ORIGINAL ARTICLE

Effect of interleukin-1β on cell proliferation

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Abstract

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Interleukin-1 (IL-1), a pro-inflammatory cytokine, has been undergoing clinical evaluation for its anti-neoplastic activity in potentiating the immune response. However, direct effects of this cytokine on cell proliferation in some cancer cells are not well understood. In this study, nonradioactive MTT proliferation assays were employed to investigate the role IL-1 in regulation of proliferation in 5 cell lines. Recombinant human IL-1β treatment resulted in inhibition of cell proliferation as well as the induction of cytotoxicity compared to the untreated control groups ($p<0.05$) in Jar, MCF-7, and HEL cell lines, whereas proliferation of Caco-2 cells was not affected. Furthermore, its stimulatory effect was observed in KB cell line. The findings in this study indicated that IL-1 has both inhibitory and stimulatory effects on cell growth and would provide more insight into the better administration of this cytokine in immunotherapy.

Key words : interleukin-1, proliferation, cytotoxicity

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บทคัดย่อ

อธิป นิลแก้ว

ผลของ interleukin-1β ต่อการเพิ่มจำนวนของเซลล์

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Interleukin-1 (IL-1) เป็นซัยโตคัยน์ที่ถูกผลิตขึ้นในระหว่างปฏิกิริยาการอักเสบ ด้วยสมบัติของซัยโตคัยน์นี้ในการกระตุ้นให้ระบบภูมิคุ้มกันทำงานได้ดีขึ้นทำให้มีการประเมินและทดสอบเพื่อใช้เป็นสารต้านมะเร็ง อย่างไรก็ตาม ผลโดยตรงของซัยโตคัยน์ชนิดนี้ต่อการเพิ่มจำนวนของเซลล์มะเร็งนั้น ยังไม่ปรากฏชัด การศึกษาครั้งนี้ได้เลือกใช้ MTT assays ในการศึกษาผลโดยตรงของ IL-1 ต่อการเพิ่มจำนวนของเซลล์ 5 ชนิด และพบว่า IL-1β สามารถยับยั้งการเพิ่มจำนวนของเซลล์และกระตุ้น cytotoxicity ในเซลล์ Jar, MCF-7 และ HEL เมื่อเปรียบเทียบกับกลุ่มที่ไม่ได้เติม IL-1β ($p<0.05$) ส่วนในเซลล์ Caco-2 นั้น ซัยโตคัยน์นี้ไม่มีผลต่อการเพิ่มจำนวนเซลล์ นอกจากนี้ยังพบว่า IL-1β สามารถกระตุ้นการเพิ่มจำนวนของเซลล์ KB ผลของการศึกษาในครั้งนี้แสดงให้เห็นว่า IL-1 มีผล ทั้งในการยับยั้งและกระตุ้นการเพิ่มจำนวนของเซลล์และสามารถใช้ข้อมูลเบื้องต้นนี้เป็นพื้นฐานในการศึกษาวิธีที่เหมาะสมในการบริหารซัยโตคัยน์ชนิดนี้เพื่อการรักษามะเร็งต่อไป

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IL-1 is a pleiotropic pro-inflammatory cytokine that is produced by activated lymphoid and non-lymphoid cells. The known activators of IL-1 production include bacterial lipopolysaccharide (LPS), complement components, and some cytokines. There are two known forms of IL-1, a membrane-bound IL-1α and secretory IL-1β, which are encoded by two distinct genes and share 27% homology (Dinarello, 1989). These forms exert similar biological effects. The binding of IL-1 to its receptor results in increased activation of transcription factor NF-κB, leading to a wide spectrum of biological effects, including local inflammation as a body defense to infection, and endocrine effect such as the induction of fever and shock (Dinarello, 1996).

IL-1 plays an important role in innate immunity as a body defense against infection (Dinarello, 1996), as well as in the reproductive system (Simon *et al*., 1994; Frank *et al*., 1995; Sawai *et al*., 1997; Kauma, 2000). It also exerts other biological effects in addition to those involved in innate immunity. For example, IL-1 acts synergistically with colony stimulating factors (CSFs) and IL-6 to stimulate hematopoiesis of stem cells. Furthermore, IL-1 also induces several factors that are important in hematopoiesis, (i.e., granulocyte-macrophage (GM)-CSF, granulocyte (G)-CSF, macrophage (M)-CSF, and IL-3) (Bagby *et al*., 1986; Zucali *et al*., 1986; Zsebo *et al*., 1988). This cytokine also play an important role in immunity to tumor and cancer cells. IL-1 treatment may increase innate defense against tumors through the activation of NK cells and lymphocytes. This, therefore, makes IL-1 a potential therapeutic cytokine in cancer patients. However, the direct effect of IL-1 on tumor cells has not been well defined. Thus, in this study, the direct effect of IL-1β on proliferation of various cell types was investigated.

