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Expression of the Functional Recombinant Interleukin-16 in *E. coli* and Mammalian Cell Lines

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Abstract The C-terminal 393 bp region of the human interleukin-16 (IL-16) gene was cloned and expressed in *E. coli* along with mammalian cell lines. Recombinant IL-16 expressed from *E. coli* was 22 kDa on SDS-PAGE and showed 260% of chemoattractant activity at a concentration of 0.1 μg/ml. HeLa, COS, and Neuro-2a cells were transduced by recombinant retrovirus vector pLNC/IL-16/IRES/TK and the intracellular and secreted amounts of IL-16 produced by HeLa/IL-16/TK, COS/IL-16/TK, and Neuro-2a/IL-16/TK cells were determined by enzyme-linked immunosorbent assay (ELISA). HeLa/IL-16/TK (1×10^5) and COS/IL-16/TK (1×10^5) cells secreted 36.1 and 13.3 ng of IL-16 for 48 h, respectively. Forty-nine ng and 86.4 ng of IL-16 remained in the cell lysates of HeLa/IL-16/TK and COS/IL-16/TK. Intracellular and secreted amounts of IL-16 from Neuro-2a/IL-16/TK (5×10^5) cells during 24 h cultivation were 50 ng and 3.3 ng, respectively. Also, HeLa and COS cells were stably transfected with mammalian expression vector pCRIII/IL-16. Both culture media and cell lysates prepared from HeLa/IL-16 cells and COS/IL-16 cells showed chemoattractant activity ranging from 190% to 460% as compared to the control experiment. Expression of the herpes simplex virus thymidine kinase (HSV-tk) gene in pLNC/IL-16/IRES/TK bicistronic retroviral expression vector was verified by performing a ganciclovir (GCV) sensitivity assay. Finally, IL-16 repressed Tat-transactivated human immunodeficiency virus type 1 long terminal repeat (HIV-1 LTR) promoter activity.

Key words: Interleukin-16, *E. coli*, mammalian cells, retrovirus producer cells, lymphocyte migration activity, HIV-1 LTR suppression

Interleukin 16 (IL-16), formerly known as lymphocyte chemoattractant factor (LCF), was originally identified and purified from the supernatants of ConA-stimulated PBMCs [3, 5]. Analysis of human IL-16 cDNA clones indicates that IL-16 mRNA encodes a precursor protein, from which a C-terminal biologically active peptide is processed and secreted [32]. IL-16 derived from peripheral blood mononuclear cells and recombinant IL-16 produced in *E. coli* form homotetramers of 14–17 kDa chains, which appears to be an essential requirement for the biological activity of IL-16 [10]. Interleukin-16 was first identified from $CD8^+$ T cells, and subsequently from other tissues such as $CD4^+$ T cells, macrophages, dendritic cells, B cells, and fibroblasts [4, 8, 27, 28].

Numerous studies have demonstrated that CD4 serves as a receptor for IL-16 and that IL-16-mediated signal transduction induces a variety of response in $CD4^+$ cells. IL-16 was initially identified as a chemoattractant factor for $CD4^+$ cells [6, 25]. Basophilic amines such as histamine or serotonin stimulate $CD8^+$ T cells to secrete IL-16 which attracts $CD4^+$ T cells, monocytes, or eosinophils to sites of inflammation amplifying immune responses. In addition to the chemotactic activity, IL-16 induces G_0 to G_1 cell cycle shift for $CD4^+$ T cells, but it is incapable of inducing cell division [6]. IL-16 also induces the expression of high affinity IL-2R on human blood $CD4^+$ T cells which are then competent to proliferate in the presence of exogenously added IL-2 [7, 21]. Recently, it was reported that human and simian IL-16 inhibited the replication of HIV-1 on $CD8^+$ depleted PBMC [1, 2, 10], and the effect of IL-16 on HIV-1 replication was shown to occur at the level of viral transcription [17, 34]. Also, IL-16 inhibited HIV-1 replication in macrophages and dendritic cells through the inhibition of viral entry [30]. In another situation, IL-16 was shown to be a possible anti-inflammatory cytokine in rheumatoid arthritis by inhibiting the production of IFN-γ, IL-1β, and TNF-α in the synovium [14], and the immunosuppressive effect of IL-16 on T cell activation was used as a local mediator to prolong graft survival [9]. As stated above, IL-

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16 is a multifunctional cytokine with many useful biological functions.

