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Comparison of the activities of granulocyte-macrophage colony-stimulating factor and interleukin-8 secretion between two lung epithelial cell lines

Shih-Chieh Lee[1], Jeng-Yuan Hsu[2], Lin-Shien Fu[1], Jao-Jia Chu[2], Sue-Jane Fan[1], Chin-Shiang Chi[1]

[1]Division of Immunology and Nephrology, Department of Pediatrics and [2]Division of Chest Medicine, Taichung Veterans General Hospital, Taichung; School of Medicine, China Medical University; and Institute of Medicine, Chung-Shan Medical University, Taichung, Taiwan

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The aim of this study was to survey the cytokine secretions in 2 human bronchial epithelial cell lines — a normal human bronchial epithelial cell line (HBEpC) and cell line A549, derived from malignant type II pneumocytes. The behavior of A549 cells is similar to epithelial cells and this line is widely used as an alternative model for studying human bronchial epithelial cell behavior. We measured the levels of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-8 (IL-8) after tumor necrosis factor-alpha (TNF-α) or interleukin-1beta (IL-1β) stimulation in the 2 cell lines. Both cell lines responded to TNF-α or IL-1β stimulation, as shown by increased GM-CSF and IL-8 secretion. The relative cost, convenience and similarity of working with these 2 cell lines suggest that A549 is preferable for use as a first-line model and that results of studies of GM-CSF and/or IL-8 secretion under various stimulation conditions with this line could be confirmed using HBEpC.

Key words: Asthma, cell lines, epithelial cells, granulocyte-macrophage colony-stimulating factor, interleukin-8

Asthma is a disorder characterized by contracted and variably obstructed airways [1]. Bronchial epithelial cells play an important role as a physical barrier in protecting the underlying tissue and maintaining the local environment in the airway [2].

These cells also play a critical role in the tissue damage and repair processes of asthma [3,4]. Bronchial epithelial cells can also synthesize and release a wide range of mediators in culture media with or without stimulation, including granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and IL-8 [5-8].

GM-CSF plays a significant role in the inflammatory cascade by stimulating cell recruitment, activation, and prolonging survival [9]. In normal airways, GM-CSF is expressed at low or undetectable levels but is significantly increased in the epithelium of asthmatics [10]. This enhanced expression may contribute to eosinophilia, a hallmark characteristic of asthma [11]. In addition, clinical studies involving segmental antigen challenge and bronchoalevolar lavage of allergic asthmatics have shown that GM-CSF remains chronically elevated after antigen challenge compared with other eosinophil-activating cytokines [12].

Another important asthma mediator secreted by lung epithelium is IL-8, a chemotactant for T-lymphocytes [13], eosinophils [14] and neutrophils [15]. Free IL-8 was been detected in the serum and bronchial tissue of subjects with severe atopic asthma but not in normal subjects or those with mild atopic asthma, suggesting that IL-8 may be a marker of severe asthma [15].

Many cell lines derived from malignant tumors of the respiratory tract have been used for studying GM-CSF and/or IL-8 secretions in human bronchial epithelium under various stimuli, including A549, H292 [16] and Hep2 [17].

The A549 cell line, derived from carcinoma cells of type II pneumocytes behaves like bronchial epithelium [18], and has therefore been widely used for studying bronchial epithelial inflammation in asthma. Virus-transformed human bronchial epithelial cell lines have also been frequently used for the same purpose [19]. However, malignant or virus-transformed bronchial epithelial cell lines are not as physiologically representative as those which are not virus transformed.

Corresponding author: Lin-Shien Fu, Department of Pediatrics, Taichung Veterans General Hospital, No.160, Sec. 3, Chung-Kang Rd., Taichung 407, Taiwan.
E-mail: linshienfu@yahoo.com.tw

In previous stimulation studies investigating mechanisms of airway inflammation, non-virus transformed bronchial epithelia were derived directly from bronchoscopic or surgical specimens, and such tissues were not as stable as human bronchial epithelial cell lines. Primary bronchial epithelial cell cultures have also been used for studying cytokine secretions. The specimen was usually collected via bronchoscope or surgery in patients with diseases, such as lung cancer [20], severe asthma [21] and chronic obstructive pulmonary disease (COPD) [22].