Materials and Methods

Cytokines, antibodies and reagents

Recombinant human IL-1β and monoclonal anti-human IL-1β antibody were purchased from Peprotech Inc. (Rocky Hill, NJ). Insulin-transferrin-selenium (ITS) growth supplement was purchased from Life Technology, Gaithersburg, MD.

Cell lines

Jar cell line is directly derived from a trophoblastic tumor of the placenta. It produces estrogen, progesterone, gonadotrophin and lacto-

gen in culture. The cells were maintained in RPMI 1640 (Life Technologies, Gaithersburg, MD) with 10% FBS (Biochrom AG Seromed®, Belin, Germany) and antibiotics (100 unit/mL of penicillin G, 100 µg/mL of streptomycin sulfate, and 0.25 µg/mL of amphotericin B) (Life Technologies, Gaithersburg, MD). Jar cell line was purchased from ATCC (Manassas, VA) and cultured at 37ºC in 5% CO_2. MCF-7 is an adenocarcinoma of mammary gland. Caco-2 cell line is a colorectal adenocarcinoma cell line. KB cell line is an epidermal carcinoma cell line derived from oral cavity. These cell lines were kindly provided by Dr.Sithichai Khuntongkaew, Faculty of Dentistry, Thammasat University. Human embryonic lung fibroblast cell line (HEL) was kindly provided by the Virology lab unit, National Institute of Health, Department of Medical Science, Ministry of Public Health, Thailand. These cells were maintained in MEM (Biochrom AG Seromed®, Berlin, Germany) with 10% FBS and antibiotics (100 unit/mL of penicillin G, 100 µg/mL of streptomycin sulfate, and 0.25 µg/mL of amphotericin B) (Life Technologies, Gaithersburg, MD) and cultured at 37ºC in 5% CO_2.

Cell proliferation assay (MTT Assay)

The assay is designed for a spectrophotometric quantitation of cell growth and viability (Hansen *et al*., 1989) as well as the assessment of cytotoxicity (Carmichael *et al*., 1987). Cells (10,000 cells/well) were cultured in collagen I (50 µg/mL) coated 96-well culture plates, in a total volume of 200 µL serum-free medium with ITS growth supplement or with 1% FBS. The cells were cultured in the presence of recombinant human IL-1β (0-100 ng/mL). Both treatment and control groups were performed in 6-8 replicate wells. The relative number of viable cell was determined at 72 hours incubation, by incubating the cells with 1 mg/mL of 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide, MTT, for 4 hours. The live cells utilized MTT resulting in the accumulation of formazan crystals, which were then solubilized with acid isopropanol (90% isopropyl alcohol, 0.004 N HCL) for 1 hour. The optical density of the solution was then measured at 595 nm. The absorption value of the solution directly correlates to the relative cell numbers. These experiments were repeated at least three times to assure the reproducibility. One-way ANOVA was used to analyze data. For neutralization study, cells will be cultured as for the MTT assay in the presence of 50 ng/mL of IL-1β with or without neutralizing antibody (5 or 10 µg/mL).

LDH detection protocol

LDH release from cells was measured at the end of the proliferation experiments. Briefly, culture plates were spun at 1200×g for 15 minutes at room temperature to ensure that all of the cells are accumulated on the bottom of the wells. The cell-free culture media (100 µL) was collected and then incubated with 100 µL of reaction mixture (Boehringer Mannheim, Indianapolis, IN) for 30 minutes at room temperature in the dark. 1 N HCl (50 µL) was then added into each well to stop the enzymatic reaction. The optical density of the solution was then measured by using ELISA plate reader with 490 nm filter.

