Induction of Interleukin-10 in the Stable Transformants of Human T-Cell Line Expressing Epstein-Barr Virus-Encoded Small RNAs

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Background : Epstein-Barr virus (EBV) is a ubiquitous virus in humans and latently infects B cells. In some individuals, however, EBV produces chronic active infection and causes life-threatening complications due probably to cytokinemia induced by EBV-infected T or natural killer (NK) cells. The role of EBV-encoded small RNAs (EBERs), which are commonly expressed in EBV-infected T and NK cells, is still unknown.

Methods : The plasmid coding EBERs was introduced into human T-lymphotropic virus-I-negative human T-cell lines in a site-directed manner, and stable transformants were established. The alteration of cytokine expression in EBERs-expressing transformants and the activation of the downstream signaling cascade from dsRNA were examined.

Results : Among three mother T-cell lines and their transformants, only the transformants from MOLT-14 cells ($\gamma\delta$ T cells) expressed EBERs. EBERs-expressing MOLT-14 cells produced a larger amount of interleukin-10 than did the mother cell line. The phosphorylation of dsRNA-dependent protein kinase (PKR) and that of IκBα which act downstream of PKR, increased in EBERs-expressing clones.

Conclusion : The $\gamma\delta$ T cell-specific production of IL-10 through EBERs expression might lead to modification of the role of $\gamma\delta$ T cells, and might play a role in human immune diseases. *Shinshu Med J 66 : 195—204, 2018*

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Key words : Epstein-Barr virus (EBV), chronic active EBV infection, EBV-encoded small RNAs, $\gamma\delta$ T cell, interleukin-10

I Introduction

Epstein-Barr virus (EBV) is a ubiquitous virus in humans. Most individuals are infected with EBV by early adulthood. Primary EBV infection is usually asymptomatic, but sometimes results in infectious mononucleosis, which is basically self-limited due to the development of EBV-specific immunity. However, some individuals in Asia, mostly in Japan, are reported to develop chronic infection with EBV. Chronic active EBV infection (CAEBV) is characterized by chronic recurrent infectious mononucleosis-like symptoms over a long period of time and by high viral loads in peripheral blood of the patients[1)2)]. CAEBV is frequently associated with the broad spectrum of EBV-associated T/natural killer (NK)-cell lymphoproliferative disorders including polyclonal, oligoclonal, and monoclonal proliferation[3)], and is a disease with a high mortality with life-threatening complications, such as virus-associated hemophagocytic syndrome, due probably to cytokinemia induced by EBV-infected lymphoid cells[4)], EBV-positive apparent leukemia/lymphoma mainly in T- and NK-cell lineage, and cardiovascular diseases and arteritis with the infiltration of EBV-positive lymphoid cells[1)5-7)].

In CAEBV, the transcripts of EBV genes are detected in T or NK cells[2)], and the in vitro infection of human T-cell lines with EBV after the enforced expression of EBV receptor in B-lineage cells, CD21, causes the enhanced production of a macrophage ac-

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tivating cytokine, tumor necrosis factor (TNF) α [8]. However, the EBV gene responsible for the upregulation of TNFα expression in T-lineage cells remains unclear. Profiles of EBV latent gene expression in EBV-infected T and NK cells show variations, and only EBV nuclear antigen-1 (EBNA1) and EBV-encoded small RNAs (EBERs) are commonly expressed in non-neoplastic infected T and NK cells in CAEBV[9]. EBNA1 enables efficient EBV episome replication, transcription, and maintenance in latently infected dividing cells[10]. Recently, EBNA1 was shown to stably interact with a ubiquitin-specific protease called USP7 and to protect cells from apoptotic challenge by lowering the p53 level[11]. However, EBNA1 has been shown to activate transcription from episomal but not integrated DNA, and would not affect the cellular gene expression[12]. Therefore, among EBV latent genes expressed in T and NK cells in CAEBV and EBV-associated hemophagocytic syndrome patients, EBERs may cause the activation of T and NK cells including the induction of cytokines, which would be responsible for the development of the diseases. EBERs cause interleukin (IL)-10 induction in human B-lineage Burkitt's lymphoma cell lines[13]. Furthermore, EBV infects a human T-lymphotropic virus-I (HTLV-I)-positive human T-cell line, MT-2[14], and EBERs induce IL-9 in EBV-infected MT-2 cells[15]. However, the HTLV-I infection activates human T cells and induces various cytokines[16], thus the alteration of cytokine expression in HTLV-I-positive T-cell lines with the superinfection of EBV might not reflect the role of EBV in vivo. In this context, the role of EBERs in HTLV-I-free human T-cell lines is still unknown.

