

## The associated regulators and signal pathway in rIL-16/CD4 mediated growth regulation in Jurkat cells

XIAO MING ZHANG, YONG HUA XU\*

*Lab of Molecular and Cellular Oncology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yueyang Road, Shanghai 200031, China*

### ABSTRACT

IL-16 is a ligand and chemotactic factor for CD4<sup>+</sup> T cells. IL-16 inhibits the CD3 mediated lymphocyte activation and proliferation. The effects of IL-16 on the target cells are dependent on the cell type, the presence of co-activators etc. To understand the regulation function and mechanism of IL-16 on target cells, we used a 130 a.a. recombinant IL-16 to study its effects on the growth of Jurkat T leukemia cells in vitro. We found that the rIL-16 stimulated the proliferation of Jurkat cells at low dose ( $10^{-9}M$ ), but inhibited the growth of the cells at higher concentration ( $10^{-5}M$ ). Results showed that  $10^{-5}M$  of rIL-16 treatment induced an enhanced apoptosis in Jurkat cells. The treatment blocked the expression of FasL, but up-regulated the c-myc and Bid expression in the cells. Pre-treatment of PKC inhibitor or MEK1 inhibitor markedly increased or decreased the rIL-16 induced growth-inhibiting effects on Jurkat cells, respectively. The results suggested that the rIL-16 might be a regulator for the growth or apoptosis of Jurkat cells at a dose-dependent manner. The growth-inhibiting effects of rIL-16 might be Fas/FasL independent, but, associated with the activation of PKC, up-regulated expression of c-Myc and Bid, and the participation of the ERK signal pathway in Jurkat cells.

**Key words:** *IL-16, Jurkat cells, growth regulation.*

### INTRODUCTION

Pro-IL-16 is a 631 amino acid precursor molecule [1],[2], which can be cleaved by caspase into two parts. The N-terminal part is kept in the cytoplasm [3], and the C-terminal is a secreted peptide containing 121 amino acids. Researches have shown that the recombinant IL-16 C-terminal 130 a.a. has same bioactivity as the natural secreted human IL-16[1], [4],[5].

The human secreting IL-16 is the natural soluble ligand of CD4 molecule[4], [6], [7]. It stimulates a series of signals mediated by CD4, resulting in the activation of p56lck, increases of intracellular Ca<sup>++</sup>

and phosphatidylinositol 1, 4, 5-trisphosphate and the increased expression of IL-2 receptor[4],[8-10]. The signals by CD4 can activate the T cell when there is another co-stimulator. The cross-linking of cell surface CD4 with anti-CD4 mAb or HIV-1 gp120 may mediate apoptosis rather than activation of the CD4<sup>+</sup> T cell[11-15]. Interestingly, another cytokine, IL-2, can also prime T cells for cell death in addition to its activity in activation and proliferation of T cells [16]. All those results indicated that the activation and cell death are conditional events, the difference is very much dependent on the amount of stimuli, cell type, and the presence of co-activators etc.

As reported here, we have investigated the possible role of rIL-16 to Jurkat T leukemia cells. We found that the rIL-16 showed the growth-stimulating activity at  $10^{-9}M/L$  to Jurkat cells, but a growth-inhibiting activity was detected at higher concentra-

\* **Corresponding author:** Prof. Yong Hua XU  
Tel/Fax: 0086-21-64711349, E-mail: yhxu@sunm.shnc.ac.cn  
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tion ( $10^{-5}M/L$ ) of IL-16 caused by an enhanced apoptosis in the cells. The IL-16 induced growth inhibition in Jurkat cells was Fas/FasL independent and may be related with the activation of c-Myc, Bid and PKC. The inhibitor of MEK1 blocked the effect of rIL-16 on Jurkat cells. The results indicated that the rIL-16 might regulate the growth and apoptosis through the up-regulation of the expression of c-Myc, Bid and the activation of PKC. The ERK pathway was closely associated to the IL-16 regulated proliferation and apoptosis in Jurkat cells.

## MATERIALS AND METHODS

### Cells and cultures

Jurkat T leukemia cells were from Shanghai Cell Bank, Chinese Academy of Sciences. The cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 25 mM HEPES buffer, 100 U/ml penicillin, and 100  $\mu$ g/ml streptomycin.

### Antibodies

Anti-IL-16 monoclonal antibody was a product from Yes Biotech Lab, Canada. Rabbit anti human CD4, CD8, FasL antibodies, and FITC-conjugated goat anti-rabbit Ig were purchased from Santa Cruz Biotech, Inc.

### Recombinant human IL-16

Human PBMCs were obtained from the venous blood of healthy normal human volunteers by density centrifugation on Ficoll-Paque (Pharmacia Fine Chemicals).

