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Epstein-Barr Virus Transactivates the Human Endogenous Retrovirus HERV-K18 that Encodes a Superantigen

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Summary

Superantigens (SAgs) are proteins produced by pathogenic microbes to elicit potent, antigen-independent T cell responses that are believed to enhance the microbes' pathogenicity. Here we show that the human lymphotropic herpesvirus Epstein-Barr virus (EBV) transcriptionally activates the *env* gene of an endogenous retrovirus, HERV-K18, that possesses SAg activity. SAg activity was demonstrated by MHC class II dependent preferential activation of TCRVB13 T cells in response to murine B cells transfected with the HERV-K18 *env* gene. This is a unique demonstration of a pathogen inducing a host-encoded Sag and accounts for the previously described EBV associated Sag activity. The T cell activation elicited by the Sag could play a central role in EBV infection and associated diseases.

Introduction

Superantigens (SAgs) are believed to play a critical role in the pathogenesis of certain microorganisms through their ability to stimulate a large fraction of primary T cells (Marrack and Kappler, 1990). They do this by associating with MHC class II molecules and binding to T cells that express particular T cell receptor (TCR) β chain variable (*TCRBV)* genes. This distinguishes them from conventional peptide antigens that bind to the groove formed by the α and β chains of the TCR and thus activate only a small population of specific T cells due to the genomic and somatic diversity of the TCR genes. It is believed that the T cell stimulation elicited by the SAg in some way benefits the pathogen because SAgs are evolutionarily conserved within a species. There are two groups of microorganisms that are known to have SAgs: bacterial and viral. Whereas a large number of bacterial SAgs have been well characterized structurally and functionally, only three families of viruses have been associated with SAg activity to date: retroviruses, rhabdoviruses, and herpesviruses (Huber et al., 1996).

We have reported previously that EBV-infected B cells express a TCRBV13-specific SAg (Sutkowski et al., 1996). The activity was characterized using an in vitro model system in which EBV-transformed B lymphoblastoid cell lines (LCLs) served as antigen-presenting cells (APCs) to autologous T cells. The EBV-infected B cells strongly and rapidly stimulated T cells, with kinetics and magnitude similar to a mitogenic response. The response was initially TCRBV13 specific (within 4–6 hr), followed by polyclonal activation (after 48 hr). The T cell stimulation was MHC class II dependent because it could be blocked using antibodies against HLA-DR and was not due to a recall antigen response; human umbilical cord blood T cells responded similarly. Finally, the TCRBV13 specificity was confirmed using a panel of chimeric murine T cell hybridomas (THys) expressing human *TCRBV* genes. We proposed that SAg-mediated T cell activation could play a role in the establishment and/or maintenance of EBV infection (Sutkowski et al., 1996), which results in lifelong viral persistence in the resting memory B cell compartment (Babcock et al., 1998). SAg driven T cell stimulation could also play a role in EBV-associated diseases.

Despite an extensive search, we have been unable to identify an EBV gene encoding this SAg. Thus, we hypothesized that EBV may transactivate an endogenous gene that has SAg activity. To this end, we noted that the human endogenous retrovirus HERV-K18 was recently localized to chromosome 1q21.2-q22 (Hasuike et al., 1999; Tonjes et al., 1999; Stauffer et al., 2001 [this issue of *Immunity*]) in the first intron of CD48, an EBV-transactivated gene (Thorley-Lawson et al., 1982). An EBV-inducible enhancer had been previously mapped to a region 1.58 kb upstream of the CD48 start site (Klaman and Thorley-Lawson, 1995). It was also shown that the IDDMK$_{1,2}$22 retrovirus (Conrad et al., 1997) is an allelic variant of HERV-K18 (Hasuike et al., 1999; Tonjes et al., 1999), designated allele 1 or K18.1 (Stauffer et al., 2001), whose *env* gene encodes SAg activity (Conrad et al., 1997). Thus, a host-encoded SAg was localized to a region of DNA in the vicinity of an EBV-inducible enhancer. These findings led us to test whether any of the HERV-K18 env alleles possessed TCRBV13 SAg activity that could be transactivated by EBV.

Here, we present evidence that this is indeed the case. We found that both HERV-K18.1 and K18.2 alleles specifically stimulated TCRBV13$^+$ and BV9$^+$ THys, but not other THys, and stimulated strong proliferation of primary T cells. We show that EBV infection leads to transcriptional activation of HERV-K18 *env* and that the EBV-associated SAg activity can be blocked with an antiserum specific for HERV-K18 Env. The implications of these findings are discussed in light of what is known about EBV biology and its transforming qualities.

Results

HERV-K18 Env Alleles and EBV-Infected B LCL Have Identical TCRBV Specificity, which Is Inhibited by HERV-K18 Env Antiserum and MHC Class II Antibodies

The HERV-K18 *env* alleles 1 and 2, K18.1 and K18.2, differ at several positions: The K18.1 Env has a stop codon at aa 153, whereas the K18.2 Env is a full-length

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553 aa protein (Stauffer et al., 2001). These are the two most common K18 *env* alleles, found in up to 90% of chromosomes tested in the Caucasion population (Stauffer et al., 2001). Because the K18.1 allele had been previously characterized (Conrad et al., 1997), we tested whether the full-length *env* of K18.2 could stimulate T cells. We cloned the entire HERV-K18.2 provirus using the chromosome 1 insertion sequences previously reported (Ono et al., 1986; Tonjes et al., 1999) as PCR primers. After sequencing, the *env* gene was subcloned into the bicistronic expression vector pCDLI (Stauffer et al., 2001), with the marker EYFP (enhanced yellow fluorescent protein) in the second cistron. Murine A20 B lymphoma cells were chosen for transfection experiments because the mouse genome does not have any HERV-related proviruses (Barbulescu et al., 1999). Stable clones expressing different levels of EYFP were selected by flow cytometry and tested for TCRBV13 T cell activation. We have previously described a system to assay for EBV-associated SAg activity (Sutkowski et al., 1996) based on the stimulation of murine THys with EBV-infected B cell lines acting as APCs. These THys bear chimeric TCRs composed of a human (h) *TCRBV* gene product with murine α chain and CD3 proteins. As shown in Figure 1A, all of the K18.2 *env* transfectants (A20/K18.2) stimulated the hTCRBV13 THy, but not the hTCRBV8 THy, whereas both THys were equally activated by CD3 crosslinking. The magnitude of the response was similar to that elicited by a LCL made from B cells from a K18.2 donor transformed by the B95-8 strain of EBV, whereas untransfected A20 cells gave no response. Similar results were obtained by transfecting K18.2 *env* into the human EBV^- B cell lymphoma BJAB (data not shown). Pretreatment of all APC lines with the phorbol ester PMA was necessary for stimulation of the THys, as was previously shown for the EBV-associated SAg activity (Sutkowski et al., 1996). In addition, HERV K18.2 Env appeared to be toxic to the B cells, because $EYFP^{hi}$ cells rapidly died off, selecting for $EYFP^{lo}$ or negative cells. These data indicate that the K18.2 *env* allele has SAg activity for TCRBV13, just like the EBV-associated SAg (Sutkowski et al., 1996).

To test whether the K18.2 *env* transfectants stimulated other T cell subsets, we used a panel of murine THys expressing different h*TCRBV* genes. In addition, we examined the response to the truncated K18.1 *env* allele transfected into A20 cells. The results, depicted in Figure 1B, show the comparison between the response obtained with a B95-8 LCL and the transfected A20 cells. The LCL and both K18 *env* alleles stimulated the hTCRBV13S1 and hTCRBV13S2 THys (Choi et al., 1990, 1991), but not the hTCRBV2, 3, 8, or 17 THys (Blank et al., 1993; Sutkowski et al., 1996), whereas A20 transfected with pCDLI vector alone did not stimulate any of the hybridomas. EBV induction of the stimulatory activity was demonstrated by comparing the APC function of the EBV^- Burkitt's lymphoma BL41 and its counterpart, BL41 infected with B95-8 EBV (Calender et al., 1987). At high APC ratios, uninfected BL41 weakly stimulated the very sensitive hTCRBV13S1 THy, most likely due to the low level of endogenous K18 *env* expression in these tumor cells (see Figure 3); however, infection with B95-8 EBV greatly enhanced stimulation. The marmoset cell line B95-8, from which the virus was derived, expresses both EBV-latent and lytic antigens but does not contain the HERV-K18 provirus (Barbulescu et al., 1999). Consistent with the idea that HERV-K18, not an EBV encoded protein, is the SAg, B95-8 cells did not stimulate the TCRBV13 THys.

At APC/responder ratios of 5:1, the K18 Env alleles and B95-8 infected BL41 and LCL also stimulated the hTCRBV9 THy (Choi et al., 1990, 1991), suggesting an additional TCR specificity for the SAg. These data show that both K18 *env* alleles have the same TCRBV specificity as the EBV-associated SAg. In this assay the truncated K18.1 Env reproducibly stimulated the TCRBV13 THys to make more IL-2 than the K18.2 Env protein. This difference likely reflects lower-level expression of the full-length K18.2 *env* due to its toxicity in the A20 cells, which was not observed with the truncated K18.1 *env* allele.