In the current study, we introduced the plasmid coding sequences of EBERs into HTLV-I-negative human T-cell lines in a site-directed manner, established stable transformants, and examined the role of EBERs in human T cells.

Ⅱ Materials and Methods

A Cell lines and culture

HTLV-I-negative human T-cell lines, Jurkat, MOLT-4, and MOLT-14 (a gift from Fujisaki Cell Center, Hayashibara Biochemical Labs., Inc., Okayama, Japan), and an EBV-positive Burkitt's lymphoma cell line, Akata (a gift from Prof. Kenzo Takada; Hokkaido University, Sapporo, Japan), were grown in RPMI 1640 medium supplemented with 10 % heat-inactivated fetal bovine serum (Cambrex Bio Science, Walkersville, MD). An EBV-positive human NK cell line, SNK6 (a gift from Dr. Norio Shimizu; Tokyo Medical and Dental University, Tokyo, Japan), was grown in Artemis-2 medium (Nihon Techno Service, Ushiku, Japan). Zeocin™ and hygromycin B used for the selection of drug-resistant clones were purchased from Invitrogen (Carlsbad, CA). A human cervical carcinoma cell line, Hela, a human mammary carcinoma cell line, MCF7, and NIH3T3 were obtained from the Health Science Research Resources Bank (Sennan, Japan), and cultured in Eagle's minimal essential medium with 10 % heat-inactivated fetal bovine serum.

B Plasmids

Flp recombinase-mediated integration kit, Flp-In™ System (including vectors, pFRT/*lac*Zeo, pOG44, and pcDNA5/FRT), was purchased from Invitrogen. The 1.0 kb *Sac*I-*Eco*RI subfragment from the *Eco*RI K fragment (EKS) of Akata EBV DNA, which corresponds to the *Eco*RI J fragment of B95-8 EBV DNA and contains EBER1 and EBER2 reading frames, was amplified, and *Bgl*II and *Kpn*I sites were added at each end of the fragment with polymerase chain reaction (PCR)[7]. The EKS fragment was subcloned into the *Bgl*II and *Kpn*I sites, between those cytomegalovirus promoter sequence locates, in pDNA5/FRT vector (pDNA5/FRT-EKS). The sequence of the insert was confirmed to be the same as the reported EBV sequence.

C Transfection and selection of transformants

Transfection-grade plasmid DNA was purified with a FlexiPrep kit (GE Healthcare, Uppsala, Sweden). Plasmids were introduced to T-cell lines by the electroporation method. 5×10^6 cells were suspended in serum-free RPMI 1640, washed twice, and resuspended in 600 μl of ice-cold serum-free RPMI 1640 containing 20 to 100 μg of plasmid DNA in a 4-mm-gap electroporation cuvette. The electroporation

was performed with the Gene Pulser II (Bio-Rad, Hercules, CA) at 1 mF, and the optimal voltage for each cell line. The voltage at electroporation was optimized for each cell line by using pEGFP-C1 and percent fluorescence-positive cells as an indicator. After electroporation, cells were cultured for 2 days without selection reagents, and subsequently 2×10^4 cells were seeded in a well of flat-bottomed 96-well culture plates with 200 μl of selection medium. Half of the medium was changed every 5 days until colonies emerged. Clones were expanded and maintained in selection medium.

D Recombinant cytokines and other reagents

Recombinant human interferon (IFN) α (rhIFNα ; 1.8×10^8 U/mg protein), recombinant human IL-1α (rhIL-1α ; 1×10^9 U/mg protein) and recombinant human TNFα (rhTNF α ; 2×10^7 U/mg protein) were purchased from PeproTechEC (London, UK). Calyculin A was purchased from Sigma-Aldrich (St. Louis, MO).

