ANTIOXIDANTS & REDOX SIGNALING
Volume 1, Number 2, 1999
Mary Ann Liebert, Inc.

Original Research Communication

Redox Control of Epstein-Barr Virus Replication by Human Thioredoxin/ATL-Derived Factor: Differential Regulation of Lytic and Latent Infection

HIROSHI SONO,[1,3] KEISUKE TESHIGAWARA,[2] TETSUROU SASADA,[1] YASUSHI TAKAGI,[1] AKIRA NISHIYAMA,[1] YUJI OHKUBO,[2] YASUHIRO MAEDA,[3] EIJI TATSUMI,[4] AKIHISA KANAMARU,[3] and JUNJI YODOI[1]

ABSTRACT

Human thioredoxin (hTRX)/adult T-cell leukemia (ATL)-derived factor (ADF) was originally reported as an interleukin-2 (IL-2) receptor-α-inducing factor produced by human T-cell lymphotropic virus-1-positive (HTLV-1$^+$) cell lines. Growing evidence indicates that hTRX/ADF plays important roles in cellular responses against oxidative stress and is involved in a variety of cellular functions. A high level of hTRX/ADF expression is also observed in other human virus-infected cell lines including those of Epstein-Barr virus (EBV) and human papillomavirus. In this report, we analyzed the effect of hTRX/ADF on lytic amplification and persistent replication of EBV as a model for lytic versus latent phase of viral replication in host cells. Addition of hTRX/ADF clearly suppressed lytic replication of EBV in Raji cells and B95-8 cells induced to the lytic phase of 12-*O*-tetradecanoylphorbol-13-acetate (TPA), and it prevented the death of these cells evoked by the lytic induction. In contrast, hTRX/ADF did not have any effect on persistent replication in the latent phase. These data indicated that hTRX/ADF prevents EBV-transformed cells from proceeding into the lytic phase and regulates cohabitation of EBV and its host cells. Antiox. Redox Signal. 1, 155–165, 1999.

INTRODUCTION

THE EPSTEIN-BARR VIRUS (EBV), a member of the human lymphotropic herpes virus group, is the causative agent of infectious mononucleosis and is closely associated with Burkitt's lymphoma and nasopharyngeal carcinoma. The virus immortalizes human B lymphocytes. These cells harbor multiple copies of predominantly circular EBV genomes and maintain them in a latent state where no virus production occurs or it occurs at a very low rate (Lindahl *et al.*, 1976). Once cells enter the viral productive cycle, viral DNA is amplified 100–1,000 times within one cell cycle and leads irreversibly to death of the host cell (Hammerschmidt and Sugden, 1988; Kawanishi, 1993). EBV-transformed cells maintain the latent state of the viral genome and suppress lytic amplification for their own survival. Thus, control of

[1]Department of Biological Responses, Institute for Virus Research, and [2]Department of Late Effects, Radiation Biology Center, Kyoto University, Kyoto, Japan.
[3]3rd Department of Internal Medicine, School of Medicine, Kinki University, Osaka, Japan.
[4]ICMR, Kobe University School of Medicine, Kobe, Japan.

latency is an important step for immortalization and cohabitation of virus and host cells. Therefore, it is important to clarify the physiological factors involved in EBV latency.

Adult T-cell leukemia (ATL)-derived factor (ADF) was originally reported as an interleukin-2 (IL-2) receptor-α-inducing factor produced by human T-cell lymphotropic virus-1-positive (HTLV-1^+) T cell lines (Teshigawara *et al.*, 1985; Tagaya *et al.*, 1988). cDNA cloning of ADF revealed that ADF protein is a polypeptide consisting of 104 amino acids, having homology with *Escherichia coli* thioredoxin (TRX), which is involved in thiol-dependent redox reactions at various steps of cellular metabolism (Tagaya *et al.*, 1989). ADF is now considered to be a human homologue of TRX (Holmgren, 1985). Human TRX/ADF (hTRX/ADF) contains a redox-active disulfide/dithiol (Cys-Gly-Pro-Cys) and regulates various signal transduction pathways by changing the intracellular redox status, such as the stabilization of the glucocorticoid receptor, the enhancement of the binding of Jun–Fox complex to the activated protein-1 (AP-1) site through the reduction of redox factor-1 (Ref-1) protein (Hirota *et al.*, 1997), and restoration of the DNA-binding activity of oxidized nuclear factor (NF)-κB (Toledano and Leonard, 1991; Schenk *et al.*, 1994). In addition, hTRX/ADF has a radical scavenging activity: recombinant hTRX/ADF protects cells from hydrogen peroxide (H_2O_2)- or tumor necrosis factor-α (TNF-α)-induced cytotoxicity, in which the involvement of oxygen radicals has been implicated (Matsuda *et al.*, 1991). The expression of hTRX/ADF has been enhanced in response to a variety of oxidative stresses, including H_2O_2, X-ray, and ultraviolet exposure (Sachi *et al.*, 1995). These observations indicate that hTRX/ADF plays an important role in cellular responses against oxidative stress. Besides HTLV-1^+ T-cell lines, a high level of hTRX/ADF expression is observed in various virus-infected cell lines and cells, including EBV and human papilloma virus (HPV) (Wakasugi *et al.*, 1990; Fujii *et al.*, 1991). It has been suggested that hTRX/ADF is involved in the mechanism for controlling cytopathic stress evoked by these virus infections, although the role of hTRX/ADF in virus infections remains to be clarified.

In this report, we investigated the effects of hTRX/ADF focused on virus and host cell cohabitation in human EBV-transformed B-cell lines. EBV-transformed B-cell lines, such as Raji cells, usually regulate their viral replication tightly, maintaining viral DNA at a low copy number, possibly to establish latent infection. The lytic replication of viral DNA can be induced in these cells by treating them with chemical inducers such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA) or sodium butyrate, or by introducing a vector expressing the BZLF-1 gene of EBV (zur Hausen *et al.*, 1978; Luka *et al.*, 1979). By using this cell system, it was possible to analyze the lytic cycle distinguished from the latent phase of viral infection. The present study has revealed the key roles of hTRX/ADF on viral DNA's replication in the latent and lytic phases.

MATERIALS AND METHODS

Cell and reagents

Raji cells and B95-8 cells, EBV-transformed B-cell lines, were cultured in RPMI 1640 (GIBCO BRL) supplemented with 10% fetal calf serum (FCS). Both cell lines are latently infected with EBV and express a high level of EBNA-1 (Reisman and Sugden, 1986). TPA was obtained from Sigma Chemical Co. *N*-acetyl-L-cysteine (NAC) and reduced-glutathione (GSH) were also purchased from Sigma Chemical Co. Recombinant hTRX/ADF was provided by Ajinomoto Co.