To test whether the SAg activity was due to K18 Env, we employed a rabbit antiserum raised against the K18 Env peptide 116-130. This antigenic peptide was selected using the hydrophilicity index of Kyte and Doolittle (1982). The K18 Env antiserum specifically blocked immune recognition of the K18.1 and K18.2 *env* alleles by the TCRBV13S1 and TCRBV13S2 THys in a dose-dependent manner, whereas the preimmune serum had no effect (Figure 2A). We then used this antiserum to prove that the TCRBV13 activation by EBV infected cells was mediated by K18 Env. As shown in Figure 2B, the Env antiserum blocked stimulation of these THys by EBV-transformed LCL and EBV-infected BL41, whereas no blocking was observed with the preimmune serum. The blocking effect was not due to toxicity by the Env antiserum because it had no effect on an anti-CD3 response. These data provide evidence that the TCRBV13-specific SAg activity associated with EBV is due to the *env* gene product of the endogenous HERV-K18 provirus.

We have previously shown that the EBV-associated SAg activation of peripheral blood T cells is dependent upon MHC class II presentation because it could be blocked by antibody specific for HLA.DR (Sutkowski et al., 1996). To determine whether the TCRBV13 activation by A20/K18.1 Env was MHC class II dependent, we preincubated the A20/K18.1 Env cells with mAb specific for MHC class I, $H\text{-}2D^d$; MHC class II, $I\text{-}A^{b/d}$ or $I\text{-}E^{k/d/p/r}$; or as a negative control, HLA.DR. The A20 cell line was derived from BALB/c mice of the $H\text{-}2^d$ haplotype. As a specificity control, B95-8 LCL were preincubated with the same antibodies. As can be seen in Figure 2C, K18.1 Env presentation in A20 cells was blocked by the anti-I-E antibody but not by antibodies specific for HLA.DR, H2-D, or I-A. The anti-I-E antibody did not inhibit SAg presentation by the B95-8 LCL, whereas, as expected, the anti-HLA.DR antibody did block. Thus, the response is MHC class II dependent, and it appears that in mouse, APC K18 Env is primarily presented by I-E molecules, the functional homolog of HLA.DR.

EBV Transcriptionally Activates HERV-K18 *env* in B LCL and Burkitt's Lymphoma with Functional Consequence

To test whether EBV could upregulate HERV-K18 *env* expression, as it does CD48 (Thorley-Lawson et al.,

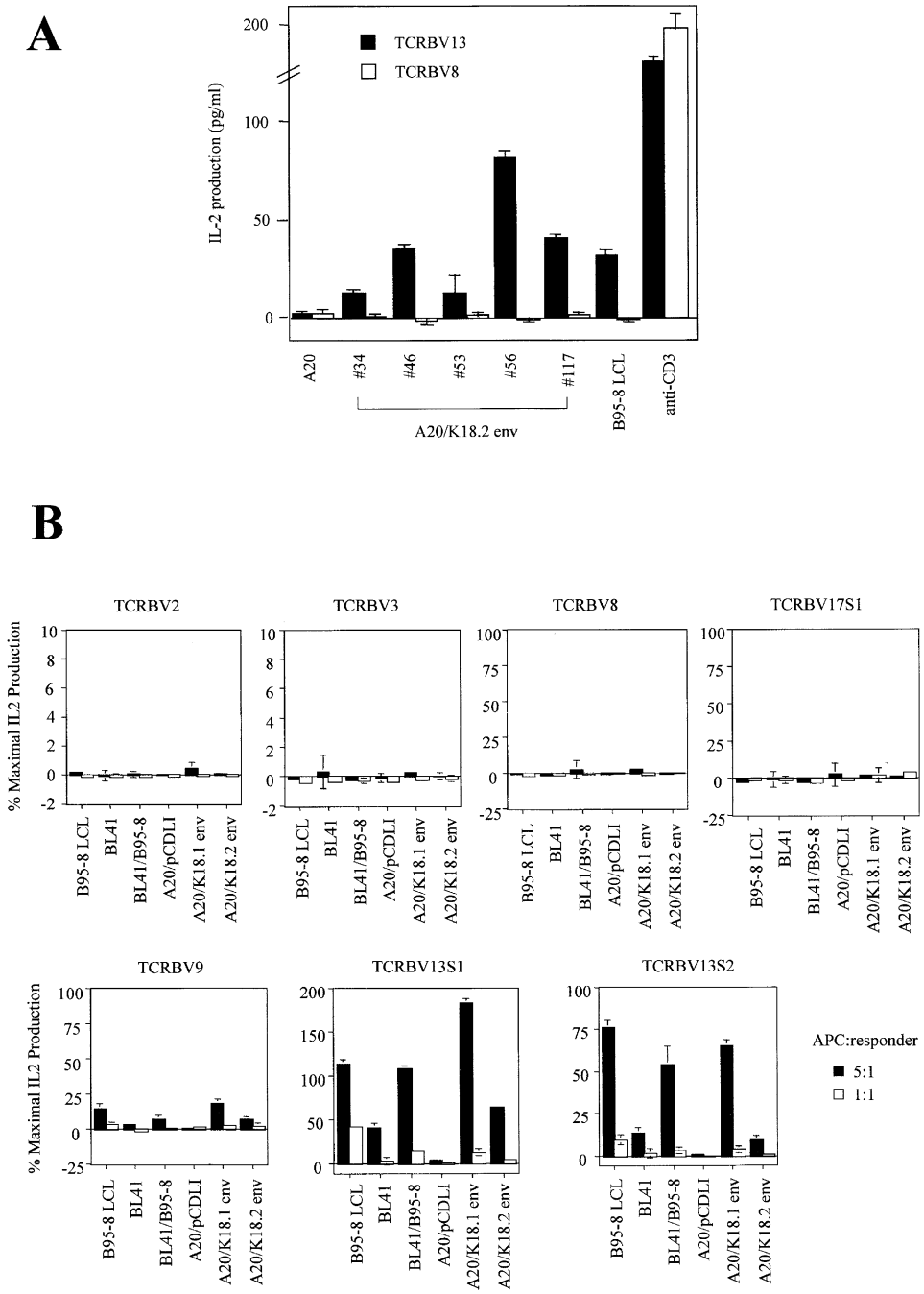
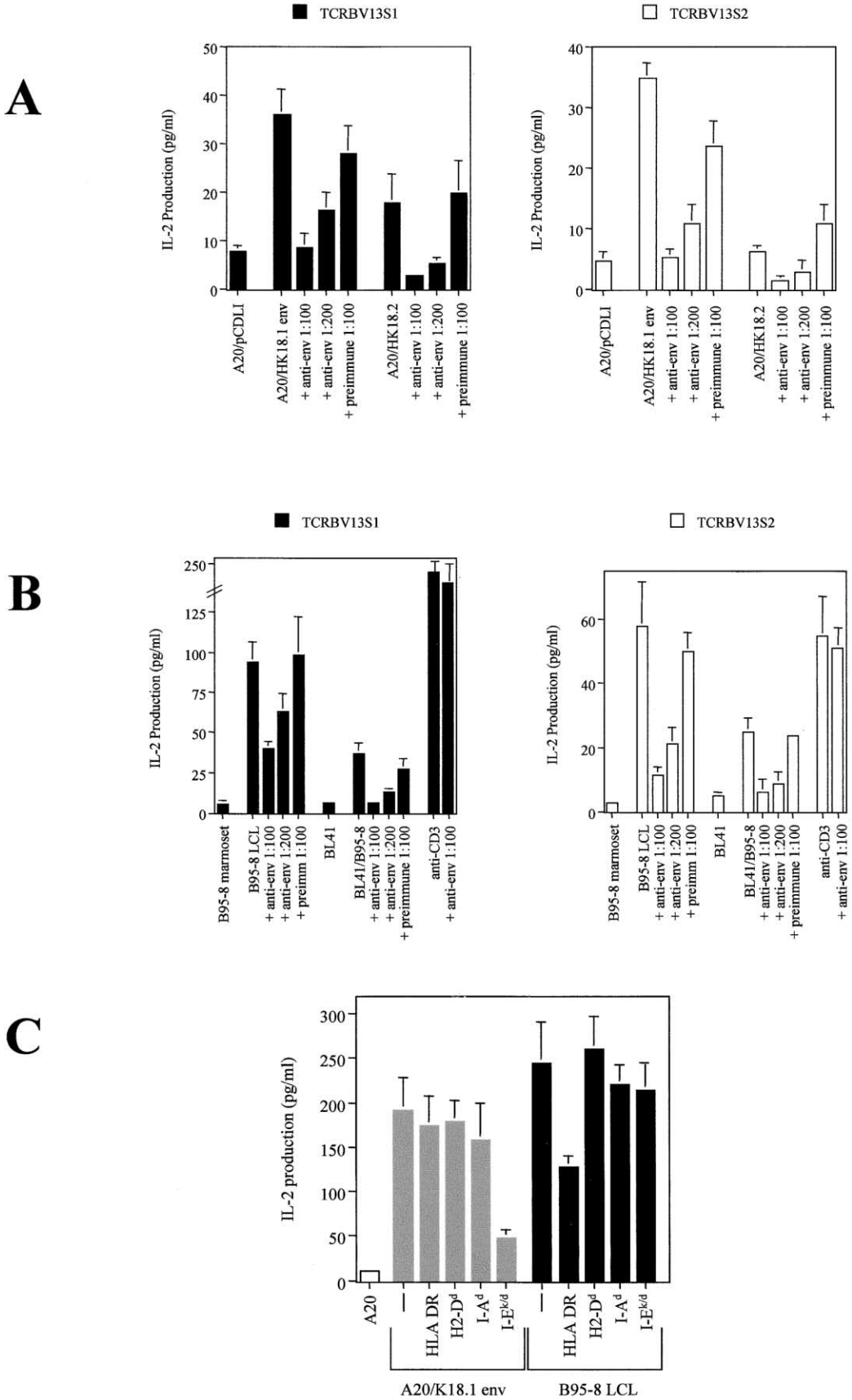