RNA was isolated from PHA-stimulated PBMC cells using Trizol (Gibco/BRL). Reverse transcription was performed in 30  $\mu$ l final volume containing 0.5  $\mu$ g total RNA which was incubated for 15 min at 65 °C before addition of 0.25 mM mix dNTPs, 1 $\times$  reverse transcriptase (RT) buffer (MBI Fermentas), RNasin (4U), DTT 10 mM, random primer 2  $\mu$ M and 10U M-MLV RT (MBI Fermentas). The reaction was allowed 1 h at 37 °C and 5 min at 95 °C. The cDNA product of reverse transcription was amplified in the presence of IL-16-specific primers flanked with a BamH 1 site at 5' end of forward and a Cla 1 site at 3' end of reverse. The primers were 5'-CGC GGA TCC ATG CCC GAC CTC AAC TCC-3' and 5'-CCA TCG ATC AGC ATG TCC TGC CTA GGA G-3'. Then, the PCR product was digested with BamH 1 and Cla 1, and cloned into pBluescript (SK) vector. After sequence confirmation, the IL-16 fragment was re-cloned into E.coli expression vector pT7-7His and expressed in BL-21 cells under IPTG induction.

### RT-PCR analysis of gene expression

We adopted semi-quantitative RT-PCR to detect the expression level of the target genes.  $\beta$ -actin was used as internal control. cDNA (1  $\mu$ l) was added to reaction mixtures (50  $\mu$ l total volume) containing 1 $\times$ PCR buffer, 200  $\mu$ M dNTPs (Pharmacia Biotech),

1  $\mu$ M of forward and reverse primers, 5U Taq DNA polymerase, and 1.5 mM MgCl<sub>2</sub>. The primers are as follows:  $\beta$ -actin (360bp): 5' - A C A C T G T G C C C A T C T A C G A G G G G - 3' and 5' - A T G A T G G A G T T G A A G G T A G T T T C G T G G A T - 3'; FasL (450bp): 5' - A T G T T T C A G C T C T T C C A C - 3' and 5' - A G A G A G A G C T C A G A T A C G - 3'; Bid (600bp): 5' - G C T G C C C A G C A T A T G G A C T G T G A G G T C A A C - 3'; and 5' - C T T C T G G A A G G A T C C G T T C A G T C C A T C C C A T T T C T G - 3'; c-Myc (442bp): 5' - G C C C A C C A C C A G C A G C G A C T C - 3', and 5' - C T T G G G G C C T T T T C A T T G T T T T C - 3'. PCR reactions were conducted in a Peltier Thermal Cycler PTC-100 (MJ Research company). The PCR involved reactions at 94 °C for 30s, annealing at 55 °C for 30 s and extension at 72 °C for 1 min. PCR products were separated by electrophoresis on 1.5% agarose gel and identified by fragment size.

### Western blot analysis

The protein extractions were analyzed by Western blot. In brief, 10 mg of total protein extract of Jurkat cells was separated by SDS-PAGE, transferred onto nitrocellulose membrane and probed with mouse anti-FasL monoclonal antibody and second antibody. Finally, the band was developed with diaminobenzidine.

### PI stain and FACS

Jurkat cells ( $5 \times 10^6/ml$ ) were washed twice with PBS and incubated for 25 min at 4 °C with 4% formaldehyde. After three washes in PBS, cells were incubated with fresh PI stain solution (5  $\mu$ g/ml PI and 50  $\mu$ g/ml RNaseA in PBS pH 7.4) for 15-20 min in dark at room temperature. Then, cells were examined in fluorescence or used for flow cytometry analysis, with FACS caliber and analysis software CELLQuest (Becton Dickinson).

### Cell counting and MTT assay

The effects of IL-16 to Jurkat cells were detected by cell counting and MTT assay. In cell counting experiment, 3  $\mu$ l of  $1 \times 10^6$  Jurkat cells/ml were cultured in different concentrations of rIL-16-containing RPMI-1640 without serum for three days. Cell numbers were counted in a haemocytometer in the presence of 0.5% trypan blue. The experiment was repeated at least 6 times.

The specific inhibitors, 0.15  $\mu$ M Bisindolylmaleimide (Bis) for PKC, 0.25  $\mu$ M PD98059 (PD) for MEK, 8 mM SB203580 (SB) for p38, were added into medium 1.5 h before rIL-16 to examine the possible regulators and associated signal pathway responsible to the rIL-16 action in the cells. After 24 h treatment, cells were harvested for MTT assay. Briefly, MTT was dissolved at a concentration of 5 mg/ml in sterile PBS. Extraction buffer was composed of 20% W/V of SDS (AMRESCO) in 50% of DMF (Fluka). pH was adjusted to 4.7 by adding 2.5% of 80% acetic acid and 2.5% of 1N HCl. Each well was added 25  $\mu$ l MTT stock solution, incubated at 37 °C for 4 h, then added 100  $\mu$ l of the extraction buffer. After an overnight incubation at 37 °C, the optical densities at 570 nm were measured using ELISA Microplate Reader (BIO-RAD Model 450).

## RESULTS

### *rhIL-16*

The recombinant human IL-16 have been expressed and purified was a 130 a.a. fragment beginning from Met at site 502 and ended until 631 of pro-IL16, which has been shown to be sufficient for the biologic activity of IL-16[17],[18].