Lytic induction of EBV replication of TPA and isolation of episomal DNA

The lytic phase of the EBV life cycle was induced by stimulating Raji cells or B95-8 cells with TPA (50 ng/ml, 10 ng/ml) in RPMI 1640 containing 5% FCS. Recombinant hTRX/ADF and TPA were added to the cell culture simultaneously. Twenty-four hours later, episomal DNA was isolated according to the method of Hirt (1967). Briefly, one million cells per sample were lysed with 400 μl of 0.6% sodium dodecyl sulfate (SDS) containing 10 m*M* EDTA. Ten minutes later, the lysate was mixed with 100 μl of 5 *M* NaCl and incubated at 4°C for 16

hr. After centrifugation at 15,000 rpm for 30 min at 4°C, the supernatant was recovered and extracted with phenol and phenol/chloroform/isoamyl alchohol. Episomal DNA in the supernatant was precipitated with ethanol. Isolated episomal DNA was subjected to Southern blot analysis.

Immunofluorescence assay

B95-8 cells were transferred to medium containing 5% FCS. TPA (10 ng/ml) and either recombinant hTRX/ADF or reduced-GSH were added to the cell culture simultaneously. After 48 hr of incubation, cells were plated onto glass slides at a density of 2×10^5 cells/ml, acetone fixed, and incubated with a 1:160 dilution of EBV(+) serum from a healthy donor (Decaussin *et al.*, 1995; Henle and Henle, 1966). EBV(−) serum was used as a negative control. Cells were washed and then incubated with 1:20 diluted fluorescein isothiocyanate (FITC)-conjugated $F(ab')_2$ fragment of rabbit anti-human immunoglobulin (IgG), specific for the γ-chain (Dako Japan). The samples were viewed on a fluorescent microscope (Oympus, BX 50WI&BX-FLA).

EBV-based vector plasmid construct

The plasmid termed as pHEBoLyt-2 was constructed previously (Teshigawara and Katsura, 1992). Briefly, the transcription unit of pcD-SRα296 (*Hin*dIII–*Sal*I fragment) was ligated into the *Hin*dIII–*Sal*I site of pHEBo. The *Bam*HI fragment of pSK^+-$lyt2^+$ containing the murine CD8α cDNA was ligated into the *Bam*HI site of the constructed vector. pHEBoLyt-2 carries the SRα promotor/enhancer, murine CD8α cDNA, the poly(A) signal, EBV Ori-P, and hygromycin B-resistant gene, as shown in Fig. 3a (below).

Transfection of plasmid DNA

Electroporation was done as reported previously. Briefly, 5×10^6 cells were washed and resuspended in 400 μl of K-PBS (30.8 m*M* NaCl, 120.7 m*M* KCl, 8.1 m*M* Na_2HPO_4, 1.46 m*M* KH_2PO_4, 5 m*M* $MgCl_2$) containing 5 μg of plasmid DNA. Electroporation was done at the condition of 500 μF, 625 V/cm using a Gene-Pulser (Bio-Rad). After the electroporation, cells were transferred into RPMI 1640 with 10% FCS. Forty-eight hours later, the transfectants were further cultured in selective medium containing 0.2 mg/ml of hygromicine B for 5 days. On day 7, recombinant hTRX/ADF was added to the cell culture. Forty-eight hours later, cells were analyzed for either the reporter gene expression by flowcytometry or the transfected plasmid's copy number by Southern blotting.

Southern blot analysis

The episomal viral DNA or transfected plasmids were cleaved with *Bam*HI endonuclease and applied to a 1% agarose gel. After electrophoresis, the DNA was transferred to nylon membranes and hybridized to a ^{32}P-labeled *Bam*HI-K fragment of EBV genome (EBNA-1) or murine CD8α cDNA. Hybridization signals were detected by autoradiography.

Flow cytometric analysis

We incubated 5×10^4 cells on ice for 15 min with or without FITC-conjugated anti-murine CD8α (Serotec). After washing cells with RPMI 1640 containing 1% FCS, cells were analyzed with a FACScan (Becton-Dickenson). The fluo-

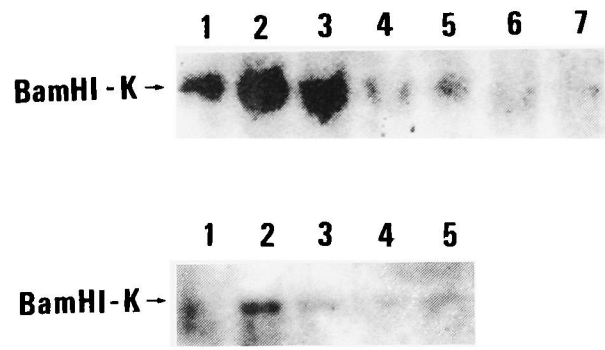
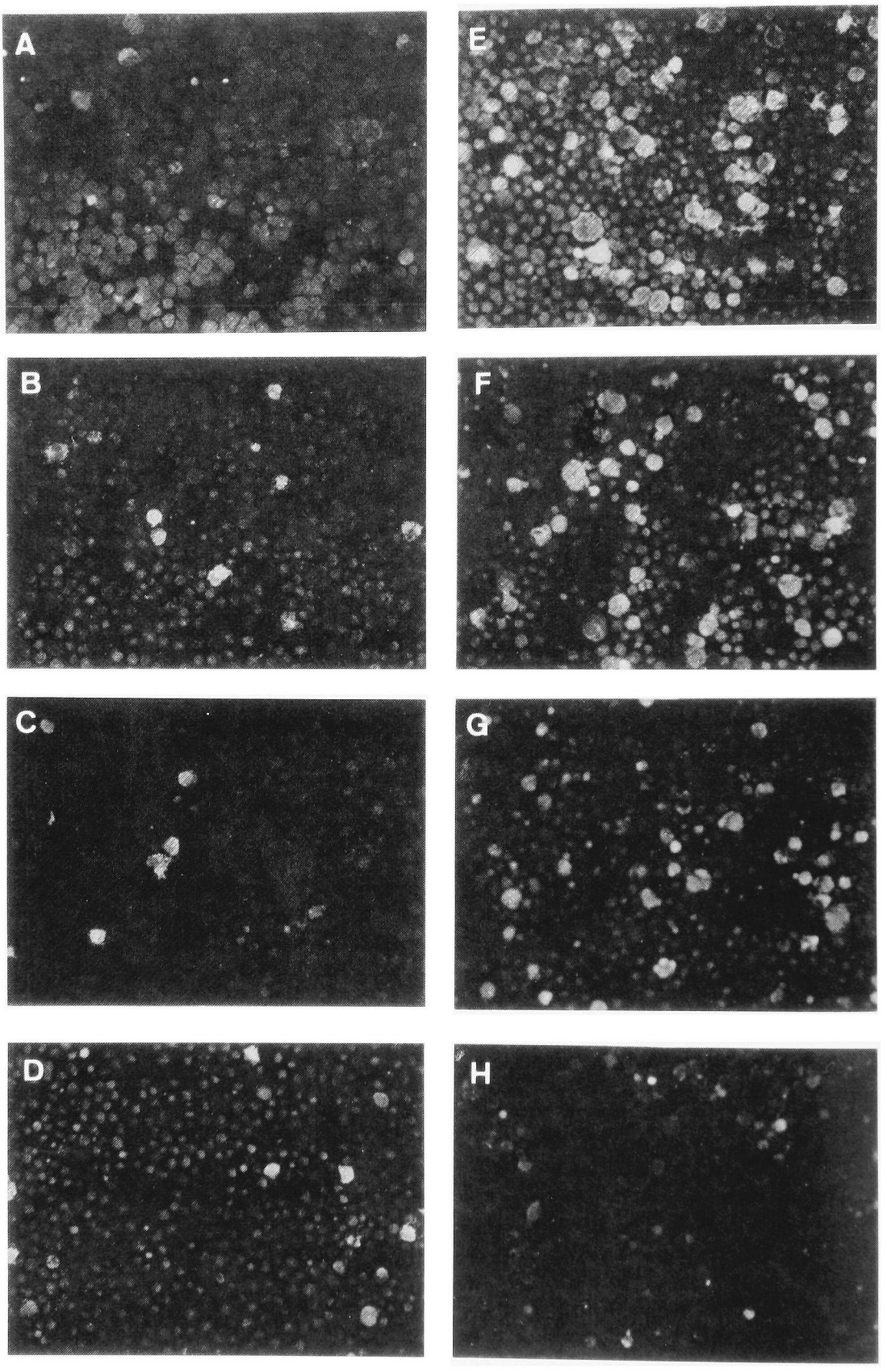


