UPREGULATION OF INTERLEUKIN-1 BY EPSTEIN–BARR VIRUS LATENT MEMBRANE PROTEIN 1 AND ITS POSSIBLE ROLE IN NASOPHARYNGEAL CARCINOMA CELL GROWTH

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Abstract: Background. Nasopharyngeal carcinoma (NPC) is associated with Epstein–Barr virus (EBV) infection. We previously found that interleukin (IL)-1α and IL-1β significantly increased in NPC tissues. This study investigated what EBV-encoded proteins were involved in such IL-1 production.

Methods and Results. IL-1α and IL-1β messenger ribonucleic acids (mRNAs) were detected in the EBV latent membrane protein 1 (LMP1) transfectant (LMP135) only by reverse transcriptase–polymerase chain reaction (RT-PCR). LMP1-mediated IL-1α and IL-1β production could be enhanced by tumor necrosis factor alpha (TNF-α), determined by enzyme-linked immunosorbent assay (ELISA). Moreover, IL-1α and IL-1β mRNAs and proteins were increased in a dose-dependent manner in epithelial cells transiently transfected by an LMP1 plasmid. Besides, immortalized human epidermal keratinocyte (RHEK-1) epithelial cells could be enhanced to proliferate by IL-1α and IL-1β determined by water-soluble tetrazolium salt (WST-1) assay.

Conclusions. EBV LMP1 is capable of upregulating IL-1α and IL-1β secretions from epithelial cells and positively modulated by TNF-α. This may consequently contribute to tumor growth in patients with NPC. © 2009 Wiley Periodicals, Inc.

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Keywords: interleukin-1; Epstein–Barr virus; latent membrane protein 1; nasopharyngeal carcinoma; cell growth

Epstein–Barr virus (EBV) is a ubiquitous human gamma herpes virus that is associated with many diseases, such as nasopharyngeal carcinoma (NPC) and Hodgkin’s disease (HD), for example.1,2 In both NPC and HD, tumor cells and infiltrating lymphocytes produce abundant cytokines, including interferon gamma (IFN-γ), interleukin (IL)-1, IL-2, IL-6, IL-8, IL-10, IL-12, and IL-13.3–5 Among these cytokines, IL-1 alpha (IL-1α) and IL-1 beta (IL-1β) are well known to play an important role in the process of inflammation and tumorigenesis.6,7 This was supported by accumulating evidence that significant expressions of IL-1α and
IL-1β were found in several human malignancies, such as breast cancer, lung cancer, and head and neck squamous cell carcinoma.5–10 Our group was the first to demonstrate that both transcript and protein expressions of IL-1α and IL-1β were specifically upregulated in NPC and metastasis lymph node biopsies compared with nasopharyngeal control tissues.3 The expressions of IL-1α and IL-1β were obviously present in the malignant epithelial cells and infiltrating CD4+ T cells, suggesting that IL-1α and IL-1β may contribute to tumor growth and lymphocyte infiltration in NPC development.

An EBV envelope glycoprotein gp350 could upregulate the synthesis of IL-1β through binding with cellular surface molecules in human monocytes/macrophages.11 Human peripheral blood neutrophils produce IL-1 after EBV infection via gp350 effects.12 Additionally, EBV latent membrane protein 1 (LMP1) could induce IL-6, IL-8, and IL-10 secretions that are likely to influence the growth of epithelial cells, lymphoblastoid cell lines, and/or Burkitt’s lymphoma cells.13,14 In vivo, many studies have shown that LMP1 induces expression of IL-8 through the nuclear factor kappa B (NF-κB) binding site in NPC biopsies and may contribute to angiogenesis in NPC.15 Therefore, it is mandatory to know the cause-and-effect relationship between viral gene products and cytokine production, to elucidate the etiological role of EBV in NPC tumorigenesis. Because increased IL-1α and IL-1β expression was found in EBV-positive tumor cells, it is important to determine which EBV product is related to the increment of IL-1α and IL-1β. In the present study, we analyzed the messenger ribonucleic acids (mRNAs) of cytokines in several epithelial cell lines and transfectants that stably expressed EBV-encoded nuclear antigen 1 (EBNA1), LMP1, or LMP2A, which were the major EBV gene products in NPC tissues. In previous studies, we detected a series of cytokines, such as tumor necrosis factor alpha (TNF-α), expressed in NPC biopsies.15 Therefore, the effect of coexisting cytokine TNF-α on LMP1-mediated IL-1 expression in epithelial cells was examined. Subsequently, the effects of IL-1α and IL-1β on epithelial cell proliferation were also investigated.