E Southern blot anaysis

DNAs were extracted from cell lines, quantified by measuring optical density $(OD)_{260}$ and stored at −20 ℃ until use. Aliquots of 10 μg of DNA were digested with *Hind*III, electrophoresed in 0.6% agarose gels and Southern blotted as previously described[17]. The 230 bp *Pvu*II/*Nco*I fragment of pFRT/*lac*Zeo was used as a template. The nonradioactive probe was labeled by random prime labeling, Southern-blotted filters were hybridized with the labeled probe, and the signals were generated by chemiluminescence as described previously[7].

F RNA extraction and Northern blot analysis

Total RNA was extracted from cell lines using Trizol reagent (Invitrogen), quantified by measuring OD_{260}, and ethanol-precipitated at −80 ℃ until use. Twenty μg of total RNA was electrophoresed in 1.5 % formaldehyde denaturing agarose gels, and Northern blotted as previously described[17]. Hybridization with nonradioactive DNA probes and signal generation were performed in the same manner as described in the previous section. The full sequence of EBER1 (167 bp) was amplified with PCR using the DNA sample extracted from Akata cells as previously described[7][13]. The 315 bp-fragment of human actin mRNA was amplified with reverse transcriptase (RT)-PCR as previously described[7][18]. After PCR amplification, the amplified segments were electrophoresed in agarose gels, cut out, purified with the JETSORB kit (GENOMED, Bad Oeynhausen, Germany) and used as templates for probe labeling reaction.

G Real time RT-PCR analyses

Real time RT-PCR analyses were performed using the TaqMan assay system (Applied Biosystems, Foster City, CA) as previously described[19]. Predeveloped TaqMan assay reagents used in this study were #NM 000575 for human IL-1α, #NM 000576 for human IL-1β, #NM 000589 for human IL-4, #NM 000600 for human IL-6, #NM 000572 for human IL-10, #NM 000594 for human TNFα, #NM 000619 for human IFNγ, and #NM 002046 for human glyceraldehyde 3-phosphate dehydrogenase (GAPDH ; all from Applied Biosystems). Serial ten-fold dilutions of the sample with the lowest threshold cycle were used to obtain the standard curve of relative amounts of each mRNA. For the real time RT-PCR analyses of EBER1 and EBER2, total RNA was treated with DNase using TURBO DNA-free™ kit (Invitrogen), and converted to cDNA with reverse-transcription. Primers and TaqMan probes were designed with Primer Express (v.1.5 ; Applied Biosystems). Primer sequences were 5'-GTGAGGACGGTGTCTGTGGTT-3' and 5'-TTGACCGAAGACGGCAGAA-3' for EBER1, and 5'-GCTACCGACCCGAGGTCAA-3' and 5'-GAGAATCCTGACTTGCAAATGCT-3' for EBER2, amplifying the 58 bp and 77 bp segment of RNA, respectively. TaqMan probe sequences were 5'-TCTTCCCAGACTCTGC-3' for EBER1 and 5'-AAGAGAGGCTTCCCGCC-3' for EBER2. Plasmid containing the EKS fragment of EBV DNA, which contains EBER1 and EBER2 reading frames, was used as standard DNA samples for absolute quantification of copy numbers of EBERs.

H Measurement of IL-10

The amounts of human IL-10 in culture supernatants of cell lines were quantitated by enzyme-linked immunosorbent assay (ELISA) using human

IL-10 ELISA kit (R&D systems Inc., Minneapolis, MN).

I Immunoblot analyses

Cells were lysed and cell lysates were separated with sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to membranes, as described previously[20], with the exception of adding phosphatase inhibitors (1 mM sodium orthovanadate, 30 mM tetrasodium pyrophosphate and 50 mM sodium fluoride) to the buffer for cell lysis. Membranes were blocked and incubated with primary antibody solutions, and signals were generated by chemiluminescence, as described previously[20], and stored as digital images using an image analyzer (LAS-4000 mini ; GE Healthcare). Antibody solutions used in this study were mouse monoclonal antibodies to β-actin (Sigma-Aldrich), IκBα and phospho-IκBα (Ser32/36), and rabbit polyclonal antibodies to dsRNA-dependent protein kinase (PKR), phospho-PKR (Thr451), and IL-1 receptor-associated kinase-1 (IRAK-1) (all from Cell Signaling Technology Inc., Beverly, MA) diluted with 5 % bovine serum albumin in Tris-buffered saline containing 0.1 % Tween 20, and rabbit polyclonal antibody to phospho-IRAK-1 (Ser376) (Abgent, San Diego, CA) diluted with Can Get Signal Solution-1 (Toyobo, Osaka, Japan).