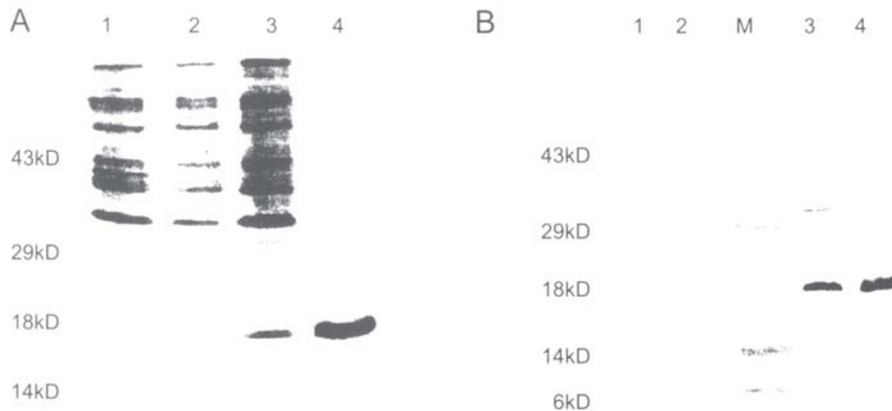
IL-16 expressing vector transformed BL21 (DE3) cells were cultured in Terric Broth medium. When the OD600 reached 0.6-0.7, 1 mM IPTG was added to induce the expression of IL-16 for another 4 h before harvest. Coomassie blue staining of SDS-PAGE of the harvested cell extract detected a protein with the proper 18 kD molecular weight (Fig 1A). Western blot confirmed the 18 kD protein specifically interact with anti-IL-16 monoclonal antibody (Fig 1B) indicating that the recombinant pro-

tein showed the predicted molecular weight and immunological activities of human IL-16.

The recombinant IL-16 was further purified by affinity chromatography on Ni<sup>2+</sup>-NTA agarose (Qiagen), and was evaluated to be more than 95% purity by silver staining (data not shown). Fractions were tested for activation of PBMCs proliferation according to Parado's method[19], and the results confirmed that the recombinant IL-16 has the human IL-16 biologic activity (data not shown).

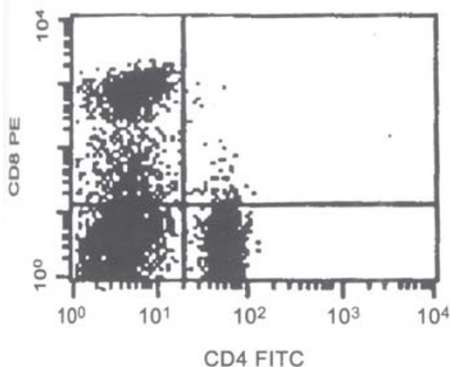
### *The effects of rhIL-16 on the growth of Jurkat T leukemia cells*

To confirm the potential response activity of Jurkat cells to IL-16 treatment, the surface marker molecules of the cells was detected by flow cytometry with anti-CD4 and anti-CD8 monoclonal antibodies. As shown in Fig 2, the Jurkat cells used in the ex-



**Fig 1.** rIL-16 expression, purification and Western blot analysis

**A.** Coomassie blue-stained 12% SDS-PAGE gel of E.coli BL-21 (DE3) cell lysate expressing recombinant human IL-16. Lane 1: untransformed BL-21 cell lysate; Lane 2: transformed by pT7-7His blank vector; Lane 3: total protein of pT7-7His-IL16 vector transformed BL-21 lysate. Cells were harvested after induction by 1 mM IPTG for 4 h; lane 4, Ni<sup>+</sup> affinity chromatography purified rIL-16 protein from lysate of Lane 3. **B.** Western blot assay of rIL-16 expression. Lane 1, 2, 3, 4, same as Fig 1A. The IL-16 expression product is about 18kD. Equal volumes of protein solutions were loaded in each lane.



Quad	Events	% Gated
UL	1351	27.02
UR	81	1.62
LL	1490	29.80
LR	2078	41.56

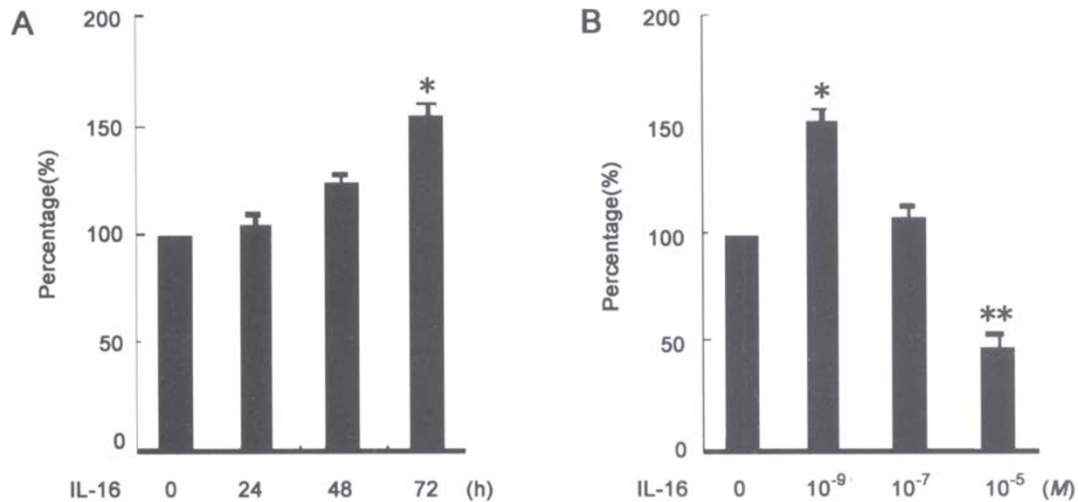
**Fig 2.** FACS analysis of surface markers of Jurkat cells