FIG. 1. Suppression of EBV genome replication by hTRX/ADF. The lytic phase of EBV was induced by stimulating (**top gel**) Raji cells with TPA (50 ng/ml) or (**bottom gel**) B95-8 with TPA (10 ng/ml) in RPMI 1640 containing 5% FCS. Recombinant hTRX/ADF was added to the medium simultaneously. **Lanes 1,** Nonstimulated; **lanes 2,** TPA(+) alone; **lanes 3,** TPA(+), hTRX/ADF 10^{-8} *M*; **lanes 4,** TPA(+), hTRA/ADF 10^{-7} *M*; **lanes 5,** TPA(+), hTRX/ADF 10^{-6} *M*; **lane 6,** TPA(−), hTRX/ADF 10^{-8} *M*; **lane 7,** TPA(−), hTRX/ADF 10^{-7} *M*. Twenty-four hours after the stimulation, episomal DNA was isolated according to the method of Hirt. The DNA was cleaved with *Bam*HI endonuclease and applied to a 1% agarose gel. After electrophoresis, the DNA was transferred to nylon membrane and hybridized to a ^{32}P-labeled *Bam*HI-K fragment of EBV genome.

a



rescence intensity was measured using a logarithmic scale.

MTT assay

On day 0, cells (1×10^4/well) were plated with 100 μl of RPMI 1640 medium containing 5% FCS in a 96-well plate. TPA (50 ng/ml) was added as well as recombinant hTRX/ADF, reduced-GSH, or NAC. On day 5, 10 μl of MTT [3-(4,5-demethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (Sigma)] at a concentration of 5 mg/ml in phosphate-buffered saline (PBS) was added to the wells. Cells were further cultured for 4 hr at 37°C. To each well was added 100 μl of *n*-propyl alcohol containing 0.04 *N* HCl. The optical densities at 590 nm were then measured with a Microplate Reader (Bio-Rad, model 3550).

RESULTS

Suppression of viral DNA's lytic amplification of hTRX/ADF

To study the effects of hTRX/ADF on amplification of viral DNA, Raji cells were induced to the lytic phase by incubation with TPA alone or TPA and recombinant hTRX/ADF. Twenty-four hours later, episomal DNA was isolated and subjected to Southern blot analysis (Fig. 1, top). The *Bam*HI-K fragment of the EBV genome is known to be conserved in various types of EBV-transformed cells, and only a single copy of this fragment is contained in one viral genome (Laux *et al.*, 1985). Therefore, the density of a detected band correlates with an episomal copy number in a

