generally exaggerated because the protein encoded by the FANCC gene modulates such responses.

Grover C. Bagby Jr

Correspondence: Grover C. Bagby Jr, Director, Oregon Cancer Center, Oregon Health Sciences University, 3181 SW Sam Jackson Park Rd, Portland, OR 97201-3098

References

1. Rathbun RK, Christianson TA, Faulkner GR, et al. Interferon-g-induced apoptotic responses of Fanconi anemia group C hematopoietic progenitor cells involve caspase 8-dependent activation of caspase 3 family members. Blood. 2000;96:4204-4211.
2. Rathbun RK, Faulkner GR, Ostroski MH, et al. Inactivation of the Fanconi anemia group C (FAC) gene augments interferon-gamma-induced apoptotic responses in hematopoietic cells. Blood. 1997;90:974-985.
3. Fagerlie SR, Diaz J, Christianson TA, et al. Functional correction of FA-C cells with FANCC suppresses the expression of interferon gamma-inducible genes. Blood. 2001;97:3017-3024.
4. Koh PS, Hughes GC, Faulkner GR, Keeble WW, Bagby GC. The Fanconi anemia group C gene product modulates apoptotic responses to tumor necrosis factor-a and Fas ligand but does not suppress expression of receptors of the tumor necrosis factor receptor superfamily. Exp Hematol. 1999;27:1-8.
5. Pang Q, Fagerlie S, Christianson TA, et al. The Fanconi anemia protein FANCC binds to and facilitates the activation of STAT1 by gamma interferon and hematopoietic growth factors. Mol Cell Biol. 2000;20:4724-4735.
6. Pang Q, Keeble W, Diaz J, et al. Role of double-stranded RNA-dependent protein kinase in mediating hypersensitivity of Fanconi anemia complementation group C cells to interferon gamma, tumor necrosis factor-alpha, and double-stranded RNA. Blood. 2001;97:1644-1652.
7. Pang Q, Keeble W, Christianson TA, Faulkner GR, Bagby GC. FANCC interacts with hsp70 to protect hematopoietic cells from IFNg/TNFa-mediated cytotoxicity. EMBO J. 2001;20:4478-4489.
8. Pang Q, Christianson TA, Keeble W, et al. The Fanconi anemia complementation group C gene product: structural evidence of multifunctionality. Blood. 2001;98:1392-1401.
9. Wang Z-Q, Stingl L, Morrison C, et al. PARP is important for genomic stability but dispensable in apoptosis. Genes Dev. 1997;11:2347-2358.
10. Guillouf C, Wang TS, Liu J, et al. Fanconi anemia C protein acts at a switch between apoptosis and necrosis in mitomycin C-induced cell death. Exp Cell Res. 1999;246:384-394.
11. Clarke AA, Philpott NJ, Gordon-Smith EC, Rutherford TR. The sensitivity of Fanconi anaemia group C cells to apoptosis induced by mitomycin C is due to oxygen radical generation, not DNA crosslinking. Br J Haematol. 1997;96:240-247.

To the editor:

Epstein-Barr virus (EBV)–DNA quantification in pediatric allogeneic stem cell recipients: prediction of EBV-associated lymphoproliferative disease

Allogeneic hematopoietic stem cell transplantation (HSCT) using a graft from a donor other than an HLA-identical family member is increasingly used in the treatment of children and adults with malignant as well as nonmalignant diseases. To prevent the occurrence of serious graft-versus-host disease (GvHD) in these patients, T-lymphocyte depletion (TCD) of the graft is generally applied as an effective tool. In addition, immunosuppressive agents (eg, antithymocyte globulins [ATG] and fludarabin) are frequently included in preparative regimens. A major side effect of this approach is a prolonged period of severely compromised immune surveillance after HSCT. Consequently, this category of HSCT recipients has an increased risk of infections or reactivations of Epstein-Barr virus (EBV) and adenovirus associated with significant morbidity and mortality.

EBV infection, mostly of donor origin, may proceed to EBV-associated lymphoproliferative disease (EBV-LPD) in up to 15% of these high-risk patients, leading to death in the majority of them.[1] Several therapeutic strategies have been reported when clinical symptoms are present, including tapering of immune suppression and administration of anti–B lymphocyte monoclonal antibodies, all with limited efficacy.[2] Restoration of T-cellular immunity seemed to be the most powerful approach as clearly demonstrated by donor lymphocyte infusions (DLI) and most elegantly by administration of EBV-specific cytotoxic T lymphocytes (CTL).[3,4] Unfortunately, DLI may result in concomitant GvHD, whereas the generation of CTL is time consuming. Notably, early stage polyclonal or oligoclonal disease is more responsive to the already described therapeutic modalities compared to monoclonal disease. Therefore, the recognition of EBV reactivation at an early stage and prevention of progression to overt clinical disease and fulminant EBV-LPD is a major challenge in the management of these HSCT recipients. Early recognition of EBV reactivation at a molecular level by real-time quantitative polymerase chain reaction (RQ-PCR) to measure EBV-DNA load might be worthwhile in this perspective. However, only limited information is available concerning the applicability of this approach in clinical decision making.