Many protein expression systems are now available including the *E. coli* expression system [22, 23, 29], insect expression system [15, 16], and mammalian expression system, and each has its own advantages and disadvantages which should be considered when choosing which one to use. Previously, IL-16 was mainly prepared from *E. coli*, but in a few cases, it was prepared from transiently or stably transfected mammalian cells [10, 17, 32, 34].

In this study, we purified recombinant IL-16 from *E. coli* utilizing the pET32 expression vector system. Also, we constructed retroviral vectors and retrovirus producer cells which efficiently produced recombinant retroviral vectors expressing IL-16 and herpes simplex virus thymidine kinase (HSV-tk). Finally, we constructed mammalian cell lines that constitutively produce a high level of human IL-16. The expressed IL-16 was found to be biologically functional in both chemoattractant activity and HIV-1 suppressive activity.

MATERIALS AND METHODS

Construction of IL-16 Expression Plasmids

Peripheral Blood Lymphocytes (PBL) were collected from human blood through Ficoll paque gradient fractionation. Total RNA of PBL was isolated by using a Qiagen RNA extraction kit (Qiagen, Germany) according to the manufacturer's instruction, and was reverse transcribed using an oligo-dT primer (Pharmacia, U.S.A.). The isolated total RNA was denatured by heating at 80°C for 3 min, and then cooling on ice immediately. A reaction mixture (20 μl) containing 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, 10 μM of oligo d(T)-16, 26 unit RNasin, dNTPs (10 mM each), and denatured RNA (11 μl) was incubated at 42°C for 1 h and the reaction was terminated by heating at 94°C for 2 min. The synthesized cDNA was used in polymerase chain reaction (PCR). The 390 bp coding sequence of IL-16 was amplified with a pair of oligonucleotides with the *Hin*dIII site introduced at their 5' ends (sense, 5'-TAA-GCTTACCATGCCCGACCTCA-AC-3' and antisense, 5'-CTAGGAGTCTCCAGCAGCTG-3'). The amplified IL-16 cDNA was inserted into pCRIII (Invitrogen, Carlsbad, U.S.A.) and named as pCRIII/IL-16. The sequence of clone was confirmed by nucleotide sequencing. Then, a 0.4 kb *Hin*dIII fragment encoding the IL-16 region was excised from the pCRIII/IL-16, and then inserted into *Hin*dIII-digested pET-32b(+) (Novagen, Madison, Wisconsin, U.S.A.), which was named as pET32b(+)/IL-16. To construct pLNC/IL-16, which is a IL-16-expressing retroviral vector, a 0.4 kb *Hin*dIII fragment of pCRIII/IL-16 was inserted into pLNCX (Clontech, U.S.A.) vector linearized by *Hin*dIII. In addition, pLNC/IL-16/IRES/TK retroviral vector, which expressed both recombinant human IL-16 and herpes simplex virus thymidine kinase (HSV-TK) gene, was constructed by inserting a *Xho*I/*Bam*HI fragment of pCRIII/IL-16 into the *Bgl*II/*Sal*I site of pLNC/IRES/TK retroviral vector [11].

Expression and Purification of IL-16 in *E. coli*

To express the C-terminal recombinant IL-16 in *E. coli*, pET32b(+)/IL16 was transformed into the *E. coli* BL21(DE3)/pLysS strain. A single colony was inoculated into 3 ml of LB broth supplemented with ampicillin (50 μg/ml) and chloramphenicol (30 μg/ml), and the broth was incubated overnight at 37°C with vigorous shaking. Then, 100 ml of LB broth with ampicillin and chloramphenicol was inoculated with 1 ml of the overnight culture and the culture was incubated until the optical density reached 0.5 to 0.7. Isopropyl-D-thiogalactopyranoside (IPTG) was added to a final concentration level of 1 mM, and the culture was further incubated for 3 h.

Cell pellet collected from a 1 ml culture was resuspended in 100 ml of 50 mM Tris-HCl (pH 8.0), 2 mM EDTA, 0.1% Triton X-100, and DNase I (10 μg/ml), and incubated until the solution became no longer viscous. The soluble and insoluble fractions were separated by centrifugation at 14,000 rpm for 15 min, and each fraction was analyzed by 12% SDS-PAGE.