Figure 1. HERV-K18 Env and the EBV-Associated SAg Preferentially Activate hTCRBV13 and hTCRBV9 THys

(A) Untransfected A20 cells, or five individual clones of A20 stably transfected with HERV-K18.2 *env*, were pretreated with PMA and then resuspended with an equal number of hTCRBV13S1 (closed bars) or hTCRBV8 THy (open bars). After a 48 hr incubation, IL-2 production was measured in the cell supernatants by ELISA. The response was compared with that obtained with PMA-treated B95-8 transformed B LCL from the HERV-K18.2 *env* donor or with anti-CD3 crosslinkage.

(B) A20 transfected with HERV-K18 *env* alleles 1, 2, or vector only (A20/K18.1 *env*, A20/K18.2 *env*, A20/pCDLI, resp.); B95-8 transformed LCL, BL41, and BL41/B95-8 infected cells were pretreated with PMA/mitomycin C and resuspended with the indicated hTCRBV THy at APC/responder ratio of 5:1 (closed bars) or 1:1 (open bars). The results are expressed as percentage maximal IL-2 production, based on the stimulation of each THy by anti-CD3 crosslinkage because the THy differ significantly. Maximal IL-2 production (pg/ml) for this assay: TCRBV2 = 389.1 ± 108.2; TCRBV3 = 255.7 ± 16.3; TCRBV8 = 497.2 ± 11.7; TCRBV9 = 34.1 ± 14.7; TCRBV13S1 = 141.2 ± 13.5; TCRBV13S2 = 19.8 ± 9.9; and TCRBV17S1 = 58.05 ± 36.9. Results are representative of at least three independent assays.

1982), we used an RNase protection assay designed to detect all of the K18 *env* alleles but not other HERV-K *env* transcripts (Stauffer et al., 2001). As can be seen in Figure 3A, the protected *env* transcripts, consisting of a 300 bp band, are readily detected in a B95-8 EBV transformed LCL and are also upregulated when EBV$^-$


A
TCRBV13S1
TCRBV13S2
IL-2 Production (pg/ml)
A20/pCDL1
A20/HK18.1 env
+ anti-env 1:100
+ anti-env 1:200
+ preimmune 1:100
A20/HK18.2
+ anti-env 1:100
+ anti-env 1:200
+ preimmune 1:100
B
TCRBV13S1
TCRBV13S2
IL-2 Production (pg/ml)
B95-8 marmoset
B95-8 LCL
+ anti-env 1:100
+ anti-env 1:200
+ preimm 1:100
BL41
BL41/B95-8
+ anti-env 1:100
+ anti-env 1:200
+ preimmune 1:100
anti-CD3
+ anti-env 1:100
C
IL-2 production (pg/ml)
A20
—
HLA DR
H2-D^d
I-A^d
I-E^k/d
A20/K18.1 env
B95-8 LCL

BL41 cells are converted to EBV$^+$ by infection with B95-8 virus (Calender et al., 1987). The human TATA binding protein (hTBP) was used as a loading control. Interestingly, treatment of the APC with PMA had no effect on K18 *env* transcription, as could be seen by measuring the densitometry ratios of the level of K18 *env* to *hTBP* at 2, 8, or 16 hr following PMA treatment. Thus, the PMA enhancement of SAg activity does not work at the level of K18 *env* transcription. It is likely that PMA affects the mode of SAg presentation required for efficient T cell activation in vitro, as has been seen in other systems (Pesando et al., 1986; Yasukawa et al., 1988; Barois et al., 1997; Anderson et al., 1999). These data show that EBV directly transactivates K18 *env* expression in EBV-infected cell lines. EBV$^-$ BL41 cells express maximal levels of CD48 transcripts, which are not further enhanced by infection with B95-8 EBV (Klaman and Thorley-Lawson, 1995). Thus, our results indicate that merely opening the chromosomal region of CD48 for transcription leads to minimal expression of HERV-K18 *env*, whereas EBV infection superinduces this transcript approximately 10-fold (Figure 3A). To confirm these results, we developed a semiquantitative RT-PCR designed to detect HERV-K18 read-through transcripts specific for chromosome 1 insertion sequences adjacent to the provirus 3′ LTR. Up to 15% of all retroviral transcripts had been shown to be read-through transcripts containing chromosomal sequences immediately downstream of the insertion site of the provirus (Herman and Coffin, 1986). RNA was isolated from primary B cells and enriched by negative selection from peripheral blood mononuclear cells from three healthy adult volunteers and also from B95-8 LCL derived from these donors. We compared HERV-K18 *env* transcription in the purified B cells directly with that in LCL because the phenotype of infected B cells 24–32 hr after EBV infection is indistinguishable from that of EBV LCL (Alfieri et al., 1991). RNA was reverse transcribed either by random or gene-specific priming using an oligo directed against the chromosome 1 insertion sequence located 293 bp downstream of the 3′LTR. PCR primers were designed to amplify a 1161 bp region of cDNA originating in the *env* gene and progressing through the 3′LTR 122 bp downstream into the adjacent chromosome 1 sequence. As an endogenous standard, primers specific for 18s rRNA were included in the reaction. The results are presented in Figure 3B. For each individual, a relative induction of HERV-K18 transcripts of approximately 2- to 4-fold after B95-8 transformation was seen, confirming that EBV transcriptionally activates HERV-K18 *env* in primary B cells.

To determine whether this transcriptional activation is functionally relevant, primary B cells were LPS activated and compared with their B95-8-transformed counterpart for activation of the TCRBV13S1 THy. LPS was added to keep the primary B cells alive throughout the 48 hr hybridoma assay. Again, EBV LCL were used in place of freshly EBV-infected B cells because the assay requires 48 hr to perform and because the phenotype of B cells 24–32 hr after EBV infection is identical to that of EBV LCL (Alfieri et al., 1991). As can be seen in Figure 3C, the B95-8 LCL from each individual elicited higher IL-2 production than the LPS activated B cells, even at lower APC/responder ratios, indicating a functional consequence to the upregulation of HERV-K18 *env* transcripts by EBV. On the other hand, B cells cultured in the presence of soluble CD40L did not lead to functional SAg expression (Stauffer et al., 2001).

HERV-K18 Env Alleles and Autologous EBV LCL Strongly and Rapidly Activate Primary T Cells, and the Stimulation Is Blocked by HERV-K18 Env Antiserum

To confirm the stimulatory activity of K18 Env on primary T cells, we measured proliferation of peripheral blood T cells induced by A20 cells that were transfected with K18.1 *env*. In all cases, APC lines were pretreated for 16 hr with PMA, washed, and treated with mitomycin C to inhibit proliferation. APCs were then extensively washed and cocultured with peripheral blood mononuclear cells at APC/responder ratios of 1:1, 1:3, or 1:10. T cell proliferation was assessed 48 hr after coculture, as measured by uptake of [^{3}H]thymidine. As can be seen in Figure 4A, PMA/mitomycin C pretreated A20/ K18.1 Env vigorously and rapidly stimulated T cells, while pretreated A20/pCDLI conferred only minimal activity. The response was comparable in both magnitude and kinetics to that elicited with autologous B95-8 transformed LCL, as was previously shown for the EBV-associated SAg activity (Sutkowski et al., 1996) or the mitogen PHA. Control wells containing APC only did not incorporate [^{3}H]thymidine. It should be noted that only T cell proliferation is measured in this assay, not activation of particular TCRBV subsets. Earlier studies had indicated that the EBV-associated SAg elicits a polyclonal T cell activation (Sutkowski et al., 1996), and we have similar findings for A20/ K18.1 Env (data not shown).