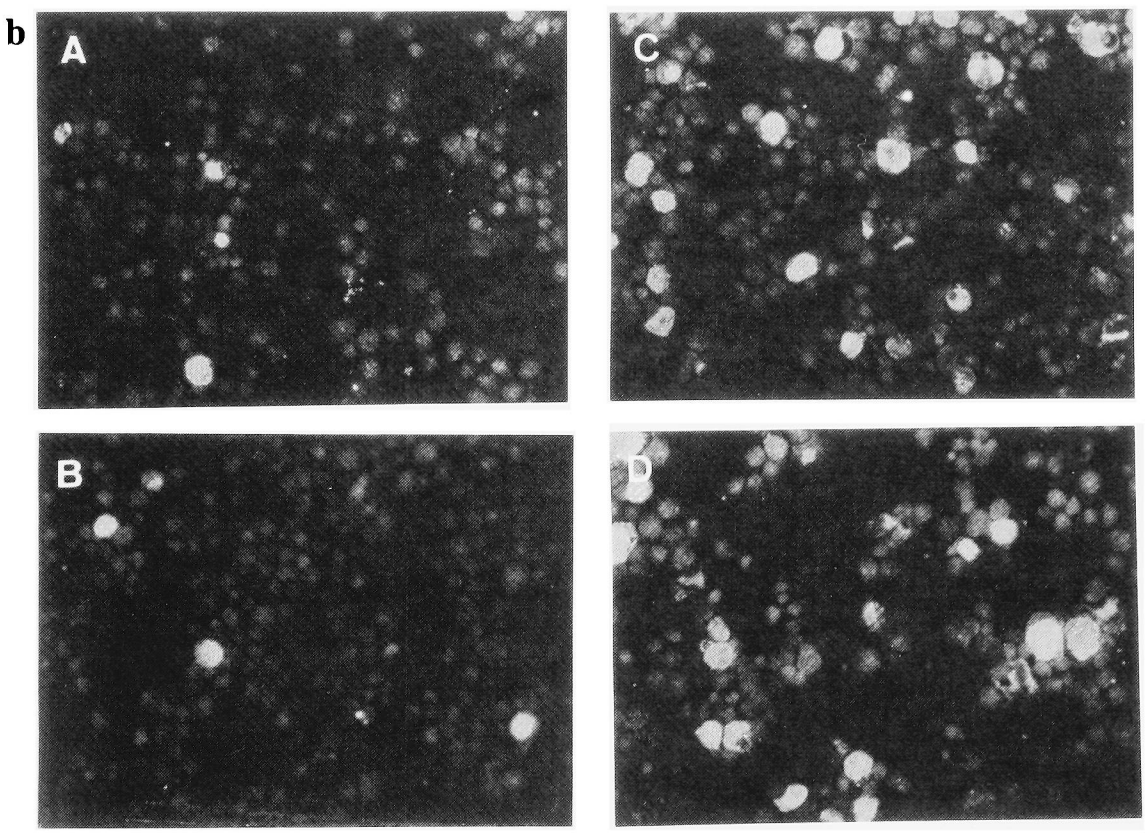


FIG. 2. Suppression of EBV lytic antigen production by hTRX/ADF. a. B95-8 cells were plated onto glass slides at a density of 2×10^5 cells/ml, acetone fixed, and incubated with a 1:160 dilution of EBV(+) serum (anti-early antigen and VCA). **A–D.** Nonstimulated. **E–H.** TPA-stimulated (10 ng/ml). Recombinant hTRX/ADF was added at a concentration of 10^{-8} *M* (**B** and **F**), 10^{-7} *M* (**C** and **G**), 10^{-6} M (**D** and **H**). **b.** The effect of GSH on lytic antigen production of B95-8 induced to the lytic phase was also evaluated. **A** and **B**. Nonstimulated. **C** and **D**. TPA-stimulated (10 ng/ml). Reduced-GSH (10^{-6} *M*) was added simultaneously (**B** and **D**).

single cell. The episomal copy number in the cells stimulated by TPA was amplified drastically, which indicates the proceeding of cells to the lytic cycle. In contrast, the addition of hTRX/ADF at 10^{-7} *M* significantly suppressed the amplification of viral DNA by TPA. B95-8 cells were also induced to the lytic phase (Fig. 1, bottom), and a similar suppression of amplifi-