To purify the recombinant IL-16, the IPTG-induced culture was harvested by centrifugation at 7,000 rpm for 5 min, resuspended in 1 ml of binding buffer (5 mM imidazole, 0.5 M NaCl, and 20 mM Tris-HCl, pH 7.9), lyzed by adding Triton X-100 (0.1%) and DNase I (10 μg/ml) as described above, and centrifuged at 14,000 rpm for 30 min. The purification of recombinant IL-16 was performed using a HIS-BIND chromatography kit (Novagen, U.S.A.) according to the manufacturer's instruction. Two ml of resin was packed in a 5-ml syringe with its tip blocked by glass wool, washed with 3 volumes of sterile distilled water, charged with 5 volumes of the buffer (50 mM $NiSO_4$), and finally washed with 3 volumes of binding buffer (5 mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl, pH 7.9). The cell lysate was loaded and the column was washed with 10 volumes of binding buffer and then with 6 volumes of the wash buffer (60 mM imidazole, 0.5 M NaCl, and 20 mM Tris-HCl, pH 7.9). Finally, recombinant IL-16 was eluted by adding 6 volumes of elution buffer (1 M imidazole, 0.5 M NaCl, and 20 mM Tris-HCl, pH 7.9). For enterokinase digestion, the eluted protein was dialyzed against enterokinase buffer (20 mM Tris-HCl, pH 7.4, 50 mM NaCl, and 2 mM $CaCl_2$). The enterokinase (Novagen) was added at a ratio of 0.5 unit per 50 μg protein and the reaction mixture was incubated overnight at 4°C. After enterokinase digestion, rIL-16 was separated from the tag protein by employing a second Ni-chromatography. The tag protein was retained in the column, while the rIL-16 was eluted. Protein concentration

was determined by the Bradford method by using the Bio-Rad protein assay kit (Bio-Rad, U.S.A.) according to the manufacturer's instruction.

Cell Culture

COS (monkey embryonic kidney cell line), Neuro-2a (murine neuroblastoma, ATCC CCL-131), PG13 [19], and Gpenvam12 [18] cells were grown on Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 100 U/ml streptomycin, and 100 μg/ml penicillin G (Life Technologies, U.S.A.). HeLa (ATCC CCL-2) cells were grown on DMEM media supplemented with 7% calf serum, and Jurkat cells were grown in RPMI media supplemented with 10% FBS. All cultures were maintained in 37°C incubator with 5% CO_2.

Construction of Retrovirus-Producing Cell Lines and Mammalian Cell Lines Expressing Recombinant IL-16

The primate packaging cell line PG13 and amphotropic Gpenvam12 were transfected with 3 μg of either pLNC/IL-16/IRES/TK or pLNC/IRES/TK by using calcium phosphate precipitation as described earlier [13], and stable transfectants were generated by G418 selection (400 μg/ml) for 2 weeks. The resulting stable transfectants were named as PG13/IL-16/TK, Gpenvamp12/IL-16/TK, PG13/TK, and Gpenvamp12/TK, respectively. HeLa and COS cells (5×10^5 cells) were plated onto 60-mm dishes and, one day after passage, cells were transduced by 1 ml of retroviral supernatants produced by PG13/IL-16/TK retroviral producer cells with polybrene (8 μg/ml). Cells were supplemented with 4 ml culture media after 4 h incubation, and stably transduced cells were generated by G418 (400 μg/ml) for 2 weeks. The resulting stable cell clones were named as HeLa/IL-16/TK and COS/IL-16/TK. Neuro-2a cells were transduced by retroviral supernatants produced by Gpenvam12/IL-16/TK and Gpenvam12/TK retroviral producer cells as described above, and stable transfectants were generated using G418 (400 μg/ml) for 2 weeks. The resulting clones were named as Neuro-2a/ IL-16/TK and Neuro-2a/TK. HeLa and COS cells were transfected with LNC/IL-16 retroviral vector, and stable clones were selected for 2 weeks using G418 (400 μg/ml). The selected clones were named as HeLa/IL-16 and COS/IL-16.

ELISA for IL-16

Amounts of IL-16 expressed from mammalian cells were quantified by using the Human IL-16 ELISA kit (Endogen, U.S.A.) according to the manufacturer's instruction. Briefly, 1×10^5 cells of HeLa and COS cell lines were plated on 60-mm dishes in which the media were changed by fresh media (5 ml) on the next day, and the cultures were incubated for 48 h. In the case of Neuro-2a cell lines, 5×10^5 cells were plated on 60-mm dishes in which the media were changed by fresh media (5 ml) on the next day, and the cultures were incubated for 24 h. After incubation, each of the culture supernatants were filtered through 0.22 μm filter, and subjected to ELISA. To measure intracellular IL-16, the incubated cells were scraped off with the policeman scraper, lyzed in 50 μl of cell lysis buffer (10 mM Tris-HCl, pH 7.4, 66 mM EDTA, 1% Triton X-100, 0.4% sodium deoxycholate), and subjected to ELISA.