Other studies used bronchial human cells from normal cadavers [23]. For studying physiology and childhood asthma inflammation, bronchial epithelial cells from normal human beings are considered most useful tissues. However, it is ethically and practically difficult to maintain a stable supply of such tissue. Thus, selection of an appropriate normal human bronchial epithelial cell line is necessary.

This study compared the secretion of GM-CSF and IL-8 after cytokine stimulation in 2 human epithelial cell lines — cell line A549 and normal human bronchial epithelial cell line (HBEpC).

Materials and Methods

Reagents

Human recombinant TNF-α and IL-1beta (IL-1β) were purchased from Becton Dickinson Bioscience (San Diego, USA) and were stored as 1 μg/mL stock solutions in phosphate-buffered saline supplemented with 0.1% bovine serum albumin at –20°C.

Cell culture

Primary cultures of HBEpCs are derived from normal human bronchial epithelium (Cell Applications Inc., San Diego, USA). HBEpCs proliferated in a T-75 flask and were cultured in Bronchial Epithelial Cell Growth Medium (Cell Applications Inc.) supplemented with bovine pituitary extract (7.5 mg/mL), hydrocortisone (0.5 μg/mL), human epithelial growth factor (0.5 μg/mL), epinephrine (0.5 mg/mL), transferrin (10 mg/mL), insulin (0.5 mg/mL), retinoic acid (0.1 μg/mL), triiodothyronine (6.5 μg/mL), gentamicin (50 mg/mL) and fatty acid-free bovine serum albumin (50 mg/mL). Growth medium was changed every other day and the T-75 flask was examined under a microscope until the cells reached 85-95% confluence.

The human alveolar epithelial cell line A549 (ATCC, Rockville, MD, USA) was grown in RPMI plus 10% fetal bovine serum, 100 U/mL penicillin, and 100 U/mL streptomycin in an atmosphere of 5% carbon dioxide and 95% air at 37°C. Cells were incubated for up to 24 h in the presence or absence of stimuli and supernatants were then collected, centrifuged and stored at –80°C until assay for GM-CSF and IL-8.

Cytokine stimulation of cells in culture

Recombinant human TNF-α and IL-1β in the dose range of 10 ng/mL, 20 ng/mL, and 100 ng/mL were added individually to confluent cell cultures of 10^6 cells in each well. After 24 h culture, the supernatants and cells were processed for immunoassay.

Measurement of GM-CSF and IL-8

The levels of GM-CSF and IL-8 were detected and quantified in the supernatants of cell culture by enzyme-linked immunosorbent assay (ELISA) kits (Becton Dickinson Bioscience). For measuring IL-8, the culture supernatants were previously diluted 100 times with diluent buffer (Becton Dickinson Bioscience)

Statistical analysis

All values of cytokine concentrations were represented as mean ± standard deviation (SD) of triplicate samples from at least 3 independent experiments. Comparisons between groups were tested for significance using 2-way analysis of variance, repeated treatment test. Differences with a probability value <0.05 were considered significant.

Results

GM-CSF

ELISA analysis of supernatants of 10^6 cells per well showed that HBEpCs and A549 cells exhibited constitutional generation of GM-CSF for 24 h. Under stimulation of TNF-α or IL-1β (Fig. 1) for 24 h, secretion of GM-CSF from 10^6 HBEpCs per well was mildly increased at 10 and 20 ng/mL and significantly increased at 100 ng/mL versus control ($p<0.05$). Under stimulation of TNF-α or IL-1β for 24 h, secretion of GM-CSF from 10^6 A549 cells per well was significantly increased at concentrations of 10, 20, and 100 ng/mL ($p<0.05$).

IL-8

ELISA analysis of supernatants of 10^6 cells per well showed that HBEpCs and A549 cells exhibited constitutional generation of IL-8 for 24 h.Under stimulation of TNF-α or IL-1β (Fig. 2) for 24 h, secretion