Results

The overall purpose of this project was to investigate direct effect of IL-1β on cell proliferation in four cancer cell lines (Jar, MCF-7, Caco-2, and KB) and one normal cell line (HEL). Jar proliferative response to IL-1 in serum-free medium containing ITS is shown in Figure 1A (bar). Data are presented as mean absorbance (λ=595 nm) ± SEM (n=6-8), which is proportional to the number viable cells in each sample. IL-1β inhibited Jar trophoblast proliferation by 57% at 100 ng/mL compared to the control groups (*$p<0.05$). Interestingly, in the presence of 1% FBS, IL-1β did not affect cell number (data not shown). This indicated that other growth factors in serum have overcome the inhibitory effect of IL-1. In related study, cytotoxic effects of IL-1β on Jar cells was determined by measuring release of cytosolic lactate dehydrogenase, as shown in Figure 1A (line). LDH concentrations in conditioned media are propor-

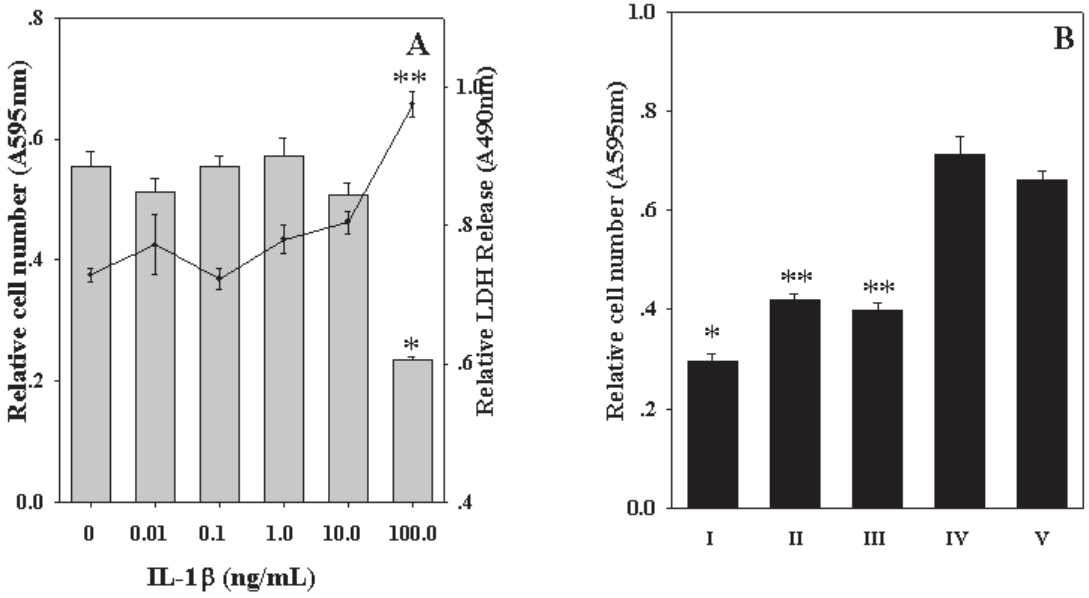

Figure 1. Jar proliferative response to IL-1 and inhibition of IL-1 effect. A. Jar cells were cultured in serum-free medium with increasing concentrations of IL-1β for 72 hours. Relative cell number (bar) was measured by MTT assay and relative LDH release (line) was measured by LDH assay. B. Jar cells were cultured in serum free medium for 72 hours in either IL-1β (50 ng/mL) alone (I), IL-1β (50 ng/mL) with anti- IL-1β antibody (5 μg/mL) (II), IL-1β (50 ng/mL) with anti- IL-1β antibody (10 μg/mL) (III), anti- IL-1β antibody (10 μg/mL) (IV), and untreated control (V). MTT assay was then performed. Asterisks (*, **p<0.05) represent significant comparisons between treatment and control groups analyzed by One Way ANOVA.

tional to cell death. Data are represented as mean absorbance (λ=490 nm) ± SEM (n=6-8). IL-1β treatment in these cells led to the increase of LDH release (**p<0.05), indicating IL-1β-promoted cytotoxicity.

