Dominant Cytotoxic T Lymphocyte Response to the Immediate-Early Trans-Activator Protein, BZLF1, in Persistent Type A or B Epstein-Barr Virus Infection

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Epstein-Barr virus (EBV), a ubiquitous human gammaherpesvirus, is the etiologic agent of infectious mononucleosis (IM) and is associated with several malignancies, including Burkitt’s lymphoma, nasopharyngeal carcinoma, and immunoblastic lymphomas, following transplants and in patients infected with human immunodeficiency virus (HIV) (reviewed in [1]). Primary infection with EBV leads to life-long persistence of virus, occurring as a latent infection of B cells [1]. In acute IM, virus replication has been detected in oropharyngeal epithelial cells [2] and in tonsillar B cells [3]. The persistent replication of virus in epithelial cells is thought to be dependent on a continual supply of virus from permissively infected B cells infiltrating the mucosal epithelium [1, 4]. Two major subtypes of EBV, types A and B, have been identified that are differentiated within the BamHIWYH and HindIIIIE regions of the EBV genome, encoding the latent antigens, Epstein-Barr nuclear antigens (EBNAs) 1–6, and latent membrane proteins (LMP) 1 and 2 [1]. The outgrowth in vivo of EBV-transformed B cells is most likely controlled by EBV-specific cytotoxic T lymphocytes (CTLs) that recognize multiple epitopes within these latent antigens [1, 5]. By contrast, the role of EBV-specific CTLs in controlling viral spread in the epithelial and B cell compartments is not yet fully understood. However, it is known that immunocompromised persons show an increased level of oropharyngeal viral shedding that correlates with a high incidence of EBV-infected B cells in the peripheral blood, and elevated levels of serum antibodies to EBV lytic antigens [6].

So far, only two CTL epitopes have been defined in the EBV lytic cycle. These CTL epitopes have been identified within the immediate-early BZLF1 protein (also termed ZEBRA, Zta, EB1, and Z) in healthy virus carriers [7] and in the BHFR1 protein in acute IM [8]. BZLF1 protein is expressed early in the lytic cycle and is pivotal in the switch of EBV from the latent to the productive cycle [1, 7]. This protein therefore is potentially a vital target for EBV-specific CTL surveillance, because lytically infected cells could be eliminated before the release of mature virions. To investigate the potential relevance of the BZLF1 protein in immune surveillance, we compared the frequency of the CTL precursor (CTLp) to the BZLF1-associated epitope RAKFKQLQ (RAK) in the peripheral blood of healthy virus carriers with the frequency of CTLp to two other well-characterized type A HLA-B8–restricted epitopes, FLRGRAYGL (FLR) and QAKWRLQTL (QAK), which are found in the latent EBV nuclear antigen 3 protein [1, 7]. This approach may provide an important baseline for further studies to determine whether individuals with EBV-associated disease or malignancy have a defective CTL response to BZLF1 protein.

Materials and Methods

Peripheral blood mononuclear cells (PBMC) were isolated from blood samples obtained from 5 healthy, long-term EBV-seropositive persons, (viral capsid antigen [VCA] IgG-positive, IgM-nega-
PBMC from each donor were serially diluted from $5 \times 10^4$ to 390 cells/well and stimulated with autologous type A LCL, or in some cases with peptide-coated PBMC, as described for the bulk assay. Cultures were fed on days 4 and 7 with medium supplemented with 10 IU of recombinant interleukin-2 and 30% (vol/vol) supernatant from MLA-144 cultures. On day 10, effectors were used in split-well assays in standard 5-h $^{51}$Cr-release assays against peptide-coated or uncoated PHA-blast target cells [9].

Donor MS consistently did not respond to the type A–specific epitopes FLR or QAK, either in the bulk CTL assay or LDA. The T cell regression assay [10] showed that donor MS, in contrast to donors BM, ISM, SP, and CR, did not have detectable memory T cells to type A virus, but instead possessed a strong memory CTL response to a type B EBV epitope (unpublished data), confirming the type B status of donor MS.

Results

Figure 1 shows representative cytotoxicity patterns of polyclonal CTLs generated from 5 HLA-B8–positive, EBV-sero-

![Figure 1](https://academic.oup.com/jid/article-abstract/176/4/1068/883704)
Figure 2. Cytotoxic T lymphocyte precursor (CTLp) frequencies to RAKFKQLLQ (RAK), FLRGRAYGL (FLR), and QAKWRLQTL (QAK) peptides in human leukocyte antigen (HLA)–B8–positive persons, as determined by limiting-dilution analysis of peripheral blood mononuclear cells following autologous type A lymphoblast cell line (LCL) stimulation or, in some cases, RAK peptide stimulation. A–E: results for EBV-seropositive carriers (BM, SP, ISM, MS, CR); F: CTLp activity to RAK only for EBV-seronegative subjects (SB, SM). Reciprocal values for responder frequencies ($f_0^{-1}$) and 95% confidence limits are as follows for FLR, QAK, and RAK (LCL-stimulated): A, 15330 (19975–11765), 30013 (40145–22435), 10990 (14290–8460); B, 8113 (10600–6210), 41650 (57200–30350), 3330 (4350–2250); C, 2230 (3090–1610), 181060 (320560–102270), 3530 (4760–2620); D, $>2.4 \times 10^6$, $>2.4 \times 10^6$, 11605 (15324–8790); E, 4300 (5640–3280), 2233 (2925–1705), 647 (870–482). Values for RAK (RAK peptide–stimulated) were 1165 (1540–880) (C); 11340 (14740–8725) (D); and 1277 (1685–968) (E). F, RAK CTLp for donors SM ($>2.4 \times 10^6$) and SB ($>2.4 \times 10^6$). stim = stimulated.