivation and posttransplantation lymphoproliferative disorder (PTLD) are complications of umbilical cord transplantation, particularly when ATG is used in the preparatory regimen[6, 11]. Based on our previously published report in which there was a high incidence of EBV viremia in recipients of an ATG containing nonmyeloablative UCB HSCT, we implemented an EBV monitoring program with preemptive therapy for this patient subset. As compared to our historical cohort, our current cohort received better HLA-matched grafts. There were similar rates of EBV-related complications. However, in the historical cohort without EBV monitoring, 5 of 6 with EBV reactivation developed PTLD

with two deaths, in contrast to 2 of 8 PTLDs in the current cohort with 1 related death. Despite small numbers, our observations suggest that in this high risk population EBV-monitoring accompanied by preemptive therapy may reduce the risk of progression to life-threatening PTLD.[14–18]

The options to treat EBV complications, in particular PTLD that often presents with disseminated disease and early, rapid organ dysfunction[22], after UCB HSCT are more limited than after non-UCB HSCT. Whereas immunosuppression can be tapered and patients may be given anti-CD20 antibodies and/or chemotherapy, there are no donor lymphocytes available from cord donor sources.[23] It is possible that in the future, with the availability of EBV specific cytotoxic T-lymphocytes[24] or UCB-derived ex-vivo expanded T-cells[25], this limitation may be overcome. An alternative strategy would be prophylactic RTX for high risk patients such as those receiving ATG or T-cell depleted grafts.[26] Although preliminary data suggest this approach may reduce the risk of PTLD, prophylactic RTX would be associated with greater cost and risk of adverse events. Therefore, at present, a low threshold to start preemptive therapy seems justified in order to prevent progression from EBV-viremia to PTLD that, once established, has a poor prognosis.[10]

Typically, HSCT recipients will develop PTLD early, at a median of two to three months after HSCT.[22] Two of our patients developed EBV complications beyond the planned 180 day window of monitoring at which time they were hospitalized for mental status changes (patient 4) and hypoxia (patient 6), respectively, resulting in the detection of EBV viremia (Table 2). Still 6 of 8 patients developed EBV viremia within 180 days, as did most patients in our historical cohort.[6] EBV-viremia qPCR monitoring is costly. Therefore, any monitoring strategy needs to consider the risk and timing of EBV complications and the cost of prophylactic RTX.[21]

In the event of a pathologic diagnosis of PTLD as opposed to isolated EBV-viremia, RTX therapy alone may not be able to eradicate rapidly growing PTLD. Immunosuppression must be reduced whenever possible and the addition of more conventional chemotherapy agents should be considered even though these agents may yield excess myelosuppression and greater toxicity.

Our results, with an incidence of PTLD now similar to other settings of UCB transplantation,[5, 6] support EBV-monitoring to day 180 and preemptive therapy for patients undergoing an ATG-containing nonmyeloablative UCB HSCT. While there are limited data on the appropriate threshold to give preemptive therapy,[14–18] and spontaneous remissions of viremia can occur, our EBV-viremia treatment threshold level and length of monitoring are covering our high risk population during a period in which there is a greater risk of EBV-related complications. Our preliminary data suggests a reduction in the risk of progression to PTLD. However, in order to draw more definitive conclusions, further follow-up of the current cohort and larger prospective multi-institutional studies to assure adequate numbers of patients are warranted.

Acknowledgments

This work was supported in part by grants from the National Cancer Institute PO1-CA65493 (J.E.W., C.G.B) and the Children's Cancer Research Fund (J.E.W.).

