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Epstein-Barr Virus-Induced Genes: First Lymphocyte-Specific G Protein-Coupled Peptide Receptors

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Since Epstein-Barr virus (EBV) infection of Burkitt's lymphoma (BL) cells in vitro reproduces many of the activation effects of EBV infection of primary B lymphocytes, mRNAs induced in BL cells have been cloned and identified by subtractive hybridization. Nine genes encode RNAs which are 4- to >100-fold more abundant after EBV infection. Two of these, the genes for CD21 and vimentin, were previously known to be induced by EBV infection. Five others, the genes for cathepsin H, annexin VI (p68), serglycin proteoglycan core protein, CD44, and the myristylated alanine-rich protein kinase C substrate (MARCKS), are genes which were not previously known to be induced by EBV infection. Two novel genes, EBV-induced genes 1 and 2 (EBI 1 and EBI 2, respectively) can be predicted from their cDNA sequences to encode G protein-coupled peptide receptors. EBI 1 is expressed exclusively in B- and T-lymphocyte cell lines and in lymphoid tissues and is highly homologous to the interleukin 8 receptors. EBI 2 is most closely related to the thrombin receptor. EBI 2 is expressed in B-lymphocyte cell lines and in lymphoid tissues but not in T-lymphocyte cell lines or peripheral blood T lymphocytes. EBI 2 is also expressed at lower levels in a promyelocytic and a histiocytic cell line and in pulmonary tissue. These predicted G protein-coupled peptide receptors are more likely to be mediators of EBV effects on B lymphocytes or of normal lymphocyte functions than are genes previously known to be up-regulated by EBV infection.

Epstein-Barr Virus (EBV) is the cause of infectious mononucleosis, a benign proliferation of infected B lymphocytes (36), and can also cause acute and rapidly progressive B-lymphoproliferative disease in severely immunocompromised patients or in experimental infection of tamarins (56). Infection of human B lymphocytes in vitro results in expression of six virus-encoded nuclear proteins (EBNAs) and two virus-encoded membrane proteins (LMPs) (44) and in substantially altered cell growth (61, 62). EBV-infected B lymphocytes recapitulate features of antigen stimulation in enlarging, increasing RNA synthesis, expressing activation antigens and adhesion molecules, differentiating toward immunoglobulin (Ig) secretion, and proliferating (10, 26, 31, 61, 79, 80). Unlike antigen-stimulated B lymphocytes, EBV-infected B lymphocytes continue to proliferate in vitro as immortalized lymphoblastoid cell lines (62).

EBV effects on lymphocytes have been studied by comparing the properties of EBV-negative [EBV(−)] Burkitt's lymphoma (BL) cell lines and EBV-positive [EBV(+)] derivatives, infected by EBV, in vitro (12, 21, 61, 66–68). EBV(−) BL cells resemble proliferating centroblasts of germinal centers, characteristically expressing CD10, CD20, CD77 (BLA), class II antigen, and the carbohydrate recognized by peanut agglutinin (12, 21, 23, 28–30, 66, 67). Both EBV(−) BL cells and centroblasts lack surface IgD and antigens associated with early phases of mitogen stimulation in vitro, including CD23, CD39, and CD30. In general, EBV(+) BL cells closely resemble EBV-infected primary B lymphocytes in not expressing CD10 or CD77 and in expressing early activation and differentiation markers, vimentin, Bac-1, Bcl-2, surface IgD, and CD44 (12, 21, 23, 29, 35, 66, 68, 70, 76, 84–86).

Experiments with single-gene transfer into EBV(−) B-lymphoma cells or with specifically mutated EBV recombinants reveal that EBNA 2, LMP 1, and EBNA 3C are essential for lymphocyte growth transformation and alter cellular or viral gene expression. Expression of EBNA 2 alone in EBV(−) BL cell lines results in enhanced transcription of CD23, CD21 (17, 85, 86), and c-*fgr* (46). EBNA 2 also transactivates the LMP promoters (22, 87). Analysis of a series of EBNA 2 mutants indicates that the ability of EBNA 2 to transactivate gene expression is tightly linked to its essential role in cell growth transformation (16). LMP 1 is also critical to the effects of EBV on cell growth. LMP 1 transforms immortalized rodent fibroblasts (5, 82, 83) and induces vimentin, Bcl-2, and many of the activation markers and adhesion molecules that EBV induces in BL cells or primary B lymphocytes (8, 35, 84, 85). In EBV(−) BL cells, EBNA 3c induces higher-level expression of CD21 (85).

Since altered B-lymphocyte gene expression is a central theme in EBV-induced changes in B-lymphocyte growth, a more complete description of the repertoire of EBV-induced genes would be advantageous before the investigation of specific genes for their role as mediators of EBV effects on cell growth. Genes induced by EBV could also be of importance in B-lymphocyte immunology because of the similar activating and differentiating effects of EBV and antigen. Previously, recognition of such genes has been based largely on increased expression of lymphocyte surface markers (12, 85, 86), defined by monoclonal antibodies derived against EBV- or antigen-activated B lymphocytes. Few of these surface markers are likely candidates for important effectors of EBV- or antigen-induced alterations in

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lymphocyte growth. The experiments described here use subtracted hybridization to identify cDNA clones of RNAs which are more abundant in an in vitro-infected EBV(+) BL cell than in the noninfected EBV(−) control BL cell.

MATERIALS AND METHODS

Cells and cell lines. BL41 and BL30 are EBV(−) BL cell lines. The BL41/B95-8 and BL41/P3HR1 cell lines were derived by infecting BL41 with the transforming EBV strain B95-8 or the nontransforming strain P3HR1, respectively (12, 23). IB4 is a latently infected B lymphoblastoid cell line established by infection of B lymphocytes with EBV (B95-8) in vitro. RHEK-1 (a generous gift from Jong Rhim, National Cancer Institute) is a human keratinocyte line derived by infection of primary foreskin epithelial cells with an adenovirus 12-simian virus 40 hybrid. K562 is a Philadelphia chromosome-positive human chronic myeloid leukemia cell line. U937 is a histiocytic lymphoma cell line with monocytic features. HL60 is a promyelocytic leukemia line. HSB-2 and Jurkat are human T lymphoblastic leukemia cell lines. TK143 was derived from a human osteosarcoma.

Human peripheral blood mononuclear cells (PBMCs) were purified from peripheral blood by centrifugation on a Ficoll cushion (Ficoll-Hypaque; Pharmacia, Vineland, N.J.). Cells were resuspended at 10^6 cells per ml in RPMI 1640 medium supplemented with 20% fetal bovine serum and were divided into parallel cultures grown for 72 h with or without 2.5 μg of pokeweed mitogen (Sigma, St. Louis, Mo.) per ml. T cells were isolated from purified PBMCs by rosetting overnight with aminoethylisothiouronium bromide (AET)-treated sheep erythrocytes at 4°C followed by centrifugation over Ficoll. Pelleted erythrocytes were lysed with ammonium chloride. The remaining T cells were resuspended at 10^6 cells per ml in RPMI 1640 medium with 20% fetal bovine serum. Phytohemagglutinin (Sigma) was added to a final concentration of 1.0 μg/ml. Cells were cultured for 72 h and harvested for extraction of total cellular RNA.