Table 1. Patient characteristics

	T-cell–depleted SCT patients without EBV-LPD (n = 20)	T-cell–depleted SCT patients with EBV-LPD (n = 6)
Age, y		
Mean (range)	7.3 (1-16)	6.6 (1-13)
Diagnosis, n		
ALL CR1	3	
ALL CR2	4	1
AML CR1	1	1
FEL	2	1
SAA	1	2
Fanconi	1	
MLD	1	
X-ALD	2	
Hurler-Scheie	1	
SCID	1	1
JMML/CML	2	
MDS	1	
Donor type, n		
MUD	12	4
Haplo	8	2
Conditioning includes, n		
ATG	3	1
Campath-1H	2	1
ATG + anti-LFA-1	3	2
Campath-1H + anti-LFA-1	11	1
Positive EBV serology, n		
D/R	20/14	6/3
Stem cell graft preparation, n		
$CD34^+$ selection	7	2
T and B immunorosetting	12	3
Other*	1	1

ALL CR1 indicates acute lymphoblastic leukemia first remission; AML CR1, acute myeloblastic leukemia first remission; FEL, familial erythrophagocytic lymphhistiocytosis; SAA, severe aplastic leukemia; MLD, metachromatic leukodystrophy; X-ALD, X-linked adrenoleukodystrophy; JMML/CML, juvenile myelomonocytic leukemia/chronic myeloid leukemia; MDS, myelodysplastic syndrome; LFA-1, lymphocyte junction-associated antigen type 1; D/R, donor recipient.

*Campath-1G in the bag and E-rosetting, respectively.

Table 2. EBV-DNA load in pediatric T-cell–depleted allo-SCT recipients (n = 26)

		EBV-DNA load (in gEq/mL)†			
	N	Less than 10^2	10^2 to 10^3	10^3 to 10^4	At least 10^4
Patients without EBV-LPD	20	19*	0	1	0
Patients with EBV-LPD					
2 to 4 weeks before EBV-LPD	6	2	1	2	1
1 to 2 weeks before EBV-LPD	6	0	0	2	4
At diagnosis of EBV-LPD	6	0	0	0	6

*Below the threshold of 50 gEq/mL in 18/19 patients.
†Highest EBV-DNA load measured in the indicated number of patients.

To study the possible value of EBV-DNA load quantification in clinical practice, we have retrospectively analyzed a group of 26 pediatric allo-SCT recipients grafted in our center after 1995 (Table 1). These children were regarded at risk for the development of EBV-LPD because they received a T-cell–depleted graft of an unrelated or haploidentical EBV-seropositive donor. Serum samples (and plasma samples in the more recent patients) obtained at least biweekly were available covering a follow-up period of 68 to 150 days (mean 88 days) after HSCT. Six individuals developed EBV-LPD (diagnosed at day 45-137 [mean 86 days] after HSCT by clinical observation, lymph node histology, and virologic methods). The remaining 20 children did not show clinical symptoms reminiscent to possible EBV-LPD. Serum samples were analyzed by RQ-PCR using the same technical approach as recently described[5] although the assay had been carried out on an iCycler iQ Multi-Color Real Time PCR Detection System (BioRad, Veenendaal, The Netherlands). Quantification has been carried out using the same EBV standard (Advanced Biotechnologies, Columbia, MD). In 18 of the 20 asymptomatic patients, RQ-PCR results were negative (threshold 50 genome equivalents/mL [gEq/mL]) for each sample at any time point (Table 2). In 2 patients, a very low level of 80 gEq/mL and of 159 and 1530 gEq/mL respectively, was measured during follow-up. In contrast, a clear and mostly rapid rise in EBV-DNA load, with levels exceeding 10^2 gEq/mL, was found in all 6 EBV-LPD patients several weeks prior to the occurrence of clinical symptoms (Table 2). Applying a cut-off level of 10^2 gEq/mL, a sensitivity of 100% and a specificity of 95% was achieved. Preemptive treatment started at this EBV-DNA load would have resulted in initiation of treatment in the EBV-LPD patients at least 2 weeks prior to clinical diagnosis. Unnecessary treatment would have been administered to only 1 of 20, ultimately asymptomatic, patients in a population regarded to be at risk for EBV-LPD. At the time EBV-LPD was clinically evident, EBV-DNA load exceeded 10^4 gEq/mL in all patients and 10^5 gEq/mL in all but 1 patient. Of these, 3 patients survived following various treatment regimens, whereas the others died, despite treatment from progressive disease. The responders to therapy could be retrospectively identified by a decrease in EBV-DNA load of at least 1 log of magnitude in the first week of treatment (data not shown), confirming the data reported by Van Esser et al.[6]