J Statistical analysis

Statistical significance was determined by the two-tailed Student's *t*-test. $p<0.01$ was considered statistically significant.

Ⅲ Results

A Establishment of stable EBERs-transformants of T-cell lines

To access the exact effects of EBERs in T-cell lines, we employed the site-directed recombination system. By comparing the expression of cytokines between T-cell clones with the FRT site alone and those with EBERs sequence integrated in the same FRT site, we could rule out the effects of the integration site in the genome.

At first, we transfected 20 μg of *Sca*I-cut pFRT/*lac*Zeo plasmids into the three human T-cell lines, Jurkat, MOLT-4, and MOLT-14, by electroporation, and the plasmid-integrated clones were selected in the culture containing Zeocin at a concentration of 100 μg/ml. After the establishment of several clones from each mother cell line, clones with a single FRT site were selected with Southern blot analyses, and we obtained two clones with a single FRT site in each mother T-cell line. We next transfected 10 μg of pcDNA5/FRT-EKS containing EBERs sequence and 90 μg of pOG44 plasmids into T-cell clones with a single FRT site by electroporation, and the cells with the recombination at the FRT site integrating pcDNA5/FRT-EKS plasmid were selected in the culture containing hygromycin at a concentration of 200 μg/ml. After the establishment of hygromycin-resistent cultures, the integration of the plasmid in the manner of site-directed recombination was confirmed by Southern blot analyses **(Fig. 1)**. Then, we examined the expression of EBER1 in the established transformants by Northern blot analyses. Among three mother T-cell lines and their transformants, only the transformants from MOLT-14 cells expressed EBER1 **(Fig. 2)**. The amount of EBER1 expressed in MOLT-14 clones was, however, far less than that in an EBV-positive Burkitt's lymphoma cell line, Akata. Furthermore, we quantitated the copy number of EBERs transcripts by real time RT-PCR analyses. In MOLT-14 clones, the amount of total EBERs transcripts was approximately 2 % of those of an EBV-positive NK cell line, SNK6 **(Table 1)**. EBER1 transcripts in MOLT-14 clones were much lower than those in SNK6, although EBER2 transcripts in MOLT-14 clones were higher than those in SNK6 **(Table 1)**.

B Alteration of cytokine expression in MOLT-14 clones

The expression of cytokines from EBERs-expressing MOLT-14 cells, which might activate macrophage-lineage cells and affect immune conditions, was examined at the mRNA level, at first.

By real time RT-PCR analyses, mRNA expression of IL-10 in EBER-expressing MOLT-14 cells was much higher than that in mother cell lines with a FRT site **(Table 2)**. The expression of IL-4 and TNF α mRNA was not apparently affected by the expres-