RNA preparation and analysis. Cytoplasmic RNA was isolated from exponentially growing cells by a modification of the acid phenol-guanidinium isothiocyanate extraction procedure followed by reprecipitation in guanidinium hydrochloride-ethanol. Total cellular RNA was extracted from 0.2- to 2-g samples of human spleen and tonsil obtained from surgical specimens and from human bone marrow obtained postmortem. Tissues were homogenized in acid phenol-guanidinium isothiocyanate by using a rotary tissue homogenizer, extracted, and precipitated. After dissolution in guanidinium hydrochloride and reprecipitation with ethanol, human tissue RNA samples were resuspended in H_2O and precipitated by addition of an equal volume of 8 M LiCl. The polyadenylated fractions of BL41 or BL41/B95-8 RNA were purified by two successive cycles of chromatography on oligodeoxythymidylate cellulose. Polyadenylated IB4 RNA was purified by a single round of oligodeoxythymidylate selection. RNA samples (12 μg per lane) were size fractionated on 0.66 M formaldehyde–1% agarose gels and transferred to charged nylon membranes (GeneScreen Plus; New England Nuclear, Billerica, Mass.) for subsequent hybridization analysis. To examine gene expression in other human tissues, we used a commercially prepared blot containing 2 μg each of polyadenylated heart, brain, placenta, lung, liver, kidney, skeletal muscle, and pancreas RNA (Multiple Tissue Northern; Clontech, Palo Alto, Calif.).

Probes were prepared from cloned cDNA inserts by using random hexamer primers and [^{32}P]dCTP. The β-actin probe was generated by using a previously described 1.4-kb cDNA (8). The glyceraldehyde phosphate dehydrogenase probe was prepared from a commercially obtained DNA fragment (Clontech). Filters were hybridized for 18 to 24 h at 47°C in a hybridization buffer consisting of 50% formamide, 6× SSPE (20× SSPE is 3.0 M NaCl, 200 mM $NaPO_4$ [pH 7.4] and 20 mM EDTA), 1% sodium dodecyl sulfate (SDS), 1× Denhardt's solution (100× Denhardt's solution is 2% bovine serum albumin, 2% polyvinylpyrrolidone, and 2% Ficoll), and 100 μg of sheared single-stranded herring testis DNA per ml. The filters were washed as specified by the manufacturer, with high stringency washes performed at 67 to 70°C in 1% SDS–0.2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and exposed to preflashed film (X-Omat AR; Kodak, Rochester, N.Y.) at −80°C for 2 h to 10 days. Autoradiographic signal intensities were quantitated by densitometric scanning with a Beckman DU-8 spectrophotometer equipped with a slab gel Compuset module. Induction factors were calculated for each probe as signal intensity ratios for EBV(+) versus EBV(−) cells, divided by the ratio of β-actin signal intensities.

cDNA library preparation. First-strand cDNA was prepared from 5 μg of polyadenylylated BL41/B95-8 RNA by using Moloney murine leukemia virus reverse transcriptase (SuperScript; Bethesda Research Laboratories, Gaithersburg, Md.) and oligodeoxythymidylate primers in a 100-μl reaction. Second-strand cDNA was synthesized by using *Escherichia coli* DNA polymerase I and RNase H. The double-stranded cDNA was blunt ended with T4 DNA polymerase and *Eco*RI methylated. After ligation of *Eco*RI linkers, the cDNA was digested with *Eco*RI and size fractionated by gel filtration chromatography on Sepharose CL-4B. The purified cDNA was ligated to phosphorylated lambda gt10 arms (Promega, Madison, Wis.) and packaged (Gigapack Gold; Stratagene, La Jolla, Calif.).

Subtractive probe preparation. Radiolabeled cDNA was prepared from 6 μg of polyadenylylated BL41 or BL41/B95-8 RNA in a 200-μl reaction containing 50 μg of random DNA hexamers per ml; 0.5 mM each dATP, dGTP, and dTTP; 25 μM unlabeled dCTP; 1.0 mCi of [^{32}P]dCTP (800 Ci/mmol; New England Nuclear); and 2,000 U of recombinant Moloney murine leukemia virus reverse transcriptase. Reactions were carried out at 42°C for 1 h. After precipitation, reaction products were resuspended in 0.1 M NaOH and incubated for 20 min at 65°C to hydrolyze RNA templates. Probes were neutralized with 0.1 M acetic acid and size fractionated on Sephadex G-50. Biotinylated RNA was prepared from polyadenylylated BL41 RNA by using a photoactivatable azidoaryl biotin reagent (Photoprobe Biotin; Vector Laboratories, Burlingame, Calif.) as specified by the manufacturer. Probe fractions were combined with 48 μg (for BL41/B95-8 probe) or 12 μg (for BL41 probe) of biotinylated BL41 RNA and precipitated with ethanol. BL41/B95-8 probes were hybridized with an eightfold excess (2 mg/ml) of biotinylated BL41 RNA, whereas BL41 control probes were hybridized with a twofold excess (0.5 μg/ml) of biotinylated BL41 RNA. Hybridizations and subtractions were performed with the Subtractor kit (Invitrogen, San Diego, Calif.) as specified by the manufacturer instructions. The precipitated cDNA-RNA mixtures were resuspended in 10 to 20 μl of H_2O and heated to 100°C for 1 min. An equal volume of 2× hybridization buffer (Invitrogen) was added, and the mixture was incubated at 65°C for 20 to 24 h. Following addition of an equal volume of HEPES buffer (10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES; pH 7.5], 1 mM EDTA), 20 μg of streptavidin was added and the mixture

was incubated on ice for 10 min. Biotinylated RNA and RNA-cDNA duplexes, complexed with avidin, were removed by repeated phenol-chloroform extractions. The single-stranded, subtracted BL41 cDNA probe which remained in the aqueous phase was used directly for in situ filter hybridizations. Aqueous-phase BL41/B95-8 cDNA probe was precipitated with ethanol and subjected to a second round of subtraction under identical conditions prior to use in filter hybridizations. Duplicate filters were made from 145-mm plates containing 6,000 recombinant bacteriophage and were hybridized in parallel to equal amounts of BL41/B95-8 or BL41 subtracted probes. The filters were hybridized at 48°C for 48 to 72 h in a buffer consisting of 50% formamide, 6× SSPE, 1% SDS, 10% dextran sulfate, 2× Denhardt's solution, 100 μg of sheared single-stranded herring testis DNA per ml, and 10 μg of poly(rA-rU) (Sigma) per ml. They were then washed at 72°C in 0.2× SSC and exposed to preflashed film (Kodak X-Omat AR) for 3 to 7 days. Differentially expressed genes were identified by overlaying films from corresponding filters. Clones selected on primary screening were rescreened once at low density to verify differential expression and for plaque purification.

Analysis of clones. DNA was extracted from bulk liquid cultures of purified lambda gt10 clones and digested with *Eco*RI. cDNA inserts were purified by agarose gel electrophoresis and subcloned into pBluescript(+). Nucleotide sequences were determined and were compared by the BLAST algorithm (3) with known sequences resident in the National Center for Biotechnology Information data bases by using the Experimental GENINFO(R) BLAST Network Service, accessed through the Molecular Biology Computer Research Resource of the Dana-Farber Cancer Institute. Multiple sequence alignments were performed by the method of Higgins and Sharp (37), using the CLUSTAL program (PCGene; IntelliGenetics, Mountain View, Calif.) with open gap and unit gap costs of 10.

Nucleotide sequence accession numbers. GenBank accession numbers are L08176 for EBI 1 and L08177 for EBI 2.

RESULTS

Identification of cDNA clones of EBV-induced RNAs by subtracted-probe hybridization. cDNA clones of RNA from an in vitro EBV-infected BL cell line, BL41/B95-8 [EBV(+) BL41], were differentially screened with an EBV(+) BL41 cDNA probe from which sequences complementary to EBV(−) BL41 cell RNA had been specifically removed and with an EBV(−) BL41 control cDNA probe. Sequences complementary to EBV(−) BL41 RNA were removed from the EBV(+) BL41 RNA cDNA probes by two subtractions with an eightfold excess of biotinylated EBV(−) BL41 RNA. Overall, 85 to 95% of the labeled EBV(+) BL41 probe was removed by the two subtractions. EBV(−) BL41 cDNA control probe was subtracted only once; this removed 60 to 85% of the probe, thereby reducing hybridization to plaques containing cDNAs from abundant RNAs so that hybridization to cDNAs from less abundant BL41 RNAs was evident.