To demonstrate that EBV induction of K18 *env* was driving this polyclonal proliferation, we again performed antibody-blocking experiments, using the rabbit antiserum raised against the K18 Env peptide (Figure 4B). The antiserum blocked peripheral blood T cells from

Figure 2. Anti-HERV K18 Env Antiserum and MHC Class II Antibodies Block Activation of THy by K18 Env Transfectants and the EBV-Associated SAg

(A) PMA/mitomycin-C-pretreated A20/K18.1 *env*, A20/K18.2 *env* were incubated with Env antiserum, diluted 1:100 or 1:200, or with preimmune serum (1:100) for 30 min prior to addition of hTCRBV13S1 THy (closed bars) or hTCRBV13S2 THy (open bars) at an APC/responder ratio of 2:1. IL-2 production was measured 24 hr later. The response was compared with A20/pCDLI (negative control).

(B) PMA/mitomycin-C-pretreated B95-8 marmoset cells, B95-8 LCL, BL41, or BL41/B95-8 were incubated with the hTCRBV13S1 THy (closed bars) or hTCRBV13S2 THy (open bars) as described above. B95-8 LCL and BL41/B95-8 were also pretreated with Env antiserum, diluted 1:100 or 1:200, or with preimmune serum at 1:100. IL-2 production was measured 24 hr later. The responses were compared with that elicited by anti-CD3 crosslinkage. As toxicity control, the Env antiserum was also added to anti-CD3 wells.

(C) PMA-pretreated A20, A20/K18.1 *env*, or B95-8 LCL were preincubated with antibodies specific for HLA.DR, H-2D^d, I-A^d, or I-E$^{k/d}$ and then added to hTCRBV13S1 at an APC/responder ratio of 1:1. IL-2 production was measured 24 hr later.

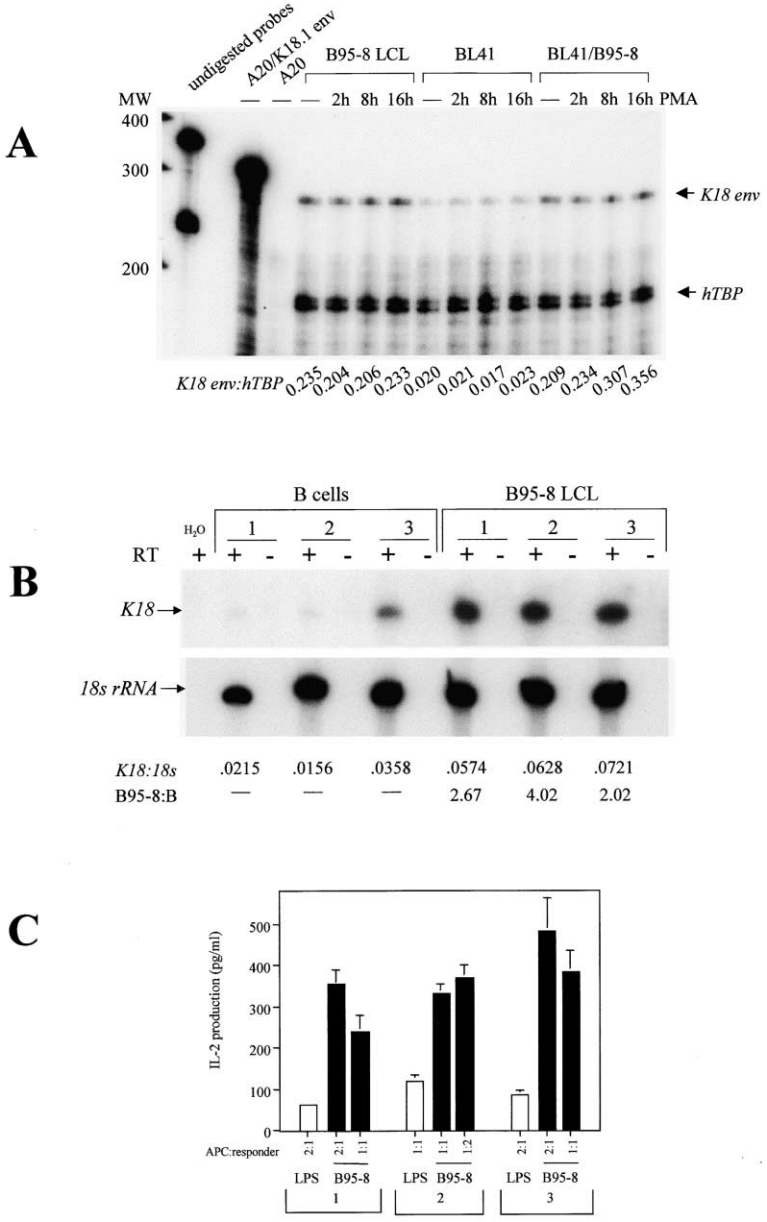


Figure 3. B95-8 EBV Transcriptionally Activated HERV-K18 *env* Expression in B Cells with Functional Significance for T Cell Activation

(A) Total RNA from B95-8 transformed LCL, BL41, or BL41/B95-8 infected cells, treated for 0, 2, 8, or 16 hr with PMA, were incubated with riboprobes specific for HERV-K18 *env* alleles and *hTBP* (loading control), and then digested with RNases, and run on a 6% polyacrylamide gel. Protected fragments for HERV-K18 *env* were detected at 300 b, and the loading control hTBP as a doublet at 161 b. As controls, RNA from A20 and A20 transfected with K18.1 *env* were included. (The A20/K18.1 *env* construct has an additional 30 b of Bluescript vector sequence that is protected by the riboprobe, accounting for the difference in size between positive control and the 300 b *K18 env* band.) Densitometry value ratios for the *K18 env: hTBP* doublet are indicated below each lane.

(B) Relative quantitative RT-PCR was performed using RNA derived from purified B cells from three different donors (1–3) and B95-8 transformed B LCL from the same three donors. Primers were designed to detect a 1161 bp HERV K18 read-through transcript that traverses the *env* gene, 3′ LTR, and adjacent chromosome 1 sequences located up to 122 bp downstream of the 3′ LTR. 25 PCR cycles were determined to yield product within the linear range. Because the read-through transcripts were extremely rare, PCR was performed in the presence of [^{32}P] α-dCTP. As endogenous standard, primers specific for an 18s rRNA 489 bp product were used in each reaction; and as negative controls, H_2O only and no RT reactions were simultaneously performed. PCR products were separated on a 6% denaturing acrylamide gel and quantified by Phosphorimaging. The ratios of *HERV K18:18s rRNA* are printed below each lane, and the fold induction of HERV K18 transcripts after B95-8 transformation is depicted for each individual (B95-8:B).

(C) Purified primary B cells from three individuals were treated with LPS (open bars) and compared with B95-8-transformed B LCL derived from the same blood donors (closed bars). Both LPS B cells and B95-8 LCL were pretreated with PMA, washed, and incubated with the hTCRBV13S1 THy at various APC/responder ratios using 2 $\times 10^4$ THy per quadruplicate well. IL-2 production was measured 48 hr later.

responding to A20/K18.1 Env in a dose-dependent manner, whereas preimmune serum was not inhibitory. On the other hand, the response to the mitogen PHA was unaffected, indicating that the antiserum was not toxic to the responding T cells.

The Env antiserum also completely blocked the strong T cell proliferative response of an EBV sero-negative donor to autologous LCL derived from in vitro transformation of B cells with B95-8 EBV, excluding the possibility that the elicited T cell proliferation was due to a potent recall response to EBV. Moreover, no response by this EBV$^-$ donor was seen to the EBV$^+$ marmoset cell line B95-8, analogous to the results depicted in Figure 2B, showing that B95-8 marmoset cells do not activate TCRBV13 THys. These experiments indicate that the polyclonal T cell proliferation elicited by the EBV LCL is a direct consequence of HERV-K18 *env* expression. Whether the polyclonal activation is a downstream event of the TCRBV13 specific response or a separate activity of the K18 *env* product is not clear.