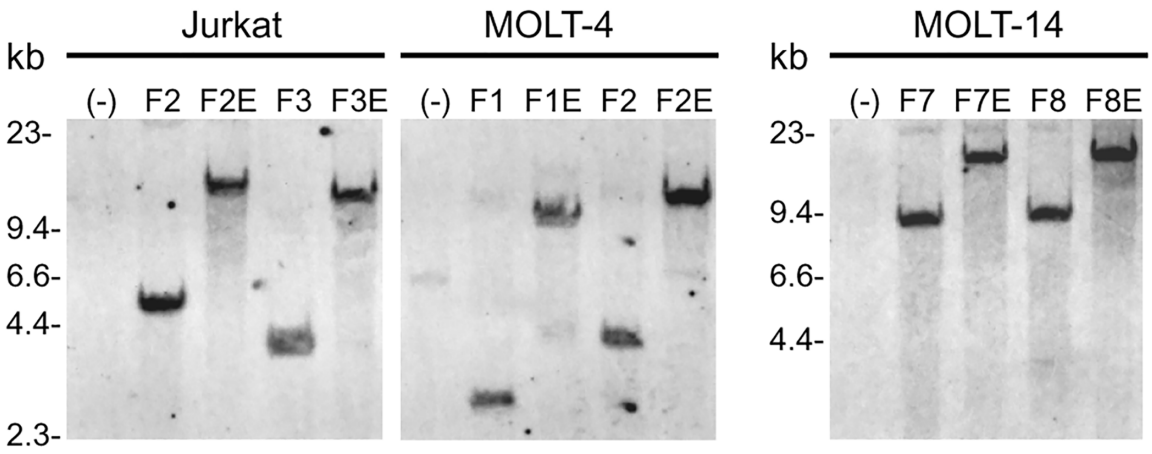

Fig. 1 Southern blot analyses for the integration of plasmids. Ten micrograms of DNA was digested with *Hind*III, electrophoresed in 0.6 % agarose gels, Southern blotted, and hybridized with DNA probe containing SV40 promoter sequence derived from integrated pFRT/*lac*Zeo plasmid, and signals were visualized as described in "Materials and Methods". DNA size markers are indicated on the left. DNA samples from each FRT clone contain a single band, and those from FRT recombinant clones with pDNA5/FRT-EKS (EBER clones) exhibit the shift of a band to a larger size, compatible with the size of the plasmid (approximately 5 kb larger). (−), mother cell line ; FN, clones containing a FRT site ; FNE, clones containing EBERs sequences with FRT recombination.

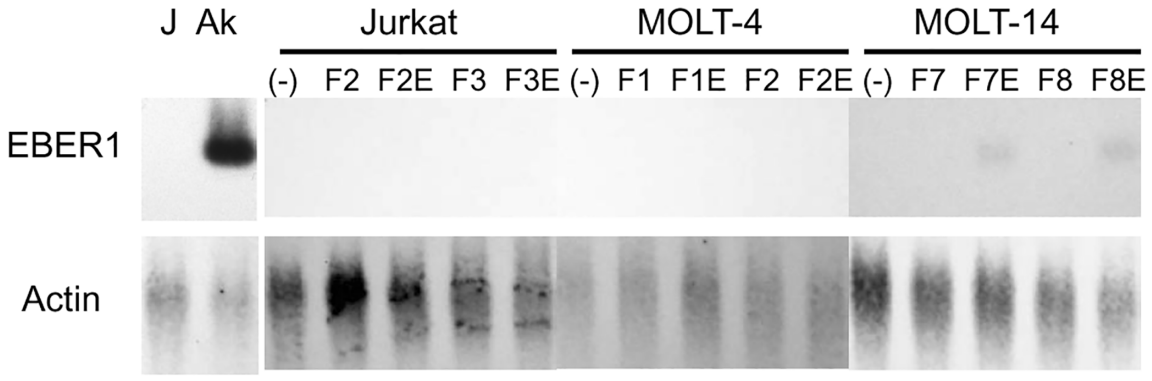

Fig. 2 Northern blot analyses of EBER1 expression in T-cell clones. Human T-cell clones were cultured for at least 7 days in the absence of selection antibiotics. Twenty micrograms of total RNA samples were applied to each well, Northern blotted, and hybridized with nonradioactive EBER1 and actin DNA probes, and signals were visualized as described in "Materials and methods". MOLT-14 clones express EBER1 at the level detectable with Northern blot, but Jurkat and MOLT-4 clones do not. RNA samples from Akata cells (Ak) served as positive control samples. J, Jurkat ; (−), mother cell line ; FN, clones containing a FRT site ; FNE, clones containing EBER sequences with FRT recombination.

sion of EBERs **(Table 2)**. The expression of IL-1α, IL-1β, IL-6 and IFNγ mRNA in these cells was extremely low, that is to say, the threshold cycles of these mRNAs were over 33 cycles with much deviation, and an exact comparison of expression level was not possible (results not shown). The change of IL-10 mRNA expression in Jurkat and MOLT-4 cell lines was not obvious after the integration of EBERs-

Table 1 Expression of EBER1 and EBER2 in cell lines analyzed by real time RT-PCR

	Copy number (copy/ng total RNA)		
Cells	EBER1	EBER2	Total
F7E	1.34×10^3	3.64×10^2	1.70×10^3
F8E	1.06×10^3	3.85×10^2	1.45×10^3
SNK6	7.26×10^4	1.14×10^2	7.27×10^4

containing plasmid without EBERs expression (results not shown). The increased IL-10 protein in 3-day culture supernatants of EBERs-expressing MOLT-14 cells, compared with that of mother cell lines, was confirmed by ELISA **(Fig. 3)**.

