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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Five healthy human leukocyte antigen-B8 (HLA-B8)–positive virus carriers were studied to investigate the CD8+ cytotoxic T lymphocyte (CTL) response to an HLA-B8–restricted peptide, RAKFKQLLQ, located in the Epstein-Barr virus (EBV) immediate-early trans-activator protein, BZLF1. Of the 5 virus carriers, 4 were infected with type A and 1 with type B EBV. Using limiting-dilution analysis of peripheral blood mononuclear cells, a high RAKFKQLLQ-specific CTL precursor frequency was demonstrated after specific peptide or autologous lymphoblastoid cell line stimulation in both type A and type B EBV carriers. The RAKFKQLLQ-specific CTL precursor frequencies in all 5 persons were at least as dominant as those observed with two other EBV-associated, HLA-B8–restricted latent epitopes, FLRGRAYGL and QAKWRLQTL. These findings show that healthy virus carriers maintain a high frequency of BZLF1-specific memory T cells, potentially to control virus spread from lytically infected cells.

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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-
tive, and EBNA IgG-positive) and 2 healthy seronegative donors (VCA IgG-negative, IgM-negative, and EBNA IgG-negative). The seropositive donors were identified as BM, ISM, SP, MS, and CR, and the seronegative donors as SB and SM. All 7 donors were HLA-B8–positive, as determined by serologic typing. RPMI 1640 with 100 IU/mL penicillin, 100 μg/mL streptomycin, and 10% (vol/vol) fetal bovine serum was used as medium in all culture work.

The memory CTL response to the BZLF1-associated epitope, RAK, and the EBNA 3–associated epitopes, FLR and QAK, was determined using a bulk CTL assay as previously described [9]. In brief, PBMC (2 × 10^6/mL) were stimulated with γ-irradiated (8000 rad) autologous type A or B lymphoblastoid cell lines (LCLs), or peptide-coated PBMC (2000 rad). After 7 days, effector cell activity was assessed in standard 5-h ^51Cr-release assays using peptide-sensitized (10 μg/mL) phytohemagglutinin (PHA)-stimulated blast cells as targets [9].

The CTLp frequencies to the epitopes RAK, FLR, and QAK were quantified using limiting-dilution analysis (LDA) [9]. In brief, PBMC from each donor were serially diluted from 5 × 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 ^51Cr-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. Recognition of EBV-associated, human leukocyte antigen (HLA)–B8–restricted peptide epitopes by polyclonal cytotoxic T lymphocyte (CTL) lines generated from HLA-B8 EBV–seropositive donors. Autologous phytohemagglutinin (auto PHA) blast cells (blasts) with or without adsorbed peptide were used as targets in standard 5-h ^51Cr-release assays: BZLF1 peptide, RAKFKQLLQ (A), EBNA3 peptide, FLRGRAYGL (B), and EBNA3 peptide, QAKWRQQL (C). Polyclonal CTL lines were generated after stimulation with type A autologous lymphocyte cell lines. (Effector-to-target ratio, 20:1.) Peripheral blood mononuclear cell donors: BM, ISM, SP, MS, CR.](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 ($f_0$) 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 ($>2.4 \times 10^6$). Stimulated.