In this retrospective study of a group of pediatric HSCT recipients considered to be at risk to develop EBV-LPD, we have demonstrated that EBV-DNA load quantification in serum or plasma can be regarded as a reliable predictive parameter to identify individuals who are ultimately proceeding to EBV-LPD. In a recent report in *Blood*,[7] a group of adult HSCT recipients was analyzed in an identical fashion. In more than 50% of the adult recipients of a TCD-HSCT EBV-DNA was detected during follow-up (threshold 50 gEq/mL). The likelihood of developing EBV-LPD was found to be correlated with the EBV-DNA load. The reported positive predictive values (PPV) of an EBV-DNA load of 10^2, 10^3, and 10^4 gEq/mL were 24%, 39%, and 50%, respectively, whereas in our study the PPV of 10^2 gEq/mL was 86%. A possible explanation for this discrepancy may be a less rigorous T- and B-cell depletion in their study. In our patients, T- and B-cell depletion was achieved by either stem cell enrichment using $CD34^+$ selection (Clinimacs, Amcell, Burlingame, CA) or T- and B-cell depletion by immunorosette technique. No antiviral prophylaxis was used in our patients. The 2 studies clearly emphasize that it is essential to validate any viral load quantification by RQ-PCR in the patient population of interest. This is particularly important when this parameter is used in clinical decision making in individual patients. Based on the kinetics of the rise in viral load in our EBV-LPD patients, frequent sampling and rapid analysis seem required to allow for a preemptive treatment strategy. Based on accurate post–HSCT EBV monitoring, therapeutic interventions can now be initiated at an earlier, still asymptomatic and probably more favorable stage of the disease course. In our specific patient group, initiation of preemptive therapy at a viral load of 10^2 gEq/mL seems justified. Anti-CD20 (Rituximab, Mabthera, Roche Pharma, Basel, Switzerland) appears to be the most attractive intervention at this moment to be used as first line therapy.[8,9] Prospective studies in well-defined patient groups and individuals will now be possible to evaluate this approach.

Arjan C. Lankester, Maarten J. D. van Tol, Jaak M. Vossen, Aloys C. M. Kroes, and Eric Claas

Correspondence: Arjan C. Lankester, Department of Pediatrics, Leiden University Medical Center, The Netherlands; e-mail: a.lankester@lumc.nl

References

1. Hale G, Waldmann H. Risks of developing Epstein-Barr virus-related lymphoproliferative disorders after T-cell-depleted marrow transplants. Blood. 1998;91:3079-3083.
2. Fischer A, Blanche S, Le Bidois J, et al. Anti-B-cell monoclonal antibodies in the treatment of severe B-cell lymphoproliferative syndrome following bone marrow and organ transplantation. N Engl J Med. 1991;324:1451-1456.
3. Papadopoulos EB, Ladanyi M, Emanuel D, et al. Infusions of donor leukocytes to treat Epstein-Barr virus-associated lymphoproliferative disorders after allogeneic bone marrow transplantation. N Engl J Med. 1994;330:1185-1191.
4. Rooney CM, Smith CA, Ng CYC, et al. Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients. Blood. 1998;92:1549-1555.
5. Niesters HGM, van Esser J, Fries E, Wolthers KC, Cornelissen J, Osterhaus ADME. Development of a real-time quantitative assay for detection of Epstein-Barr virus. J Clin Microbiol. 2000;38:712-715.
6. Van Esser JWJ, Niesters HGM, Thijsen SFT, et al. Molecular quantification of viral load in plasma allows for fast and accurate prediction of response to therapy of Epstein-Barr virus-associated lymphoproliferative disease after allogeneic stem cell transplantation. B J Haematol. 2001;113:814-821.
7. Van Esser JWJ, van der Holt B, Meijer E, et al. Epstein-Barr virus (EBV) reactivation is a frequent event after allogeneic stem cell transplantation (SCT) and quantitatively predicts EBV-lymphoproliferative disease following T-cell-depleted SCT. Blood. 2001;98:972-978.
8. Faye A, van den Abeele T, Peuchmaur M, Matheu-Boue A, Vilmer E. Anti-CD20 monoclonal antibody for post-transplant lymphoproliferative disorders. Lancet. 1998;352:1285.
9. Kuenhle I, Huls H, Liu Z, et al. CD20 monoclonal antibody (rituximab) for therapy of Epstein-Barr virus lymphoma after hemopoietic stem-cell transplantation. Blood. 2000;95:1502-1505.