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No Correlation in Epstein-Barr Virus Reactivation Between Serological Parameters and Viral Load

Epstein-Barr virus (EBV) has been identified as a cofactor in the pathogenesis of a significant proportion of human immunodeficiency virus (HIV)-related or transplantation-related lymphoproliferative disorders (2, 3). Furthermore, EBV reactivation occurs more frequently in patients on hemodialysis due to the uremic immunodeficiency (5). Currently, EBV reactivations are almost exclusively diagnosed by antibody assays. We investigated whether the indirect detection of EBV by serological assays is correlated with the direct detection of the EBV viral DNA load in patients with immunosuppression (30 HIV-positive individuals, 30 transplant recipients, 23 patients on hemodialysis) and in 30 blood donors as controls. EBV primary infections and seronegatives were excluded.

First, immunofluorescence analysis of immunoglobulin G (IgG), IgM, and IgA antibodies to virus-capsid antigen (VCA), early antigen (EA-IgG), and EBV nuclear antigens 1 and 2 (EBNA1-IgG and EBNA2-IgG, respectively) were carried out. Serological reactivations were detected when at least one of the following parameters was found: VCA-IgM titer, >32; VCA-IgA titer, >32, EA-IgG titer, >64; EBNA1-IgG ≤ EBNA2-IgG (5).

EBV DNA present in peripheral leukocytes was quantified by a competitive nested PCR assay of EBV-p23 (1). The detection limit was determined as 20 EBV copies in 10^5 EBV-negative leukocytes. In the latent stage, not more than 5 cells in 10^6 leukocytes are EBV infected; thus, latent infections were not detectable with this PCR (4).

Serological reactivation and EBV DNA content were not correlated, since serological reactivations were found with almost the same frequency in PCR-positive as in PCR-negative individuals (Table 1). No PCR-positive subjects were present in controls; however, 13% exhibited serological markers for reactivation. Furthermore, no differences were found when single serological reactivation parameters such as EA-IgG or EBNA1-IgG ≤ EBNA2-IgG were correlated with PCR. Finally, PCR results failed to correlate with the number of different reactivation markers in individual subjects. Four patients with a high viral load (>10^6 copies/μg of DNA) were found in the group of PCR-positive subjects. Even among these patients, no serological reactivations were detected. In contrast, 4 of 11 patients (36%) with moderate (10^4 to 10^6 copies/μg of DNA) and 6 of 7 patients (85%) with low viral load (<10^4 copies/μg of DNA) showed such serological reactivations, suggesting that antibody production and the loss of antibodies are individually different, particularly in immunosuppressed patients.

In summary, we suggest that the serological parameters that are currently employed as an indicator for EBV reactivation may be of limited use as they fail to correlate with viral load.

TABLE 1. Comparison of serological reactivations detected by antibody assays and EBV DNA by PCR in leukocytes

Patient group	EBV PCR	*n*	No. (%) of patients with serological reactivation	*P*[a]
HIV	Positive	12	9 (75)	0.31
	Negative	18	16 (89)	
Hemodialysis	Positive	4	2 (50)	0.23
	Negative	19	4 (21)	
Transplantation	Positive	15	4 (27)	0.36
	Negative	15	2 (13)	
Overall[b]	Positive	31	15 (48)	0.59
	Negative	52	16 (32)	
Blood donors	Negative	30	4 (13)	

[a] Statistical analysis by chi-square test.
[b] HIV-infected, hemodialysis, and transplantation groups.

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