Lymphocyte Migration Assay

The lymphocyte migration assay was performed using a modified Boyden chamber technique [6]. Briefly, 500 μl of lymphocytes (5×10^6 cells/ml in RPMI) were placed in the upper compartment of chemotactic chambers, separated from 200 μl of samples by 8 mm pore nitrocellulose filters. Migration experiments were carried out for 3 h at 37°C in a 5% CO_2 atmosphere.

Ganciclovir Sensitivity Test

Ten-thousand cells of the Neuro-2a, Neuro-2a/TK, and Neuro-2a/IL-16/TK were plated on 12-well plates, and various concentrations (0, 0.01, 0.1, 1, 10, and 20 μM) of GCV (Sigma, U.S.A.) were added on the next day. Then, cells were incubated for 4 days and viable cells were enumerated after trypan blue staining.

Luciferase Assay

Jurkat cells (1×10^6) were co-cultivated with 5×10^5 cells of Neuro-2a/IL-16/TK or Neuro-2a/TK in 6-well plates for 24 h, and then transiently transfected with TARluc (10 μg; [13]) and pTat120K or pUC19SXLTR (5 μg; [12]) by electroporation at the capacitance of 1170 μF and 260 volts/0.4 cm. After 48-h incubation, cells were harvested and subjected to luciferase assay by using the Luciferase Assay System kit (Promega, U.S.A.) and a Lumat LB9501 Luminometer (Berthold, U.S.A.) according to the manufacturer's instruction.

Results and Discussion

Construction of Various IL-16 Expression Vectors

The C-terminal 393 bp coding region that has been shown to be sufficient for the biological activity of IL-16 was amplified by RT-PCR and was inserted into the pCRIII vector (Fig. 1A). The sequence of PCR-amplified IL-16 gene was verified. Then, a *Hin*dIII fragment containing the C-terminal IL-16 gene was inserted into the *Hin*dIII site of pET32b(+) and pLNCX to construct pET32b(+)/IL-16 (Fig. 1B) and pLNC/IL-16 (Fig. 1C), respectively. Also, the pLNC/IL-16/IRES/TK vector (Fig. 1D) was prepared by ligating the *Xho*I/*Bam*HI fragment of pCRIII/IL-16 into the *Bgl*II/*Sal*I site of pLNC/IRES/TK. Retroviral vector pLNC/IL-16/IRES/TK can express both interleukin-16 and herpes simplex virus thymidine kinase (HSV-tk) simultaneously by using encephalomyocarditis virus (EMCV) internal ribosome entry sites (IRES).

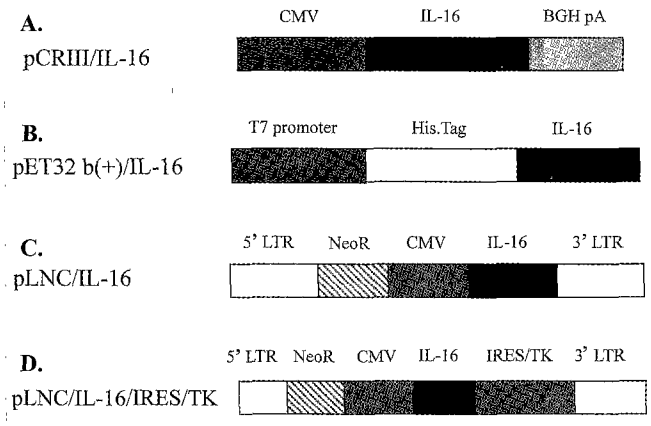

Fig. 1. Vectors used in the cloning and expression of human IL-16.
pCRIII/IL-16; eukaryotic expression vector which was used in the cloning and the expression of IL-16 in mammalian cell lines. pET32b(+)/IL-16; prokaryotic expression vector expressing rIL-16 as a histidine-tagged form in *E. coli*. pLNC/IL-16; IL-16 expressing retroviral vector. pLNC/IL-16/IRES/TK; bicistronic retroviral vector which expresses the HSV-TK gene as well as IL-16 using an encephalomyocarditis virus (EMCV) internal ribosomal entry site (IRES) element. pCRIII/IL-16, pLNC/IL-16, and pLNC/IL-16/IRES/TK vectors harbor neomycin resistance gene to select stable integrants of the gene of interest. CMV, human cytomegalovirus promoter; pA, bovine growth hormone polyadelylation sequences; LTR, long terminal repeats; NeoR, neomycin resistance gene.