Seventy-five phage cDNA clones differentially hybridized to the EBV(+) BL41 probe on the first screen of 75,000 recombinant phage. Twenty-five clones were consistently positive on rescreening. The 18 clones which demonstrated the greatest reactivity with the EBV(+) versus the EBV(−) BL41 cDNA probes were selected for nucleotide sequencing and RNA blot hybridization.

TABLE 1. Summary of EBV-induced RNA-DNA clones

Clone	Gene	cDNA size (kb)	RNA size (kb)	Induction[a]
1.1	CD44	1.3	1.6, 2.2, 5.0	>100×
3.3, 7.3	CD21	2.1, 1.8	4.8	
6.5	MARCKS	2.6	2.9	30×
8.2	Cathepsin H	1.5	1.7	6×
10.4, 11.4	Serglycin	1.1, 1.1	1.4	3.5×
12.3	Annexin VI	2.3	3.0	5×
12.5, 13.0	Vimentin	1.0, 1.8	2.0	
6.4	EBI 1	1.2 (2.14)[b]	2.4	21×
3.2	EBI 2	1.64	1.9	>200×
	β-Actin		2.2	3×[c]

[a] Induction levels were calculated as ratio of signal intensities (BL41/B95-8 to BL41) for individual probes, divided by the ratio of signal intensities for the β-actin probe.

[b] The 1.2-kb EBI 1 clone identified on the initial screen was incomplete. Rescreening of the cDNA library resulted in isolation of several additional full-length clones, the largest of which was 2.14 kb.

[c] Induction of β-actin RNA was calculated as the ratio of actin signal intensities (BL41/B95-8 to BL41) divided by the ratio of signal intensities for glyceraldehyde phosphate dehydrogenase probe.

Nucleotide sequences of EBV-induced cDNAs. The first 12 clones are described in Table 1. Ten clones matched seven previously characterized genes: two independent clones each of the genes for complement receptor type 2 (CD21), the serglycin proteoglycan core protein, and vimentin; and one clone each of the genes for cathepsin H, annexin VI (p68), the myristylated alanine-rich protein kinase C substrate (MARCKS), and the lymphocyte hyaluronic acid receptor (CD44). The 2.6-kb MARCKS cDNA precisely matched the previous 1.58-kb human MARCKS cDNA clone (32) at its 5′ end (7a). The 3′ untranslated region of the new clone is highly homologous to bovine MARCKS cDNA (75).

The two remaining clones are from novel RNAs, EBV-induced genes 1 (EBI 1) and 2 (EBI 2), whose nucleotide sequences can be predicted to encode G protein-coupled peptide receptors. The complete nucleotide and deduced amino acid sequences of the EBI 1 and EBI 2 cDNAs are shown in Fig. 1A B, respectively. Because the first EBI 1 cDNA was 1.2 kb, significantly shorter than the 2.4-kb RNA, 20 other cDNA clones were obtained by using the initial cDNA as a probe. The largest clone is 2,153 nucleotides (nt) and has a 1,134-nt open reading frame (Fig. 1A). This clone is probably nearly full length, since it is close to the expected size, considering that it has only a short poly(A) tail. Translation is likely to initiate from either of two AUGs, at nt 64 to 66 or nt 82 to 84, the first of which conforms to a consensus translational initiation sequence (49). An in-frame stop codon at nt 10 to 12 is consistent with translational initiation at nt 64 to 66. The polypeptide encoded by the sequence beginning at nt 64 has a predicted molecular mass of 42.7 kDa and includes eight hydrophobic domains likely to mediate membrane insertion. The first hydrophobic domain begins at the amino terminus and ends at a predicted signal peptidase cleavage site. The seven remaining hydrophobic domains are characteristic of the G protein-coupled receptor family. A potential asparagine-linked glycosylation site is encoded as part of the extracellular amino-terminal segment and as part of the third extracellular loop.

Since the initial EBI 2 cDNA was 1,643 nt and approximated the size expected from a 1.9-kb polyadenylated RNA, further cDNA clones were not obtained. The EBI 2 cDNA contains a 1,083-nt open reading frame with two methionine