Discussion

We have shown that EBV infection of B cells leads to transactivation of HERV-K18 *env* alleles, which express a TCRBV13 specific SAg activity previously identified

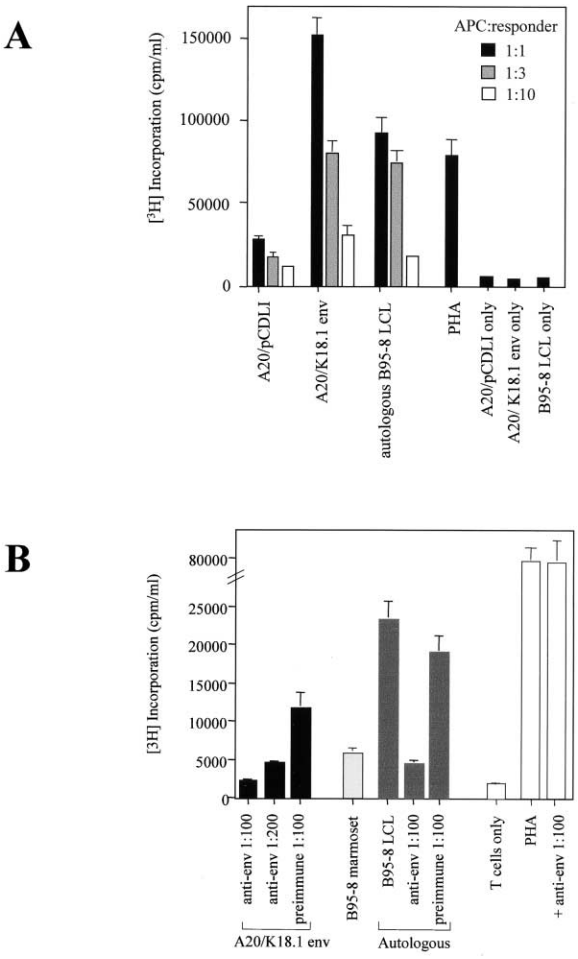


Figure 4. The EBV-Associated SAg, HERV-K18 Env, Strongly Stimulated Peripheral Blood T Cells with Rapid Kinetics

(A) PMA/mitomycin-C-treated A20/K18.1 *env* or A20/pCDLI and autologous B95-8 transformed LCL were used as APC in 48 hr T cell proliferation assays, as measured by the incorporation of [^{3}H]thymidine. APC/responder ratios of 1:1 (closed bars), 1:3 (gray bars), and 1:10 (open bars) show that T cell proliferation is dependent upon antigen dose. The response was compared with the mitogen PHA, and APC only were shown for comparison.

(B) Preincubation with HERV-K18 Env antiserum at 1:100 and 1:200 dilutions blocked 48 hr T cell proliferation to PMA/mitomycin-C-treated A20/K18.1 Env, whereas preimmune serum, added at 1:100, was noninhibitory. In addition, T cell proliferation to autologous B95-8 transformed LCL from an EBV-seronegative donor was blocked by the Env antiserum, but not by the preimmune serum, whereas the Env antiserum had no effect on T cell proliferation due to PHA. The B95-8 marmoset cell line, which produces high titer EBV, was not stimulatory to the EBV seronegative donor T cells.

as an EBV-associated SAg (Sutkowski et al., 1996). This represents a demonstration of a microbial pathogen, EBV, inducing an endogenous SAg in humans. SAgs are believed to provide an advantage for the pathogen, facilitating its transmission in the host (Huber et al., 1996; Acha-Orbea et al., 1999). Therefore, it is conceivable that EBV makes use of the SAg-induced T cell stimulatory activity to establish persistent infection in vivo, ultimately residing in the memory B cell pool (Babcock et al., 1999). Because B cell memory formation is clearly a T cell dependent event (Gray et al., 1997; Liu and Arpin, 1997; Liu and Banchereau, 1997), the SAg-elicited T cell help would fulfill this requirement. This is particularly tantalizing because it has been observed that marmosets, New World primates that do not contain HERV-K18 in their genome (Barbulescu et al., 1999), are unable to establish long-term, persistent EBV infection even when efficiently infected with EBV in vivo (D.A.T.-L., unpublished data).

Other herpesviruses have been associated with SAg-like activities. Herpesvirus *saimiri* Orf-14 was identified as a viral protein having homology to the MMTV SAg, Mls-1 (Yao et al., 1996). Orf-14 was reported to activate T cells polyclonally (Yao et al., 1996), similar to a mitogen. Interestingly, deletion of *orf-14* from the *saimiri* genome resulted in a virus with reduced ability to transform primate T cells in vivo (Duboise et al., 1998). Infection with cytomegalovirus was associated with a TCRBV12 T cell response; however, no gene has been identified encoding this activity (Dobrescu et al., 1995). It is possible that this herpesvirus transactivates another HERV gene that has TCRBV12-restricted SAg activity. The murine γ-herpesvirus is associated with a mouse TCRBV4-restricted T cell response but does not entail the traditional characteristics of a SAg (Tripp et al., 1997; Coppola et al., 1999), and, again, no gene has been identified that encodes this T cell stimulatory activity. These observations imply that herpesviruses may have evolved different mechanisms enabling them to elicit T cell help in the infected host. This may be analogous to the various mechanisms used by these viruses to avoid recognition by cytotoxic T cells, such as downregulating MHC class I expression (Ploegh, 1998). In the scenario of EBV-transactivating HERV-K18 expression, it will be interesting to study the interplay of biological activity that has allowed the evolutionary retention of an endogenous retrovirus that potentially benefits a persistent herpesvirus.

In vitro detection of the HERV-K18 SAg activity required a highly defined system whereby murine transfectants presented the K18 *env* gene product to hTCRBV-specific THys. The chimeric human/mouse TCR of the THys revealed the preference for *TCRBV 13S1*, *13S2*, and *9* gene products. In primary cells, the EBV-associated T cell response is also TCRBV13 restricted initially; it then rapidly becomes polyclonal (Sutkowski et al., 1996). We have shown here that K18 Env alone induces this polyclonal response in peripheral blood T cells when presented by transfected mouse APC (Figure 4). We saw no evidence of TCRBV-specific expansions in peripheral blood T cells, despite the massive T cell proliferation (data not shown). It is possible that the polyclonal activation could be a direct downstream consequence of the early TCRBV13-specific effect. Alternatively, it could be a separate property of the *env* gene product that masks the TCRBV13-specific effect at later times. Similar polyclonal effects have been seen with bacterial SAgs in toxin titration experiments (Fleischer et al., 1996); namely, above a particular threshold, TCRBV restriction was lost, and all T cells were activated. In our experimental system, it was difficult to control the amount of HERV-K18 Env protein that was presented to T cells. We accomplished this by varying the APC/responder ratios; however, this is very different from adding known amounts of soluble protein, as is done with the bacterial toxins.

PMA treatment of the APCs was required to detect SAg activity in all transfected clones and EBV lines. Clues to the mechanism of PMA enhancement are suggested by the MMTV system, which employs the best-analyzed viral SAg, Mls-1. We obtained comparable findings with Mls-1 transfectants, where preincubation of the APCs with IL-4 yielded a more efficient T cell stimulation read-out, although there was no increase in *mls-1* mRNA levels (Beutner et al., 1992a, 1992b). Instead, the IL-4 was likely boosting the level of MHC class II transcription (Roehm et al., 1984; Finn et al., 1991), allowing more efficient presentation of Mls-1 on newly synthesized class II molecules. PMA, like IL-4, initiates MHC class II expression (Pesando et al., 1986; Yasukawa et al., 1988). Although Mls-1 has been studied for years, it is still not clear how it associates with MHC class II nor in what cellular compartment (Hsu et al., 2001). It is known that Mls-1 presentation to T cells requires both glycosylation and proteolytic processing by arginine-specific, and possibly other, proteases (Mix and Winslow, 1996; Denis et al., 2000). We show here that PMA treatment of the APCs also does not affect the level of HERV-K18 *env* transcription (Figure 3). By analogy to the MMTV system, PMA treatment may facilitate presentation of the EBV SAg by affecting posttranslational modification or trafficking of the SAg. For example, it has been recently shown that protein kinase C phosphorylation directly affects MHC class II trafficking and antigen presentation pathways in APCs (Barois et al., 1997; Anderson et al., 1999).

Mls expression in B cells from mice naturally infected with MMTV is even lower than that derived from an endogenous SAg, requiring a highly sensitive PCR reaction coupled with Southern blotting for detection (Beutner et al., 1994). This level is sufficient to facilitate the transmission of the infectious virus without the noticeable disturbance to the immune system associated with a strong superantigen response. In fact, no obvious T cell activation in naturally MMTV-infected animals is observed, despite the complete dependence on the superantigen stimulation pathway for transmission of the virus from the gut, the site of primary infection, to the mammary tissue. It is likely that EBV-induced HERV-K18 *env* SAg activity is also minimal in vivo, sufficient for the EBV-infected B cells to receive T cell help without overt stimulation of the immune system. This scenario fits very well with the fact that EBV persists in humans throughout our lifetime, causing problems only when the immune system is suppressed.