**MATERIALS AND METHODS**

**Cells.** Three cell lines—including RHEK-1, 293, and NPC-Tw-01—and their stable transfectants that were previously established in this laboratory were used in this study. In detail, RHEK-1 is a nonmalignant human epithelial cell line immortalized from foreskin keratinocytes with adenovirus type 12-simian virus 40. LMP135 and RR3 are clones derived from LMP1 and control vector transfected RHEK-1 cells, respectively.16 In addition, several EBV protein-expressing stable clones and their paired vector controls were used, including EBNA1/RHEK-1 cells and Vector-1, EBNA1/293 cells and Vector-2, and LMP2A/293 cells and Vector-3, respectively.16 NPC-Tw-01, an EBV-negative epithelial cell line, is derived from an NPC biopsy.17

**RNA Extraction and Reverse Transcriptase-Polymerase Chain Reaction.** The methods to extract RNA and to amplify specific transcript signals from the samples were previously described.3 Sequences of the primers used in the reverse transcriptase–polymerase chain reaction (RT-PCR) to detect expression of cytokines IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, TNF-α, transforming growth factor beta (TGF-β), and INF-γ and expression of receptors IL-1RI and IL-1RII were also shown in the same report. Briefly, single-stranded complementary deoxyribonucleic acid (cDNA) was generated in the presence of M-MLV Reverse Transcriptase RNase H Minus (Promega, Madison, WI). RT-PCR for β-actin served as RNA quality and quantity control. All positive controls of RT-PCR were derived from peripheral blood mononuclear cells stimulated with 5 μg/mL phytohemagglutinin (PHA). Negative controls were carried out in the same way but without reverse transcriptase.

**Real-Time Quantitative Reverse Transcriptase-Polymerase Chain Reaction.** Quantitative reverse transcriptase–polymerase chain reaction (RT-Q-PCR) for IL-1α, IL-1β, and internal control glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed by amplifying the cDNA derived from 100 ng deoxyribonucleic acid (DNase I) pretreated total RNA in a TaqMan PCR Core Reagents Kit (PerkinElmer, Waltham, MA) in a reaction volume of 50 μL, except the Taq enzyme was replaced by Dynazyme II DNA polymerase (Finzymes Oy, Espoo, Finland). The reaction was initiated by heating at 50°C for 2 minutes, then 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds, and terminated by 60°C for 1 minute in a PerkinElmer Applied Biosystems 7700 Sequence Detector.
EBV Latent Membrane Protein 1 and IL-1 Expression

(PerkinElmer). Each sample was analyzed in duplicate. The IL-1α system consisted of the amplification primers: sense 5'-GAC CTG GCC ATC GC-3'; antisense 5'-TTT CAC ATT GCT CAG GAAG-3', and the dual-labeled fluorescent probe 5'-VIC CTC AGA GGA AGA AAT CAT CAA GCC TAG GTC AGC (TAMRA)-3'. The IL-1β system consisted of the amplification primers: sense 5'-TCG CCC TCT GGA TGG CG-3'; antisense 5'-TCG CTG AAG CCC TTG CTG-3', and the dual-labeled fluorescent probe 5'-VIC CAT CCA GCT ACG AAT CTC CGA CCA CC (TAMRA)-3'. IL-1 cutoff values of the samples were normalized by the average cutoff value of GAPDH. The relative fold increase or decrease of IL-1 mRNA was standardized using the IL-1 cutoff values of untransfected RHEK-1 as reference.

