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Nonresponsiveness to an immunodominant Epstein–Barr virus-encoded cytotoxic T-lymphocyte epitope in nuclear antigen 3A: Implications for vaccine strategies

(viral immunity/herpesvirus/T-cell repertoire)

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ABSTRACT **An immunodominant Epstein–Barr virus (EBV)-encoded cytotoxic T lymphocyte (CTL) epitope has been mapped to the EBV nuclear antigen 3A. The epitope, represented by the peptide sequence AWNAGFLRGRAYGLD (hereafter termed AWNA), is restricted through the HLA-B8 allele and is expressed by type A but not type B-infected transformants. Herein, we show that EBV-specific memory CTLs from an HLA-B8$^+$ healthy virus carrier, JS, did not respond *in vitro* to AWNA, even though that individual's endogenously infected transformants processed and presented the natural equivalent of this peptide to AWNA-specific CTLs from another B8$^+$ individual. Instead, an epitope, represented by the peptide sequence QLSDTPLIPLTIFVGENTGV, was the dominant EBV-specific CTL epitope in donor JS. This epitope mapped to EBV nuclear antigen 2A, was restricted by an HLA-A2 subtype, and specifically associated with type A strains of EBV. No AWNA-specific CTL precursors were detected by limiting dilution analysis of peripheral blood mononuclear cells from donor JS whereas the precursor frequency of AWNA-specific CTLs from a responder donor, LC, was estimated at 1:4500. The presentation *in vivo* of an immunogenic epitope–HLA antigen complex is clearly insufficient to guarantee an effective memory CTL response to that foreign epitope. Thus, vaccination strategies based on peptides inducing CTL responses may need to take into account not only the polymorphism of HLA antigens but also possible allelic variation in the repertoires of T-cell receptors.**

Epstein–Barr virus (EBV), a human herpesvirus, is tropic for B cells *in vitro* and cells transformed by the virus grow as lymphoblastoid cell lines (LCLs) that express a limited number of viral gene products. These latency proteins include a family of EBV nuclear antigens (EBNAs) 1, 2, 3, 4, and 6, leader protein, and latent membrane proteins (1–3). EBV persists systemically as a latent infection in B cells and EBV-specific memory cytotoxic T lymphocytes (CTLs) from virus carriers can be reactivated *in vitro* by stimulation of peripheral blood mononuclear cells (PBMCs) with the autologous LCL. Activated memory cells are predominantly CD8$^+$ class I-restricted CTLs (4).

The discovery that virus-encoded nuclear proteins act as targets for CTL recognition (5) suggested that virtually all viral proteins were potential sites of self–nonself discrimination. Foreign proteins can be processed to yield immunogenic oligopeptides, each of which features an agretope region that binds to an appropriate major histocompatibility complex (MHC) determinant within the endoplasmic reticulum and an epitope region that is recognized by the T-cell receptor (TCR) of responsive T cells after the expression of the MHC–peptide complex at the surface of infected cells (for review, see ref. 6). In theory, any suitably presented peptide, not resembling self and present in sufficient quantity, could be expected to evoke an immune response. In practice, few CTL viral epitopes have been defined by oligopeptide sequences and certain viral proteins have been characterized by a high frequency of nonresponsiveness (7). Since each CTL epitope is typically restricted by a specific MHC determinant, their apparent paucity for a given individual may simply reflect the restrictions imposed by an extensive MHC polymorphism.

There are two types of EBV, A and B, that show DNA sequence divergence within the *Bam*HI YWH and E regions of the genome (8) and allelic polymorphism in each of the EBNA proteins encoded from these regions (9, 10). We have exploited the allelic polymorphism in the EBNA family of proteins to generate EBV-specific CTL clones that discriminate between autologous B cells transformed by type A or type B virus (11) and shown that EBNA proteins are an important source of epitopes for EBV-specific CTLs. EBNA-encoded epitopes have been mapped to gene regions unique to type A isolates (12) or regions shared between type A and type B (13). These oligopeptides function as recognition determinants at the induction and effector level of a CTL response and are restricted through different HLA class I alleles (14). An immunodominant CTL epitope in EBNA 3A, relevant to the present study, was originally referred to as peptide 68 (12). The natural equivalent of this synthetic peptide is presented functionally by type A but not type B transformants and CTL recognition of the epitope is restricted by the HLA-B8 allele (12). More recently, we have shown (15) that the natural equivalent of peptide 68 is not recognized on autologous B95-8 transformants by CTL clones stimulated with type A wild-type transformants. This apparent anomaly can be explained by mutations in the B95-8 epitope region resulting in sequence divergence between the B95-8 peptide and the equivalent peptide found in type A transformants infected with the wild-type IARC-BL74 or IARC-BL36 isolates (46). In the present study, we have used the 15-residue peptide AWNAGFLRGRAYGLD (hereafter termed AWNA), since this peptide contains the relevant epitope found in type A wild-type transformants. We now report that an HLA-B8$^+$ healthy individual showed no detectable CTL memory to AWNA, even though the natural equivalent of this peptide was presented by that individual's endogenously infected B cells in association with the appropriate MHC determinant. Instead, the dominant CTL response against EBV was specific for an epitope in EBNA 2A and was restricted by a subtype of HLA-A2. The mapping of