To confirm the inhibitory effect of IL-1 on Jar cell proliferation, blocking of proliferation inhibition was studied by using anti-human IL-1β monoclonal antibody. Neutralizing activity of anti-human IL-1β monoclonal antibody was studied by incubating the antibody with IL-1β at least one hour prior to the addition to the cell culture. The MTT assay was then performed after 72 hours incubation. As shown in Figure 1B, IL-1β (50 ng/mL) inhibited Jar cells by 55% (I, *p<0.05) compared to the control groups (V). Anti-human IL-1β antibody 5 and 10 βg/mL blocked IL-1β activity by 34 % and 28 %, respectively (II, III, **p<0.05). Although monoclonal antibody could partially inhibit IL-1 activity, these findings confirmed the effect of IL-1 and indicated the existence of biological activity in this cytokine purchased. Inhibition of IL-1 activity in other cell lines in this research project, therefore, was not performed.

When cultured in the presence of FBS, IL-1β treatments did not affect relative cell number (bar) in MCF-7, KB, and Caco-2 (Figure 2A, 2B, and 2C, respectively). However, HEL cell proliferation could be significantly inhibited by IL-1β concentrations as low as 10 pg/mL (*p<0.05), and the cell number decreased to the maximum of 14.6% with 100 ng/mL of IL-1β (Figure 2D). Further analysis on LDH release (line) showed that IL-1β did not affect LDH release in KB, Caco-2 and HEL cell lines, indicating that this cytokine does not induce cytotoxicity in these cells.