**Transfection of Epithelial Cells.** To verify the relationship between LMP1 and IL-1, NPC-Tw-01 was transiently transfected with LMP1 plasmid of different concentrations. NPC-Tw-01 cells were seeded in 6-well plates for 20 hours before transfection. Just before liposome transfection, the cells were fed with fresh Dulbecco's modified Eagle's medium (DMEM; Gibco BRL/Life Technologies, Grand Island, NY) containing 10% fetal calf serum (FCS) for 4 hours. The transfection was accomplished by using Gene PORTER 2 Transfection Reagent (Gene Therapy System, San Diego, CA) in accord with the manufacturer's instructions. Briefly, liposome and plasmids were premixed for 20 seconds, and the mixtures were incubated at room temperature for 20 minutes before dropping into the cells. After 37°C for 4 hours, the same volume of DMEM with 20% FCS was added and incubated at 37°C for 30 hours. Lysates and culture supernatant of the transfected cells were collected for the RT-Q-PCR analyses and enzyme-linked immunosorbent assays (ELISA), respectively. Expressions of IL-1α and IL-1β transcripts and the amounts of secreted IL-1α and IL-1β proteins were measured.

**WST-1 Cell Growth Assay.** To test whether IL-1α and IL-1β can promote RHEK-1 cells to grow, cell proliferation assays using water-soluble tetrazolium salt (WST-1; Boehringer Mannheim GmbH, Mannheim, Germany) reagent was performed. Roughly 2000 RHEK-1 cells were seeded in the well of a 96-well plate overnight. The next day, culture medium was changed by serum-free medium DMEM supplied with various concentrations of purified recombinant human IL-1α or IL-1β (R&D Systems, Minneapolis, MN) for 5 days. During treatments, the culture media were refreshed every 2 days. Each experiment was set in triplicate. The fifth day, cultures of the IL-1α- and IL-1β-stimulated cells were reacted with 10 μL of cell proliferation reagent WST-1 for 30 minutes, and the samples were measured at a 450-nm wavelength using a Metertech 960 microplate reader (Metertech, Taipei, Taiwan), with a blank as the background control.

**ELISA for Interleukin-1.** All the sample cells tested for secretion of interleukin-1 (IL-1) were cultured in 10% FCS DMEM for 24 or 48 hours. Supernatant (1.5 mL) of the culture medium was collected and concentrated to 150 μL by a Microcon YM-10 centrifugal filter device (Millipore, Bedford, MA). Secreted IL-1α or IL-1β was detected by a specific Quantikine ELISA kit (R&D Systems) as the manufacturer described.

**Tumor Necrosis Factor-α Treatment.** LMP135 cells were cultured in the presence of 2000 units/mL of tumor necrosis factor alpha (TNF-α; R&D Systems) at 37°C for 6 hours. The IL-1α and IL-1β were detected by RT-Q-PCR and cytokine ELISA kits.

**Statistical Analysis.** The means and standard errors of all data were calculated, and the differences between experiments were determined by the Wilcoxon signed-rank test, in which p < .05 is regarded as significant. All statistical analytic work was accomplished with Statistical Analysis Software (SAS Institute, Cary, NC).

**RESULTS**

**IL-1α and IL-1β mRNAs Exclusively Detected in LMP1-Expressing Cells.** Several cytokines and receptors in previously established epithelial transfectants, including RHEK-1 cells or 293 cells, which stably express EBNA1, LMP1, or LMP2A, were analyzed by RT-PCR.18,19 The results are shown in Table 1. Three expression patterns were found: (1) TGF-β was detected in all cells examined; (2) certain cytokines were expressed in a cell-type–specific way, regardless of the transfected plasmids; they were IFN-γ in 293 cells and IL-1 receptor type I (IL-1RI) in...
RHEK-1 cells; and (3) IL-1α and IL-1β were exclusively present in the LMP1-expressing RHEK-1 cells (LMP135). Figure 1 shows the cDNA fragments of IL-1α and IL-1β transcripts from the RT-PCR products. The 808 base pairs (bps) IL-1α cDNA and 295 bps IL-1β cDNA were detected only in LMP135 cells, but not in the vector-transfected RHEK-1 control cells (RR3), EBNA1/RHEK-1, EBNA1/293, or LMP2A/293 cells. The remaining cytokines, including IL-2, IL-4, IL-5, IL-6, IL-10, and TNF-α, and receptor IL-1RII were not detected in any of the transfectants.