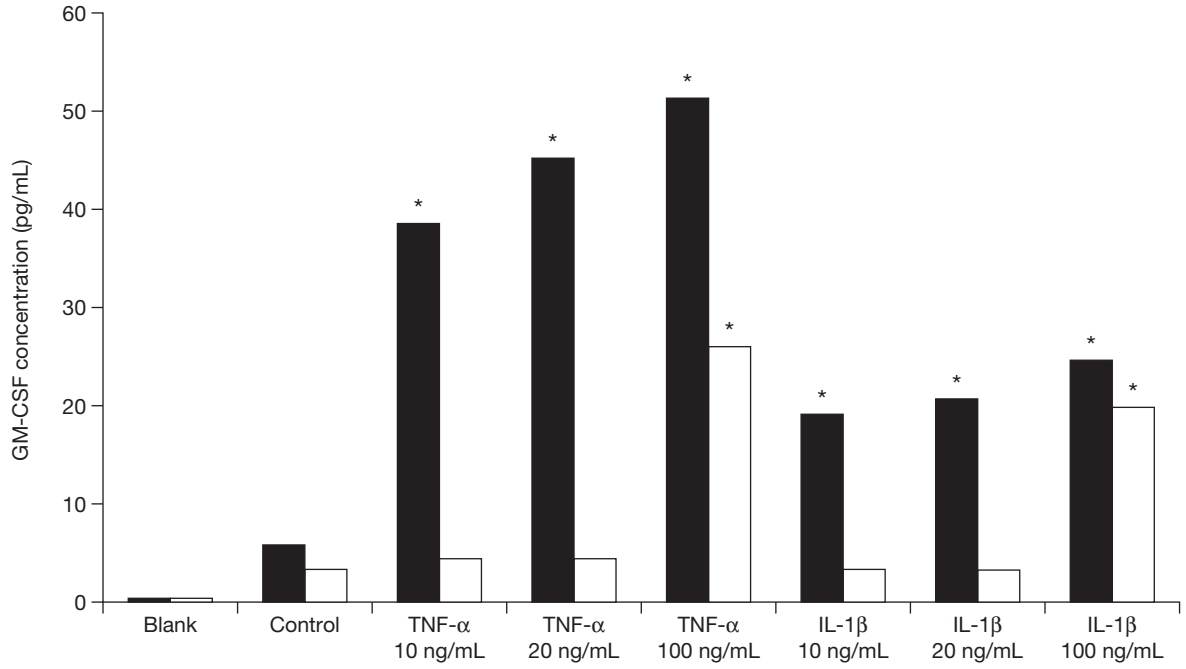

Fig. 1. Granulocyte-macrophage colony-stimulating factor (GM-CSF) concentrations in A549 (■) and human bronchial epithelial cells (□) under the conditions of blank, control, and stimulation with tumor necrosis factor-alpha (TNF-α) or interleukin-1beta (IL-1β) at concentrations of 10, 20 and 100 ng/mL for 24 h. The symbol "*" indicates significantlly elevated concentrations versus control ($p<0.05$).

of IL-8 from 10^6 HBEpCs per well was mildly increased at 10 and 20 ng/mL and significantly increased at 100 ng/mL versus control ($p<0.05$). Under stimulation of TNF-α or IL-1β for 24 h, secretion of IL-8 from 10^6 A549 cells per well was significantly increased at 10, 20, and 100 ng/mL ($p<0.05$).

Discussion

HBEpCs are derived from the surface epithelium of normal human bronchi. They are cryopreserved at the end of primary culture. Due to lack of immortality or virus transformation, these cells grow quite slowly. According to the product specification sheet, the cell viability is 84%, and doubling time is 32.8 h.

We found that this cell line grew more slowly than reported, even though we followed fully the suggested conditions and procedures. We therefore added certain nutrients and supplements in order to achieve better growth in HBEpCs.

In our study, HBEpCs and A549 cells released many mediators including GM-CSF and IL-8 spontaneously and also after adequate stimulation. Furthermore, the secretion from HBEpCs was enhanced significantly by 100 ng/mL TNF-α or 100 ng/mL IL-1β individually, and the secretion from A549 cells was enhanced significantly by 10, 20 and 100 ng/mL TNF-α or 10, 20 and 100 ng/mL IL-1β individually.

Concentrations of TNF-α and IL-1β can be as high as 150 ng/mL in patients with status asthmaticus [24]. In previous studies [25,26], immortal bronchial epithelial cell lines were stimulated with TNF-α and/or IL-1β at concentrations of 10, 20 and 100 ng/mL to induce IL-8 and GM-CSF production. Also, immortal A549 cells exhibited a much higher level of expression for both cyclin A mRNA and protein than did normal cells [27]. Thus, the non-virus-transformed cell line in our study possessed the characteristics of slower growth and higher TNF-α and IL-1β concentrations needed for stimulation compared with A549 cells.