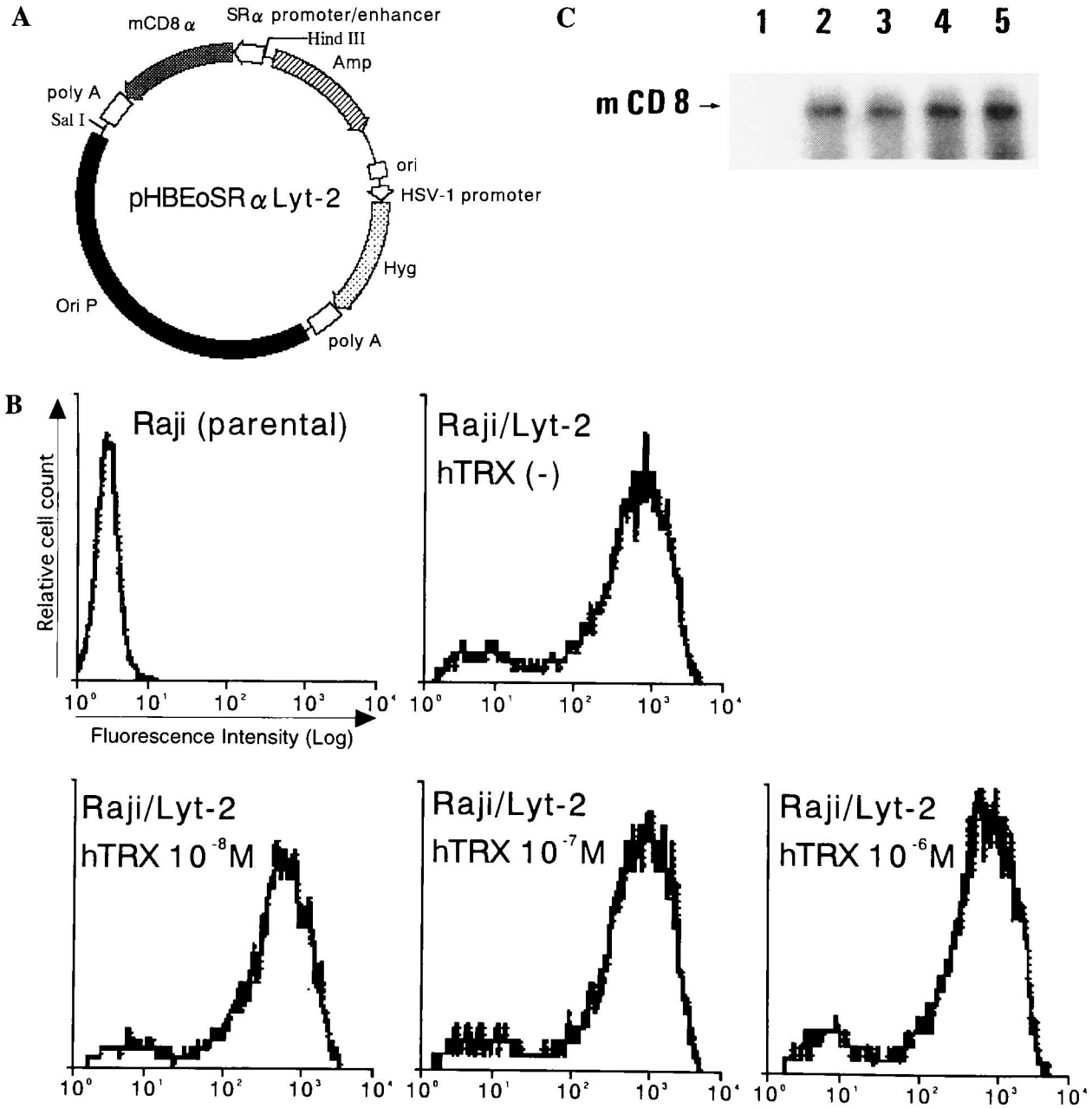


FIG. 3. Effect of hTRX/ADF in the persistent replication of EBV. A. pHEBoSRαLyt-2 carries SRα promotor/enhancer, murine CD8α cDNA, poly(A) signal, EBV OriP, and a hygromycin-resistant gene. **B.** pHEBoSRαLyt-2 was introduced to Raji cells by electroporation. Forty-eight hours after the transfection, the transfectants were further cultured in selective medium containing hygromicin B for 5 days. On day 7, recombinant hTRX/ADF was added to the medium at indicated concentrations and incubated for 48 hr. After the incubation, we performed a flow cytometric analysis of murine CD8α expression. **C.** Raji/Lyt-2 cells were cultured in medium containing 5% FCS with recombinant hTRX/ADF. **Lane 1,** Parental Raji cells; **lanes 2–5,** Raji/Lyt-2 cells; **lane 2,** hTRX/ADF(−); **lane 3,** 10^{-8} *M*; **lane 4,** 10^{-7} *M*; **lane 5,** 10^{-6} *M*). Twenty-four hours later, episomal DNA was isolated according to the method of Hirt (1967). The DNA was cleaved with *Bam*HI endonuclease and applied to a 1% agarose gel. After electrophoresis, the DNA was transferred to nylon membrane and hybridized to a ^{32}P-labeled murine CD8α cDNA.

cation of viral DNA by exogenous hTRX/ADF was observed. Moreover, inhibition of lytic antigen expression in B95-8 cells was revealed by EBV(+) serum [anti-early antigen and viral capsid antigen (VCA)] using fluorescent microscopy (Fig. 2a). We also tested for the inhibitory effect of GSH, one of the typical intracellular redox factors, on lytic antigen production; however, no significant suppression was seen at similar concentrations as used with the recombinant hTRX/ADF (Fig. 2b).

hTRX/ADF has no effects on viral DNA replication in the latent phase

We established an EBV-based vector system as a model for EBV latency to strictly determine the effect of hTRX/ADF in the latent phase. In transformed B-cell lines, a low percentage of cells spontaneously proceed to the lytic cycle (Laux *et al.*, 1988), and the results shown in Fig. 1, top (lanes 6 and 7) may not reflect the latency. Raji cells were transfected with pHBEoSRαLyt-2. The flow cytometric analysis revealed that expression of the reporter gene is not influenced by hTRX/ADF (Fig. 3B). The effect of hTRX/ADF was also evaluated on copy numbers of the transfected plasmids. As reported previously, the expression of transfected plasmid correlates with its copy number in the target cell. Southern blot analysis showed no suppression of the transfected plasmid's replication by hTRX/ADF (Fig. 3C). These findings suggested that hTRX/ADF does not inhibit viral DNA replication in the latent phase

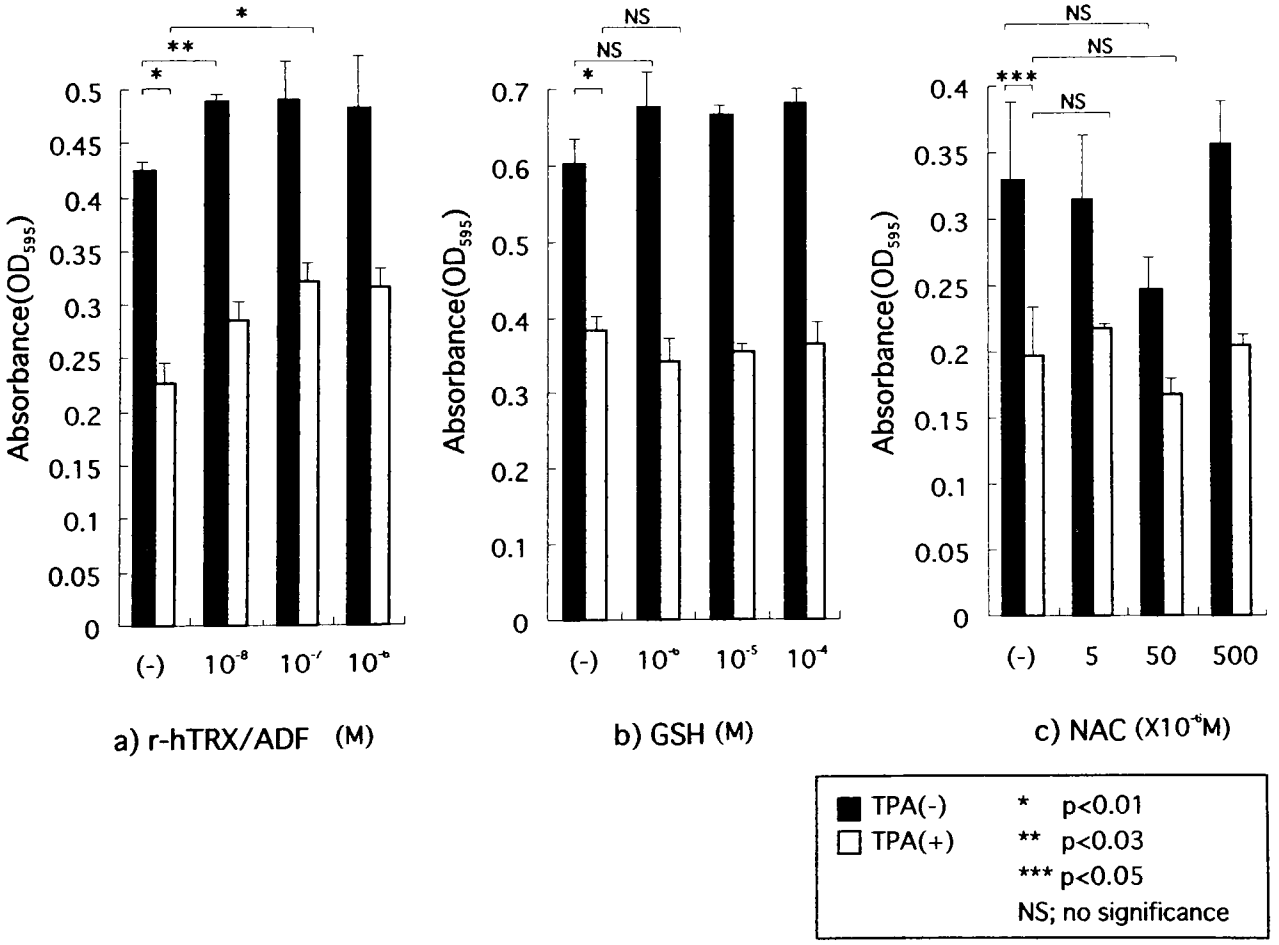


FIG. 4. Inhibition of the lytic cell death by hTRX/ADF. On day 0, cells (1×10^4/well) were plated with 100 μl of RPMI 1640 medium containing 5% FCS in a 96-well plate. TPA (50 ng/ml) was added along with recombinant hTRX/ADF **(a)**, reduced-GSH **(b)**, or NAC **(c)**. On day 5, an MTT assay was performed. hTRX/ADF, human thioredoxin/ATL-derived factor, GSH, glutathione; NAC, *N*-acetyl-L-cysteine.

of EBV infection sufficiently as it does in the lytic phase.

hTRX/ADF prevents lytic cell death by TPA

Viabilities of Raji cells in the lytic phase were evaluated by MTT assay, which was performed after cultivating the cells in medium containing TPA with or without recombinant hTRX/ADF, reduced-GSH, or NAC for 5 days. Viability in cells chemically induced to the lytic phase was reduced approximately 50%. Human TRX/ADF was shown to reverse the cell death partially, while neither GSH nor NAC had any effect on the lytic cascade in Raji cells (Fig. 4).