C Increased phosphorylation of PKR and IκBα in EBER-expressing MOLT-14 clones

To demonstrate that the EBERs expressing in MOLT-14 cells were actually functioning, we examined whether the downstream signaling cascade from dsRNA was activated by immunoblot analyses for the phosphorylated signaling proteins involved in the cascade.

PKR is the first-described dsRNA sensor molecule, and is phosphorylated and activated with the viral dsRNA in infected cells[21]. We thus examined at first the alteration of phosphorylation of PKR with EBERs expression. The phosphorylation of PKR increased along with the expression of EBERs in our T-cell system **(Fig. 4A)**, although the protein expression of PKR was not clearly changed **(Fig. 4A)**. To confirm the increased activation of PKR in EBERs-expressing T-cell clones, we next examined the phosphorylation of IκBα, which acts downstream of PKR in the signaling cascade[22], and revealed a slightly increased phosphorylation of IκBα in EBERs-expressing clones without alteration of protein expression **(Fig. 4B)**.

Furthermore, to evaluate the involvement of innate immune system in the EBERs-induced IL-10 expression, we next examined the phosphorylation of IRAK-1, which is a critical signaling mediator of innate immunity[23] and is involved in the LPS-induced IL-10 expression[24], by immunoblot analyses. MOLT-14 clones containing a FRT site varied in protein expression and phosphorylation of IRAK-1 and the change induced by the expression of EBERs was not uniform in these two clones **(Fig. 4C)**. One clone (F7) showed up-regulated expression of IRAK-1 protein following EBERs expression and the other (F8) showed down-regulated expression **(Fig. 4C)**.

Ⅳ Discussion

In the current study, we established stable transformants of MOLT-14 cells expressing EBERs and revealed the increased production of IL-10 from these EBERs-expressing MOLT-14 cells.

Table 2 Real time RT-PCR analyses of the expression of cytokines in EBERs-expressing MOLT-14 clones

Cells	IL-4	IL-10	TNFα
F7E	0.43 +/− 0.06*	28.2 +/− 13.0	1.45 +/− 0.46
F8E	0.62 +/− 0.02	6.64 +/− 1.01	1.83 +/− 0.17

*Relative expression level of mRNA compared to that in the mother line containing the FRT site alone, which are standardalized with the expression of mRNA of GAPDH. The data are presented as the mean value of three independent experiments plus/minus standard deviation.

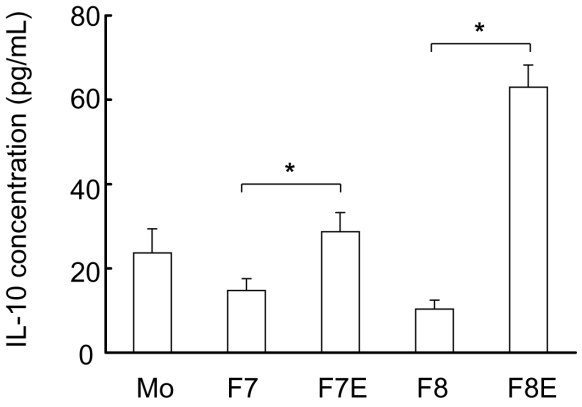

Fig. 3 Concentration of IL-10 protein in the culture of MOLT-14 clones. After the 3-day culture of cell lines, the supernatants were harvested by centrifugation and stored at −80℃. The concentration of IL-10 was measured by ELISA. The bars represent the mean values of triplicate from two independent supernatant preparations plus/minus standard deviation.
*P<0.01. Mo., mother cell line ; FN, clones containing a FRT site ; FNE, clones containing EBERs sequences with FRT recombination.