Expression and Purification of IL-16

When total cell proteins of IPTG-treated *E. coli* BL21(DE3)/pLysS harboring pET32b(+)/IL-16 were analyzed on 12% SDS-PAGE, the induced recombinant IL-16 band was easily detectable (Fig. 2A, lane 2). The expressed recombinant protein was purified using Ni-affinity chromatography (Fig. 2A, lane 3). The purified 37.7 kDa recombinant protein (Fig. 2B, lane 2) was digested with enterokinase to remove accompanying tag protein and a second Ni-affinity chromatography was performed to separate rIL-16 from the tag protein (Fig. 2B, lane 3). A small amount of eluted tag band was seen below the IL-16 band. When *E. coli* cells harboring pET32b(+)/IL-16 were induced by IPTG for 3 h, the portion of recombinant IL-16 nearly made up 50% of the total cell proteins (Fig. 2A, lane 2), and when soluble and insoluble fractions of the induced cell proteins were analyzed in 12% SDS-PAGE, most rIL-16 existed in the soluble fraction (data not shown). As rIL-16 belonged to the soluble fraction, there were few difficulties in purifying rIL-16 by the commercial Ni-column chromatography. The purified recombinant IL-16 was biologically active when tested in the lymphocyte migration assay (described below).

Construction of Retroviral Producer Cell Lines and Mammalian Cell Lines Expressing IL-16

We made retrovirus producer cell lines by transfection with the retroviral vector pLNC/IL-16/IRES/TK and selected by G418 (400 μg/ml) treatment for two weeks. PG13/IL-16/TK is a primate packaging cell line that produces retroviruses

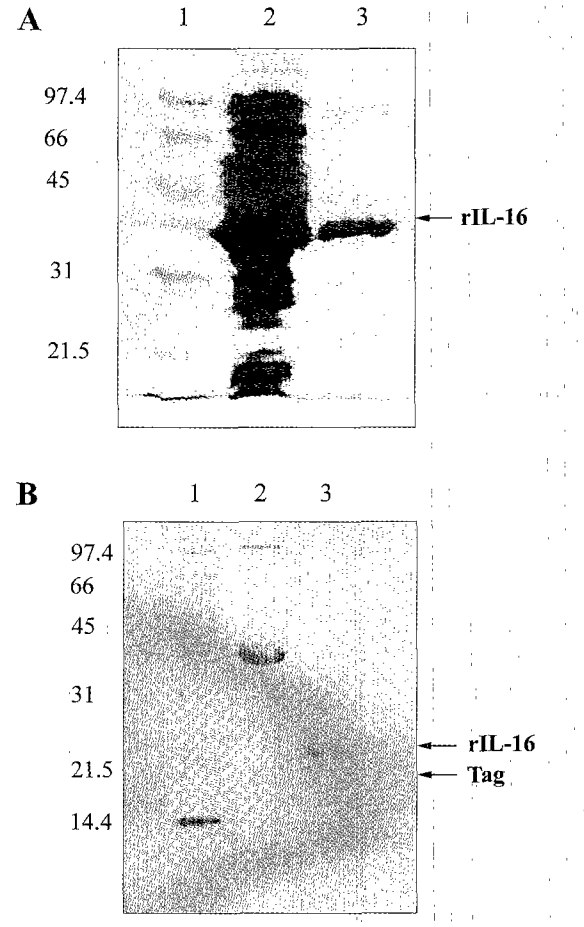

Fig. 2. Expression and purification of recombinant IL-16.
(A) SDS-PAGE gel of *E. coli* cells expressing recombinant human IL-16. Lane 1: protein size marker (Pharmacia, USA); lane 2: *E. coli* whole cell lysate; lane 3: Ni-column purified recombinant IL-16 before enzymatic digestion. (B) Enterokinase digestion of purified rIL-16. Lane 1: standard protein size marker; lane 2: undigested rIL-16; lane 3: rIL-16 digested by enterokinase and eluted through Ni-column chromatography again. A minor amount of tag band is seen below the rIL-16 band.

infecting primate species including humans. Gpenvam12/IL-16/TK is an amphotropic packaging cell line that produces retroviruses infecting both humans and mice. We transduced HeLa, COS, and Neuro-2a cell lines with the recombinant retroviruses produced by PG13/IL16/TK and Gpenvam12/IL-16/TK, and constructed HeLa/IL-16/TK, COS/IL-16/TK, and Neuro-2a/IL-16/TK stable mammalian cell lines by selection with G418 (400 μg/ml) treatment for two weeks. We also constructed the Neuro-2a/TK cell line as a control for the Neuro-2a/IL-16/TK cell line. Meanwhile, we constructed HeLa/IL-16 and COS/IL-16 mammalian cell lines by transfection with the pCRIII/IL-16 vector and selection of stable clones by using G418.