Both IL-1α and IL-1β mRNAs and Proteins Upregulated by LMP1 and Enhanced by TNF-α in LMP135 Cells. LMP1-induced IL-1α and IL-1β synthesis in the LMP135 epithelial cells was further confirmed by RT-Q-PCR and ELISA, as shown in Figure 2. An approximately 18.5-fold increase in expression of IL-1α and 14.5-fold increase in expression of IL-1β mRNA (Figures 2A and 2B) were obtained in LMP135 cells compared with the parental RHEK-1 cells. Furthermore, secreted IL-1α and IL-1β proteins were also significantly increased from these stable transfectants. The results revealed that LMP135 produced 389 pg/mL IL-1α and 306 pg/mL IL-1β in comparison with trivial amounts of IL-1α and IL-1β (<72 pg/mL) in parental and vector control cells (Figures 2C and 2D). After treatment with 2000 U/mL TNF-α for 6 hours, LMP1-mediated IL-1α and IL-1β synthesis was enhanced (black bars in Figures 2A–2D). There were no effects on IL-1α and IL-1β expression in parental RHEK-1 or RR3 cells.

IL-1α and IL-1β Expression Dose-Dependently Correlated with LMP1. Expression of IL-1α and IL-1β transcripts and the amount of secreted IL-1α and IL-1β proteins of transiently LMP1 transfected NPC-Tw-01 cells is shown in Figures 3A–3D. Data were derived from 5 independent experiments. IL-1α and IL-1β were increased in a dose-dependent manner at both transcriptional and translational levels. There was statistical significance (p < .05; Wilcoxon signed-rank test) between data of 5 μg LMP1 and 5 μg vector in all 4 panels. Similar results were also obtained in RHEK-1 cells transiently transfected by the LMP1 plasmid (data not shown).

IL-1α and IL-1β Enhanced Growth of RHEK-1 Epithelial Cells. The results of WST-1 assay testing whether IL-1α and IL-1β can promote RHEK-1 to grow are shown in Table 2. The number of RHEK-1 epithelial cells increased in parallel with the concentrations of IL-1α and IL-1β. Cell numbers were increased with statistical significance after treating with 10 and 100 U/mL IL-1α and 500 U/mL IL-1β compared with untreated RHEK-1 cells (p < .05; Wilcoxon signed-rank test).

DISCUSSION

Cells. Because NPC is a tumor of epithelial origin, RHEK-1 was considered to express EBV proteins. Besides taking RHEK-1 for transient expression experiments, several stable cell lines have been previously established in our

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Table 1. Expression of cytokines and receptors in stable clones by RT-PCR* analysis.

<table>
<thead>
<tr>
<th></th>
<th>IL-1α</th>
<th>IL-1β</th>
<th>IL-1RI</th>
<th>IL-1RII</th>
<th>IL-2</th>
<th>IL-4</th>
<th>IL-5</th>
<th>IL-6</th>
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<td>LMP2A</td>
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Abbreviations: RT-PCR, reverse transcriptase–polymerase chain reaction; IL, interleukin; IL-R, interleukin receptor; TNF-α, tumor necrosis factor alpha; TGF-β, transforming growth factor beta; IFN-γ, interferon gamma; RHEK-1, immortalized human epidermal keratinocyte; LMP, latent membrane protein; EBNA1, Epstein–Barr Virus-encoded nuclear antigen 1.