There have been few studies of IL-8 and GM-CSF secretion in HBEpCs [28-31]. Previous reported studies used normal human primary bronchial epithelial cells (NHBE) purchased from Clonetics (San Diego, CA, USA). In comparison with A549, NHBE also showed much less IL-8 secretion on IL-1β stimulation [29], and also in the conditioned media from *Mycobacterium tuberculosis*-infected human monocytes [30]. NHBE also showed higher IL-8 secretion after swine dust stimulation, with at least a 5-fold higher concentration compared to A549 [32]. Our data also showed similar results in IL-8 secretion among these 2 cell lines.

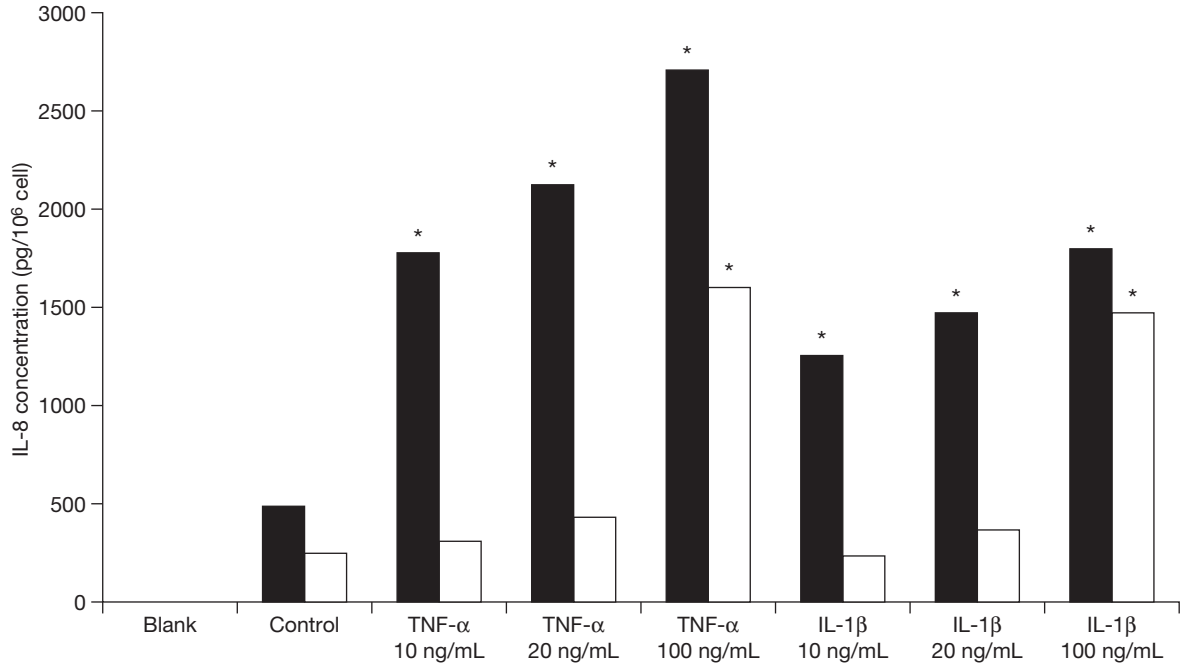

Fig. 2. Interleukin-8 (IL-8) concentrations in A549 (■) and human bronchial epithelial cells (□) under the conditions of blank, control, and stimulation with tumor necrosis factor-alpha (TNF-α) or interleukin-1beta (IL-1β) at concentrations of 10, 20 and 100 ng/mL for 24 h. The symbol "*" indicates significantly elevated concentrations versus control ($p<0.05$).

Only 1 previous study [28] demonstrated that NHBE can produce GM-CSF and IL-8 after house dust mite allergen stimulation. This study provides parallel data for A549 which were previously lacking.

This study suggests that HBEpC is a stable, readily available non-virus-transformed source in studying inflammatory roles of bronchial epithelial cell in vitro. Its convenience and cost make it attractive for use as a screening tool for IL-8 and/or GM-CSF secretion under various stimulations, to be followed by confirmation.

Acknowledgments

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