Finally, EBV is a highly oncogenic virus, and there is good evidence that activated T-helper cells contribute to viral pathogenesis. Extensive T cell infiltrates are characteristic of the EBV-associated tumors, Hodgkin's lymphoma and naso-pharyngeal carcinoma, as well as transplant-associated lymphomas (PTLD). SCID mice reconstituted with peripheral blood mononuclear cells (PBMC) from EBV^+ donors develop EBV^+ lymphomas, providing a model for PTLD (Mosier et al., 1988). In this system, it was shown that human CD4 T cells greatly increase the development of EBV^+ B cell lymphomas (Veronese et al., 1992; Veronesi et al., 1994). Furthermore, massive lymphocytosis is characteristic of acute infectious mononucleosis (IM). Although it has been reported that viral-specific CTL clones are greatly expanded in IM and account for much of the lymphocytosis (Callan et al., 1998; Blake et al., 2000), murine studies have demonstrated that administration of a bacterial SAg to virally infected animals resulted in an augmented and protracted viral CTL response (Coppola and Blackman, 1997). Hence, an EBV-induced SAg activity may not only be critical at the early stages of EBV infection facilitating transmission of the virus, but could also play a role in enhancing the EBV-specific CTL response. We are currently testing these models in vitro and in vivo in the SCID system.

Experimental Procedures

Cell Lines, Antibodies, and DNA Constructs

All cell lines were grown in RPMI supplemented with 10% FCS, glutamine, HEPES, Na pyruvate, and β-mercaptoethanol (all tissue culture reagents from GIBCO Life Technologies). A20 is a murine B cell lymphoma (BALB/c background). Burkitt's lymphoma lines BL-41 and BL-41/B95-8 (Calender et al., 1987) were a gift from Gilbert Lenoir. PBMC were obtained from the whole blood of healthy adult volunteers by density gradient separation on Ficoll-Paque Plus (Pharmacia). B95-8 virus was derived by 0.4-μm filtering 5 day cell supernatants of confluent B95-8 marmoset cells (Miller and Lipman, 1973). Viral supernatants were either used immediately or frozen in aliquots at −80°C. B95-8 LCL were obtained by incubating 10^7 freshly isolated PBMC with 1 ml of B95-8 supernatant that was diluted 1:1 in media for 2 hr at 37°C. Cells were then diluted in 10 ml media with the addition of cyclosporin A (1 μg/ml) and placed at 37°C for 2–3 weeks. The medium was changed during this time every 5 days. Once the growing LCL became confluent, cyclosporin A was removed. All THy lines were described previously (Sutkowski et al., 1996) and were kindly donated by Philippa Marrack or Rafick-Pierre Sekaly. Briefly, the TCRBV2 cell line was TAL 8.1.9 (Blank et al., 1993); TCRBV3 THy was 58α-β-/HAI-7α/β; TCRBV8 THy was YLβ8#24; TCRBV9 THy was DS3-A; TCRBV13S1 THy was hVβ13.1-1; TCRBV13S2 THy was hVβ13.2-1 (Choi et al., 1990, 1991); and TCRBV17 THy was YLβ17.1#5. THy lines were maintained by occasional sorting for CD4 and CD3ε bright cells (antibodies: FITC-L3T4 and PE-145 2C11; Pharmingen), using a MoFlo cytometer (Cytomation, Fort Collins, CO). Sorted THy were expanded, and aliquots were frozen as early as possible to minimize the spontaneous loss of CD3 and CD4 during cell division, which leads to reduced IL-2 production.

HERV K18.2 *env* was cloned by two-step PCR using genomic DNA obtained from LCL of different sources. First-step PCR primers were specific for the chromosome 1 insertion sequences flanking the HERV-K18 provirus (Ono et al., 1986; Tonjes et al., 1999). The sense primer was 5′TACAACATAAGCGGAATCTGAGACTG3′, and the antisense primer was 5′CCCAAACCTTTAAATATTGTCTCATG3′. The entire provirus was amplified using Expand Long Template PCR system (Boehringer Mannheim) according to manufacturer's protocol. The second-step PCR used as a template the amplification product from step 1, diluted 1:100. Primers were specific for the full-length *env* gene and included SpeI and NotI restriction enzyme sites for subcloning into the pCDLI bicistronic expression vector (Stauffer et al., 2001), which has the SRα promoter driving expression and the *EYFP* marker gene encoded in the second cistron. The sense primer was 5′ATACTAGTGAGATGGTAACACCAGTCACATGG3′, and the antisense primer was 5′AAGCGGCCGCTCATGGCCCGTTCTCGATGTCACT3′. PCR was performed using the Expand High Fidelity PCR system (Boehringer Mannheim). PCR products were sequenced after subcloning into the pCDLI expression vector. The subcloning of HERV K18.1 *env* DNA into pCDLI was previously described (Stauffer et al., 2001); pCDLI/I550 transfectants were used for THy assays, and IDDM465 was used for RNase protection assays. These constructs differ by inclusion of 550 or 465 nucleotides of K18.1 *env*. *pCDLI/K18.1* (15 μg) or *K18.2 env* (15 μg), or 15 μg *pCDLI* DNA and 0.5 μg of a hygromycin-carrying plasmid, *DZ17*, were then cotransfected by electroporation into 2×10^7 A20 cells in 0.4 ml of Optimem (GIBCO), using a 0.4 cm cuvette, at 960 μF

and 300 V. Cells were resuspended in 20 ml media overnight and were then single-cell subcloned in selective media containing hygromycin (0.5 mg/ml). Two weeks later, individual clones were assayed for EYFP expression by flow cytometry using a FACSCalibur. Because K18.2 env transfectants rapidly lost Env expression, likely due to toxicity, these cells were not maintained in vitro; instead, aliquots were frozen as quickly as possible after expansion. K18.1 *env* transfectants were much more stable.

The anti-HERV-K18 antiserum was obtained by immunizing rabbits with a HERV-K18 Env peptide consisting of amino acids 116–130, CPKEIPKGSKNTEVL, conjugated to KLH (Sigma-Genosys). Rabbits were immunized on day 0, 14, 28, 42, 56, and 70. Final bleeds were performed within 2 weeks of the last boost. The antibody titer, as reported by the manufacturer's peptide-based ELISA, was ~1:100,000. The following antibodies were used in the MHC class II blocking studies: purified anti-HLA.DR, D1-12 (IgG2a) (a gift from R.-P. Sekaly); anti-I-$E^{k/d/p/r}$, IE-D6 (IgG2a) (American Research Products, Belmont, MA); anti-I-$A^{b/d}$, IA-C6 (IgM) (American Research Products); and anti-H-$2D^{d/u/p}$, KD-B4 (IgM) (American Research Products). All antibodies were low endotoxin, sodium azide free, and in PBS.

T Hybridoma Assays and IL-2 ELISA

EBV cell lines and stable A20 transfectants expressing HERV-K18.1 or K18.2 *env*, growing in log phase, were treated overnight with PMA (10 ng/ml, Calbiochem) at 37°C. Cells were washed once and then treated with mitomycin C (0.1 mg/ml PBS, 10^7 cells/ml; Sigma) for 1 hr at 37°C and washed extensively in PBS (4×, 50 ml). Frozen aliquots of $CD3^{hi}/CD4^{hi}$ THys were thawed no more than 1–2 days prior to assay to minimize spontaneous loss of CD3. Cells were counted and resuspended with THy in 96-well, round-bottom plates, with 10^5 or 2×10^4 APC and 2×10^4 THy/well in quadruplicates. After 24–48 hr at 37°C, the plates were frozen at −80°C to lyse the cells, and thawed supernatants were tested for the presence of mIL-2 by ELISA (see below). As positive control, the THys were crosslinked with plate-bound anti-CD3. Wells were coated with protein A (100 μg/ml PBS; ICN Biomedicals) for 2 hr at 37°C and then with anti-CD3 (145 2C11 culture supernatant diluted 1:150 in PBS) for 2 hr at 37°C. Wells were washed three times with PBS before addition of THy. IL-2 production for each THy was expressed either in pg/ml or as percentage maximal based on the response to anti-CD3 crosslinking.

The murine IL-2 ELISA was performed using as capture antibody purified anti-mouse IL-2 (2 μg/ml, diluted in 0.1 M Na_2HPO_4 [pH 9.0]; Pharmingen #18161D) and as detection antibody biotin anti-mouse IL-2 (2 μg/ml diluted in blocking buffer: PBS/2% BSA/0.05% Tween 20; Pharmingen #18172D). Visualization at 405 nm was with Neutravidin-AP (0.1 μg/ml in blocking buffer) and PNPP substrate solution (Pierce). Experimental samples were compared with values from a standard curve, using known amounts of rIL-2 (R&D Systems).

Antiserum blocking studies were performed as described above, except that APC were preincubated for 30 min at 37°C with rabbit anti-Env peptide 116–130 antiserum, diluted 1:100 or 1:200 in medium, or preimmune serum diluted 1:100 in medium. The APC/responder ratio was 2:1, with 2×10^4 THy per well. Plates were frozen after 24 hr, and thawed supernatants were tested for mIL-2 by ELISA as described above.

MHC class II blocking studies were performed as described above, except that APC were preincubated for 30 min at 37°C with anti-HLA.DR, anti-I-A, anti-I-E, or anti-H-2D antibodies at 50 or 5 μg/ml. Blocking was obtained with anti-HLA.DR at 50 μg/ml and with anti-I-E at 5 μg/ml, whereas no blocking was seen with anti-I-A or H-2D at either concentration. The APC/responder ratio was 1:1 with 2×10^4 Thy per well. Plates were frozen after 24 hr, and thawed supernatants were tested for mIL-2 by ELISA as described above.