*The primer sequences and methods of RT-PCR for cytokines were as described in Huang et al.3 RNA extracts from peripheral blood mononuclear cells stimulated with 5 μg/mL phytohemagglutinin were used as positive controls of cytokines and receptors.

†Stable transfectants were established in either RHEK-1 or 293 cells. Each transfectant had its own plasmid vector-control cells, eg, RR3 is the vector control for LMP135/RHEK-1 cells; Vector-1 for EBNA1/RHEK-1 cells; Vector-2 for EBNA1/293 cells; Vector-3 for LMP2A/293 cells.

‡‘‘†‘‘ indicates that PCR products are visible in an ethidium bromide–stained gel; ‘‘–‘‘ indicates that PCR products are visually undetectable in the gel.
laboratory. LMP135 is a stable clone of the RHEK-1 transfectant, which can express LMP1. RR3 is a vector transfectant of RHEK-1 as a control clone.\textsuperscript{18} To compare LMP1 with other EBV latent proteins, clone EBNA1/RHEK-1, which can stably express EBNA1 and its vector control clone Vector-1, was selected. The 293 cells are originally derived from human embryonic kidney cells that were transformed with sheared adenovirus 5 DNA. Because of its ease of growth and transfection, the cells have been widely used in research work. Cloned 293-derived cell lines were collected for associated analyses, including EBNA1/293 cells, LMP2A/293 cells, and their vector-control cells, respectively. Total cellular RNAs were isolated from cell lines, and IL-1\(\alpha\) and IL-1\(\beta\) transcripts were determined by reverse transcriptase–polymerase chain reaction (RT-PCR) analysis. Products of \(\beta\)-actin served as the RNA quality control. Lane (–) was derived from RHEK-1 parental cells. Lane (+) was extracted from phytohemagglutinin (PHA)-stimulated human peripheral blood mononuclear cells.

**FIGURE 1.** Detection of interleukin-1 alpha (IL-1\(\alpha\)) and interleukin-1 beta (IL-1\(\beta\)) messenger ribonucleic acids (mRNAs) in transfectants expressing Epstein–Barr Virus (EBV)-encoded latent proteins. Two clones from immortalized human epithelial keratinocyte (RHEK-1) epithelial cells, ie, latent membrane protein 1 (LMP1) stable clone-LMP135, EBV-encoded nuclear antigen 1 (EBNA1) stable clone; 2 clones from 293 cells (ie, EBNA1 stable clone), LMP2A clone, and their vector-control cells, respectively. Total cellular RNAs were isolated from cell lines, and IL-1\(\alpha\) and IL-1\(\beta\) transcripts were determined by reverse transcriptase–polymerase chain reaction (RT-PCR) analysis. Products of \(\beta\)-actin served as the RNA quality control. Lane (–) was derived from RHEK-1 parental cells. Lane (+) was extracted from phytohemagglutinin (PHA)-stimulated human peripheral blood mononuclear cells.

**FIGURE 2.** Measurement of expression levels of interleukin-1 alpha (IL-1\(\alpha\)) and interleukin-1 beta (IL-1\(\beta\)) using quantitative reverse transcriptase–polymerase chain reaction (RT-Q-PCR) and enzyme-linked immunosorbent assay (ELISA) in immortalized human epithelial keratinocyte (RHEK-1) parental cells, vector-control cells (RR3), and latent membrane protein 1 (LMP1) stable transfectant cells (LMP135). (A–D) IL-1\(\alpha\) messenger ribonucleic acids (mRNAs), IL-1\(\beta\) mRNA, IL-1\(\alpha\) protein, and IL-1\(\beta\) protein, respectively. Gray bars indicate experiments under basal condition; black bars indicate cells cultured in the presence of 2000 U/mL of tumor necrosis factor alpha (TNF-\(\alpha\)) at 37 \(^\circ\)C for 6 hours. Each experiment was carried out >4 times. The data showed significant difference (\(p < .05\); Wilcoxon signed-rank test) in LMP135 versus RHEK-1 and LMP135 versus RR3 in both TNF-\(\alpha\) untreated and treated groups and also between TNF-\(\alpha\) untreated and treated LMP135 cells.