The expression of EBERs in MOLT-14 clones was much lower than that of an EBV-positive NK cell line, SNK6, and the ratio of EBER1 to EBER2 in MOLT-14 clones was greatly different from that in SNK6. This is not surprising because these variations in EBERs expression were reported in EBV-positive B-lineage cell lines[25]. Furthermore, EBER2 induces IL-6 and plays a role in EBV-induced B-cell growth transformation dominantly over EBER1[26]. Therefore,

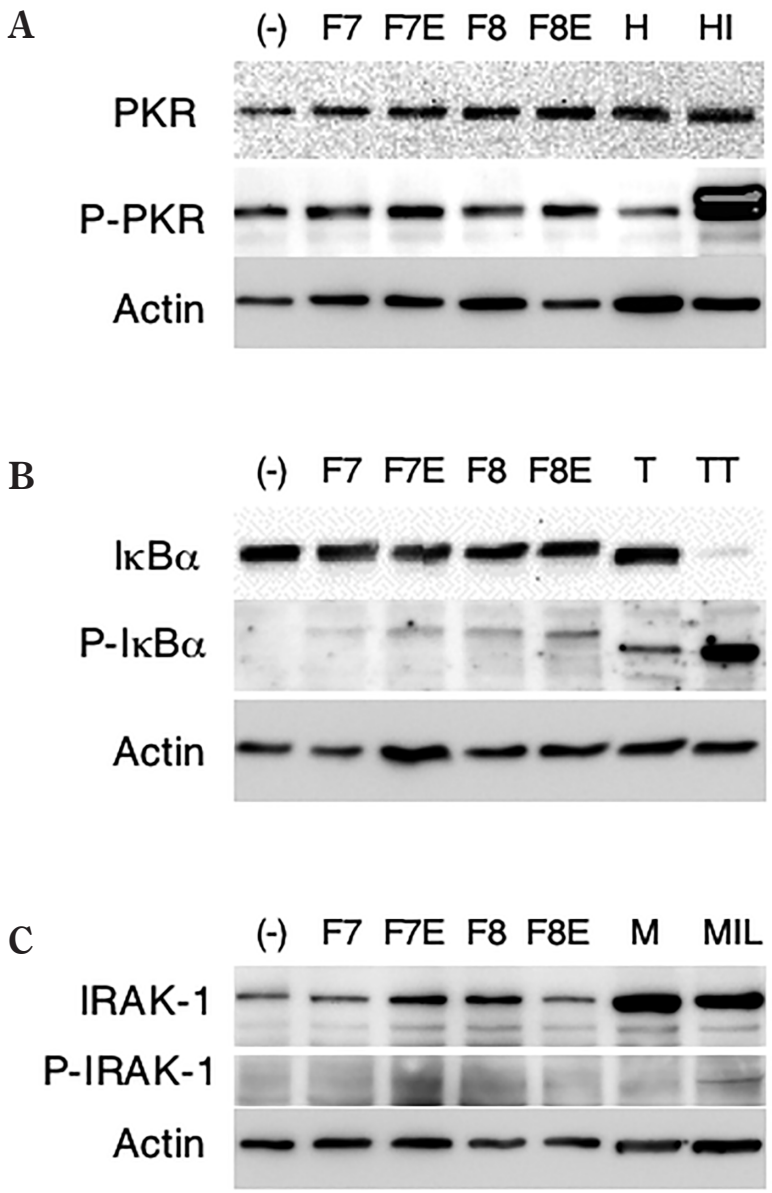

Fig. 4 Expression and phosphorylation of signaling molecules, such as PKR (**A**), IκBα (**B**), and IRAK-1 (**C**), in MOLT-14 clones. MOLT-14 clones were cultured for at least 7 days in the absence of selection antibiotics. Subconfluent cultures of Hela, NIH/3T3, and MCF7 cells were incubated with rhIFNα (1,000 U/ml for 18 hrs) and subsequently with calyculin A (100 nM for 15 min), with rhTNFα (1,000 U/ml for 10 min), and with rhIL-1α (20 ng/ml) and calyculin A (100 nM) for 5 min, respectively. Cells were harvested, 500 μl of cell lysates were prepared from 5×10^6 cells, and a 10-μl (for β-actin) or 30-μl (for signaling molecules) of aliquot of each sample was applied to each well. After electrophoresis in 12 % (β-actin and IκBα) or 10 % (PKR and IRAK-1) polyacrylamide gels, proteins were transferred to a polyvinylidene difluoride membrane, incubated with the antibodies, and visualized as described in "Materials and methods". (−), mother cell line ; FN, clones containing a FRT site ; FNE, clones containing EBERs sequences with FRT recombination ; H, Hela ; HI, Hela treated with IFNα and calyculin A ; T, NIH3T3 ; TT, NIH3T3 treated with TNF α ; M, MCF7 ; MIL, MCF7 treated with IL-1α and calyculin A.