LPS B cells were prepared by adding lipopolysaccharide from Salmonella typhimurium (50 μg/ml) (Sigma) to B cells purified from the peripheral blood of three healthy adult donors. B cells were prepared by negative selection using the StemSep Antibody Cocktail for enrichment of human B cells (Stem Cell Technologies Inc., Vancouver, Canada), according to manufacturer's protocol. Non-B lineage cells were removed with StemSep™ magnetic colloid, using a 5 Tesla magnet. The resulting population of enriched B cells for each donor was ~85% $CD20^+$, as assessed by flow cytometry using FITC-labeled anti-CD20 (BD Pharmingen, San Diego, CA). B95-8 LCL, previously transformed from the same blood donors, and 24 hr LPS-treated B cells were treated with PMA (10 ng/ml), and cells were incubated overnight and then washed extensively and resuspended with TCRBV13S1 THy at various APC/responder ratios using 2×10^4 THy per well. Plates were frozen after 48 hr, and thawed supernatants were tested for mIL-2 by ELISA as described above.

RNase Protection Assay and Semi-Quantitative PCR

A total of 2×10^8 BL41, BL41/B95-8, or B95-8 LCL were treated for 0, 2, 8, or 16 hr with PMA (10 ng/ml), and total RNA was prepared with Trizol (Gibco BRL). The RNase protection assay was performed as previously described (Staufer et al., 2001) but with 100 μg total RNA/lane. As controls, 100 μg RNA from untransfected A20 cells and 20 μg RNA from A20 transfected with HERV-K18.1 *env* (IDDM465) were loaded on the gel. Densitometry values were obtained by scanning the autoradiograph with a Biorad Gel Doc 1000, using the Molecular Analyst program. The ratio of K18 *env:hTBP* (human TATA binding protein) was determined.

Total RNA was prepared from purified B cells derived using Trizol from three healthy adult donors (see above for B cell enrichment) or from B95-8 LCL transformed from the same blood donors. RNA was DNase I treated (Roche) according the manufacturer's protocol and then phenol/chloroform treated to remove the enzyme. cDNA was prepared from 1 μg RNA for each sample in 20 μl reactions, using Superscript II reverse transcriptase (Gibco BRL), either by random priming or with a gene-specific primer, according to the manufacturer's protocol. In all cases, a no-RT control was simultaneously performed. The RT primer was 5′ GTGGCAAATTCACTTGG CTGA 3′. This primer is specific for the chromosome 1q23.1-q24.1 (Accession NT_003229) sequence located 293 bp downstream of the HERV-K18 3′LTR. Both random priming and gene-specific priming yielded similar PCR results (data not shown). The PCR sense primer was 5′ TCCGAAGAGACAGTGACAT 3′, which is directed against a HERV K18 *env*-specific sequence; the PCR antisense primer was 5′ CTGGCTATGTAAGT 3′, which is directed against a chromosome 1q23.1-q24.1 sequence located 122 bp downstream of the 3′ viral LTR. The 50 μl PCR reaction consisted of Platinum Taq PCR Supermix (GIBCO), 1 μM (final) of each primer, and 0.5 μl of [^{32}P]α-dCTP (10 μCi/μl) and 2 μl of cDNA (1/10 volume). As an endogenous standard, primers against 18s rRNA were included in the reaction from the Ambion Gene Specific Relative RT-PCR kit (Ambion Inc., Austin, TX). Because the HERV-K18 read-through transcripts were expected to be extremely rare compared with the 18s rRNA transcripts, 18s Competimers (Ambion) were added at a primer:competimer ratio of 1:9. PCR was performed on an Eppendorf Mastercycler using a hot start of 4 min at 94°C, then 25 cycles of 30 s at 94°C, 1.5 min at 72°C, and 1 min at 55°C, followed by a 7 min extension at 72°C, which yielded PCR products within a linear range. PCR products were separated on a 6% denaturing acrylamide gel, and HERV-K18 read-through transcripts were quantified by Phosphorimaging (Molecular Dynamics). Induction was measured after normalization against the 18s rRNA product.

T Cell Proliferation Assays

PBMC were obtained from healthy adult volunteers, plated overnight at 37°C in 10% FCS/complete RPMI media to allow monocytes to adhere, and then used as a source of T cells. In all cases, autologous T cells were plated with B95-8 LCL. A20 transfected with HERV-K18.1 *env* or pCDLI, B95-8 LCL, or B95-8 marmoset cells were treated overnight with PMA (10 ng/ml) and then with mitomycin C (0.1 mg/ml) for 1 hr and washed extensively with PBS, as described in the THy assays. APC and T cells were resuspended at APC/responder ratios of 1:1, 1:3, or 1:10, always using 10^5 T cells per well in quadruplicate using 96-well, round-bottom plates. After 48 hr at 37°C, cells were pulsed with [^{3}H]thymidine (1 μCi/well) for 12–16 hr and harvested and counted for [^{3}H] incorporation.

Antiserum blocking studies were performed identically; however, prior to addition of T cells, APC were preincubated for 30 min at 37°C with anti-HERV-K18 Env peptide 116-130 antiserum, diluted 1:100 or 1:200 in medium, or preimmune serum diluted 1:100 in medium.

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References

Acha-Orbea, H., Finke, D., Attinger, A., Schmid, S., Wehrli, N., Vacheron, S., Xenarios, I., Scarpellino, L., Toellner, K.M., MacLennan, I.C., and Luther, S.A. (1999). Interplays between mouse mammary tumor virus and the cellular and humoral immune response. Immunol. Rev. *168*, 287–303.

Alfieri, C., Birkenbach, M., and Kieff, E. (1991). Early events in Epstein-Barr virus infection of human B-lymphocytes. Virology *181*, 595–608.

Anderson, H.A., Bergstralh, D.T., Kawamura, T., Blauvelt, A., and Roche, P.A. (1999). Phosphorylation of the invariant chain by protein kinase C regulates MHC class II trafficking to antigen-processing compartments. J. Immunol. *163*, 5435–5443.

Babcock, G.J., Decker, L.L., Freeman, R.B., and Thorley-Lawson, D.A. (1999). Epstein-barr virus-infected resting memory B cells, not proliferating lymphoblasts, accumulate in the peripheral blood of immunosuppressed patients. J. Exp. Med. *190*, 567–576.

Babcock, G.J., Decker, L.L., Volk, M., and Thorley-Lawson, D.A. (1998). EBV persistence in memory B cells in vivo. Immunity *9*, 395–404.

Barbulescu, M., Turner, G., Seaman, M.I., Deinard, A.S., Kidd, K.K., and Lenz, J. (1999). Many human endogenous retrovirus K (HERV-K) proviruses are unique to humans. Curr. Biol. *9*, 861–868.

Barois, N., Forquet, F., and Davoust, J. (1997). Selective modulation of the major histocompatibility complex class II antigen presentation pathway following B cell receptor ligation and protein kinase C activation. J. Biol. Chem. *272*, 3641–3647.

Beutner, U., Frankel, W.N., Cote, M.S., Coffin, J.M., and Huber, B.T. (1992a). Mls-1 is encoded by the long terminal repeat open reading frame of the mouse mammary tumor provirus *Mtv-7*. Proc. Natl. Acad. Sci. USA *89*, 5432–5436.

Beutner, U., Kraus, E., Kitamura, D., Rajewsky, K., and Huber, B.T. (1994). B cells are essential for Murine Mammary Tumor Virus transmission, but not for presentation of endogenous superantigens. J. Exp. Med. *179*, 1457–1466.

Beutner, U., Rudy, C., and Huber, B.T. (1992b). Molecular characterization of Mls-1. Int. Rev. Immunol. *8*, 279–288.

Blake, N., Haigh, T., Shaka'a, G., Croom-Carter, D., and Rickinson, A. (2000). The importance of exogenous antigen in priming the human CD8 (+) T cell response: lessons from the EBV nuclear antigen EBNA1. J. Immunol. *165*, 7078–7087.

Blank, U., Boitel, B., Mege, D., Ermonval, M., and Acuto, O. (1993). Analysis of tetanus toxin peptide/DR recognition by human T cell receptors reconstituted into a murine T cell hybridoma. Eur. J. Immunol. *23*, 3057–3065.

Calender, A., Billaud, M., Aubry, J.P., Banchereau, J., Vuillaume, M., and Lenoir, G.M. (1987). Epstein-Barr virus (EBV) induces expression of B-cell activation markers on in vitro infection of EBV-negative B-lymphoma cells. Proc. Natl. Acad. Sci. USA *84*, 8060–8064.

