Brief report

Prompt versus preemptive intervention for EBV lymphoproliferative disease

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Posttransplantation lymphoproliferative disorders (PTLDs) caused by uncontrolled expansion of Epstein-Barr virus (EBV)–infected B cells after hematopoietic stem cell transplantation (HSCT) can be predicted by an increase in EBV DNA in peripheral blood mononuclear cells. We used real-time quantitative polymerase chain reaction (RQ-PCR) analysis to determine whether frequent monitoring of EBV DNA to allow preemptive treatment is truly of value in patients after HSCT. More than 1300 samples from 85 recipients were analyzed. No patient with consistently low EBV DNA levels developed PTLD. Nine patients had a single episode with a high EBV load (more than 4000 EBV copies/μg peripheral blood mononuclear cell [PBMC] DNA), and 16 patients had high EBV loads detected on 2 or more occasions. Only 8 of these developed symptoms consistent with PTLD, and all were promptly and successfully treated with EBV-specific cytotoxic T cells or CD20 monoclonal antibody. Hence, quantitative measurement of EBV DNA may best be used to enable the prompt rather than the preemptive treatment of PTLD. (Blood. 2004;103: 3979-3981)

Introduction

For the first year after hematopoietic stem cell transplantation (HSCT), Epstein-Barr virus (EBV)–induced posttransplantation lymphoproliferative disorders (PTLDs) are the most common neoplastic diseases among patients.[1] They may occur after any HSCT, but they are more common if donor and recipient are HLA-mismatched or if T-cell depletion is used for graft-versus-host disease (GVHD) prophylaxis.[1] The clinical diagnosis of PTLD may be difficult because it is a spectrum of heterogenous histologic and clinical entities. It may present as an infectious mononucleosis-like illness, with fatigue and lymphadenopathy, or as a febrile illness with leukopenia. Almost all organs may be affected by disease. Because of the progressive nature of PTLD, the key to management may be early or even preemptive treatment with either anti–B-cell monoclonal antibodies[2-4] or donor-derived EBV-specific cytotoxic T lymphocytes (CTLs).[5-7] Preemptive treatment is feasible because the onset of EBV-associated PTLD is foreshadowed for several weeks by an increase in EBV load (more than 4000 EBV copies/μg peripheral blood mononuclear cell [PBMC] DNA) in the peripheral blood of patients receiving a transplant from an HLA-mismatched family member or an HLA-matched unrelated donor.[7-10]

The study of HSCT patients has revealed that preemptive intervention with antiviral agents when cytomegalovirus (CMV) DNA levels increase is an effective way to prevent the onset of overt CMV disease, which retains high morbidity and mortality rates.[11] Hence, careful monitoring of CMV DNA levels after HSCT reveals a group of patients to whom treatment can be given preemptively with the greatest benefit. We questioned whether a similar strategy was of value in guiding the treatment of patients with EBV reactivation after HSCT, or whether the detection of high EBV DNA levels was better used to confirm clinically overt EBV PTLD and to facilitate prompt rather than preemptive treatment.

We used accurate real-time quantitative polymerase chain reaction (RQ-PCR) amplification in HSCT patients who were receiving a transplant from an unrelated donor or a mismatched family member and who were at high risk for the development of EBV-associated PTLD. We tried to determine whether detecting high EBV DNA levels using this technique was sufficiently sensitive and specific to support preemptive intervention. We also determined the outcome of prompt treatment of established EBV PTLD. Our results suggest that, in contrast to measurements of CMV DNA, EBV DNA levels are best used to confirm diagnoses of EBV PTLD, thereby permitting intervention that is early and effective rather than preemptive but unnecessary.