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Abbreviations: EBV, Epstein–Barr virus; EBNA, EBV nuclear antigen; CTL, cytotoxic T lymphocyte; LCL, lymphoblastoid cell line; PBMC, peripheral blood mononuclear cell; TCR, T-cell receptor; CTLp, CTL precursor; PHA, phytohemagglutinin; MHC, major histocompatibility complex; rIL-2, recombinant interleukin 2; mAb, monoclonal antibody.

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this CTL peptide epitope, QLSDTPLIPLTIFVGENTGV (hereafter termed QLSD)(EBNA 2A residues 64–83), was facilitated by studies using recombinant vaccinia viruses carrying EBNA 2 gene deletions (16). Nonresponsiveness to AWNA was clearly not due to inappropriate "determinant selection" (17) resulting from defective processing or presentation of AWNA. Rather, the absence of a memory CTL response could indicate (*i*) a "hole in the T-cell repertoire" (18) due to either clonal deletion or an imperfection in the TCR gene repertoire, (*ii*) clonal anergy caused by active suppression or induced tolerance, or (*iii*) hierarchical T-cell interactions limiting the precursor CTL (CTLp) frequency against selected viral epitopes.

MATERIALS AND METHODS

Cell Donors. PBMCs from EBV-seropositive donors JS (HLA-A1,2; B8,w51) and LC (HLA-A1,−; B8,18) were isolated from heparinized blood by centrifugation on Ficoll/Paque (Pharmacia) and used for the establishment of LCLs, phytohemagglutinin (PHA) blasts, and the generation of EBV-specific CTLs.

EBV Isolates and Establishment of Cell Lines. EBV isolates were prepared from the type A cell lines IARC-BL74 (designated BL74) (8) and IARC-BL36 (designated BL36) (19) and the type B cell lines Ag876 (1) and L4 (9). LCLs were established by transformation of B cells with exogenous type A or type B EBV isolates as described (11) and a spontaneous LCL was established from donor JS using cyclosporin A (a kind gift from Sandoz, Basel). PBMCs were stimulated with PHA as described (12). Cell lines were routinely maintained in growth medium consisting of RPMI 1640 medium, glutamine (300 mg/liter), penicillin (100 international units/ml), streptomycin (100 μg/ml), and 10% (vol/vol) heat-inactivated fetal calf serum.

Selection and Synthesis of Peptides. A series of oligopeptides (20–25 amino acids) was synthesized by the method of Houghten (20) based on the known EBNA protein sequences of the prototype B95-8 strain of virus. However, AWNA was synthesized on the basis of PCR and DNA sequence data of transformants infected with BL74 or BL36 wild-type isolates. Sequences selected within EBNA 2A spanned the major part of the protein but concentrated on regions that were predicted sites (21, 22). The sequence, purity, and concentration of AWNA and QLSD were checked directly by Edman degradation (model 473A Protein sequencer, Applied Biosystems).

Activation of Memory CTLs with Peptide Epitopes. Autologous PHA blasts or LCLs were precoated with peptide (100–200 μg/ml) for 1 h at 37°C, γ-irradiated (8000 rad for LCL cells or 2000 rad for blasts; 1 rad = 0.01 Gy), washed twice, and added as antigen-presenting cells to 2×10^6 PBMCs in 24-well plates (Linbro, Flow Laboratories) in a final volume of 2 ml. A stimulator/responder cell ratio of 1:20 (LCL cells) or 1:2 (blasts) was used routinely. Cultures were incubated at 37°C in 5% CO_2/95% air and fed biweekly with growth medium. In certain experiments, memory CTLs were activated with various concentrations of peptide epitopes, as described above, and human recombinant interleukin 2 (rIL-2) (Cetus) was added to cultures on specified days. The rIL-2 used was highly purified from *Escherichia coli* and was an altered form created using site-directed mutagenesis (23, 24). Cells were harvested on day 10.