FACS analysis was performed as described in Material and Method. Up-left (UL) represents CD4<sup>+</sup>CD8<sup>+</sup> cells; Up-right (UR) represents CD4<sup>+</sup>CD8<sup>-</sup> cells; Low-left (LL) represents CD4<sup>-</sup>CD8<sup>-</sup> cells; Low-right (LR) represents CD4<sup>+</sup>CD8<sup>-</sup> cells.

periments were a population containing 41.56% of CD4<sup>+</sup> cells, 27.02% of CD8<sup>+</sup> cells and 1.54% of CD4<sup>+</sup> CD8<sup>+</sup> cells. IL-16 is a native ligand to CD4; the rIL-16 would be able to react with CD4<sup>+</sup> Jurkat cells in the population.

The regulation effects of IL-16 on the growth of Jurkat cells were examined by cell counting and MTT assay. In the presence of 10<sup>-9</sup> M IL-16/L, the

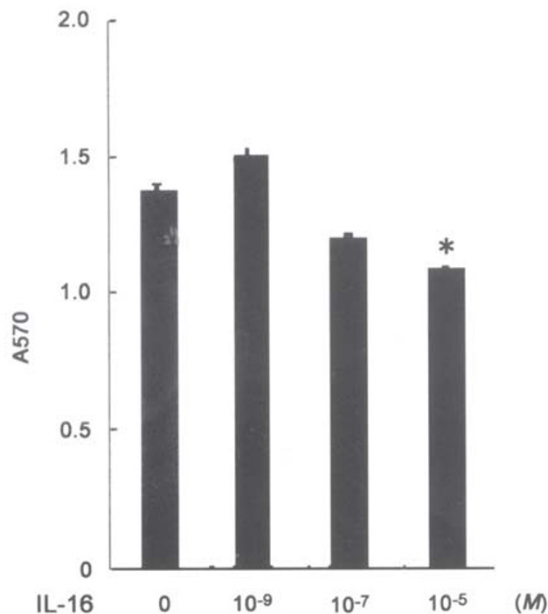
growth of Jurkat cells was estimated by cell counting at 24 h intervals for three days. To normalize the result, rectified average was calculated as following: Cell Number (with rIL-16 or not)/Cell number (without rIL-16). Control was defined as 100%. As shown in Fig 3A, cells number increased gradually in the presence of 10<sup>-9</sup> M rIL-16 compared with control showing the stimulating activity of rIL-16 to



**Fig 3.** Effects of rIL-16 on Jurkat cell growth.

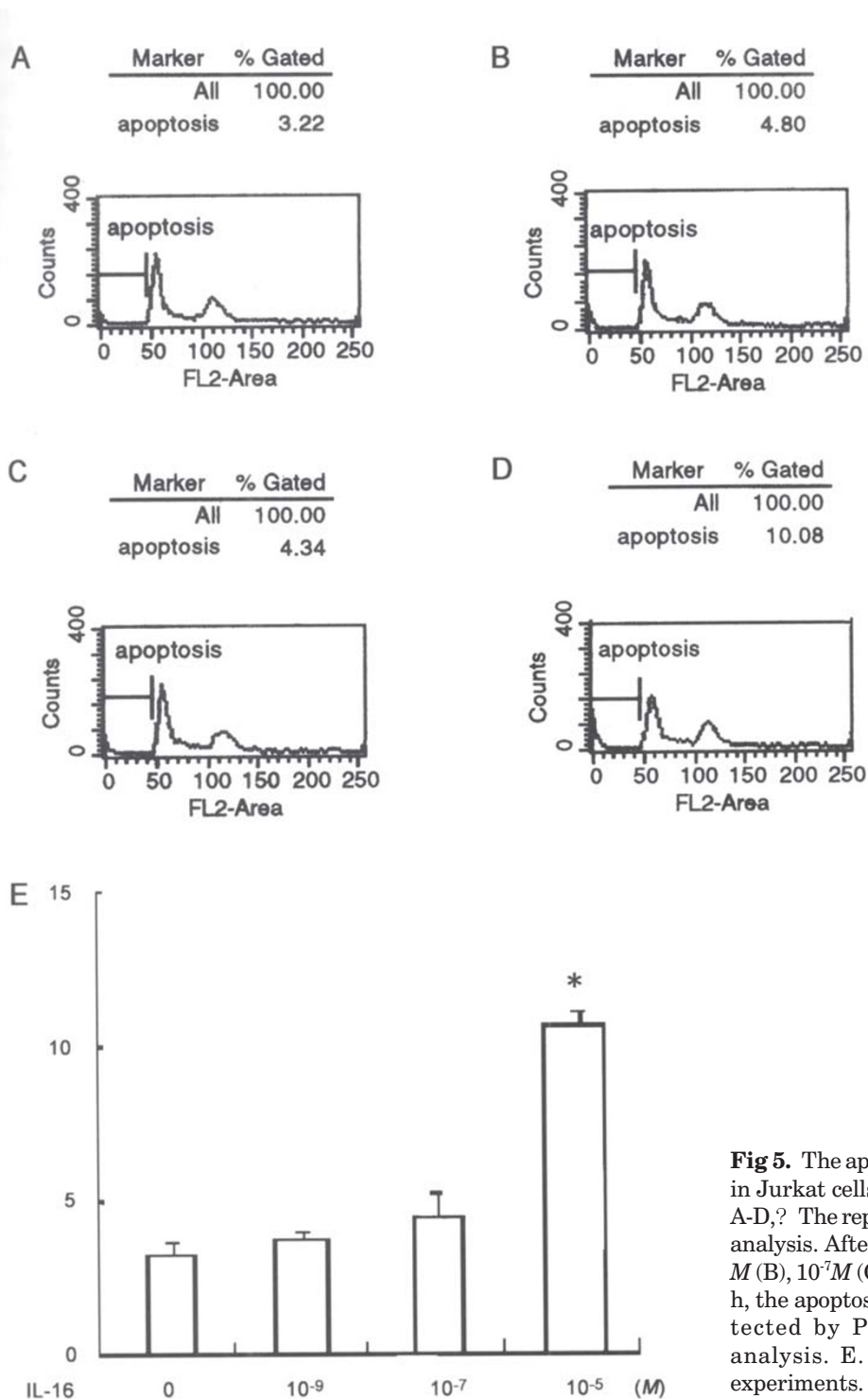
**A.** Cell number was detected by cell counting in the presence of 10<sup>-9</sup> M rIL-16 for 0, 24, 48, 72 h. Rectified average of three experiments showed a significant increase of cell proliferation at 72 h.