ELISA

We quantified the amount of IL-16 produced from HeLa/IL-16/TK, COS/IL-16/TK, and Neuro-2a/IL-16/TK cells by using

Table 1. Amounts of intracellular and secreted interleukin-16.

	Secreted IL-16	Intracellular IL-16
HeLa/IL-16/TK[a]	7.22 ng/ml	49 ng
HeLa[a]	ND[c]	ND
COS/IL-16/TK[a]	2.66 ng/ml	86.3 ng
COS[a]	ND	ND
Neuro-2a/IL-16/TK[b]	0.665 ng/ml	50 ng
Neuro-2a/TK[b]	ND	0.17 ng

[a]HeLa, HeLa/IL-16/TK, COS, and COS/IL-16/TK cells were incubated for 48 h before ELISA.
[b]Neuro-2a/IL-16/TK and Neuro-2a/TK cells were incubated for 24 h before ELISA.
[c]Not detected.

the ELISA kit (Table 1). The amounts of IL-16 secreted by HeLa/IL-16/TK (10^5) and COS/IL-16/TK (10^5), cultured for 48 h, were 7.22 ng/ml and 2.66 ng/ml, respectively. Neuro-2a/IL-16/TK (5×10^5) cells secreted 0.665 ng/ml IL-16 into the culture medium during the 24-h incubation period. In contrast, no IL-16 was observed in the culture media of HeLa, COS, and Neuro-2a/TK cell lines. We also investigated the amounts of intracellular IL-16 that was not secreted into the culture media. The concentration of intracellular IL-16 was 49 ng, 86.3 ng, and 50 ng for 1×10^5 cells of HeLa/IL-16/TK, COS/IL-16/TK, and 5×10^5 cells of Neuro-2a/IL-16/TK, respectively. No IL-16 was detected in the cell lysates of HeLa and COS cells. However, to our surprise, IL-16 existed in the cell lysate of Neuro-2a/TK cells at the concentration of 0.71 ng per 5×10^5 cells incubated for 24 h. As shown in Table 1, the majority of rIL-16 expressed in the mammalian cells was not secreted and remained inside the cell. IL-16 is not known to have a signal peptide and the mechanism involved in the distribution and secretion of IL-16 remains to be elucidated [33].

Lymphocyte Migration

We tested the bioactivity of the recombinant IL-16 that was produced from *E. coli* or mammalian cells, using the lymphocyte migration assay. The recombinant IL-16 (0.1 μg/ml) stimulated a motile response compared to RPMI media alone, and when treated at the level of 1 μg/ml, rIL-16 showed 2.6-fold higher migration activity (Fig. 3A). The conditioned media that were prepared from mammalian cell lines expressing the C-terminal IL-16 were more effective than the control media in the chemotactic activity on Jurkat cells. The COS/IL-16 conditioned medium showed 4.6-fold higher migration activity than RPMI media alone, and the HeLa/IL-16 conditioned medium showed 1.9-fold higher migration activity (Fig. 3B). IL-16 prepared from HeLa/IL-16 cell lysate showed 4.5-fold higher migration activity than control buffer, and COS/IL-16 cell lysate showed 2.2-fold higher migration activity on Jurkat cells (Fig. 3C). In our system, the conditioned media obtained from HeLa/IL-16 or COS/IL-16 showed higher chemotactic activity