Agar Cloning of T Cells. T-cell clones were generated as described (25). Briefly, PBMCs from donors JS and LC were activated by stimulation with the irradiated autologous BL74 LCL or BL36 type A LCL at a stimulator/responder cell ratio of 1:100. After 3 days, dispersed cells were seeded in 0.35% agarose (SeaPlaque, FMC) containing RPMI 1640 medium, 10% fetal calf serum, 25% (vol/vol) supernatant from MLA-144 cultures, and rIL-2 (30 units/ml). Colonies were harvested after a further 3 days and amplified in culture with biweekly restimulation with rIL-2 and specific LCLs.

Cytotoxicity Assay. Polyclonal and clonal CTLs from donors JS and LC were tested for cytotoxicity in standard 5-h ^{51}Cr-release assays. Effector populations were assayed against peptide-coated (100 μg/ml) or untreated autologous LCL cells and PHA-blast target cells. Target cells were incubated with 100 μCi of ^{51}Cr (Amersham; 1 Ci = 37 GBq), with and without the appropriate peptide, for 90 min. A variation of this assay was used when screening large numbers of peptides in that peptides were added directly to ^{51}Cr-labeled targets (final concentration, 20 μg/ml) and remained present throughout the assay. The mean spontaneous lysis for targets in culture medium was <20%, the mean maximum lysis in 0.5% SDS was >90% of total uptake, and the variation about the mean specific lysis was <5%.

Limiting Dilution Analysis. PBMCs were distributed in graded numbers from 2×10^4 to 6×10^4 cells per well (responder JS) or 2.5×10^3 to 5×10^4 cells per well (responder LC) in round-bottomed microtiter plates. Approximately 1×10^4 γ-irradiated (8000 rad) autologous type B Ag876 LCL cells, preincubated with the appropriate peptide as described above, were added to give a total volume of 100 μl. Thirty or 60 replicates were used at each concentration in each experiment. Cultures were fed on days 4 and 7 with 50 μl of medium supplemented with 10 units of rIL-2 and 10% (vol/vol) supernatant from MLA-144 cultures. On day 10, washed cells were split into two replicates and used as effectors in a standard 5-h ^{51}Cr-release assay against autologous PHA-blasts, precoated with the appropriate peptide or left uncoated (control). Wells were scored as positive when the percent specific chromium release exceeded by the mean release from the control targets by 3 SDs. Limiting dilution analysis was performed by the method of maximum likelihood estimation (26). Data from all experiments were compatible with the hypothesis of single-hit kinetics ($P > 0.4$) and precursor estimates are given with 95% confidence limits.

RESULTS

Both JS polyclonal (Fig. 1*A*) and clonal (Fig. 1*B*) CTLs specifically recognized autologous PHA blasts coated with the exogenous peptide QLSD. The minimal sequence tested that retained full activity was the hydrophobic 10-mer DTPLIPLTIF (data not shown). The EBV-associated memory CTL response in JS was biased to the EBNA 2A-derived peptide epitope in that 10 out of 16 CTL clones selected from this individual were specific for QLSD. The cytotoxicity data in Fig. 2 show a typical pattern of lysis obtained with a QLSD-specific clone 18 from donor JS. This clone lysed type A, but not type B, LCLs unless the type B Ag876 LCL targets were precoated with exogenous QLSD. An allogeneic type A LCL target (BG) that was matched with the autologous donor for HLA-A2 was lysed whereas HLA-B8-matched type A LCL targets (AD and LC) were refractory to lysis. No lysis was observed in a wide panel of type A LCLs derived from allogeneic donors sharing HLA-A1, -B8, or -Bw51 alleles. These restriction patterns were supported by a family study in which the clonal and bulk CTL response to QLSD segregated with the HLA-A2-Bw51 maternal haplotype (data not shown). Certain HLA-A2-matched LCL targets were not lysed by QLSD-specific CTL clones from donor JS (e.g., donor AD), suggesting the importance of A2 subtypes in modulating CTL recognition.