**B.** Cell number was detected by Cell counting with or without the presence of rIL-16 (0, 10<sup>-9</sup> M, 10<sup>-7</sup> M, 10<sup>-5</sup> M). Over three days, cell number was compared by rectified average with control. Rectified average showed a significant decrease in 10<sup>-5</sup> M rIL-16 treated cells. \**P* < 0.05, \*\**P* < 0.01.



**Fig 4.** Effects of rIL-16 treatment for 24 h on Jurkat cell growth

Cell growth was detected by MTT assay in the presence or not of rIL-16 (0, 10<sup>-9</sup> M, 10<sup>-7</sup> M, 10<sup>-5</sup> M). After 24 h treatment, cell viability was detected at OD570. Column chart showed an inhibition of cell viability in the highest rIL-16 (10<sup>-5</sup> M) treatment. \**P* < 0.05.



**Fig 5.** The apoptosis induced by rIL-16 in Jurkat cells  
 A-D, The representative data of FACS analysis. After treatment of 0 (A),  $10^{-9}$  M (B),  $10^{-7}$  M (C),  $10^{-5}$  M (D) rIL-16 for 24 h, the apoptosis of Jurkat cells was detected by PI staining and FACS analysis. E. The average of three experiments. \* $P < 0.01$ .

Jurkat cells. Similar as cell counting, MTT assay also showed the time-dependent stimulating activity of  $10^{-9}$  M rIL-16 on Jurkat cells (data notshown). However, increased amount of IL-16 (up to  $10^{-5}$  M/L) in the medium indicated an inhibiting action to

Jurkat cells as shown in Fig 3B. The results suggested that the rIL-16 affected the proliferation of Jurkat cells in a dose dependent manner. The cells number decreased 45% under the treatment of  $10^{-5}$  M/L of IL-12 for three days in contrast to control



cells. Further evidence for the inhibiting effect of rIL-16 on Jurkat cell viability was also obtained by MTT assay. Under 24 h treatment of  $10^{-5}M$  rhIL-16, the growth of Jurkat cells was markedly inhibited. The result demonstrated that rIL-16 in higher dose ( $10^{-5}M$ ) had the inhibiting effect to the cells (Fig 4).

#### Higher concentration rIL-16 treatment resulted in an enhanced apoptosis

To Jurkat T leukemia cells, rIL-16 showed either growth-stimulating activity at low dose ( $10^{-9}M$ ) or growth-inhibiting activity at high dose ( $10^{-5}M$ ). The effects of  $10^{-5}M$  rIL-16 to Jurkat cells were further examined by DNA measurement of the cells. After treatment, the cells were stained with Propidium Iodide and run on FACS Calibur (Becton Dickinson, America) and analyzed by CELLQuest (Becton Dickson).

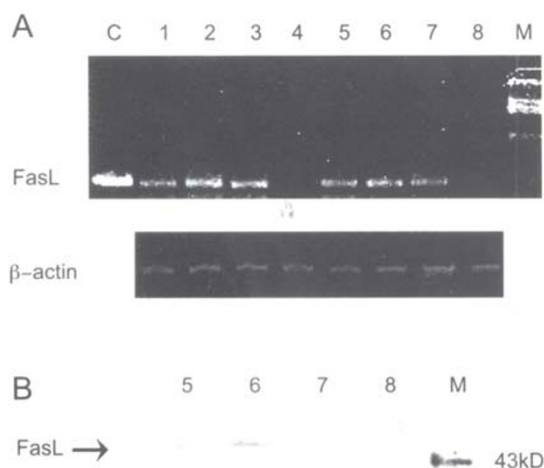
The FACS assay indicated a low ratio of spontaneous apoptosis of Jurkat cells in serum-free medium (Fig 5A). Low concentration ( $10^{-9}M$  and  $10^{-7}M$ ) of rIL-16 treatment showed no significant change of cell apoptosis in 24 h (Fig 5B, C). But, under  $10^{-5}M$  rIL-16 treatment for 24 h, apoptotic cells ratio was increased to 10.08% (Fig 5D). Fig 5E shows the average result of three experiments. It has been reported recently that the activation of  $CD8^+$  T cells induced the expression of CD4[20]. We have not

examined the induction of CD4 molecule on  $CD8^+$  cells in Jurkat cells in the presence of rIL-16. In rIL-16 treated Jurkat cell population, the real apoptotic cells of total  $CD4^+$  target cells might be more than 10.08%.