A

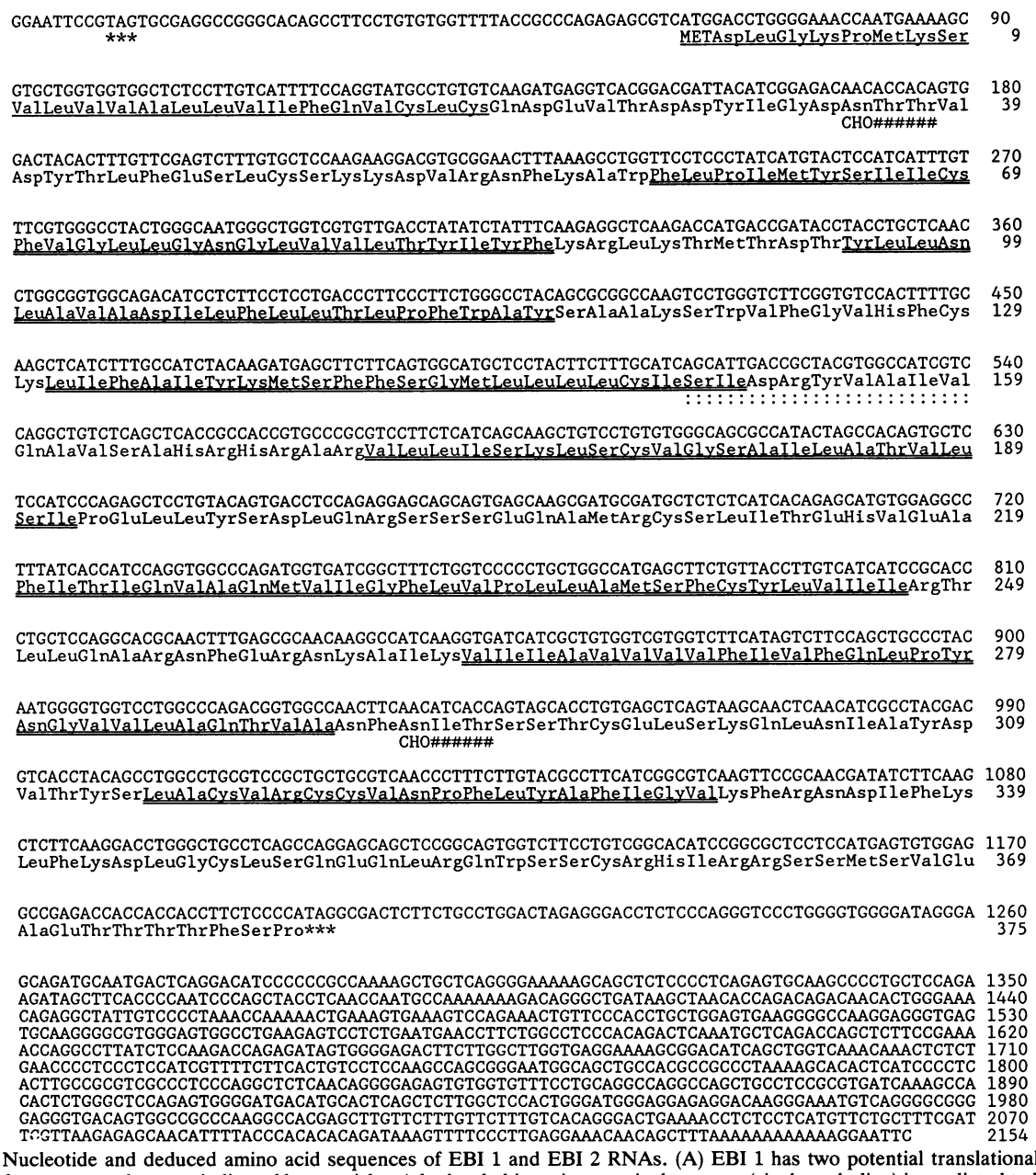

FIG. 1. Nucleotide and deduced amino acid sequences of EBI 1 and EBI 2 RNAs. (A) EBI 1 has two potential translational initiation codons. In-frame stop codons are indicated by asterisks. A hydrophobic amino-terminal segment (single underline) is predicted to be a signal peptide for membrane translocation. Seven other highly hydrophobic segments are predicted to form membrane-spanning domains and are delineated by double underlines. Potential asparagine-linked glycosylation sites (CHO######) are present in the extracellular amino-terminal segment and third extracellular loop. The sequence motif S-(I/V)-D-R-(Y/F)-X-X-X-X (where X represents consecutive hydrophobic residues) is highly conserved among a large number of G protein-coupled receptors and is indicated at the end of the third transmembrane domain (::::). (B) EBI 2 has two possible initiator methionine codons. Predicted transmembrane domains are indicated (double underlines). No signal sequence was identified.

codons at nt 34 to 36 and 46 to 48 (Fig. 1B). Although neither methionine codon is in a favored initiation context (49), the presence of an upstream in-frame termination codon and the absence of other potential open reading frames are consistent with translation initiation at the first or second methionine codon. Initiation at the first would result in a 41.2-kDa protein. The deduced amino acid sequence predicts seven hydrophobic transmembrane segments in the characteristic configuration of a G protein-coupled receptor. In contrast to the EBI 1 protein, EBI 2 lacks a signal peptide. The amino-terminal putative extracellular domain has a potential N-linked glycosylation site. Although the EBI 2 cDNA lacks

B

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GGAATTCCCTGATATACACCTGGACCACCACCAATGGATATACAAATGGCAAACAATTTTACTCCGCCCTCTGCAACTCCTCAGGGAAAT   90
        ***                          METAspIleGlnMETAlaAsnAsnPheThrProProSerAlaThrProGlnGlyAsn   19
                                                                CHO######

GACTGTGACCTCTATGCACATCACAGCACGGCCAGGATAGTAATGCCTCTGCATTACAGCCTCGTCTTCATCATTGGGCTCGTGGGAAAC  180
AspCysAspLeuTyrAlaHisHisSerThrAlaArgIleValMetProLeuHisTyrSerLeuValPheIleIleGlyLeuValGlyAsn   49

TTACTAGCCTTGGTCGTCATTGTTCAAAACAGGAAAAAAATCAACTCTACCACCCTCTATTCAACAAATTTGGTGATTTCTGATATACTT  270
LeuLeuAlaLeuValValIleValGlnAsnArgLysLysIleAsnSerThrThrLeuTyrSerThrAsnLeuValIleSerAspIleLeu   79

TTTACCACGGCTTTGCCTACACGAATAGCCTACTATGCAATGGGCTTTGACTGGAGAATCGGAGATGCCTTGTGTAGGATAACTGCGCTA  360
PheThrThrAlaLeuProThrArgIleAlaTyrTyrAlaMetGlyPheAspTrpArgIleGlyAspAlaLeuCysArgIleThrAlaLeu  109

GTGTTTTACATCAACACATATGCAGGTGTGAACTTTATGACCTGCCTGAGTATTGACCGCTTCATTGCTGTGGTGCACCCTCTACGCTAC  450
ValPheTyrIleAsnThrTyrAlaGlyValAsnPheMetThrCysLeuSerIleAspArgPheIleAlaValValHisProLeuArgTyr  139
                                              :::::::::::::::::::::::::::

AACAAGATAAAAAGGATTGAACATGCAAAAGGCGTGTGCATATTTGTCTGGATTCTAGTATTTGCTCAGACACTCCCACTCCTCATCAAC  540
AsnLysIleLysArgIleGluHisAlaLysGlyValCysIlePheValTrpIleLeuValPheAlaGlnThrLeuProLeuLeuIleAsn  169

CCTATGTCAAAGCAGGAGGCTGAAAGGATTACATGCATGGAGTATCCAAACTTTGAAGAAACTAAATCTCTTCCCTGGATTCTGCTTGGG  630
ProMetSerLysGlnGluAlaGluArgIleThrCysMetGluTyrProAsnPheGluGluThrLysSerLeuProTrpIleLeuLeuGly  199

GCATGTTTCATAGGATATGTACTTCCACTTATAATCATTCTCATCTGCTATTCTCAGATCTGCTGCAAACTCTTCAGAACTGCCAAACAA  720
AlaCysPheIleGlyTyrValLeuProLeuIleIleIleLeuIleCysTyrSerGlnIleCysCysLysLeuPheArgThrAlaLysGln  229

AACCCACTCACTGAGAAATCTGGTGTAAACAAAAAGGCTCTCAACACAATTATTCTTATTATTGTTGTGTTTGTTCTCTGTTTCACACCT  810
AsnProLeuThrGluLysSerGlyValAsnLysLysAlaLeuAsnThrIleIleLeuIleIleValValPheValLeuCysPheThrPro  259

TACCATGTTGCAATTATTCAACATATGATTAAGAAGCTTCGTTTCTCTAATTTCCTGGAATGTAGCCAAAGACATTCGTTCCAGATTTCT  900
TyrHisValAlaIleIleGlnHisMetIleLysLysLeuArgPheSerAsnPheLeuGluCysSerGlnArgHisSerPheGlnIleSer  289

CTGCACTTTACAGTATGCCTGATGAACTTCAATTGCTGCATGGACCCTTTTATCTACTTCTTTGCATGTAAAGGGTATAAGAGAAAGGTT  990
LeuHisPheThrValCysLeuMetAsnPheAsnCysCysMetAspProPheIleTyrPhePheAlaCysLysGlyTyrLysArgLysVal  319

ATGAGGATGCTGAAACGGCAAGTCAGTGTATCGATTTCTAGTGCTGTGAAGTCAGCCCCTGAAGAAAATTCACGTGAAATGACAGAAACG 1080
MetArgMetLeuLysArgGlnValSerValSerIleSerSerAlaValLysSerAlaProGluGluAsnSerArgGluMetThrGluThr  349

CAGATGATGATACATTCCAAGTCTTCAAATGGAAAGTGAAATGGATTGTATTTTGGTTTATAGTGACGTAAACTGTATGACAAACTTTGC 1170
GlnMetMetIleHisSerLysSerSerAsnGlyLys***                                                      361

AGGACTTCCCTTATAAAGCAAAATAATTGTTCAGCTTCCAATTAGTATTCTTTTATATTTCTTTCATTGGGCGCTTTCCCATCTCCAACT 1260
CGGAAGTAAGCCCAAGAGAACAACATAAAGCAAACAACATAAAGCACAATAAAAATGCAAATAAATATTTTCATTTTTATTTGTAAACGA 1350
ATACACCAAAAGGAGGCGCTCTTAATAACTCCCAATGTAAAAAGTTTTGTTTTAATAAAAAATTAATTATTATTCTTGCCAACAAATGGC 1440
TAGAAAGGACTGAATAGATTATATATTGCCAGATGTTAATACTGTAACATACTTTTTAAATAACATATTTCTTAAATCCAAATTTCTCTC 1530
AATGTTAGATTTAATTCCCTCAATAACACCAATGTTTTGTTTTGTTTCGTTCTGGGTCATAAAACTTTGTTAAGGAACTCTTTTGGAATA 1620
AAGAGCAGGATGCTGCGGAATTC                                                                       1644
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FIG. 1—*Continued.*

a polyadenylate tail, a canonical polyadenylation signal (AATAAA) near the 3′ end is consistent with the conclusion that the cDNA is essentially complete.