**LMP1 Mediated IL-1\(\alpha\) and IL-1\(\beta\) Production.** Transcripts of at least 4 EBV latent proteins (ENBA1, LMP1, LMP2A, and LMP2B) were detectable in EBV-positive NPC biopsies and the transplantable NPC cell line, C15.\textsuperscript{20} Many reports have shown that EBV infection modulates the synthesis of a variety of cytokines.\textsuperscript{13,14,21,22} However, no previous study directly indicated that EBV latent proteins could modulate the expressions of IL-1\(\alpha\) and IL-1\(\beta\). Most recent data showed that reactive nitrogen species–dependent DNA damage was found in LMP1-expressing cancer cells of patients with NPC, resulting from nuclear accumulation of epidermal growth factor receptor (EGFR) and IL-6–induced activation of signal transducer and activator of transcription 3 (STAT3).\textsuperscript{23} Following this line of thought, the unique expression of IL-1\(\alpha\) and IL-1\(\beta\) in NPC tumors, as we initially found, might be induced by a certain EBV-encoded protein as well. From our results, we provided evidence that stably or transient transfection of LMP1 upregulates mRNA synthesis and protein secretion of IL-1\(\alpha\) and IL-1\(\beta\) from the epithelial cells. In contrast, other EB viral latent proteins, such as EBNA1 and LMP2, have no effect on IL-1\(\alpha\) and IL-1\(\beta\)
production, as seen in Table 1. Furthermore, the result from Figure 2 indicates that the coexisting TNF-α might have an additive effect on LMP1-mediated IL-1 secretion in the EBV-reactivated microenvironment of NPC tumor.

**IL-1α and IL-1β Enhance the Growth of RHEK-1 Epithelial Cells.** Considering that RHEK-1 cells constitutively express IL-1RI (Table 1), the production of IL-1α and IL-1β might have an autocrine effect on the cell growth. The results in Table 2 show that the number of RHEK-1 epithelial cells increased in parallel with the concentrations of IL-1α and IL-1β, indicating that IL-1α and IL-1β could enhance proliferation of the epithelial cells. Therefore, it might be the secreted cytokines in the milieu that promote growth. This result gives evidence to complete a logical growth loop for proliferation of NPC cells.

**Possible Mechanism by LMP1 Mediation.** Increased expression of IL-1α and IL-1β mRNAs does not guarantee increased production of IL-1α and IL-1β proteins. For example, the complement component C5a enhances only the level of IL-1β mRNA, but not the expression of IL-1β protein. However, LMP1 significantly induced both mRNA transcription and protein secretions of IL-1α and IL-1β in this study.

IL-1β expression was regulated by many inducers, including phorbol esters, bacterial lipopolysaccharides, IL-1, and dibutyryl cyclic adenosine monophosphate (cAMP). Its proximal element of promoter necessary for recruiting transcription initiation factors consists of 1 NF-IL-6 recognizing element, 2 transcription factor Spi-1/PU.1 binding sites, and 2 NF-κB recognized regions. The promoter of the IL-1α gene also contains similar nuclear-factor binding sites, such as NF-κB and activator protein 1 (AP-1) recognized regions. On the other hand, there are 2 important C-terminal activation regions (CTAR) of LMP1 for signal transduction. CTAR1 is for binding of TNF receptor-associated family proteins (TRAFs) and induces both NF-κB activation and EGFR expression. CTAR2 is responsible for activation of AP-1 and is the major NF-κB activation domain. LMP1 has been shown to induce expression of IL-8 through the NF-κB binding site within its promoter in NPC biopsies, and might contribute to angiogenesis in NPC.

Besides, IL-1-receptor–associated kinase 1 was also critical for LMP1-induced NF-κB activation. Therefore, LMP1 may possibly activate signaling of IL-1α and IL-1β via NF-κB and

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**Table 2.** Proliferation status of RHEK-1 cells upon stimulation with IL-1α or IL-1β.