Study design

Patients

We studied 111 patients who underwent HSCT from closely HLA-matched unrelated donors or HLA-mismatched family members between May 1998 and July 2002 (Table 1).[12,13] All patients received fully ablative conditioning regimens that included ATG or alemtuzumab (Campath)[12,13] and either unmanipulated marrow or peripheral blood stem cells (PBSCs), a T-cell–depleted product, or a T- and B-cell–depleted product (Table 1). Eighty-five of these 111 patients had EBV that had been monitored on at least 4 occasions and were included in this analysis. The remaining 26

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Table 1. Demographics of 85 patients

	No. patients (%)
Sex	
Male	48 (56)
Female	37 (44)
Diagnosis	
Acute lymphocytic leukemia	30 (35.3)
Acute myelocytic leukemia	16 (18.8)
Non-Hodgkin leukemia	5 (5.9)
Chronic myelocytic leukemia	2 (2.4)
Hodgkin disease	1 (1.2)
Aplastic anemia	13 (15.3)
Myelodysplastic syndrome	7 (8.2)
Severe combined immunodeficiency	1 (1.2)
Wiskott-Aldrich syndrome	1 (1.2)
Virus-associated hemophagocytic syndrome	6 (7.6)
Metabolic disease (Hurler disease)	3 (3.5)
Transplantation regimen	
Full conditioning including ATG, no T or B cell depletion	25 (24.5)
Full conditioning including ATG, T cell depletion	8 (9.9)
Full conditioning including ATG, T and B cell depletion	31 (42.0)
Full conditioning including alemtuzumab, no T or B cell depletion	21 (23.5)
Graft type	
Unrelated donor	
Matched (6 of 6)	44 (51.8)
Mismatched (5 of 6)	26 (30.6)
Related donor	
Matched (6 of 6)	1 (1.2)
Mismatched (5 of 6, 4 of 6)	9 (10.6)
Haploidentical (3 of 6)	5 (5.9)

Median patient age was 7 years (range, 3 months to 36 years). Full-conditioning regimens included cyclophosphamide, Ara C, and total body irradiation (TBI),[12] busulphan, cyclophosphamide, and Ara C, or cyclophospahmide and TBI.[13] ATG indicates antithymocyte globulin.

patients underwent fewer than 4 analyses because of early relapse, death before day 100, or graft failure. In all patients, EBV DNA load was serially monitored every 2 weeks after transplantation.

Quantification of EBV DNA in PBMCs using RQ-PCR

PBMCs were enriched from heparinized blood samples of patients by standard density centrifugation and DNA isolated from PBMCs and quantitated as previously described.[14] For Epstein-Barr viral load measurements, an RQ-PCR assay specific for the highly conserved EBER 1 region of the EBV genome was used as described previously.[14-16] In total, 1361 patient samples were included in the analysis.

Generation and adoptive transfer of EBV-specific CTL

EBV-specific CTL were prepared as described in detail elsewhere.[15]

Statistical analysis

For comparison of different patient groups, χ^2 analysis was used. A P value less than .05 was regarded as statistically significant. Calculations were performed using SPSS 8.0 for Windows (SPSS, Richmond, CA).

Results and discussion

EBV load in HSCT recipients

There was wide variability in the EBV load over time, both between and within patients. In 60 of 85 patients, the EBV load remained less than 4000 EBV copies/μg PBMC DNA. None of these patients ever had any signs or symptoms of lymphoproliferation. Twenty-five (29.4%) of 85 patients had an EBV load of more than 4000 EBV copies/μg PBMC DNA on one or more occasion. In 9 of these 25 patients, the EBV load was elevated only once, was not accompanied by clinical symptoms, and normalized spontaneously. Sixteen patients had EBV DNA levels exceeding 4000 copies/μg PBMC DNA on 2 or more occasions. Eight of these patients had no other symptoms or evidence of PTLD on imaging studies. They were followed up conservatively, and none developed EBV PTLD. The remaining 8 patients developed prolonged fever, lymphadenopathy, or other symptoms or imaging findings consistent with PTLD (Table 2). Four of these patients had received T-cell–depleted marrow, 2 received T- and B-cell–depleted marrow, and 2 received unmanipulated product. Hence, the detection of 2 or more levels of EBV DNA above 4000 copies/μg had a sensitivity of 100% for the prediction of early PTLD but a specificity of only 50% (8 of 16).