DISCUSSION

One particularly effective antivirus host defense is to eliminate infected cells by apoptosis. Several groups reported that viral proteins prevent infected cells from apoptosis by interacting with various host cellular pathways (McFadden, 1998). A unique selenoprotein, a gene product of the molloscum contagiosum virus, has been shown to have an antioxidant activity preventing death of infected cells (Shisler *et al.*, 1998). Viral infections evoke oxidative stress, which switches on apoptotic cascades in host cells. Therefore, we suspect that viruses utilize the host cell's antioxidant systems to evade the host's defense mechanisms. There is strong evidence indicating the hTRX/ADF-mediated antioxidant system plays an important role in virus–host cohabitation. As previously reported (Yodoi and Truz, 1991), exogenous hTRX/ADF promotes cell proliferation of EBV-transformed cell lines such as 3B6 and HTLV-1-transformed cell line ATL-2. Recent study has revealed that HTLV-1 Tax transactivates the expression of hTRX/ADF (Masutani *et al.*, 1996). A competitive inhibitor of hTRX/ADF reductase, 13-*cis*-retinoic acid induces apoptosis in HTLV-1 transformed T cells, but not in non-transformed T cells (Masutani *et al.*, 1992; Furuke *et al.*, 1997). In HPV, there is a remarkable correlation between hTRX/ADF production and the presence of viral DNA in human cervical carcinoma tissues. There are also several reports suggesting the relationship between other antioxidants and viral infection. For example, pyrrolidine-dithiocarbamate (PDTC) is known to induce changes of the AP-1 transcription complex and selectively downregulate HPV transcription (Rosl *et al.*, 1997). Moreover, human immunodeficiency virus (HIV) replication and the death of infected cells are inhibited by NAC, GSH precursor (Roederer *et al.*, 1990; Malorni *et al.*, 1993), or lecithinized superoxide dismutase (Premanathan *et al.*, 1997). Although these reagents were used at extremely high concentrations (over 5 m*M*), these reports collectively indicate the possibility that latency of these viral infections are highly dependent on intracellular redox regulations.

In the present study, we demonstrated the suppressive effect of recombinant hTRX/ADF on the lytic amplification of EBV, giving growth advantage to the host cell at a physiological concentration. As hTRX/ADF blocks overt EBV replication and is more advantagous to the growth of host cells than to GSH or NAC, there may exist hTRX/ADF-specific target molecule(s) in the inhibitory mechanism of the viral DNA's lytic replication. Another important finding is that hTRX/ADF could not inhibit EBV replication in the latent phase. The distinct effects of hTRX/ADF on latent and lytic EBV replication suggest that hTRX/ADF plays an important role in the establishment of latent infection.

In EBV infection, latent replication proceeds from ori-P, which is composed of two domains (Puglielli *et al.*, 1996). Region I contains 20 tandemly repeated binding sites for EBNA-1 and functions as an EBNA 1-dependent enhancer whose activity is important for both ori-P replication and transactivation of the *Bam*HI-C latency promoter. Region II contains two pairs of overlapping EBNA-1-binding sites and is the site of initiation of latency replication. Virus replication in the latent phase is tightly regulated, possibly to permit the cohabitation of virus and host cells. On the other hand, lytic DNA replication proceeds from ori-Lyt (Fixman *et al.*, 1992). In the prototype EBV genome, there are two copies of ori-Lyt, one in DS-L and one in DS-R. It contains two binding sites for Rta (BRLF1) and one for Zta (BZLF-1, ZEBRA). It has been indicated that the initial even in the

lytic cycle is activation of BZLF-1 transcription (Schepers *et al.*, 1993; Sarisky *et al.*, 1996). The BZLF-1 promotor (Zp) region has two distinct TPA-response elements and subsequently increases AP-1-binding activity. The initial responses of Zp to changes in the activity of cellular factors are likely to play a crucial role in the disruption of viral latency.

Activation of the BZLF-1 gene by chemical inducers requires *de novo* cellular protein synthesis. The candidate of hTRX/ADF-target during expression of EBV-specific proteins in TPA-treated Raji cells might be redox-sensitive cellular molecules involved in controlling the activation of the EBV immediate-early gene. ZEBRA is related to the AP-1 family of regulatory proteins with transcription factor activity. Since hTRX/ADF regulates the binding of Jun–Fos complex to the AP-1 site, it is likely that hTRX/ADF influences the functions of ZEBRA. There is also an interest report that indicates inhibition of ZEBRA functions by NF-κB p65 and physical interaction between ZEBRA and p65 both *in vitro* and *in vivo* (Gutsch *et al.*, 1994; Hong *et al.*, 1997). In most cell types, NF-κB is retained in cytoplasm as an inactive complex with its inhibitory protein I-κB. However, in B cells, a certain amount of NF-κB is present in the nucleus constitutively as a heterodimer of the p50 and p65 (Lenardo and Baltimore, 1989). Human TRX/ADF restores the DNA binding activity of oxidized NF-κB in cell-free systems. However, it has been reported that exogenous hTRX/ADF suppresses TPA-induced DNA binding of NF-κB. Therefore, another possible mechanism for the inhibitory effect of hTRX/ADF on chemically induced lytic amplification of EBV may be by its indirect enhancing of the p65/ZEBRA-interaction.

Our findings strongly support the hypothesis that hTRX/ADF is one of the important host cellular factors involved in virus and host cohabitation. Further investigations are in progress to clarify the mechanism of redox regulation by hTRX/ADF in lytic amplification of EBV and the establishment of latency. Understanding a critical regulatory role of hTRX/ADF in EBV infection may lead to a new therapeutic strategy for treating EBV and other cytopathic viral infections including AIDS.