Surprisingly, EBV-specific polyclonal CTLs and CTL clones that were screened from the HLA-$B8^+$ donor JS failed to lyse blasts coated with AWNA derived from EBNA 3A. This peptide represents an immunodominant B8-restricted CTL epitope (12) and in view of the lack of reported subtype

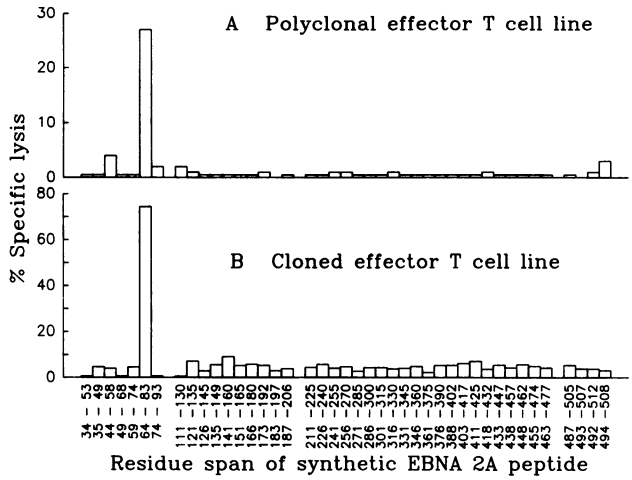

FIG. 1. QLSD (residues 64–83) represents a dominant EBV-encoded CTL epitope in donor JS and maps to EBNA 2A. Polyclonal (*A*) and cloned (*B*) CTL lines were established in culture from donor JS by stimulation of PBMCs with the autologous spontaneous LCL (*A*) or BL74 type A LCL (*B*). CTLs were tested against autologous PHA blasts coated with peptides spanning the outlined regions of EBNA 2A. The cytotoxicity data are expressed as the percentage specific lysis of ^{51}Cr-labeled PHA blasts preincubated with peptide (100 μg/ml) for 1 h prior to their use as targets in a standard 5-h chromium-release assay. Effector/target ratio, 5:1.

microheterogeneity in the B8 allele (27), it was expected that memory CTLs from JS would recognize this oligopeptide. To test the possibility that the lack of a memory CTL response to AWNA was simply due to the absence of a natural peptide equivalent in autologous B cells infected with the endogenous EBV strain, a spontaneous line from JS was generated *in vitro* and screened with an AWNA-specific CTL clone. Clone 13 from the B8-compatible donor LC strongly lysed the autologous type A LCL as well as the JS spontaneous LCL target. This clone was specific for AWNA in that it did not lyse LCL infected with the B95-8 strain, which shows genetic drift within this region. PHA blasts coated with exogenous AWNA were lysed by clone 13 whereas uncoated blasts were only poorly lysed (Fig. 3). Thus, the failure of EBV-specific CTLs from JS to recognize AWNA was not due to the lack of expression of a natural peptide equivalent by transformants infected with the endogenous strain of EBV. Further,

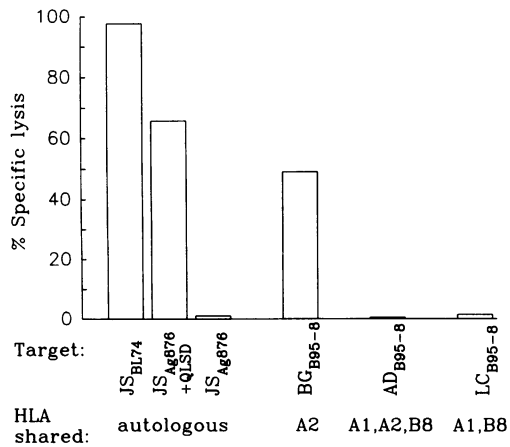

FIG. 2. Percentage specific lysis by QLSD-specific clone 18 from donor JS. This clone was initially stimulated with the autologous BL74 type A LCL and tested against a target panel consisting of the autologous BL74 type A LCL and the Ag876 type B LCL (with and without adsorbed QLSD) and the HLA-matched allogeneic (BG, AD, and LC) B95-8 type A LCL. JS Ag876 LCL targets were incubated with QLSD as described in Fig. 1. Effector/target ratio, 5:1.