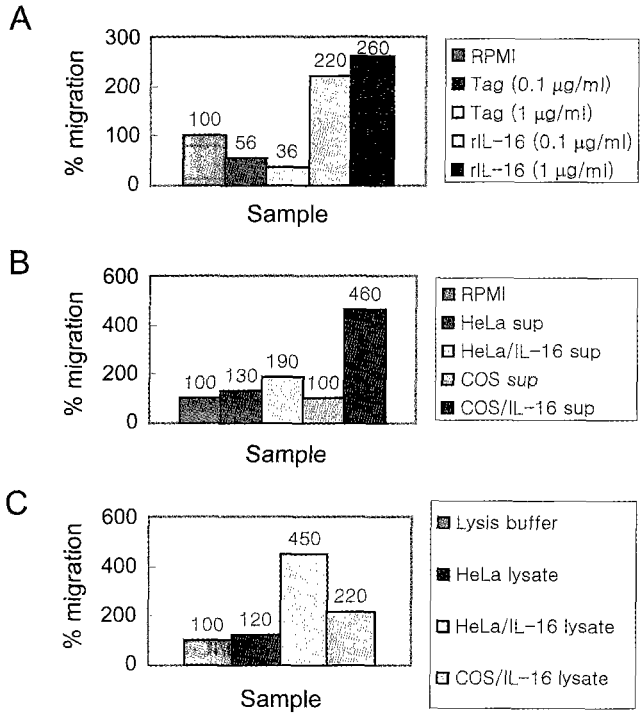

Fig. 3. Lymphocyte migration activity of various sources of IL-16. Lymphocyte migration assay was performed as described in Materials and Methods. (A) Lymphocyte migration activity of rIL-16 purified from *E. coli*. (B) Lymphocyte migration activity of IL-16 secreted from HeLa/IL-16 and COSM6/IL-16 clones. (C) Lymphocyte migration activity of IL-16 prepared from cell lysates of HeLa/IL-16 and COS/IL-16 cell clones. The results are means of duplicate experiments.

than the recombinant IL-16 expressed from *E. coli* (Fig. 3), even in the lower concentration level. The higher biological activity of rIL-16 expressed in HeLa/IL-16 and COS/IL-16 cell lines might be explained by the differences in the protein folding efficiency or posttranslational modification. In C-terminal IL-16, there are three putative N-glycosylation sites (Asp 5, Ser 6, and Ser 7) that could influence natural folding when expressed in *E. coli* [33]. However, we could not observe direct correlation between the amount of IL-16 produced by HeLa and COS cell lines (Table 1) and the lymphocyte migration activity (Figs. 3B and 3C).

Sensitivity of Neuro-2a/TK and Neuro-2a/IL-16/TK Cell Lines to GCV

When Neuro-2a, Neuro-2a/TK, and Neuro-2a/IL-16/TK cell lines were treated with various concentrations of ganciclovir (0, 0.1, 1.0, 10, and 20 μg/ml), the cell viability of Neuro-2a/TK and Neuro-2a/IL-16/TK was reduced dramatically along with the increasing concentrations of GCV, while the survival rate of the control Neuro-2a cells was not affected by the treatment of GCV (Fig. 4). This result showed that the HSV-tk gene behind the IRES element was effectively translated and expressed. HSV-tk catalyzes the phosphorylation of the antiviral nucleoside ganciclovir (GCV) by ATP to

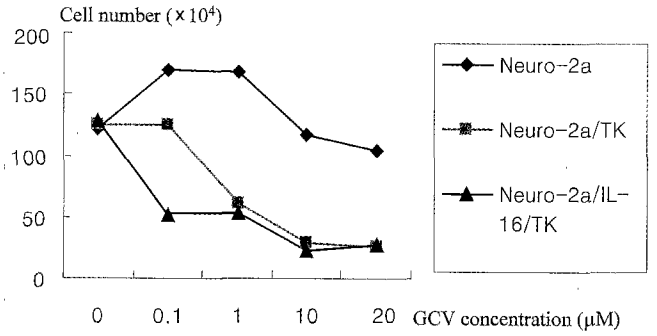

Fig. 4. Expression of the herpes simplex virus thymidine kinase (HSV-tk) gene as determined by the ganciclovir (GCV) cytotoxicity test.

Briefly, 10^4 cells of Neuro-2a, Neuro-2a/TK, and Neuro-2a/IL-16/TK cells were plated on 6-well dishes and increasing amounts of GCV (0, 0.01, 0.1, 1, 10, and 20 μM) were added on the next day. After 4 days incubation, the viable cells were enumerated after trypan blue staining. Over 80% of Neuro-2a/TK and Neuro-2a/IL-16/TK cells were killed by 10 μM GCV, while Neuro-2a cells were not affected.

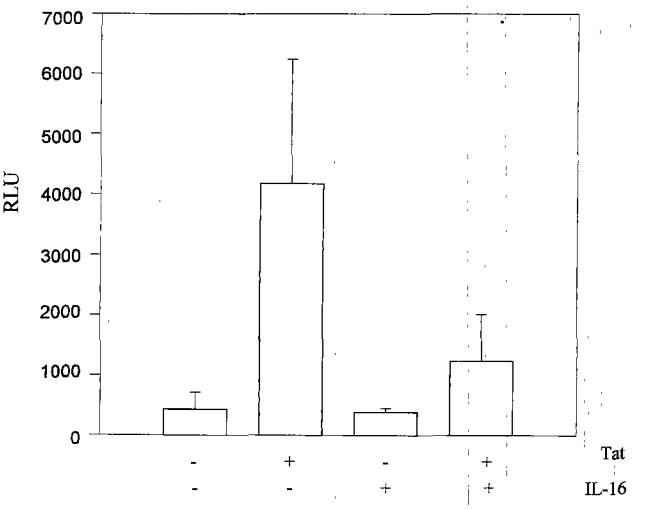

Fig. 5. IL-16 represses Tat-transactivated HIV-1 LTR promoter activity in Jurkat cells.

