

## Disruption of cytoskeleton by cytochalasin E strongly induces interleukin-8 production

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### Abstract

Using an enzyme immunoassay, cytochalasin E was found to strongly induce interleukin-8 production in the cervical carcinoma cell line, Hela; this induction was accompanied by morphological changes. Interleukin-8 production in Hela cells cultured with cytochalasin E was completely blocked in the presence of a protein kinase C inhibitor, Go6976. These results indicate that cytochalasin E activates protein kinase C, leading to the induction of interleukin-8 production in Hela cells.

**Key words** : cytochalasin E, interleukin-8 (IL-8), Hela cells, protein kinase C (PKC)

### Introduction

Cytochalasins are fungal metabolites that affect mammalian cell motility by binding to the barbed end of actin filaments. Cytochalasins also inhibit general cell functions, such as chemotaxis, cytokinesis, phagocytosis and receptor cap formation<sup>1-4)</sup>.

On the other hand, cytochalasins have also been found to enhance both the antigen and mitogen induced proliferation of human T cells. For example, when added to T cells, low concentrations of cytochalasins dramatically enhance the DNA-synthesis to mitogens, resulting in the enhancement of T-cell proliferation, which in turn increases cellular cyclic AMP (cAMP) levels, intracellular  $Ca^{2+}$ , and the turnover of phosphatidylinositol 3 ( $IP_3$ ), thereby activating protein kinase C (PKC)<sup>5,6)</sup>.

Several reports have indicated that cytochalasins also enhance the production of immunological mediators, such as interleukin-2 (IL-2)<sup>7)</sup>, prostaglandin  $E_2$  ( $PGE_2$ )<sup>8)</sup> by T cells, osteoblastic cells and the hepatocyte carcinoma cell line, HepG2<sup>9)</sup>. The production of these factors results from phosphorylation of particular kinases, such as protein-kinase C (PKC), protein tyrosine kinase (PTK) and protein kinase A (PKA). However, the mechanism(s) underlying

changes in cell morphology and differentiation induced by cytochalasins, especially the relationship between the cytoskeletal network and cytokine production, are not known in detail.

Recently, we found that cytochalasin E (CyE) strongly induces the production of interleukin-8 (IL-8), a chemoattractant for neutrophils. In the present study, we examined the mechanism of IL-8 production induced by disruption in the cytoskeleton as a result of treatment with CyE.

### Materials and methods

#### Reagents

Go6976 was purchased from Funakosi Co. (Tokyo, Japan). Phorbol myristate acetate (PMA) and cytochalasin E (CyE) were purchased from Sigma Chemical Co. (St Louis, MO). The enzyme-linked immunoassay (EIA)-kit for interleukin-8 (IL-8) was purchased from TFB Co. (Tokyo, Japan).

#### Cell line

The cervical carcinoma cell line, Hela used in the present

study was supplied by the Japanese Research Resource Bank (Tokyo, Japan). The cells were cultured in RPMI 1640 medium (GIBCO) supplemented with 10 mM Hepes buffer, 2 mM glutamine and 10% fetal calf serum (FCS) (hereafter referred to as "complete medium"). At confluence, the cells were detached using 0.12% trypsin (GIBCO) in 1 mM EDTA and washed three times with RPMI 1640 medium.

#### Cultures of Hela cells with substances

Fully grown Hela cells in culture dishes (Sumitomo Co. Tokyo, Japan) were washed three times with phosphate-buffered saline (PBS); complete medium was then added to the dishes. CyE (200 ng/ml) or PMA (100 ng/ml) was added in the presence or absence of a PKC inhibitor (optimum concentration), and the plates were incubated at 37°C for 24 hr. Thereafter, morphological changes in the cells were analyzed using phase-contrast microscope (Olympus, Tokyo, Japan). Culture supernatant, obtained under the same conditions, was subjected to IL-8 detection.

#### IL-8 assay of culture supernatant

The IL-8 amount of the supernatant from Hela cells cultured with CyE (200 ng/ml) or PMA (100 ng/ml) was assayed using the EIA-kit.

#### Statistical analysis

The statistical analysis was performed using a Student *t* test. A *P* value of less than 0.05 was considered to be statistically significant.

### Results

#### Morphological changes in Hela cells cultured with CyE

The morphological features of Hela cells cultured with CyE (200 ng/ml) were observed using a phase-contrast microscope. CyE was found to induce dramatic morphological changes in the Hela cells (Fig.1). The cytoskeletal network of Hela cells cultured with CyE (200 ng/ml) was completely disrupted (data not shown).

#### IL-8 production in Hela cells cultured with CyE

The IL-8 amount of culture supernatants from Hela cells cultured with or without CyE was assayed using the EIA-kit. Figure 2 shows that CyE strongly induced IL-8 production ( $156.5 \pm 8.1$  pg/ml, on average), as measured in the culture supernatant of Hela cells after culturing for 24 hr. The amount of IL-8 produced by cultures in the presence or absence of CyE differed significantly ( $P < 0.01$ ). PMA (100 ng/ml) also produced a much larger amount of IL-8 after 24 hr ( $1525.5 \pm 71.1$  pg/ml, on average) (data not shown).