ACKNOWLEDGMENTS

We are grateful to E. Klein, W. Droege, and H. zur Hausen for critical reading of the manuscript and to Y. Yamaguchi for her technical assistance and Y. Kanekiyo for manuscript preparation. This work is supported by Grants-in-Aid for Research for the Future Program from the Japan Society for the Promotion of Science and the Smoking Research Foundation.

ABBREVIATIONS

ADF, ATL-derived factor; AIDS, acquired immunodeficiency syndrome; ATL, adult T-cell leukemia; AP-1, activated protein-1; EBV, Epstein-Barr virus; FCS, fetal calf serum; FITC, fluoroisothiocyanate; GSH, glutathione; H_2O_2, hydrogen peroxidase; HIV, human immunodeficiency virus; HPV, human papilloma virus; HTLV-1, human T-cell lymphotropic virus-1; IgG, immunoglobulin G; IL-2, interleukin-2; MTT, 3-(4,5-demethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NAC, *N*-acetyl-L-cysteine; NF-κB, nuclear factor kappa B; PBS, phosphate-buffered saline; PDTC, pyrrolidine-dithiocarbamate; Ref-1, redox factor-1; SDS, sodium dodeyl sulfate; TNF-α, tumor necrosis factor-α; TPA, tumor promoting agent or phorbol ester; TRX, thioredoxin; VCA, viral capsid antigen.

REFERENCES

DECAUSSIN, G., LECLERC, V., and OOKA, T. (1995). The lytic cycle of Epstein-Barr virus in the nonproducer Raji line can be rescued by the expression of a 135-kilodalton protein encoded by the BALF2 open reading frame. J. Virol. **69,** 7309–7314.

FIXMAN, E.D., HAYWARD, G.S., and HAYWARD, S.D. (1992). Trans-acting requirements for replication of Epstein-Barr virus ori-Lyt. J. Virol. **66,** 5030–5039.

FUJII, S., NANBU, Y., NONOGAKI, H., KONISHI, I., MORI, T., MASUTANI, H., and YODOI, J (1991). Co-expression of adult T-cell leukemia-derived factor, a human thioredoxin homologue, and human papillomavirus DNA in neoplastic cervical squamous epithelium. Cancer **68,** 1583–1591.

FURUKE, K., SASADA, T., UEDA-TANIGUCHI, Y., YAMAUCHI, A., INAMOTO, T., YAMAOKA, Y., MASUTANI, H., and YODOI, J. (1997). Role of intracellular redox status in apoptosis induction of human T-cell leukemia virus type I-infected lymphocytes by 13-cis-retinoic acid. Cancer Res. **57,** 4916–4923.

GUTSCH, D.E., HOLLEY-GUTHRIE, E.A., ZHANG, Q., STEIN, B., BLANAR, M.A., BALDWIN, A.S., and KENNEY, S.C. (1994). The bZip transactivator of Epstein-Barr virus, BZLF-1, functionally and physically interacts with the p65 subunit of NF-κB. Mol. Cell. Biol. **14,** 1939–1948.

HAMMERSCHMIDT, W., and SUGDEN, B. (1988). Identification and characterization of oriLyt, a lytic origin of DNA replication of Epstein-Barr virus. Cell **55,** 427–433.

HENLE, G., and HENLE, W. (1966). Immunofluorescence in cells derived from Burkitt's lymphoma. J Bacteriol. **91,** 1248–1256.

HIROTA, K., MATSUI, M., IWATA, S., NISHIYAMA, A., MORI, K., and YODOI, J. (1997). AP-1 transcriptional activity is regulated by a direct association between thioredoxin and Ref 1. Proc. Natl. Acad. Sci. USA **94,** 3633–3638.

HIRT, B. (1967). Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. **26,** 365–369.

HOLMGREN, A. (1985). Thioredoxin. Annu. Rev. Biochem. **54,** 237–271.

HONG, Y., HOLLEY-GUTHRIE, E., and KENNEY, S. (1997). The bZip dimerization domain of the Epstein-Barr virus BZLF-1 (Z) protein mediates lymphoid-specific negative regulation. Virology **229,** 36–48.

KAWANISHI, M. (1993). Epstein-Barr virus induces fragmentation of chromosomal DNA during lytic infection. J. Virol. **67,** 7654–7658.

LAUX, G., FREESE, U., and BORNKAMM, G.W. (1985). Structure and evolution of two related transcription units of Epstein-Barr virus carrying small tandem repeats. J. Virol. **56,** 987–995.

LAUX, G., FREESE, U., FISCHER, R., POLACK, A., KOFLER, E., and BORNKAMN, G.W. (1988). TPA-inducible Epstein-Barr virus genes in Raji cells and their regulation. Virology **162,** 503–507.

LENARDO, M.J., and BALTIMORE, D. (1989). NF-κB: A pleiotropic mediator of inducible and tissue-specific gene control. Cell **58,** 227–229.

LINDAHL, T., ADAMS, A., BJURSELL, G., BORNKAMM, G.W., KASCHKA-DERICH, B., and JEHN, U. (1976). Covalently closed circular duplex DNA of Epstein-Barr virus in a human lymphoid cell line. J. Mol. Biol. **102,** 511–530.

LUKA, J., KALLIN, B., and KLEIN, G. (1979). Induction of the Epstein-Barr virus (EBV) cycle in latently infected cells by n-butyrate. Virology **91,** 228–231.

MALORNI, W., RIVABENE, R., SANTINI, T., and DONELLI, G. (1993). N-acetylcysteine inhibits apoptosis and decreases viral particles in HIV-chronically infected U937 cells. FEBS Lett. **327,** 75–78.

MASUTANI, H., NAKAMURA, H., UEDA, Y., KITAOKA, Y., KAWABE, T., IWATA, S., MITSUI, S., and YODOI, J. (1992). ADF (adult T-cell leukemia-derived factor)/human thioredoxin and viral infection: Possible new therapeutic approach. Microbiol. Infect. 265–274.

MASUTANI, H., HIROTA, K., SASADA, T., UEDA-TANIGUCHI, Y., TANIGUCHI, Y., SONO, H., and YODOI, J. (1996). Transactivation of an inducible anti-oxidative stress protein, human thioredoxin by HTLV-I Tax. Immunol. Lett. **54,** 67–71.

MATSUDA, M., MASUTANI, H., NAKAMURA, H., MIYAJIMA, S., YAMAUCHI, A., YONEHARA, S., UCHIDA, A., IRIMAJIRI, K., HORIUCHI, A., and YODOI, J. (1991). Protective activity of adult T cell leukemia-derived factor (ADF) against tumor necrosis factor-dependent cytotoxity on U937 cells. J. Immunol. **147,** 3837–3841.