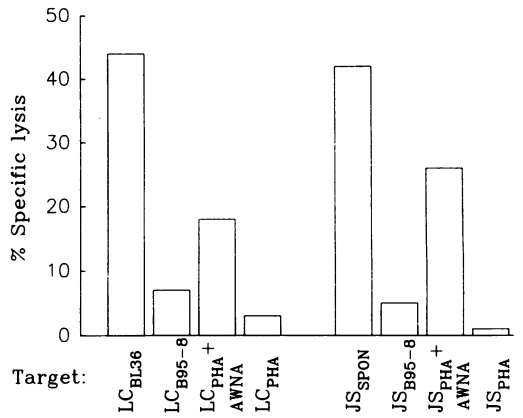

FIG. 3. Spontaneous LCL derived from donor JS expresses and presents the natural equivalent CTL epitope of AWNA. An AWNA-specific CTL clone 13 from donor LC was generated in culture after stimulation with the autologous BL36 type A LCL. Clone 13 was tested against a target panel consisting of autologous BL36 and B95-8 LCL, allogeneic (from a B8-matched donor JS) spontaneous and B95-8 LCL, and autologous and allogeneic PHA blasts, with and without adsorbed AWNA. PHA blasts were incubated with AWNA as described in Fig. 1. The cytotoxicity data are expressed as the percentage specific lysis of ^{51}Cr-labeled targets in a standard 5-h chromium-release assay. Effector/target ratio, 5:1.

the JS B8 determinant was effective in presenting the natural or synthetic peptide to peptide-specific CTLs showing that the absence of a CTL response to AWNA was not due to an inappropriate peptide–MHC interaction.

To exclude the possibility that competition among clones was involved in restricting the CTL response to AWNA *in vitro*, polyclonal CTLs were generated in cultures stimulated with autologous type B LCLs or PHA blasts, precoated with AWNA. Since in donor JS the EBV-specific memory CTL response is restricted to the type A strains of EBV and both AWNA and QLSD peptide sequences are coded by genome regions that differ between type A and type B EBV, the autologous type B LCL can be used to present select peptide epitopes encoded by type A strains of EBV. On the other hand, PHA blasts can effectively present individual oligopeptides to EBV-specific memory CTLs without background effects from endogenous virus and multiple CTL epitopes expressed by EBV transformants. The data in Fig. 4 show the outcome of a typical experiment in which the EBV-specific

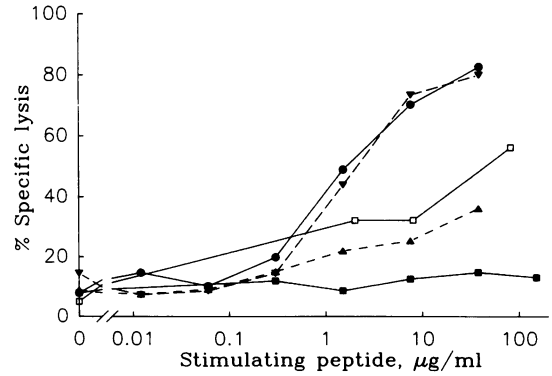

FIG. 4. Percentage specific lysis by polyclonal CTLs generated in culture for 10 days from donors JS and LC after stimulation with a peptide-coated autologous type B Ag876 LCL. Stimulator LCL cells from donor JS were preincubated with QLSD (●) or AWNA (■) and from donor LC were preincubated with AWNA (□), at the indicated peptide concentrations. In certain experiments, CTLs from donor JS were assayed in the presence of anti-class I mAb (▲) or anti-class II mAb (▼). The targets in this assay were the autologous spontaneous LCL cells. Effector/target ratio, 5:1.

memory CTLs from donor JS were reactivated in culture by stimulation with peptide-coated autologous type B Ag876 LCLs. These antigen-presenting cells were preincubated with a broad concentration range of stimulating peptide in case the absence of a CTL response to AWNA was due to a tolerogenic dose effect. JS polyclonal CTLs that had been stimulated with LCL adsorbed with QLSD but not AWNA lysed the autologous spontaneous LCL. Cytotoxicity was restricted by class I antigens as shown by the significant level of inhibition of lysis in the presence of anti-class I monoclonal antibody (mAb) whereas anti-class II mAb had no effect. Similar cytotoxicity patterns were obtained by using peptide-coated autologous PHA blasts as the antigen-presenting cells (data not shown). By contrast, LC polyclonal CTLs lysed the autologous spontaneous LCL after their stimulation with the LCL adsorbed with AWNA. The lack of a response to AWNA in donor JS has been observed consistently over a period of 2 years.