#### Apoptosis induced by rIL-16 may be Fas/FasL pathway independent, but Bid and c-Myc associated in Jurkat cells

To identify the death signal molecules activated in apoptosis caused by rIL-16, we first analyzed the possible role of Fas/FasL pathway. Because Fas was constitutively expressed on the lymphocyte cell surface[21], we measured the FasL expression level in the Jurkat cells under the treatment of IL-16 by RT-PCR. As shown in Fig 6A, the Jurkat cells endogenously expressed FasL, and maintained the expression level in low dose rIL-16-containing medium. However, incubation with  $10^{-5}M$  rIL-16 for 24 h demonstrated a markedly inhibition of FasL mRNA expression in the cells.

Western blot showed a similar results (Fig 6B) indicating the FasL protein could only be detected in control cells and the  $10^{-9}M$  rIL-16 treated cells, but not in the  $10^{-7}M$  and  $10^{-5}M$  rIL-16 treated cells. Our results suggested that Fas/FasL death receptor mechanism seems not to be responsible for the apoptosis induced by rIL-16 in Jurkat cells.



**Fig 6.** Effects of rIL-16 on FasL expression in Jurkat T cells

Jurkat cells were incubated with rIL-16 for 15 and 24h. The FasL expression was detected by RT-PCR(A) and Western Blot assay (B).

**A.** Upper panel showed FasL expression. Lane C, positive control; Lanes 1, 2, 3, 4 represent 0,  $10^{-9}$ ,  $10^{-7}$ ,  $10^{-5}$  M rIL-16 treatment for 15 h; Lanes 5, 6, 7, 8, represent 0,  $10^{-9}$ ,  $10^{-7}$ ,  $10^{-5}$  M rIL-16 treatment for 24 h. M was Markers. The lower panel was the PCR result by  $\beta$ -actin primer as loading control.

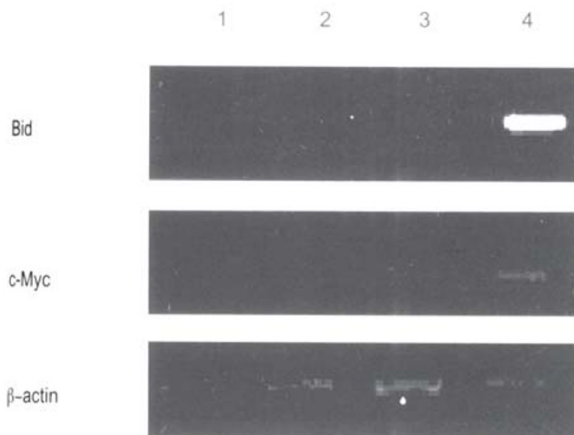
**B.** FasL protein expression. Jurkat T cells were treated with rIL-16 at various doses. Lanes 5, 6, 7, 8, represent 0,  $10^{-9}$ ,  $10^{-7}$ ,  $10^{-5}$  M rIL-16 treatment for 24 h. Equal amount of protein was loaded on a 12% SDS-PAGE. The expression level was analyzed by anti-FasL antibody. M was 43 Kd protein marker.

Bcl-2 family members bearing only the BH3 domain are essential inducers of apoptosis. The BH3-only protein Bid is one of the most important molecules in programmed cell death. We analyzed the expression of Bid in rIL-16 treated Jurkat cells. RT-PCR results definitely revealed the stimulation of higher expression of Bid in Jurkat cells after  $10^{-5}M$  rIL-16 treatments for 24 h (Fig 7) as compared with un-detectable signal in control cells and low dose ( $10^{-9}M$ ) rIL-16 treated cells.

As a transcript factor, c-Myc is a critical molecule in transmitting up-stream signals into proliferation or apoptosis effects by a series of regulating genes. The c-Myc expression in rIL-16 driven Jurkat cells was also examined by RT-PCR. As shown in Fig 7, c-myc transcripts were undetectable in the control cells and  $10^{-9}M$  or  $10^{-7}M$  of rIL-16 treated cells, but up-regulated expression of c-myc occurred in  $10^{-5}M$  rIL-16 induced Jurkat cells. We, therefore, postulated that c-Myc over-expression in rIL-16 treated Jurkat cells might be also responsible to the apoptosis of the cells.