Treatment of HSCT recipients with incipient PTLD

Because our previous studies suggested that early treatment of overt PTLD was safe and effective,[2,5,17] we elected not to institute preemptive treatment in any of the 16 patients with high EBV DNA levels but to wait instead until clinical symptoms (eg, adenopathy or fever) appeared. Eight of the 16 patients were so affected (Table 2). Two received CTL infusions (1 patient 2×10^7 CTLs/m^2 once; 1 patient 2×10^7 CTLs/m^2 twice within 62 days), 5 patients were given rituximab (1-4 doses of 375 mg/m^2), and 1 patient received CTLs (2×10^7 CTLs/m^2 once) and rituximab (1 dose of 375 mg/m^2) (Table 2). In all 8 patients, clinical symptoms associated with increased EBV load disappeared. Elevated EBV DNA levels decreased to normal in 7 patients. In the eighth patient, who received 2 CTL infusions, clinical symptoms resolved but the elevated EBV load persisted for 14 months before normalizing.

Table 2. Details of patients who received treatment

Patient	Donor	T cell depletion	Symptoms	EBV DNA	Treatment	Outcome
J31	6 of 6 URD	Ex vivo T-cell depletion	Fever, pneumonia	40 000	CTLs	Symptoms resolved, and EBV DNA returned to normal
J23	6 of 6 URD	Ex vivo T-cell depletion	Fever	4 000	CTLs and rituximab	Symptoms resolved, and EBV DNA returned to normal
2110	6 of 6 URD	Ex vivo T-cell depletion	Lymphadenopathy	20 000	Rituximab	Symptoms resolved, and EBV DNA returned to normal
J28	6 of 6 URD	Ex vivo T-cell depletion	Cough, fever	36 539	CTLs	Symptoms resolved, EBV DNA decreased but remained above normal for several months
J180	6 of 6 URD	Ex vivo T- and B-cell depletion	Abdominal pain, after ATG for GVHD	176 009	Rituximab	Symptoms resolved, and EBV DNA returned to normal
J152	4 of 6 mother	Ex vivo T- and B-cell depletion	Fever	29 807	Rituximab	Symptoms resolved, and EBV DNA returned to normal
J383	6 of 6 URD	In vivo alemtuzumab	Fever	74 346	Rituximab	Symptoms resolved, and EBV DNA returned to normal
J366	5 of 6 mother	In vivo alemtuzumab	Fever	103 695	Rituximab	Symptoms resolved, and EBV DNA returned to normal

URD indicates unrelated donor.

Is routine monitoring required?

We and others have previously shown that elevated EBV DNA levels are highly predictive for the development of EBV PTLD in recipients of T cell–depleted transplants.[8,9] The inference is that regular monitoring of EBV DNA levels after HSCT will allow preemptive treatment of patients before overt EBV PTLD appears. Such a strategy has been beneficial when applied to CMV, another latent herpesvirus that frequently causes disease after HSCT. However, preemptive treatment of CMV is desirable because therapy for established disease still has a high failure rate. Overt EBV PTLD, by contrast, may be more readily amenable to treatment.[2,5,15] Moreover, the predictive power of detecting EBV DNA levels higher than 4000 on more than 2 occasions was only 50% in this series, indicating that preemptive treatment would expose half the recipients to unnecessary therapy. Accordingly, we treated patients only when a persistently high EBV DNA level was coupled with clinical symptoms and signs of EBV PTLD. All patients responded completely. We suggest that the most effective way to prevent morbidity and mortality from EBV PTLD after HSCT is to maintain a high index of suspicion for the disease and to confirm a clinical assessment by measuring EBV DNA using accurate RQ-PCR. This approach allows prompt treatment while avoiding needless preemptive intervention.

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