<table>
<thead>
<tr>
<th>Interleukin</th>
<th>Units/mL</th>
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<tr>
<td>0</td>
<td></td>
<td>7.67 ± 0.19</td>
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<tr>
<td>10</td>
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<tr>
<td>100</td>
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<tr>
<td>IL-1β</td>
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<tr>
<td>0</td>
<td></td>
<td>7.10 ± 0.39</td>
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<tr>
<td>10</td>
<td></td>
<td>9.06 ± 0.42</td>
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<tr>
<td>100</td>
<td></td>
<td>8.98 ± 0.53</td>
</tr>
<tr>
<td>500</td>
<td></td>
<td>10.67 ± 1.90^†</td>
</tr>
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</table>

Abbreviations: RHEK-1, immortalized human epidermal keratinocyte; IL-1α, interleukin 1 alpha; IL-1β, interleukin 1 beta.

*RHEK-1 cell proliferation assays were performed by using WST-1 (water-soluble tetrazolium salt) reagent to elucidate the effects of IL-1α and IL-1β on cell growth. Each experiment was in triplicate. Data are presented in the average and the SDs.

*a Cell numbers were obviously increased with statistical significance after treating with 10 and 100 U/mL IL-1α and 500 U/mL IL-1β compared with untreated RHEK-1 cells (p < .05; Wilcoxon signed-rank test).
AP-1 binding to their promoters. Furthermore, our preliminary study on 2 LMP1 deletion mutants, LMP1-pCdel155 and pCdel199, indicated that deletion of 199 bps from the cytoplasmic end of LMP1, an NF-kB signaling site, would significantly decrease the ability of LMP1-mediated IL-1α and IL-1β production (data not shown). Thus, to identify the detailed intracellular signal transduction pathway would be interesting for future research.

**Biological Function of IL-1α and IL-1β in NPC.** In the microenvironment of an NPC tumor, infiltrating lymphocytes have been shown to produce various cytokines, including IL-1α and IL-1β, that would reinforce lymphoid infiltration. Importantly, IL-1α and IL-1β might induce activation of immediate-early transcription factors and genes that enhance the proliferation and survival of epithelial cells. IL-1 could also act through regulation of IL-6, and indirectly enhance the proliferation of human oral squamous cell carcinoma cells. IL-1β was highly expressed and was demonstrated to increase cell growth of an EBV-associated gastric carcinoma cell line in vitro. Thus, there may be 2 possible functions of IL-1α and IL-1β in NPC tissues in the cancer-developing process: promotion of lymphocyte infiltration and tumor growth.

**Inflammation and Cancer.** LMP1 expressed in epithelial cells was involved in the activation of cellular signal transduction including the NF-kB pathway. Data obtained from 2 cancer models suggested that the NF-kB pathway has dual actions in tumor promotion, first by preventing the death of cells with malignant potential and, second, by stimulating the production of proinflammatory cytokines from inflammatory cells in the tumor mass. A recent study also showed that LMP1 induced IL-1α and IL-1β expression in a keratinocyte cell line (SCC12F cells) by using microarray analysis. These findings are well in accord with our results. They suggest that alterations in the inflammatory cytokines IL-1α and IL-1β signaling network may be responsible for many of the changes in host–cell gene expression induced in response to LMP1. Eventually, the mechanisms by which this oncoprotein LMP1 influences cellular pathways of terminal differentiation and inflammation will be discovered. Speculatively, with respect to NPC as a cancer associated with EBV infection, these new results may suggest that anti-inflammatory therapy might be helpful during the early stages of cancer development.

In conclusion, IL-1α and IL-1β were produced spontaneously from cultured LMP1-expressing epithelial cells, and were able to act as autocrine factors to promote cell growth. The abundant production of IL-1α and IL-1β by EBV-containing epithelial cells may contribute to lymphocyte infiltration and tumor growth during NPC development.

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