#### Signal transduction pathway involved in rIL-16 action on Jurkat cells

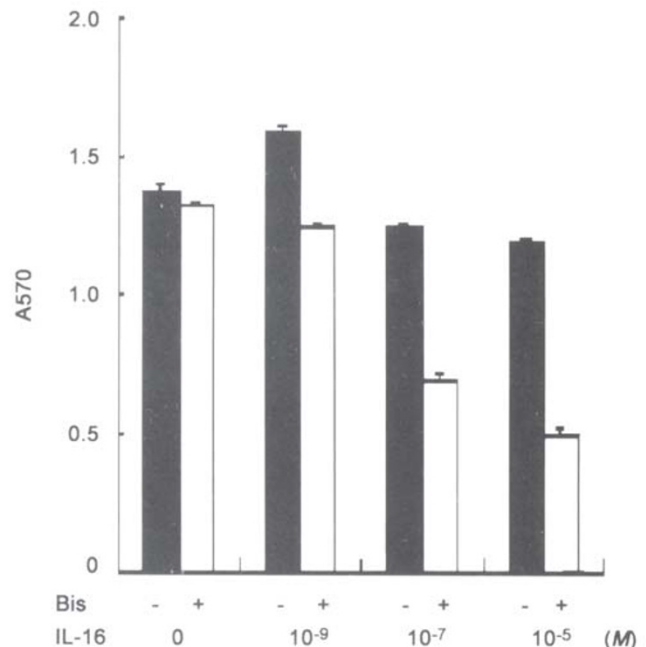
To understand the signal pathways involved in the growth-stimulating or growth-inhibiting activities of rIL-16 on Jurkat cells, the possible role of PKC and ERK were investigated.



**Fig 7.** rIL-16 induced expression of Bid and c-Myc detected by RT-PCR

Jurkat cells were cultured for 24 h in the presence or not of rIL-16. Lanes 1, 2, 3, 4 represent 0,  $10^{-9}$ ,  $10^{-7}$ ,  $10^{-5}M$  rIL-16 treatment for 24 h, respectively. Expression level of Bid and c-myc were quantified by RT-PCR using specific primers.  $\beta$ -actin levels were used to monitor the equal amount template being used.

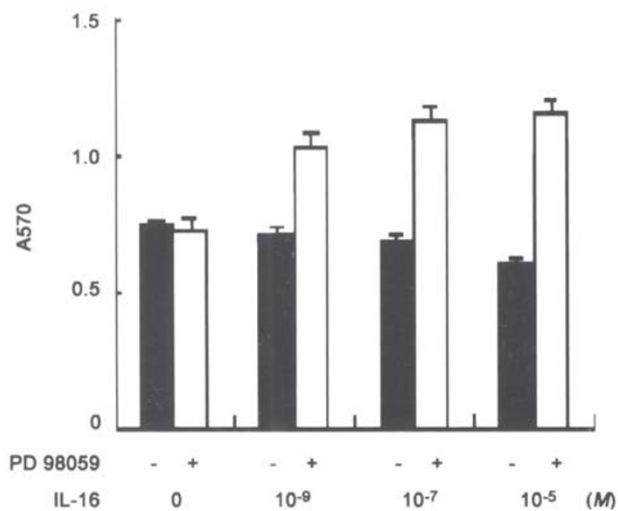
PKC is a  $Ca^{2+}$  dependent Ser/Thr kinase. It is activated in cell proliferation, differentiation, and migration[10]. We used the PKC specific inhibitor Bisindolymaleimide (Bis) to analyze its action in rIL-16 treated Jurkat cells. In the presence of  $0.15 \mu M$  Bis, cells viability under the treatment of different concentration of rIL-16 was measured by MTT assay. The data showed in Fig 8 indicated that the proliferation of cells dropped rapidly in the presence of Bis in all of the rIL-16 treated cells, but little change was found in rIL-16 not treated cells. This result suggested that the growth regulation activity of rIL-16 on Jurkat cells might be associated with the activation of PKC in the cells. The blocking of PKC activity by Bis inhibited partially the proliferation of rIL-16 treated Jurkat cells, in comparison, no effect was found in control cells without rIL-16 treatment. The results reflected the dominant inhibitory effect of Bis on cell proliferation in this situation indicating PKC must take a role in triggering proliferation in the Jurkat cells induced by rIL-16.



**Fig 8.** Block of rIL-16 treated Jurkat cell proliferation by Bisindolymaleimide (Bis)

PKC inhibitor Bis was added 1.5 h before rIL-16 induction to block the action of PKC in the regulation of Jurkat cell growth. MTT assay measured the decrease of cell growth in rIL-16 treated Jurkat cells.

Mitogen-activated protein kinase (MAPK) cascades have been shown to play a key role in transduction of extracellular signals to cellular responses. At least three MAPK families have been found and characterized: extra-cellular signal-regulated kinase (ERK), Jun kinase (JNK/SAPK) and p38 MAPK. We used the MEK1-specific inhibitor, PD 98059, and the p38 specific inhibitor, SB 203580, to analyze the mechanism of IL-16 induced apoptosis in Jurkat cells. Jurkat cells were incubated with 50  $\mu$ M PD98059 or 8  $\mu$ M SB203580 before IL-16 treatment, the cell growth was determined by MTT assay. Fig 9 showed that the addition of PD98059 dominantly prevented the cells from apoptosis induced by IL-16. No effect was detectable in the SB203580 treated cells (data not shown). The result implied that the MEK1 might be involved in apoptosis signal delivery in Jurkat cells.