Comparison of EBI 1 and 2 with other G protein-coupled receptors. The EBI 1 and EBI 2 nucleotide and predicted amino acid sequences were compared with the GenBank (release 72 and updates), EMBL (release 31), GenBank translation, Swiss protein (release 22) and Protein Identification Resource (PIR; release 33) data bases by using the BLAST algorithm (3). EBI 1 and EBI 2 are homologous to G protein-associated receptors. EBI 1 is highly homologous to the human high- or low-affinity interleukin 8 (IL-8) receptors at both the nucleotide (data not shown) and amino acid (Fig. 2A) sequence levels. These IL-8 receptors are not expressed on lymphocytes (38, 59). Excluding the putative EBI 1 signal peptide, the overall amino acid identity among the three proteins exceeds 30%, with conservative changes observed at many of the nonidentical residues. The identity increases to 40% when EBI 1 is compared with either IL-8 receptor individually. Additional similarities with the IL-8 receptors include a high proportion of serine and threonine near the carboxy terminus and a highly acidic amino-terminal extracellular domain. The IL-8 receptor acidic residues are implicated in binding IL-8 basic amino acids (38, 59).

The EBI 2 gene does not have such a close homolog. EBI 2 has 24% amino acid identity with the thrombin receptor (Fig. 2B; data not shown) (81). Less extensive homologies are observed with a number of other G protein-coupled receptors, including the receptors for vasoactive intestinal polypeptide, somatostatin (type 1), and angiotensin II, as well as with the low-affinity IL-8 receptor (Fig. 2B). EBI 2 also exhibits more distant homologies with EBI 1 and the high-affinity IL-8 receptor. Significantly, these are the same proteins which, in a different order, exhibit the closest homologies with the EBI 1 protein. Together they constitute a subfamily of G protein-coupled peptide receptors. The

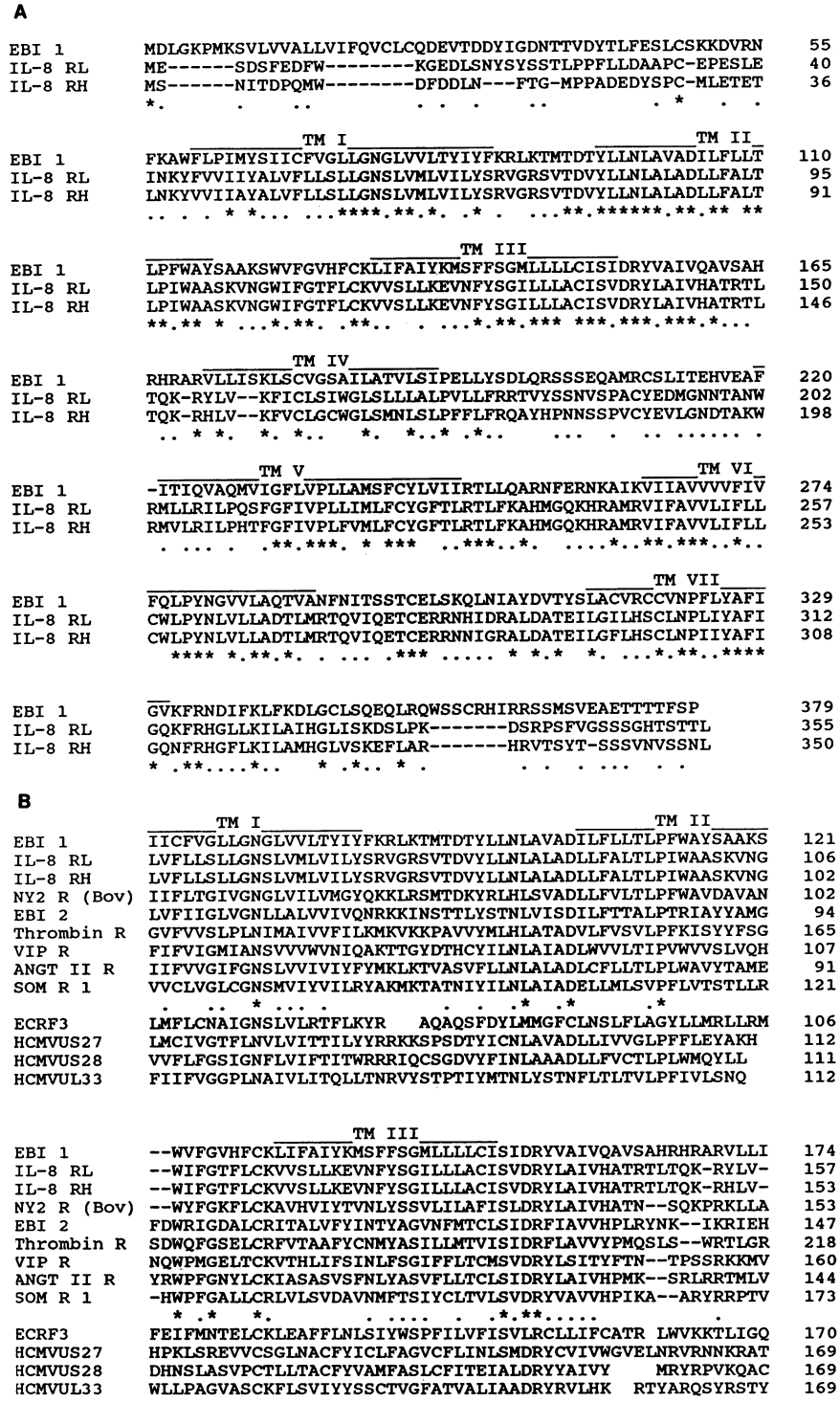

FIG. 2. Protein sequence homologies between EBI 1 and EBI 2 and previously identified G protein-coupled receptors (74). Positions of predicted transmembrane domains I through VII (TM I through TM VII) are indicated by horizontal lines above the corresponding sequences. Amino acids identical in all aligned sequences are indicated by an asterisk. Conservative changes are indicated by a dot. (A) Alignment of EBI 1 with high-affinity (IL-8 RH) and low-affinity (IL-8 RL) IL-8 receptors. (B) Alignment of EBI 2 with EBI 1 and receptors for thrombin (81), IL-8 (IL-8 RL, IL-8 HL), vasoactive intestinal polypeptide (VIP R) (71), angiotensin II (ANGT II R) (19), bovine neuropeptide Y [NY2 R (Bov)] (64), and somatostatin receptor type 1 (SOM R 1) (89). A proposed alignment with the G protein-coupled receptor homologs of herpesvirus saimiri (ECRF3) (60) and of human cytomegalovirus (HCMVUS27, HCMVUS28, and HCMVUS33) (14) is shown below.