The frequency of memory CTLps responding to individual peptide epitopes was estimated by limiting dilution analysis. Peptide-specific CTLps were reactivated *in vitro* by stimulation of PBMCs with the autologous type B Ag876 LCL precoated with the appropriate peptide and autologous PHA blasts precoated with the corresponding peptide were used as target cells. The representative data in Fig. 5 show that a high frequency (1 in 15,000 ± 4000) of memory CTLs from donor JS responded to QLSD, but no response was detected to AWNA (<1 in 6×10^6, assuming 100% cloning efficiency). By contrast, memory CTLs from donor LC responded particularly well to AWNA and the frequency of the CTLps for this peptide was estimated at 1 in 4500 ± 1000.

DISCUSSION

Normal EBV carriers have been shown to possess an efficient EBV-specific CTL memory component that apparently controls the persistent EBV infection (28). The induction *in vivo* of protective CTL memory after vaccination with oligopeptides has shown that CTL peptide epitopes may be useful in the design of viral vaccines (29, 30). Since the EBV genome codes for at least seven latent proteins, there is an enormous array of potential epitopes available for CTL surveillance, presumably involving restriction through a wide range of MHC molecules. Nonetheless, the high degree of polymorphism in human MHC molecules and the fact that for a given MHC molecule only a limited number of epitopes provide functional immunity (7) present well-recognized problems for peptide immunization at an individual level. In this study we have described a further problem in that an individual may fail to respond to an endogenous viral peptide that is processed and presented by the appropriate MHC, even though that peptide is strongly immunogenic in suitably MHC-matched individuals.

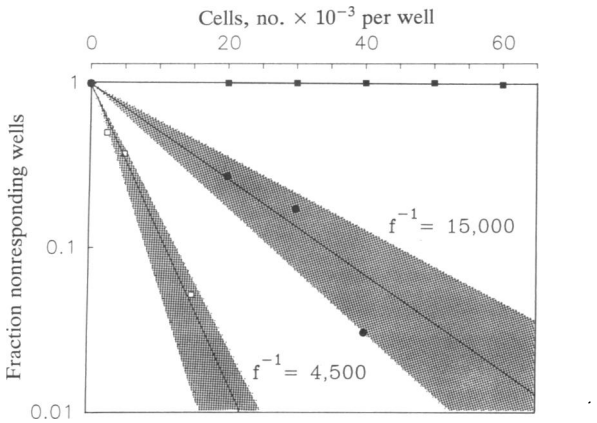

FIG. 5. Comparative CTLp frequencies in PBMCs from donor JS for QLSD (●) or AWNA (■) and from donor LC for AWNA (□), using limiting dilution analysis. The stippled area indicates 95% confidence limits. Reciprocal values of responder frequencies (f^{-1}) are indicated.

In the present study, a seropositive $B8^+$ donor, JS, failed to respond to a CTL peptide epitope, AWNA, recognized by 3 of 3 other normal $B8^+$ immune donors tested (14). It is most unlikely that this nonresponsiveness stems from inappropriate epitope presentation by infected cells, since (*i*) direct sequencing of PCR-amplified DNA shows that a spontaneously derived LCL from donor JS is identical in this gene region to all type A isolates tested (46), (*ii*) the donor's B8 molecule accepts exogenous AWNA for recognition by AWNA-specific CTL clones and bulk cell lines in an identical fashion to all other (>20) $B8^+$ donors tested, and (*iii*) most importantly, the spontaneous LCL from donor JS is also recognized by AWNA-specific CTL clones, indicating that the endogenously infected B cells process and present the natural epitope immunogenically to CTLs from other $B8^+$ individuals. Therefore, nonresponsiveness to AWNA is not due simply to inadequate determinant selection since the peptide–MHC complex is immunogenic for responder CTLs.

The outcome of immune competition between two peptides (QLSD and AWNA) could favor one peptide over the other (31). This potential problem was avoided in culture by using autologous type B LCLs and PHA blasts to present individual peptide epitopes for CTL induction. Moreover, the peptide epitopes map to separate EBNA proteins (QLSD to EBNA 2A and AWNA to EBNA 3A); they show no sequence homology and are restricted by different MHC alleles (QLSD by A2 and AWNA by B8). A family study has confirmed that the restriction allele for QLSD is linked with the HLA-A2-Bw51, and not the HLA-A1-B8, maternal haplotype. Thus, there are no *a priori* reasons to implicate *in vitro*-induced mutual competition effects for the lack of an immune response to AWNA.