References

1. Hoshino Y, Kimura H, Tanaka N, et al. Prospective monitoring of the Epstein-Barr virus DNA by a real-time quantitative polymerase chain reaction after allogenic stem cell transplantation. Br J Haematol. 2001; 115:105–111. [PubMed: 11722419]
2. Hoshino Y, Kimura H, Kuzushima K, et al. Early intervention in post-transplant lymphoproliferative disorders based on Epstein-Barr viral load. Bone Marrow Transplant. 2000; 26:199–201. [PubMed: 10918431]
3. Gartner BC, Schafer H, Marggraff K, et al. Evaluation of use of Epstein-Barr viral load in patients after allogeneic stem cell transplantation to diagnose and monitor posttransplant lymphoproliferative disease. J Clin Microbiol. 2002; 40:351–358. [PubMed: 11825941]
4. Juvonen E, Aalto SM, Tarkkanen J, et al. High incidence of PTLD after non-T-cell depleted allogeneic haematopoietic stem cell transplantation as a consequence of intensive immunosuppressive treatment. Bone Marrow Transplant. 2003; 32:91–102.
5. Barker JN, Martin PL, Coad JE, et al. Low incidence of Epstein-Barr virus-associated posttransplantation lymphoproliferative disorders in 272 unrelated-donor umbilical cord blood transplant recipients. Biol Blood Marrow Transplant. 2001; 7:395–399. [PubMed: 11529490]
6. Brunstein CG, Weisdorf DJ, DeFor T, et al. Marked increased risk of Epstein-Barr virus related complications with the addition of anti-thymocyte globulin to a nonmyeloablative conditioning prior to unrelated umbilical cord blood transplantation. Blood. 2006; 108:2874–2880. [PubMed: 16804113]
7. Curtis RE, Travis LB, Rowlings PA, et al. Risk of lymphoproliferative disorders after bone marrow transplantation: a multi-institutional study. Blood. 1999; 94:2208–2216. [PubMed: 10498590]
8. Gross TG, Steinbuch M, DeFor T, et al. B cell lymphoproliferative disorders following hematopoietic stem cell transplantation: risk factors, treatment and outcome. Bone Marrow Transplant. 1999; 23:251–258. [PubMed: 10084256]
9. Yoo KH, Lee SH, Sung KW, et al. High incidence of posttransplant lymphoproliferative disorder after unrelated cord blood transplantation [abstract #5401]. Blood. 2006; 108
10. Ballen KK, Cutler C, Beow YY, et al. High Rate of Second Malignancies after Reduced Intensity Double Cord Blood Transplants in Adults. Blood. 2008; 112:1958.
11. Cohen J, Gandhi M, Naik P, et al. Increased incidence of EBV-related disease following paediatric stem cell transplant with reduced-intensity conditioning. Br J Haematol. 2005; 129:229–239. [PubMed: 15813851]
12. Dobbelstein CE, Kamal HM, Dammann E, Hertenstein B, Weissinger EM, Ganser A. Atypical presentations of EBV reactivation and EBV-associated PTLD post-HSCT: Implication for routine EBV surveillance after allogeneic HSCT? [abstract #5364]. Blood. 2005; 106
13. Clave E, Agbailka F, Bajzik V. Epstein-Barr virus reactivation in allogeneic stem-cell transplantation: Relationship between viral load, EBV-specific T-cell reconstitution and rituximab therapy. Transplantation. 2004; 77:76–84. [PubMed: 14724439]
14. Dominietto A, Tedone E, Soracco M, et al. Epstein-Barr virus reactivation after allogeneic hematopoietic stem cell transplant based on molecular monitoring is predictive of lymphoproliferative disease. Bone Marrow Transplant. 2004; 33:S192.
15. van Esser JW, Niesters HG, van der Holt B, et al. Prevention of Epstein-Barr virus-lymphoproliferative disease by molecular monitoring and preemptive rituximab in high-risk patients after allogeneic stem cell transplantation. Blood. 2002; 99:4364–9. [PubMed: 12036863]
16. Ahmad I, Cau NV, Kwan J, et al. Preemptive management of Epstein-Barr virus reactivation after hematopoietic stem cell transplantation. Transplantation. 2009; 87:1240–1245. [PubMed: 19384173]

17. Meerbach A, Wutzler P, Hafer R, Zintl F, Gruhn B. Monitoring of Epstein-Barr virus load after hematopoietic stem cell transplantation for early intervention in post-transplant lymphoproliferative disease. J Med Virol. 2008; 80:441–454. [PubMed: 18205222]

18. Gruhn B, Meerbach A, Hafer R, Zell R, Wutzler P, Zintl F. Preemptive therapy with rituximab for prevention of Epstein-Barr virus-associated lymphoproliferative disease after hematopoietic stem cell transplantation. Bone Marrow Transplant. 2003; 31:1023–1025. [PubMed: 12774054]

19. Barker JN, Weisdorf DJ, Defor TE, Blazar BR, Miller JS, Wagner JE. Rapid and complete donor chimerism in adult recipients of unrelated donor umbilical cord blood transplantation after reduced-intensity conditioning. Blood. 2003; 102:1915–1919. [PubMed: 12738676]

20. Brunstein CG, Weisdorf DJ, DeFor T, Barker JN, Tolar J, van Burik JA, Wagner JE. Marked increased risk of Epstein-Barr virus-related complications with the addition of antithymocyte globulin to a nonmyeloablative conditioning prior to unrelated umbilical cord blood transplantation. Blood. 2006; 108(8):2874–80. [PubMed: 16804113]