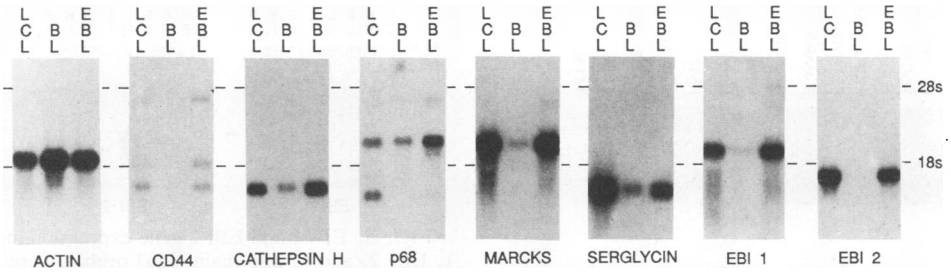

FIG. 3. RNA blot hybridization analysis of EBV-induced cellular gene expression. Polyadenylated RNA (4 to 12 μg per lane) was size fractionated on formaldehyde-agarose gels, transferred to charged nylon membranes, and hybridized with the probes indicated at the bottom of each autoradiograph panel. RNA samples used are indicated at the top of each lane (lane LCL, EBV-immortalized primary B lymphoblastoid cell line, [IB4]; lane BL, EBV-negative BL cell line [BL41]; EBL, EBV-infected BL cell line [BL41/B95-8] derived by in vitro infection of BL41). Dashes indicate positions of rRNA bands (18S, 28S). The band detected at 1.5 kb in lane LCL by the P68 probe is due to residual signal from a prior hybridization.

greatest conservation of residues among these proteins extends from the first transmembrane domain (Fig. 2B; TM I) to the second intracellular loop. Because of the particular conservation of an amino acid sequence among these G protein-coupled receptors, we were able to identify a new highly conserved sequence motif at the carboxy end of TM III and the adjacent second intracellular loop. This motif, S-(I/L)-D-R-(Y/F)-X-X-X-X, with X being a hydrophobic amino acid, is present in a wide variety of G protein-coupled receptors but is not present in other proteins in the data bases surveyed. Other highly conserved features of G protein-coupled receptors in EBI 1 and EBI 2 include the asparagine in TM I, the proline in TM II, the aspartate in the first intracellular loop, and the tryptophan and cysteine in the first extracellular loop. This cysteine has been postulated to be involved in disulfide linkage to a conserved cysteine present in the second extracellular loop in several other receptors, including the β-adrenergic and thrombin receptors.

Analysis of induced gene expression by RNA blot hybridization. Probes from seven of the nine EBV-induced cDNAs (vimentin and CD21 were previously shown to be EBV induced and were not further evaluated) were hybridized to identical blots of polyadenylated RNA from the EBV(+) or EBV(−) BL41 cell lines or from the EBV-transformed lymphoblastoid cell line IB4 (Fig. 3). The RNAs loaded in the EBV(+) BL41 and EBV(−) BL41 lanes were standardized with respect to β-actin reactivity. Significantly less IB4 cell RNA was used because of the high abundance of the putative induced-gene RNAs in these cells (Fig. 3, actin probe). Probes from each of the cDNA clones detected RNAs which are significantly more abundant in both IB4 and EBV(+) BL41 cells than in EBV(−) BL41 cells. Induction factors indicated in Table 1 were determined by quantitative densitometric scanning of autoradiographs and reflect the fold enhancement of signal intensities in EBV(+) BL41 cells over those in EBV(−) BL41 cells, corrected for the ratio of actin reactivities. Standardization by actin reactivity, however, significantly underestimates the absolute induction levels since actin is induced threefold by EBV infection of BL41 cells relative to glyceraldehyde phosphate dehydrogenase (data not shown) or total RNA amounts quantitated spectrophotmetrically. So that the actin signal intensities would be equal, threefold more EBV(−) BL41 than EBV(+) BL41 RNA was loaded per lane. Importantly, each of the RNAs was at least as abundant relative to glyceraldehyde phosphate dehydrogenase in IB4 cells as in EBV(+) BL41 cells (Fig. 3; data not shown).

The EBI 1, EBI 2, CD44, and MARCKS genes are the most highly induced of the seven genes, being induced 21, >200, >100, and 30 times background, respectively, relative to actin (Table 1). The CD44 gene encodes three distinct RNAs of 1.6, 2.2, and 4.8 kb in both IB4 and EBV(+) BL41 cells. No CD44 RNA was detected in EBV(−) BL41 cells even after prolonged autoradiographic exposures. EBI 2 RNA was also undetectable in EBV(−) BL41 cells.

Expression of EBI 1 and EBI 2 in human cell lines and tissues. The expression of EBI 1 and EBI 2 in human cell lines and tissues was evaluated by hybridizing actin, EBI 1, or EBI 2 probes to blots of cell line or tissue RNAs. Although EBI 1 is weakly expressed in BL41, EBI 2 is not; also, neither EBI 1 nor EBI 2 is expressed in another EBV(−) BL cell line, BL30 (Fig. 4). EBI 1 and EBI 2 RNAs are abundant in primary human lymphocytes transformed by EBV in vitro and propagated as continuous lymphoblastoid cell lines for several years (IB4) or for less than 1 year (LCL-W91) (Fig. 4). EBI 1 RNA is faintly detectable in the human T-cell line Jurkat and is abundantly expressed in a second T-cell line, HSB-2 (Fig. 4). EBI 2 RNA is not detected in either of these T-cell lines (Fig. 4) or in a third T-cell line, Molt-4 (data not shown). EBI 1 is not expressed in the human promyelocytic line HL60, the chronic myelogeneous leukemia cell line K562, the epithelial cell line RHEK-1, the fibroblast-like osteosarcoma cell line TK143, or the monocytic cell line U937 (Fig. 4). EBI 2, however, is expressed weakly, relative to actin, in HL60, U937 (U937 RNA is partially degraded), and HeLa (Fig. 4; data not shown) cells.

EBI 1 and EBI 2 RNAs are abundant in human spleen tissue, somewhat less abundant relative to actin in tonsil tissue, and undetectable in bone marrow (Fig. 4). Both genes were expressed in resting PBMCs at levels comparable to those in IB4 or LCL-W91 B lymphoblastoid cells (Fig. 4). Expression increased in parallel cultures stimulated for 72 h with pokeweed mitogen, although actin expression also increased after pokeweed mitogen treatment (Fig. 4). The EBI 1 and EBI 2 RNA in stimulated and nonstimulated PBMC cultures is likely to be mostly in B lymphocytes since EBI 1 RNA is present at low levels and EBI 2 RNA is absent in phytohemagglutinin-stimulated, PBMC-derived T lymphocytes (Fig. 4). These findings are consistent with the expression patterns observed in T-cell lines.

EBI 1 and EBI 2 RNA levels in a variety of nonhematopoietic human tissues were also evaluated. The EBI 1 probe detects small amounts of RNA in both lung and pancreas tissues (Fig. 5). Rehybridization of this blot with an Ig mu

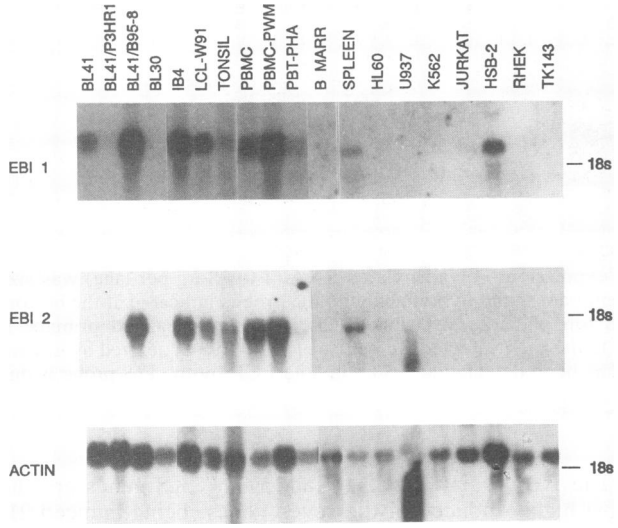

FIG. 4. Expression of EBI 1 and EBI 2 receptor genes in human lymphoid tissues and cell lines. ^{32}P-labeled probes indicated at the left of each panel were hybridized to blots containing RNA from the cell lines indicated at the top of each lane. BL41 and BL30 are EBV-negative BL cell lines; BL41/P3HR1 is infected with a nontransforming EBV strain, P3HR1; BL41/B95-8 is infected with a transforming EBV strain; IB4 is a cell line derived by infecting primary B lymphocytes with EBV of the B95-8 strain; LCL-W91 is a recently established cell line transformed with EBV W91; TONSIL is unfractionated cells from surgically excised human tonsils; PBMC is unfractionated peripheral blood mononuclear cells; PBMC PWM is PBMC stimulated for 72 h with pokeweed mitogen (2.5 μg/ml); PBT PHA is T cells purified from PBMC by sheep erythrocyte rosetting, stimulated for 72 h with phytogemagglutinin (1 μg/ml); B MARR is postmortem bone marrow; SPLEEN is unfractionated cells from surgically excised spleen; HL60 is a promyelocytic leukemia cell line; U937 is a monocytic leukemia cell line; K562 is a chronic myelogenous leukemia cell line; JURKAT is a T-cell leukemia cell line; HSB-2 is a T-cell acute lymphoblastic leukemia cell line; RHEK-1 is an adenovirus-simian virus 40-transformed human keratinocyte; TK143 is a osteosarcoma cell line. Each panel is a composite prepared from autoradiographs of two separate blots for each probe.