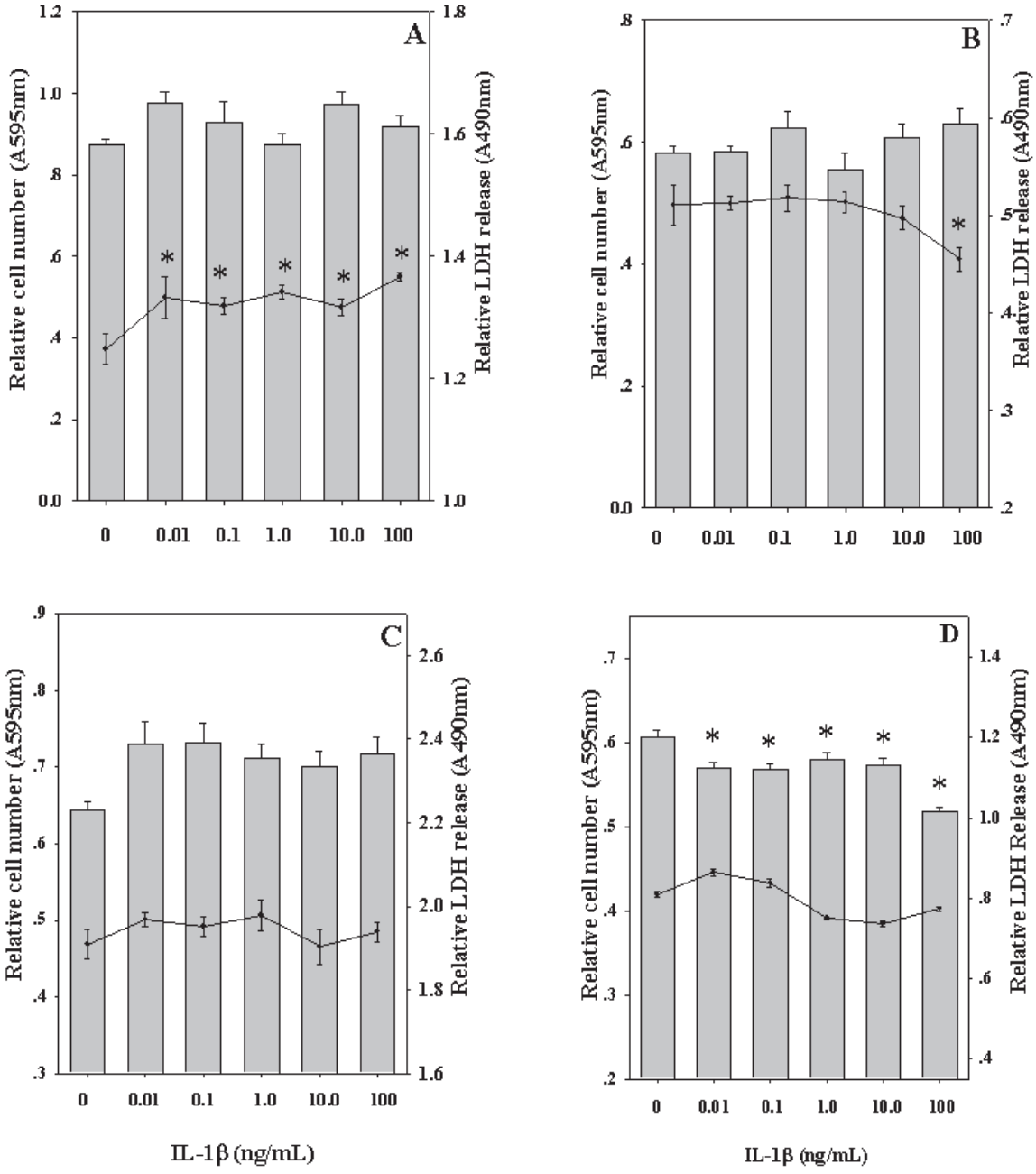

Figure 2. Effect of IL-1 on cell proliferation in FBS-containing media. MCF-7 (A), KB (B), Caco-2 (C), and HEL (D) cells were cultured in medium containing 1% FBS with increasing concentrations of IL-1β for 72 hours. Relative cell number (bar) was measured by MTT assay and relative LDH release (line) was measured by LDH assay. Asterisks (*p<0.05) represent significant comparisons between treatment and control groups analyzed by One Way ANOVA.