even the lower expression of total EBERs with considerable expression of EBER2 may play a role in the induction of an immune-modulating cytokine, IL-10.

The induction of IL-10 in T-lineage cells by EBERs is compatible with the increased IL-10 in the sera of patients with T-cell type CAEBV[2], although EBERs did not induce a cytokine with macrophage-activat-

ing activities, such as TNFα, IFNγ, and IL-1β. MOLT-14 is a T-cell receptor γ/δ-bearing human T-cell line and exhibits NK-like cytotoxic activity to K562 cells after treatment with rIL-2 or phorbol myristate acetate[27], and thus, shares the characteristic features of EBV-infected cells in CAEBV patients[2]. Therefore, MOLT-14 cells would be suitable for the model cells of functional analyses of EBV genes. On the other hand, Jurkat and MOLT-4 cell lines are basically T-cell receptor α/β-bearing human T-cell lines[28], although MOLT-4 lacks the T-cell receptor α gene[29]. Therefore, the cell line-dependent expression of EBERs in T-lineage cell lines **(Fig. 2)** might be useful for the analysis of transcription mechanisms of EBERs.

PKR is phosphorylated and activated with the viral dsRNA in infected cells[21]. As for the interaction with EBERs, however, the activation of PKR is inhibited by EBERs[30], and in Burkitt's lymphoma cell lines, EBERs induce the inactivation of PKR and cause resistance to IFN-induced apoptosis[31]. Thus, the increased phosphorylation of PKR along with the expression of EBERs in our T-cell system **(Fig. 4A)** is not consistent with these previous reports. On the other hand, Ruf et al reported that EBERs do not inhibit PRK during the protection from IFN-induced apoptosis[32]. Furthermore, the low-level expression of HIV-I TAR RNA causes the phosphorylation and activation of PKR, although the higher expression of the same RNA inactivates PKR[33]. Thus, the low-level expression of EBERs observed in the current T-cell system would cause the phosphorylation of PKR, like HIV-I TAR RNA, but anyway, the dose-dependent effects of EBERs on the activation of PKR must be clarified.

Increased phosphorylation of IκBα **(Fig. 4B)** indicates increased transcription of the genes controlled by nuclear factor κB, which includes a variety of cytokines, but does not explain the increased cytokine expression specific for IL-10. As a candidate for the IL-10-specific regulatory molecule, we evaluated the possible involvement of IRAK-1[23)24)]. However, the protein expression and phosphorylation of IRAK-1 were not associated with EBERs expression and subsequent IL-10 induction **(Fig. 4C)**. Thus, the involvement of the innate immune system in this process[23] is uncertain and the mechanism for the specific up-regulation of IL-10 is still unclear.

The induction of IL-10 by EBERs is common to both B-[13] and T-lineage cells. IL-10 has two functions in the immune system, inhibitory effects on cellular immunity, such as the inhibition of cytotoxic T lymphocyte induction, and accelerating effects on humoral immunity, such as the promotion of B cell growth and differentiation. In the latter aspect, IL-10 seems to be associated with human autoimmune diseases, such as systemic lupus erythematosus[34] and autoimmune hemolytic anemia[35]. Since EBERs are non-polyadenylated, untranslated RNAs and subsequently escape from host immune responses, continuous stimulation of B-cell growth would be permitted through the development of diseases. Therefore, although EBERs in T-lineage cells did not seem to play a role in the development of hemophagocytic syndrome, which is a major complication of CAEBV, the induction of IL-10 by viral small RNAs, such as EBERs, might play a role in the development of human immune diseases with B cell hyper-reactivity.

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