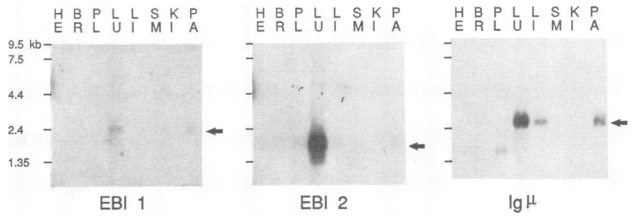

FIG. 5. EBI 1 and EBI 2 gene expression in human tissues. EBI 1, EBI 2, and Ig mu chain (Igμ) probes were hybridized to RNA samples from the following human tissues: heart (HE), brain (BR), placenta (PL), lung (LU), liver (LI), skeletal muscle (SM), kidney (KI), and pancreas (PA). Numbers at the left indicate positions and sizes (in kilobases) of RNA markers. Specific RNA bands are indicated by arrows to the right of each panel. The EBI 1 probe detects faint 2.4-kb bands in lung and pancreas RNA. The EBI 2 probe detects an abundant 1.9-kb band in lung RNA and a faint 1.9-kb band in pancreas RNA. The 2.7-kb Ig mu RNA is detected in lung, liver, and pancreas preparations. The 1.5-kb band in placental RNA hybridized with Ig mu probe is residual signal from a previous hybridization.

chain probe (Fig. 5, Ig μ probe) indicated that these tissue preparations contained significant amounts of Ig RNA, probably because of the presence of B lymphocytes in the tissues. Since EBI 1 RNA is abundant in peripheral blood lymphocytes, the EBI 1 RNA in the lung and pancreas tissues is likely to be due to B lymphocytes. Similarly, the low level of EBI 2 RNA detected in pancreas tissue is probably due to infiltrating B lymphocytes (Fig. 5). However, the abundance of EBI 2 RNA in lung tissue is too great to attribute to lymphocyte contamination and is more likely to be due to specific expression in pulmonary epithelial cells or macrophages (Fig. 5).

DISCUSSION

The present studies were undertaken to elucidate the mechanisms of EBV transformation of B lymphocytes by identifying cell genes whose expression is increased by EBV infection. By identifying genes induced in EBV(+) versus EBV(−) BL cells, the effects of EBV infection were observed in human B lymphocytes, the natural target of EBV infection and growth transformation. Differences in gene expression that were not due to EBV infection were minimized as a result of the isogenic background of these cells. Further, since the full repertoire of EBV genes expressed in growth-transformed B lymphocytes is expressed in the infected BL41 cells, many effects of these genes on cell gene expression are evident. Consequently, a large number of EBV-induced genes are being identified for the first time, including some novel genes likely to be mediators of B-lymphocyte growth or differentiation.

This report describes the identification of nine EBV-induced genes, CD21 and vimentin genes being the only two which were previously known to be EBV induced (8, 12). CD21 is the EBV receptor, and CD21 mRNA is induced within 36 h following in vitro infection or antigen stimulation of normal peripheral blood B cells (2, 72). EBV induction of CD21 may be a consequence of activating and differentiating effects. Gene transfer experiments in EBV(−) BL cells indicate that CD21 expression is increased by EBNA 2 or EBNA 3C expression (17, 85, 86). CD21 is also the receptor (CR2) for the C3d fragment of complement (88), associates with Ig in the B lymphocyte plasma membrane (77), and may mediate the stimulating effects of antigen-antibody and complement complexes on uninfected B lymphocytes (53).

Vimentin expression is likely to be a consequence of EBV activating effects. Vimentin is the predominant intermediate filament protein in lymphocytes and is expressed at high levels in EBV-transformed B lymphocytes (8). It associates with LMP 1 at the cell periphery (50). Infection of BL41 or BL30 cells in vitro increases vimentin mRNA and protein production (8). In gene transfer studies, LMP 1 alone can induce vimentin expression in EBV(−) Loucke or BL41 BL cells (8).

Similarly, induction of MARCKS, serglycin, cathepsin H, annexin VI, and CD44 is likely to be a consequence of EBV-activating or -differentiating effects on B-lymphocyte structures. Ig cross-linking in normal murine peripheral blood B cells results in specific phosphorylation and dramatically increases the synthesis of MARCKS protein (39, 40). Steady-state MARCKS RNA levels may be regulated in part by mRNA stability (11). The unusually high degree of homology among three prime untranslated regions of the human and bovine MARCKS RNAs (75) suggests that these sequences may play a regulatory role, possibly in mRNA stability. Although its function is unknown, MARCKS pro-

tein localizes to substratum contact points in macrophages in physical association with vinculin, talin, and cytoskeletal actin (65, 78). MARCKS protein also binds to calmodulin with high affinity and inhibits calmodulin-mediated activation of phosphodiesterase (27, 55). This interaction is disrupted by protein kinase C-mediated MARCKS phosphorylation. MARCKS-regulated interaction with calmodulin is of particular interest in light of the role of calmodulin in lymphocyte apoptosis (54) and the ability of EBV or LMP 1 to protect cells from apoptosis (35). MARCKS may be a mediator of the anti-apoptotic effects of EBV infection.

Annexin VI is a Ca^{2+} binding protein of the lipocortin family (18) and is phosphorylated in response to growth factor stimulation (43). It may associate with CD21 (6). In vitro membrane reconstitution studies indicate that annexin VI may regulate the release of Ca^{2+} from intracellular stores (34). Annexin VI is expressed only in mantle zone B cells and is not detectable in GC cells (15).

Recent experiments indicate that annexin VI plays a critical role in the formation and budding of clathrin-coated pits by a process which may be triggered by specific phosphorylation (51). These findings are of particular significance since cathepsin H and serglycin may also be intracellular vesicle constituents. Cathepsin H is a lysosomal cysteine protease (9), whereas serglycin is a core protein of proteoglycans. Although not previously known to be expressed in lymphocytes, serglycin is stored in granulocyte secretory granules, where it may neutralize hydrolytic enzymes (45, 47).

CD44 binds hyaluronic acid (4) and is the lymphocyte homing receptor for high endothelial venules. Its expression increases following anti-Ig stimulation of murine B cells (13). In contrast with other EBV-induced genes, high-level CD44 expression in vivo is observed primarily in germinal-center B lymphocytes (24). CD44 may associate with the lymphocyte cytoskeleton via an ankyrinlike molecule (42). Transfection studies with Daudi and BL41 cell lines indicate that LMP 1 is responsible for CD44 induction (85).