We can postulate that the CTLps specific for the AWNA–B8 complex were absent when memory to EBV was established in donor JS. This can occur if no T cells exist with the appropriate TCR for AWNA and this would then constitute a hole in the T-cell repertoire, a situation that has been described for class II antigens in the mouse model to explain the nonresponsiveness of MHC-restricted T cells to particular antigens (18, 32). A hole in the TCR repertoire could result from an inherited lack of specific variable (V), joining (J), or diversity (D) region alleles essential to the formation of a functional TCR specific for the AWNA–MHC complex. Human TCR genes are polymorphic, showing allelic variation within the species (33, 34). The TCR V gene family involved in the recognition of AWNA has not been defined. Alternatively, the clonal deletion of peptide-specific T cells during thymic maturation could also contribute to a hole in the TCR repertoire. T-cell clones could conceivably be deleted either by negative selection if they expressed self-reactivity or during positive selection if their TCR did not achieve critical binding with the relevant MHC residues (35, 36). Because of the extensive TCR diversity generated by VDJ rearrangements, it would be unlikely that holes in the TCR repertoire would arise by the chance failure to generate the appropriate random rearrangements (37). It is interesting that, in murine models, CTL clones can utilize a limited TCR V gene repertoire in response to select viral antigens (38, 39), making it more plausible that a hole in the TCR repertoire could effectively delete an epitope-specific CTL response.

Nonresponsiveness of CTLps to AWNA could be due to clonal anergy manifested in the periphery by suppressed or tolerant mature T cells. A state of anergy can be induced by engaging the TCR with inadequately presented antigen and, in some cases, T-cell function can be rescued with exogenous

rIL-2 (40, 41). Since donor JS can mount an effective *in vitro* response to QLSD, but not to AWNA, in the presence or absence of exogenous rIL-2, it is unlikely that the lack of a response to AWNA is due to a dysfunction in the production of accessory factors critical for T-cell activation. Therefore, donor JS contrasts strongly with certain HLA-B8$^+$ Sjogren syndrome patients whose anergic EBV-specific CTLs fail to respond *in vitro* to AWNA unless exogenous rIL-2 is added during memory-cell reactivation (I.S.M., C.S., and S. Whittingham, unpublished data).

We have described the response of CTLs from donor JS upon rechallenge, rather than the initial inducution of memory. Thus, the experimental data alone do not directly imply that this donor always lacked a CTL repertoire appropriate to the AWNA–B8 complex, only that such CTLs are currently undetectable. It is possible that CTLps for AWNA were present *in vivo* at the time of the primary infection, but memory was never stimulated or was lost, despite a persistent infection sustaining a strong memory to QLSD. EBV persists *in vivo* and EBV-specific CTLs are, presumably, constantly rechallenged during reinfection or recrudescence of latent virus. It has been postulated that restimulation is an essential requirement for the continued existence of memory T cells (42), and one could predict from the balance of growth model (43) that there is a selective advantage for T-cell clones that interact more frequently or avidly with their target cells. In the present context, this could occur (*i*) if EBNA 2 was expressed significantly earlier than EBNA 3 in latently infected cells *in vivo*, as has been suggested from *in vitro* studies (44), or (*ii*) if the TCR repertoire in donor JS was skewed in avidity toward QLSD rather than AWNA. Both of these possibilities depend on the state of expression of the EBV genome *in vivo*, about which little is known. In support of the first possibility, it has been shown that a frequency hierarchy can occur in the memory CTL response *in vitro* to individual influenza virus gene products (45). The second possibility requires that T-cell surveillance so effectively reduces the number of antigen-expressing cells that restimulation of T cells is a rare event. On the basis of spontaneous LCL data, it is estimated that 1 in 100,000 PBMCs from seropositive donors is infected with EBV. Thus, the frequency of encounter between any given CTL and an infected cell would be expected to be high. A skewed TCR repertoire would lead to dominance of one epitope over another only if few infected cells expressed latent genes.

One key element in all the above models is the absence in donor JS of CTLps with sufficiently high affinity for antigen to generate an effective primary response or to maintain a memory response specific for the AWNA–B8 complex. Our data show unequivocally that an individual may lack a response to a defined epitope that is appropriately presented *in vivo*. This has important implications for the effectiveness of strategies in which peptide vaccines are tailored strictly to individual MHC alleles.

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