21. Balfour HH Jr, Holman CJ, Hokanson KM, et al. A prospective clinical study of Epstein-Barr virus and host interactions during acute infectious mononucleosis. J Infect Dis. 2005; 192:1505–1512. [PubMed: 16206064]

22. Shapiro RS, McClain K, Frizzera G, et al. Epstein-Barr virus associated B cell lymphoproliferative disorders following bone marrow transplantation. Blood. 1988; 71:1234–1243. [PubMed: 2833957]

23. Papadopoulos EB, Ladanyl M, Emanuel D. Infusions of donor leukocytes to treat epstein-barr virus-associated lymphoproliferative disorders after allogeneic bone marrow transplantation. NEJM. 1994; 330:1185–1191. [PubMed: 8093146]

24. Haque T, Wilkie GM, Jones MM, et al. Allogeneic cytotoxic T-cell therapy for EBV-positive posttransplantation lymphoproliferative disease: Results of a phase 2 multicenter clinical trial. Blood. 2007; 110:1123–1131. [PubMed: 17468341]

25. Mazur MA, Davis CC, Szabolcs P. Ex vivo expansion and Th1/Tc1 maturation of umbilical cord blood T cells by CD3/CD28 costimulation. Biol Blood Marrow Transplant. 2008 Oct; 14(10): 1190–6. [PubMed: 18804050]

26. Small TN, Kernan NA, Jakubowski A, et al. Pilot trial of rituximab for the prevention of Epstein-Barr virus B cell lymphoproliferative disorder (EBV-LPD) following T cell depleted (TCD) unrelated or HLA-mismatched related hematopoietic stem cell transplantation. Blood. 2006; 108:827a. Abstract.

Table 1

Demographic characteristics of EBV-monitored patients as compared to historical controls

Factors	Historical Controls	Current Cohort	P-value
Number of patients	30	34	**NA**
Median age (year)	55 (19–69)	56 (36–67)	.24
Median weight (kg)	85 (50–134)	80 (52–111)	.08
CMV-positive recipients	12 (40%)	24 (71%)	.02
Median infused nucleated cell dose × 10^8/kg	0.35 (0.21–0.68)	0.35 (0.22–0.59)	.94
Median infused $CD34^+$ cell dose × 10^6/kg	0.44 (0.07–1.66)	0.38 (0.14–1.32)	.27
Median time to follow up (day)	219 (7–1056)	339 (31–765)	.59
HLA match (worst match)			**<.01**
6 of 6	0	1 (3%)	
5 of 6	0	16 (47%)	
4 of 6	30 (100%)	17 (50%)	
No. of UCB recipients (%)			1.00
Single unit	1 (3%)	2 (6%)	
Double unit	29 (97%)	32 (94%)	
No. with malignant diagnosis at Tx (%)	28 (93%)	33 (97%)	.60
EBV subgroups	6	8	.10
Viremia	1 (3%)	6 (18%)	
PTLD	5 (17%)	2 (6%)	

P values for the comparison of categorical and continuous variables were performed from the Fisher's exact test and wilcoxon singed rank test, respectively.

Table 2

Characteristics of patients who developed EBV-related complications

Pt no.	Dx	Age, years	Time to acute GVHD (days)/ Maximun Grade	Time to EBV event, days	EBV event	Method of Diagnosis	Peak EBV titer (copies/mL whole blood)	Doses of Rituximab	Time EBV reactivation to clearance, days	Outcome/Cause of Death
1	AML	61	20/2	89	PTLD	Tonsil biopsy	4,300	4	5	Alive at 12 mos
2	AML	59	100/1	77	Viremia	PCR	12,500	2	7	Alive at 28 mos
3	ALL	36	None	73	Viremia	PCR	102,400	1	27	Death
4	CLL	65	None	243	Viremia	PCR	3,100	0	310	Alive, at 24 mos
5	AML	67	44/3	100	Viremia	PCR	311,300	3	21	Death
6	MDS	68	76/2	342	Viremia	PCR	22,500	3	26	Alive, at 16 mos
7	PNH	39	none	72	PTLD	Lung biopsy	20,800	7	Not achieved	Death/PTLD
8	MDS	58	31/2	71	viremia	PCR	42,300	1	Not achieved	Death

Pt indicates patient; Dx, diagnosis; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; CLL, chronic lympocytic leukemia; MDS, myelodysplastic syndrome; PNH, paroxysmal nocturnal hemoglobinuria; NMA, nonmyeloablative; w, with; ATG, antithymocyte globulin; PTLD, posttransplant lymphoproliferative disease; PCR, polymerase chain reaction; EBV, Epstein Barr virus

Biol Blood Marrow Transplant. Author manuscript; available in PMC 2014 July 28.