McFADDEN, G. (1998). Even virus can learn to cope with stress. Science **279,** 40–41.

PREMANATHAN, M., NAKASHIMA, H., IGARASHI, R., MIZUSHIMA, Y., and YAMADA, K. (1997). Lecithinized superoxide dismutase: An inhibitor of human immunodeficiency virus replication. AIDS Res. Hum. Retroviruses. **13,** 283–290.

PUGLIELLI, M.T., WOISETSCHLAEGER, M., and SPECK, S.H. 91996). oriP is essential for EBNA gene promoter activity in Epstein-Barr virus-immortalized lymphoblastoid cell lines. J. Virol. **70,** 5758–5768.

REISMAN, D., and SUGDEN, B. (1986). Trans-activation of an Epstein-Barr viral (EBV) transcriptional enhancer by the EBV nuclear antigen 1. Mol. Cell. Biol. **6,** 3838–3846.

ROEDERER, M., STAAH, F.J.T., RAJU, P.A., ELA, S.W., HERZENBERG, L.A., and HERZENBERG, L.A. (1990). Cytokine-stimulated human immunodeficiency virus replication is inhibited by N-acetyl-L-cysteine. Proc. Natl. Acad. Sci. USA **87,** 4884–4888.

ROSL, F., DAS, B.C., LENGERT, M., GELENTNEKY, K., and ZUR HAUSEN, H. (1997). Antioxidant-induced changes of the AP-1 transcription complex are paralleled by a selective suppression of human papillomavirus transcription. J. Virol. **71,** 362–370.

SACHI, Y., HIROTA, K., MASUTANI, H., TODA, K., OKAMOTO, T., TAKIGAWA, M., and YODOI, J. (1995). Induction of ADF/TRX by oxidative stress in keratinocytes and lymphoid cells. Immunol. Lett. **44,** 189–193.

SARISKY, R.T., GAO, Z., LIEBERMAN, P.M., FIXMAN, E.D., HAYWARD, G.S., and HAYWARD, S.D. (1996). A replication function associated with the activation domain of the Epstein-Barr virus Zta transactivator. J. Virol. **70,** 8340–8347.

SCHENK, H., KLEIN, M., EEDBRUGGER, W., DRÖGE, W., and SCHULZE-OSTHOFF, K. (1994). Distinct effects of thioredoxin and antioxidants on the activation of transcription factors NF-κB and AP-1. Proc. Natl. Acad. Sci. USA **91,** 1672–1676.

SCHEPERS, A., PICH, D., and HAMMERSCHIMIDT, W. (1993). A transcription factor with homology to the AP-1 family links RNA transcription and DNA replication in the lytic cycle of Epstein-Barr virus. EMBO J. **12,** 3921–3929.

SHISLER, J.L., SENKEVICH, T.G., BERRY, M.J., and MOSS, B. (1998). Ultraviolet-induced cell death blocked by a selenoprotein from a human dermatotropic poxvirus. Science **279,** 102–104.

TAGAYA, Y., OKADA, M., SUGIE, K., KASAHARA, T., KONDO, N., MATSUSHIMA, K., DINARELLO, A.C.,

and YODOI, J. (1988). IL-2 receptor/Tac(p55) inducing factor: Purification and characterization of ATL-derived factor (ADF). J. Immunol. **140,** 2614–2620.

TAGAYA, Y., MAEDA, Y., MITSUI, A., KONDO, N., MATSUI, H., HAMURO, J., BROWN, N., ARAI, K., YOKOTA, K., WAKASUGI, H., and YODOI, J. (1989). ATL-derived factor (ADF), an IL-2 receptor/Tac inducer homologous to thioredoxin; Possible involvement of dithiol-reduction in the IL-2 receptor induction. EMBO J. **8,** 757–764.

TESHIGAWARA, K., and KATSURA, Y. (1992). A simple and efficient mammalian gene expression system using an EBV-based vector transfected by electroporation in G2/M phase. Nucleic Acids Res. **20,** 2607.

TESHIGAWARA, K., MAEDA, M., NISHINO, K., NIKAIDO, T., UCHIYAMA, M., TSUDO, M., WANO, M., and YODOI, J. (1985). Adult T leukemia cells produce a lymphokine that augments interleukin 2 receptor expression. J. Mol. Cell. Immunol. **2,** 17–26.

TOLEDANO, M.B., and LEONARD, W.J. (1991). Modulation of transcription factor NF-κB binding activity by oxidation-reduction in vitro. Proc. Natl. Acad. Sci. USA **88,** 4328–4332.

WAKASUGI, N., TAGAYA, Y., WAKASUGI, H., MITSUI, A., MAEDA, M., YODOI, J., and TURSZ, T. (1990). ADF/thioredoxin produced by both HTLV-I and EBV transformed lymphocytes acts as an autocrine growth factor[ ]and synergizes with IL-1 and IL-2. Proc. Natl. Acad. Sci. USA **87,** 8282–8286.

YODOI, J., and TRUSZ, T. (1991). ADF, a growth-promoting factor derived from adult T-cell leukemia and homologous to thioredoxin: Involvement in lymphocyte immortalization by HTLV-I and EBV. Adv. Cancer, Res. **57,** 381–411.

ZUR HAUSEN, H., O'NEILL F., and FREESE, U. (1978). Persisting oncogenic herpes virus induced by the tumor promoter TPA. Nature **276,** 270–272.

Address reprint requests to:
Dr. J. Yodoi
Department of Biological Responses
Institute for Virus Research, Kyoto University
53 Kawahara-cho, Shogoin, Sakyo
Kyoto, 606, Japan

This article has been cited by:

1. Hajime Nakamura, Norihiko Kondo, Kiichi Hirota, Hiroshi Masutani, Junji YodoiThiols and Thioredoxin in Cellular Redox Control **20035754**, . [CrossRef]
2. T Sasada. 2002. Thioredoxin-mediated redox control of human T cell lymphotropic virus type I (HTLV-I) gene expression. *Molecular Immunology* **38**:10, 723-732. [CrossRef]
3. Yumiko Nishinaka , Hajime Nakamura , Noriko Okada , Hidechika Okada , Junji Yodoi . 2001. Redox Control of EBV Infection: Prevention by Thiol-Dependent Modulation of Functional CD21/EBV Receptor ExpressionRedox Control of EBV Infection: Prevention by Thiol-Dependent Modulation of Functional CD21/EBV Receptor Expression. *Antioxidants & Redox Signaling* **3**:6, 1075-1087. [Abstract] [PDF] [PDF Plus]