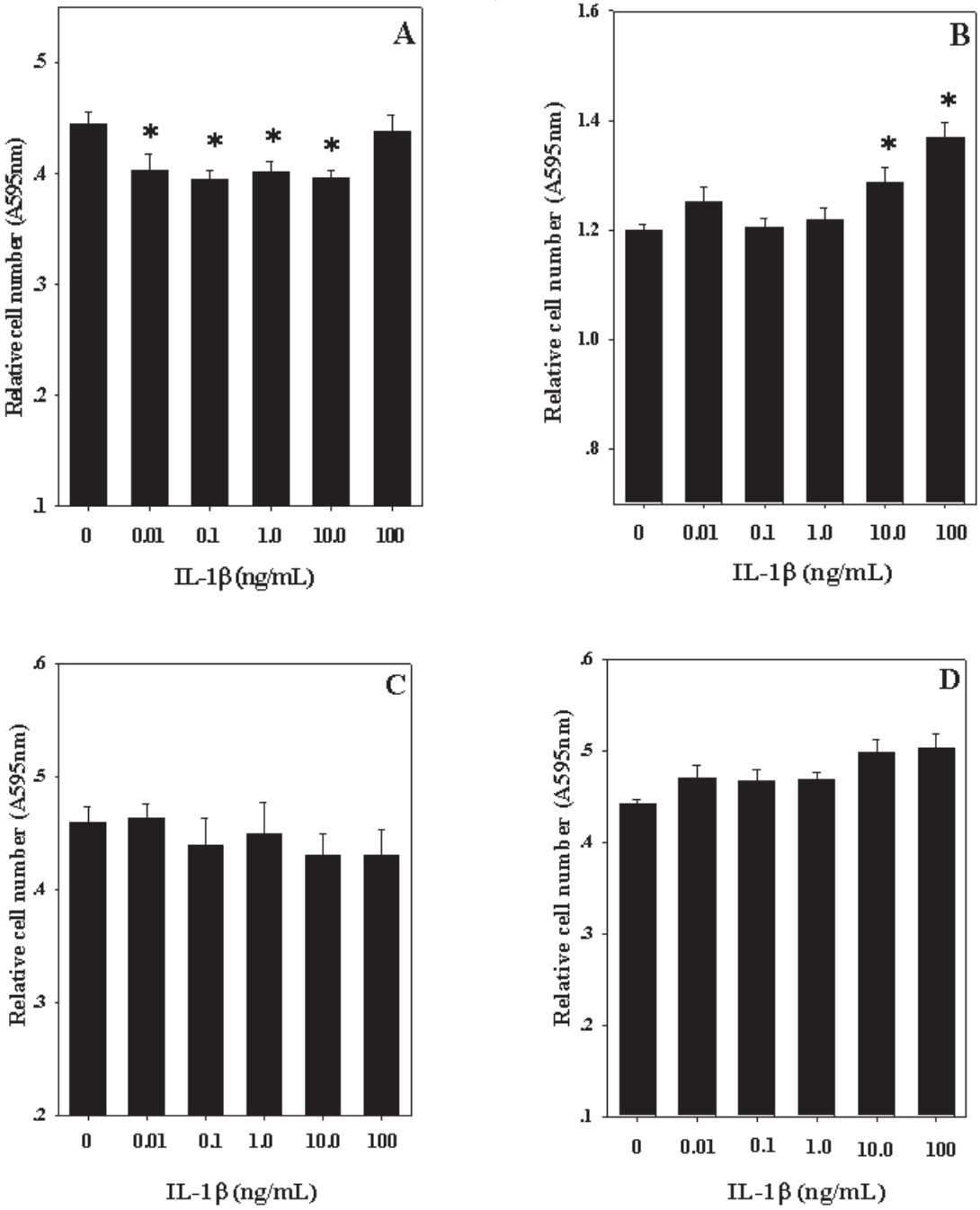

Figure 3. Effect of IL-1 on cell proliferation in ITS-containing media. MCF-7 (A), KB (B), Caco-2 (C), and HEL (D) cells were cultured in serum free medium containing ITS with increasing concentrations of IL-1β for 72 hours. Relative cell number was measured by MTT assay. Asterisks (*p<0.05) represent significant comparisons between treatment and control groups analyzed by One Way ANOVA.

Interestingly, as low as 10 pg/mL of IL-1β seemed to induce the increase of LDH (line) in culture media (*p<0.05) in MCF-7, indicating that IL-1 induces cytotoxicity in this cell line.

When cultured in serum-free media supplemented with ITS, IL-1β, as low as 10 pg/mL inhibited cell proliferation by 9% (*p<0.05) in MCF-7 cell (Figure 3A). Interestingly, at higher concentration (100 ng/mL) of IL-1, the relative cell number was not altered. In KB cells (Figure 3B), IL-1β was found to upregulate cell proliferation at the concentration of 10 ng/mL (*p<0.05). This finding indicated that IL-1 could act as a growth regulator or growth factor for KB cells. However, this cytokine did not affect cell proliferation in Caco-2 and HEL cell in serum-free media. This could be attributed to the low doses of IL-1 used in this study.

Discussion

The main purpose of this research project was to investigate the direct biological activity of IL-1 on regulation of cell proliferation on several cell types especially cancer cells, as this inflammatory cytokine has become an interesting target to use for cancer treatment in the form of adjuvant or as an immunopotentiator. Although IL-1 would provide detrimental effects as the induction of inflammation could provide pathology, modification of IL-1 molecule would be beneficial. Recently, a nonapeptide of IL-1β has been employed as an adjuvant for immunization without giving rise to inflammatory effect, but still has immunostimulatory function (Boraschi and Tagliabue, 1999).

The experimental design in this project has provided a new insight of IL-1β activity on cell lines in 2 conditions, in the presence or in the absence of fetal bovine serum. In these systems, IL-1β did not affect Caco-2 proliferation in either condition; however, previous study revealed that IL-1 treatment in this cell type led to decreased cell proliferation via the stimulation of cyclooxygenase-2 (Dommels *et al*., 2003). In Jar cell line, IL-1β could downregulate cell proliferation in the absence of FBS by inducing cytotoxicity, or perhaps cell death or the induction of cell differentiation. However, the presence of FBS could overcome the inhibitory effect of this cytokine in Jar cells. The inhibitory effect of IL-1 is consistent with the previous studies using macrophage-derived IL-1, which could suppress Jar cell growth (Steller *et al*., 1994). On the contrary, some studies have shown that IL-1 does not affect Jar proliferation by using other methods to study proliferation (Yagel *et al*., 1989), (Marth *et al*., 1995). Although the mechanism of IL-1 inhibition of Jar proliferation in our studies was unknown, it is likely to be mediated by the induction of cell death. Furthermore, IL-1 inhibition of cell growth was also observed in MCF-7 and is consistent with the previous studies (Liu and Gudas, 2002), (Tsai and Gaffney, 1986) and this may due to the production of nitric oxide (Reveneau *et al*., 1999). IL-1β also inhibited proliferation in a non-cancerous human embryonic lung fibroblast. In addition to the inhibitory effect, IL-1 exerts its stimulatory effect on KB cell growth indicating that this cytokine can act as a growth factor for this cell line. In conclusion, in this project using MTT assays, IL-1β was found to act as a growth regulator for the cell lines studied by inhibiting or stimulating cell proliferation. In addition, the inhibitory effect on cell proliferation of this cytokine may have a potential therapeutic use in choriocarcinoma and breast cancer.

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