The most significant outcome of this study is the discovery of the first G protein-coupled peptide receptors expressed in lymphocytes exclusively (as with EBI 1) or predominantly (as with EBI 2). Both genes are expressed at high levels in EBV(+) BL cells and EBV-transformed lymphoblastoid cell lines but are expressed at low or undetectable levels in two different EBV(−) BL lines. Expression of neither gene is entirely EBV specific, and EBI 1 and EBI 2 are likely to function normally as tissue-specific mediators of polypeptide cytokine effects. EBI 1 and EBI 2 RNAs are present in PBMCs at levels comparable to their levels in lymphoblastoid cell lines; they are also detected in RNA from unfractionated tonsil tissue, which consists mostly of B lymphocytes. The much higher EBI 2 RNA level in B-lymphocyte tissues and cell lines than in phytohemagglutinin-stimulated T cells and the T-cell lines Jurkat, HSB-2, and Molt-4 is evidence that EBI 2 expression in vivo is predominantly B- rather than T-lymphocyte restricted. An intermediate level of EBI 2 RNA was observed in HL60 and U937 cell lines and in pulmonary tissue, suggesting that EBI 2 may also be expressed in monocytes, granulocytes, and pulmonary tissue in vivo. In contrast, EBI 1 is expressed in B- and T-lymphocyte lines but not in nonlymphoid tissues or cell lines; it thus appears to be entirely lymphocyte restricted. The level of EBI 1 RNA in phytohemagglutinin-stimulated peripheral blood T cells and in T-cell lines indicates that both B and T lymphocytes may contribute to the overall expression observed in unfractionated PBMC, tonsil, and spleen cell RNA preparations. Experiments are now in progress to delineate the roles of EBI 1 and EBI 2 in normal B- and T-lymphocyte development and immune responses.

The relatively high EBI 1 and EBI 2 mRNA levels in PBMCs, spleen tissue, and, to a lesser extent, tonsil tissue is surprising in light of the low levels in BL cells. EBI 1 and EBI 2 expression may be restricted to particular stages of lymphocyte differentiation or activation. EBV(−) BL cells may correspond to a differentiation state in which expression of both genes is characteristically low. In latent EBV infection, EBV gene products may act individually or in concert to activate expression or to maintain high levels of expression in host cells which already transcribe EBI 1 and EBI 2. Preliminary data indicate that EBI 1 is induced in EBV(−) BL cells converted to LMP 1 or EBNA 2 expression by single-gene transfer. EBI 2 expression may be specifically induced by EBNA 2 or EBNA LP since expression is up-regulated in BL41 cells transfected with an EBNA LP and EBNA 2 expression vector.

The extent of EBI 1 and EBI 2 homology to G protein-coupled peptide receptors makes it probable that EBI 1 and EBI 2 are receptors which transduce ligand-binding signals through heterotrimeric GTP-binding proteins (G proteins). Homologies between EBI 1 and the high- and low-affinity IL-8 receptors are particularly striking and are apparent at both the nucleotide and amino acid levels. In fact, EBI 1 is the closest known homolog of the IL-8 receptors. The neuropeptide Y receptor type 2, for which only the bovine form has thus far been cloned, also exhibits a high degree of homology to these proteins (64). These four proteins appear to constitute a closely related subfamily within the superfamily of G protein-coupled receptors. EBI 2 exhibits more distant homology to these proteins and appears to be most closely related to the thrombin receptor.

We have identified a previously unrecognized, highly conserved G protein-coupled receptor motif at the junction of the third transmembrane domain and second intracellular loop. This motif, (A/S)-(I/V)-D-R-(Y/F)-X-X-X-X, where X represents hydrophobic residues, is characteristic of even distantly related G protein-coupled receptors, including photoreceptor opsins and receptors from phylogenetically divergent species such as *Drosophila* species. The motif is not present in other proteins. The restricted presence of this sequence in G protein-coupled receptors is most consistent with an important role in G protein-coupled receptor function. This region is implicated in receptor interaction with G proteins (48). The sequence includes five continuous hydrophobic amino acids, and ligand binding may alter the disposition of the hydrophobic portion of this sequence with respect to the adjacent plasma membrane, effecting changes which modulate G protein interaction.

Ligands for EBI 1 or EBI 2 are likely to be polypeptide cytokines since EBI 1 and EBI 2 are most closely related to G protein-coupled receptors which have polypeptide ligands. Further, a third transmembrane domain aspartate residue is highly conserved among G protein-coupled biogenic amine receptors but is absent from EBI 1 and EBI 2, excluding membership in that family (73). The close similarity between EBI 1 and the IL-8 receptors is evidence that the EBI 1 ligand is a polypeptide proinflammatory "intercrine" factor similar to ACT-2, GRO/MGSA or RANTES, or IL-8 (63).

The expected action of EBI 1 and EBI 2 is through G proteins and secondary messenger pathways by the activation of effector molecules such as adenyl cyclase, cyclic AMP phosphodiesterase, phospholipase C, or various ion

channels (20). In general, G protein-coupled peptide receptors regulate specialized functions of target cells, such as secretion of hormones in endocrine cells, membrane depolarization in neural cells, or chemotactic migration and activation of phagocytic cells. Ligand binding may also alter growth properties. Expression of the serotonin 1c receptor in immortalized rodent fibroblasts results in ligand-dependent oncogenic transformation (41). The melanoma growth-stimulatory activity protein (MGSA) binds to a G protein-coupled receptor and supports melanoma cell growth. MGSA can compete with IL-8 for binding to the IL-8 receptor, suggesting that the MGSA receptor may be closely related to the IL-8 receptors (58).

Indirect evidence indicates that a lymphocyte G protein-coupled receptor may affect phospholipase C activity and EBI 1 or EBI 2 may be the receptor that interacts in this pathway. Nonhydrolyzable GTP analogs constitutively activate both G proteins and lymphocyte polyphosphoinositide breakdown by phospholipase C (25, 33). Further, some experiments have demonstrated constitutive inhibition of phospholipase C activation by pertussis toxin, which blocks G proteins by ADP-ribosylation of their alpha subunits (25, 33, 57). Moreover, a G protein isoform regulates phospholipase C activity in brain tissue (69).

The finding that EBV induces these two putative G protein-coupled peptide receptors is also of interest in light of the recent findings that human cytomegalovirus and herpesvirus saimiri have incorporated homologs to G protein-coupled receptors into their genomes (60). Cytomegalovirus has three such genes (14). This virus is unusual among herpesviruses in its transient stimulation of cell DNA synthesis early in lytic replication and in establishing infection in precursor cells in bone marrow (52). Herpesvirus saimiri is a New World primate herpesvirus and is the herpesvirus most closely related to the EBV group of Old World primate lymphotropic herpesviruses (1). Herpesvirus saimiri can transform human T lymphocytes in vitro and can produce tumors in New World primates (7). The role of the G protein-coupled receptor homolog in herpesvirus saimiri infection has not been investigated. The finding that EBV induces G protein-coupled receptors and that cytomegalovirus and herpesvirus saimiri have incorporated G protein-coupled receptors into their genomes is compatible with the hypothesis that these G protein-coupled receptors mediate similar functions for these herpesviruses. However, comparison of the cytomegalovirus and herpesvirus saimiri homologs with EBI 1 and EBI 2 and the G protein-coupled peptide receptors reveals significant differences between the cytomegalovirus or herpesvirus saimiri G protein-coupled receptor homologs and the G protein-coupled peptide receptor group (Fig. 2B).

The identification of 9 different EBV-induced mRNAs among the first 12 clones and of 5 new and different mRNAs among 6 additional clones in various stages of sequence determination is evidence indicating that a larger group of EBV-induced genes can be identified by this approach. Other novel B-lymphocyte genes which may encode mediators of B-lymphocyte growth, differentiation, or activation or of EBV-induced growth transformation are likely to be identified.

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