**Fig 9.** Block of rIL-16 induced growth-suppressing effect by PD 98059 in Jurkat cells  
The MEK1 inhibitor PD98059 was added 1.5 h before rIL-16 treatment. MTT assay was used to measure the growth of the cells.

## DISCUSSION

Human IL-16 functions as a pleiotropic regulator stimulating the proliferation, activation, chemotaxis and programmed cell death on T lymphocytes. The signals to T cell are all mediated by its natural receptor CD4. The role of CD4 in the regulation of cell activation and death is pretty complex.

We report here the recombinant IL-16 may stimu-

late the proliferation of Jurkat T leukemia cells at  $10^{-9}$  M and potentially inhibit the cell growth accompanied by an increased apoptosis under  $10^{-5}$  M of IL-16 induction. The inhibiting effects of IL-16 on CD3-dependent lymphocyte activation and proliferation were previously reported[8],[22]. Cross-linking of the CD4 molecule by anti-CD4 mAb or by HIV envelope protein gp120 has been shown to prime resting CD4<sup>+</sup> T lymphocytes for apoptosis[24],[25]. While the opposite effects on activated T cell was also observed, ligation of CD4 by anti-CD4 mAb or HIV-1 gp120 drastically inhibited subsequent AICD (Activation Induced Cell Death) of human T cell clones triggered through CD3/TCR, due to the prevention of up regulation of FasL[25],[26]. Similarly, CD3/CD4 co-ligation was shown to inhibit T-cell activation in the absence of a costimulus in mouse lymphocytes[27]. In a word, the presence of costimulus and the subsequent events of stimulus may direct the cell to activation or death. Based on these former results, it could be postulated that IL-16 may also function as a stimulus for apoptosis besides its activation effect on CD4<sup>+</sup> T cells.

Various members of the interleukin family, like IL-2, have been shown to possess both anti-apoptotic and pro-apoptotic stimulation activity[16]. IL-2 can induce proliferation in most circumstances, but it has also been reported that IL-2 can down-regulate the caspase-8 inhibitor c-FLIP[23], and up-regulate FasL[16], both molecules can prime cells for death.

Several studies have shown the expression and interaction of Fas and FasL can result in the autocrine stimulation of Fas/FasL death pathway in AICD in T hybridoma cells and activated T cells. Because Jurkat cells constitutively express Fas at their surface and are sensitive to Fas-induced apoptosis[21], our research focuses on FasL expression. Surprisingly, rIL-16 doesn't up-regulate FasL, which correlate with AICD in Jurkat cells caused by most stimulators[21], [29-32], but does down regulate its expression on both RNA and protein level. Although several studies have shown that the expression and interaction of Fas and FasL is required for AICD of activated T cells. But, Fas/FasL pathway is not an absolute requirement for death control[33]. These results also hint that there may be another apoptosis pathway functioning in the IL-16 mediated effects.

We found that c-myc and Bid expression level were



up regulated by IL-16. This result suggested that c-Myc might be involved in the process to activate certain cell death associated genes, but not FasL in this case. The Bcl-2 family proteins consist of both antagonists and agonists that regulate the apoptosis by competing through dimerization. Over-expression of Bcl-2 suppresses Fas-mediated apoptosis in human hepatocellular carcinoma BEL-7404 cells and Trail-induced apoptosis in Jurkat cells[34],[35]. Bid, one of the BH3 domain-containing molecules, promotes cell death after binding with Bcl-2[34],[36]. The up-regulation of Bid in IL-16 treated Jurkat cells implied the involvement of Bid molecule in the cell death through other death receptors, tumor necrosis factor or Trail etc.

To figure out the special pathways associated with the regulation activity of IL-16 in Jurkat cells, we found that PKC was activated after IL-16 treatment, and ERK pathway is also required for control of rIL-16 induced Jurkat cell death. It has been reported that the ERK pathway is activated during AICD upon stimulation of the T cell receptor-CD3 complex [37]. However, the IL-16 activates the stress activated protein kinase (SAPK) pathway and p38 MAPK, but, not ERK, in CD4<sup>+</sup> macrophages. The IL-16 mediated activation of SAPKs and p38 in macrophages alone does not induce a detectable apoptotic cell death [38]. Our results suggest that, in Jurkat T leukemia cells, the ERK pathway is responsible for the rIL-16-induced cell death. PKC pathway and ERK pathway have a common regulated molecule, nur77[37], [39], which is an orphan nuclear steroid receptor [40]. It transmitted the signal to apoptosis related gene through protein-protein interaction [41]. We have not elucidated every molecule in these signal pathways, but the signal is probably transduced by both PKC and ERK pathway and regulated the effect to proliferation or apoptosis. From our result, the high concentration of IL-16 ( $10^{-5}M$ ) can induce CD4<sup>+</sup> T cell apoptosis without any other pre-stimulation, although no significant difference was observed in the lower concentration IL-16 ( $10^{-9}M$ ) treatment. So a dual role happened to IL-16 in Jurkat cells. The cytokine can stimulate cells proliferation and activation, but also induce apoptosis under certain conditions. It might be a kind of dose effect, but the signal pathway participation makes it looks like a special biological activity. So the function of

IL-16 in CD4<sup>+</sup> T cells needs to be further elucidated.

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