Callan, M.F., Tan, L., Annels, N., Ogg, G.S., Wilson, J.D., O'Callaghan, C.A., Steven, N., McMichael, A.J., and Rickinson, A.B. (1998). Direct visualization of antigen-specific CD8+ T cells during the primary immune response to Epstein-Barr virus In vivo. J. Exp. Med. *187*, 1395–1402.

Choi, Y.W., Herman, A., DiGiusto, D., Wade, T., Marrack, P., and Kappler, J. (1990). Residues of the variable region of the T cell-receptor beta-chain that interact with S. aureus toxin superantigens. Nature *346*, 471–473.

Choi, Y.W., Kotzin, B., Lafferty, J., White, J., Pigeon, M., Kubo, R., Kappler, J., and Marrack, P. (1991). A method for production of antibodies to human T cell receptor beta-chain variable regions. Proc. Natl. Acad. Sci. USA *88*, 8357–8361.

Conrad, B., Weissmahr, R.N., Boni, J., Arcari, R., Schupbach, J., and Mach, B. (1997). A human endogenous retroviral superantigen as candidate autoimmune gene in type I diabetes. Cell *90*, 303–313.

Coppola, M.A., and Blackman, M.A. (1997). Bacterial superantigens reactivate antigen-specific CD8+ memory T cells. Int. Immunol. *9*, 1393–1403.

Coppola, M.A., Flano, E., Nguyen, P., Hardy, C.L., Cardin, R.D., Shastri, N., Woodland, D.L., and Blackman, M.A. (1999). Apparent MHC-independent stimulation of CD8+ T cells in vivo during latent murine gammaherpesvirus infection. J. Immunol. *163*, 1481–1489.

Denis, F., Shoukry, N.H., Delcourt, M., Thibodeau, J., Labrecque, N., McGrath, H., Munzer, J.S., Seidah, N.G., and Sekaly, R.P. (2000). Alternative proteolytic processing of mouse mammary tumor virus superantigens. J. Virol. *74*, 3067–3073.

Dobrescu, D., Ursea, B., Pope, M., Asch, A.S., and Posnett, D.N. (1995). Enhanced HIV-1 replication in V beta 12 T cells due to human cytomegalovirus in monocytes: evidence for a putative herpesvirus superantigen. Cell *82*, 753–763.

Duboise, M., Guo, J., Czajak, S., Lee, H., Veazey, R., Desrosiers, R.C., and Jung, J.U. (1998). A role for herpesvirus saimiri orf14 in transformation and persistent infection. J. Virol. *72*, 6770–6776.

Finn, P.W., Kara, C.J., Grusby, M.J., Folsom, V., and Glimcher, L.H. (1991). Upstream elements of the MHC class II E beta gene active in B cells. J. Immunol. *146*, 4011–4015.

Fleischer, B., Necker, A., Leget, C., Malissen, B., and Romagne, F. (1996). Reactivity of mouse T cell hybridomas expressing human Vbeta gene segments with staphylococcal and streptococcal superantigens. Infect. Immun. *64*, 987–994.

Gray, D., Bergthorsdottir, S., van Essen, D., Wykes, M., Poudrier, J., and Siepmann, K. (1997). Observations on memory B-cell development. Semin. Immunol. *9*, 249–254.

Hasuike, S., Miura, K., Miyoshi, O., Miyamoto, T., Niikawa, N., Jinno, Y., and Ishikawa, M. (1999). Isolation and localization of an IDDMK1,2–22-related human endogenous retroviral gene, and identification of a CA repeat marker at its locus. J. Hum. Genet. *44*, 343–347.

Herman, S.A., and Coffin, J.M. (1986). Differential transcription from the long terminal repeats of integrated avian leukosis virus DNA. J. Virol. *60*, 497–505.

Hsu, P.-N., Bryant, P.W., Sutkowski, N., McLellan, B., Ploegh, H.L., and Huber, B.T. (2001). Association of Mouse Mammary Tumor Virus Superantigen with MHC class II during biosynthesis. J. Immunol. *166*, 3309–3314.

Huber, B.T., Hsu, P.N., and Sutkowski, N. (1996). Virus-encoded superantigens. Microbiol. Rev. *60*, 473–482.

Klaman, L.D., and Thorley-Lawson, D.A. (1995). Characterization of the CD48 gene demonstrates a positive element that is specific to Epstein-Barr virus-immortalized B-cell lines and contains an essential NF-kappa B site. J. Virol. *69*, 871–881.

Kyte, J., and Doolittle, R.F. (1982). A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. *157*, 105–132.

Liu, Y.J., and Arpin, C. (1997). Germinal center development. Immunol. Rev. *156*, 111–126.

Liu, Y.J., and Banchereau, J. (1997). Regulation of B-cell commitment to plasma cells or to memory B cells. Semin. Immunol. *9*, 235–240.

Marrack, P., and Kappler, J. (1990). The staphylococcal enterotoxins and their relatives. Science *248*, 1066.

Miller, G., and Lipman, M. (1973). Release of infectious Epstein-Barr virus by transformed marmoset leukocytes. Proc. Natl. Acad. Sci. USA *70*, 190–194.

Mix, D., and Winslow, G. (1996). Proteolytic processing activates a viral superantigen. J. Exp. Med. *184*, 1549–1554.

Mosier, D.E., Gulizia, R.J., Baird, S.M., and Wilson, D.B. (1988). Transfer of a functional human immune system to mice with severe combined immunodeficiency. Nature *335*, 256–259.

Ono, M., Yasunaga, T., Miyata, T., and Ushikubo, H. (1986). Nucleotide sequence of human endogenous retrovirus genome related to the mouse mammary tumor virus genome. J. Virol. *60*, 589–598.

Pesando, J.M., Graf, L., and Hoffman, P. (1986). HLA-DP can be expressed with or without -DR molecules on a malignant B cell line. J. Immunol. *137*, 1932–1936.

Ploegh, H.L. (1998). Viral strategies of immune evasion. Science *280*, 248–253.

Roehm, N.W., Leibson, H.J., Zlotnik, A., Kappler, J., Marrack, P., and Cambier, J.C. (1984). Interleukin-induced increase in Ia expression by normal mouse B cells. J. Exp. Med. *160*, 679–694.

Stauffer, Y., Marguerat, S., Meylan, F., Ucla, C., Sutkowski, N., Huber, B., Pelet, T., and Conrad, B. (2001). Interferon-α-induced endogenous superantigen: a model linking environment and autoimmunity. Immunity *15*, this issue, 591–601.

Sutkowski, N., Palkama, T., Ciurli, C., Sekaly, R.P., Thorley-Lawson, D.A., and Huber, B.T. (1996). An Epstein-Barr virus-associated superantigen. J. Exp. Med. *184*, 971–980.

Thorley-Lawson, D.A., Schooley, R.T., Bhan, A.K., and Nadler, L.M. (1982). Epstein-Barr virus superinduces a new human B cell differentiation antigen (B-LAST 1) expressed on transformed lymphoblasts. Cell *30*, 415–425.

Tonjes, R.R., Czauderna, F., and Kurth, R. (1999). Genome-wide screening, cloning, chromosomal assignment, and expression of full-length human endogenous retrovirus type K. J. Virol. *73*, 9187–9195.

Tripp, R.A., Hamilton-Easton, A.M., Cardin, R.D., Nguyen, P., Behm, F.G., Woodland, D.L., Doherty, P.C., and Blackman, M.A. (1997). Pathogenesis of an infectious mononucleosis-like disease induced by a murine gamma-herpesvirus: role for a viral superantigen? J. Exp. Med. *185*, 1641–1650.

Veronese, M.L., Veronesi, A., D'Andrea, E., Del Mistro, A., Indraccolo, S., Mazza, M.R., Mion, M., Zamarchi, R., Menin, C., Panozzo, M., et al. (1992). Lymphoproliferative disease in human peripheral blood mononuclear cell-injected SCID mice. I. T lymphocyte requirement for B cell tumor generation. J. Exp. Med. *176*, 1763–1767.

Veronesi, A., Coppola, V., Veronese, M.L., Menin, C., Bruni, L., D'Andrea, E., Mion, M., Amadori, A., and Chieco-Bianchi, L. (1994). Lymphoproliferative disease in human peripheral-blood-mononuclear-cell-injected scid mice. II. Role of host and donor factors in tumor generation. Int. J. Cancer *59*, 676–683.

Yao, Z., Maraskovsky, E., Spriggs, M.K., Cohen, J.I., Armitage, R.J., and Alderson, M.R. (1996). Herpesvirus saimiri open reading frame *14*, a protein encoded by T lymphotropic herpesvirus, binds to MHC class II molecules and stimulates T cell proliferation. J. Immunol. *156*, 3260–3266.

Yasukawa, M., Shiroguchi, T., Inatsuki, A., and Kobayashi, Y. (1988). Antigen presentation in an HLA-DR-restricted fashion by B-cell chronic lymphocytic leukemia cells. Blood *72*, 102–108.