Jurkat cells (10^6 cells) were co-cultivated with 2×10^5 Neuro-2a/IL-16/TK or Neuro-2a/TK cells for 24 h and transfected with 5 μg of TARluc and 3 μg of pTat120K by electroporation as described in Materials and Methods. Cells were harvested 48 h posttransfection and subjected to luciferase assay. The relative luciferase activity was normalized by total protein contents and the above result is representative of at least three experiments performed independently. Error bars represent standard deviation. RLU; relative luciferase unit.

GCV-monophosphate that cause cell death by blocking DNA polymerization. The anticancer effect of the HSV-tk/GCV system has been demonstrated both *in vitro* and in diverse animal models [26], and phase I clinical trials using retrovirus or adenovirus vectors have been performed with no serious toxicity [20, 24]. In this regard, our PG13/IL-16/TK, PG13/TK, Gpenvam12/IL-16/TK, and Gpenvam12/TK cell lines can be useful in preparing recombinant retroviruses for HSV-tk/GCV based cancer gene therapy.

Suppression of the HIV-1 LTR Promoter by IL-16

Next, we checked the bioactivity of the C-terminal IL-16 expressed by mammalian cells by examining the ability to repress the HIV-1 LTR promoter. To supply mammalian cell-derived rIL-16 continuously, we co-cultivated Neuro-2a/IL-16/TK cells or control Neuro-2a/TK cells with Jurkat cells. As shown in Fig. 5, IL-16 derived from Neuro-2a/IL-16/TK cells significantly suppressed the HIV-1 LTR promoter activity transactivated by Tat (Fig. 5). While the basal level of HIV-1 LTR promoter activity was not affected (440 RLU in the case of pUC19SXLTR with Neuro-2a/TK vs. 385 RLU in the case of pUC19SXLTR with Neuro-2a/TK), transactivation of the HIV-1 LTR promoter by Tat was suppressed dramatically by IL-16 (4166 RLU in the case of pTat120K with Neuro-2a/TK vs. 1234 RLU in the case of pTat120K with Neuro-2a/IL-16/TK).

In this report, we constructed various IL-16 expression vectors including *E. coli* (pEF32b(+)/IL-16), mammalian (pCRIII/IL-16), monocistronic retroviral (pLNC/IL-16), and bicistronic retroviral (pLNC/IL-16/TK) expression vectors (Fig. 1). Recombinant IL-16 was easily expressed in *E. coli* (Fig. 2) and was functional in a lymphocyte migration activity test (Fig. 3A). Retrovirus-transduced mammalian cells (HeLa/IL-16/TK and COS/IL-16/TK) produced and secreted IL-16 in the 1-10 ng/ml range (Table 1). Both culture media and cell lysates were also functional in the lymphocyte migration assay (Figs. 3B and 3C). The bicistronic retrovirus vector pLNC/IL-16/TK expressed the HSV-tk gene efficiently, as shown by the GCV sensitivity test (Fig. 4). Also, IL-16 suppressed the Tat-transactivated HIV-1 LTR promoter activity significantly (Fig. 5).

The purified rIL-16 from *E. coli* and the IL-16 expressed in mammalian cell lines can be utilized in various applications. As IL-16 augments the bioactivity of IL-2, rIL-16 can be used in immune therapeutic treatments such as anticancer immunotherapy [21]. The recombinant IL-16 can also be used for potent anti-HIV-1 therapeutics [2, 10, 17, 31]. Retroviral packaging cell lines producing recombinant retrovirus expressing the IL-16 might be used to reconstitute the cell population of HIV-1-infected individuals by recombinant virus transduction, i.e. *ex vivo* gene therapy protocol. pLNC/IL-16/TK retrovirus vector produced by PG13/IL-16/TK or Gpenvam12/IL-16/TK might be used in antitumor gene therapy using the HSV-tk/GCV system. Also, IL-16-producing cell lines might be used in transplantation to reduce local immune rejection [9].

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