#### IL-8 production in Hela cells cultured with CyE is mediated by PKC

To identify the signaling pathway(s) involved in CyE-induced IL-8 production in Hela cells, we examined the metabolic requirements for this induction using a PKC inhibitor, Go6976 ( $1.3 \mu\text{M}$ ). Figure 2 shows that Go6976 completely blocked IL-8 production in Hela cells cultured with CyE (200 ng/ml). The amount of IL-8 produced by the CyE and the CyE+Go6976 cultures were significantly different ( $P < 0.01$ ).

### Discussion

In the present study, IL-8 production by Hela cells cultured with CyE which disrupts the cytoskeletal network was strongly induced at the protein level. In addition, the PKC pathway appeared to be involved in the production of IL-8 in Hela cells cultured with CyE.

Generally, cytochalasins inhibit several cellular functions, including chemotaxis, cytokinesis, phagocytosis, and receptor capping<sup>1-4)</sup>. However, cytochalasins also enhance the proliferation of chemotactic peptide-induced respiratory burst and antigen- or mitogen-induced T cells<sup>5,6,10)</sup>. In fact, CyE strongly induces the expression of CD23 on the cell surface of the human monocyte-like cell line, U937; this mechanism is mediated by PTK<sup>11)</sup>. These findings suggest that cytochalasins trigger a target reaction or receptor molecule on the cell surface membrane and also indicate that the disruption of the cytoskeleton by CyE is closely associated with cell activation and/or proliferation in various immune responses.

Cytochalasins can strictly regulate cytokine production, such as IL-2, PGE<sub>2</sub>, etc<sup>7-9)</sup>. In this study, CyE strongly induced IL-8 production. These findings suggest that the disruption of cytoskeletal rearrangement by treatment with cytochalasins directly or indirectly affects the expression of cytokine genes; furthermore, the mechanism for the induction of cytokine production by cytochalasins depends on the activation of phosphorylation kinases, such as PKC and PTK. In this study, we obtained evidence that CyE strongly induces IL-8 mainly via a PKC-mediated pathway because IL-8 production was completely blocked by the addition of a PKC inhibitor, Go6976.

IL-8 is one of the most potent chemoattractants for neutrophils and plays an important role in various immune responses and angiogenesis. IL-8 is induced by various substances, such as lipopolysaccharides (LPS), zymosan, both Gram negative and positive bacteria, viruses, certain cytokines, PMA, etc<sup>12-14)</sup>. Although little is known about the detailed mechanism(s) of the functional association between cytoskeletal network rearrangement and IL-8 production, this study provided evidence that IL-8 is induced by CyE via PKC activation.

In conclusion, CyE strongly induced IL-8 production in Hela cells mainly via a PKC-mediated pathway. Further molecular analyses will be necessary for a better understanding of the detailed mechanism(s) underlying this immune response, which is triggered by cytoskeletal network rearrangement.

### References

- 1) Lin, S., Lin, D.C., Flanagan, M.D.: Specificity of the effects of cytochalasin B on transport and motile processes. *Proc. Natl. Acad. Sci. U.S.A.* 75: 329 - 333, 1978.
- 2) Yahara, I., Harada, F., Sekita, S., et al.: Correlation between effects of 24 different cytochalasins on cellular structures and cellular events and those on actin in vitro. *J. Cell Biol.* 92: 69-78, 1982.
- 3) Forscher, P., Smith, S.J.: Actions of cytochalasins on the organization of actin filaments and microtubules in a neural growth cone. *J. Cell Biol.* 107: 1505-1516, 1988.
- 4) Shinji, H., Akagawa, K.S., Yoshida, T.: Cytochalasin D inhibits lipopolysaccharide-induced tumor necrosis factor production in macrophages. *J. Leukoc. Biol.* 54: 336-342, 1993.
- 5) Matsuyama, T., Yamada, A., Deusch, K., et al.: Cytochalasins enhance the proliferation of CD4 cells through the CD3-Ti antigen receptor complex or the CD2 molecule through an effect on early events of activation. *J. Immunol.* 146: 3736-3741, 1991.
- 6) Jasper, J.R., Post, S.R., Desai, K.H., et al.: Colchicine and cytochalasin B enhance cyclic AMP accumulation via postreceptor actions. *J. Pharmacol. Exp. Ther.* 274: 937-942, 1995.
- 7) Valentine, M.A., Vaughan, J.A.: Augmentation of interleukin-2 release by cytochalasins. *Cell. Immunol.* 101: 651-658, 1986.
- 8) Yeh, C.K., Rodan, G.A.: Microtubule disruption enhances prostaglandin E<sub>2</sub> production in osteoblastic cells. *Biochem. Biophys. Acta.* 927: 315-323, 1986.
- 9) Lewitt, M.S., Baxter, R.C.: Cytochalasin B stimulates insulin-like growth factor-binding protein-1 production by Hep G2 cells. *Mol. Cell Endocrinol.* 77: 149-157, 1991.
- 10) Lehmyer, J.E., Snyderman, R., Johnson, R.B.: Stimulation of neutrophil oxidative metabolism by chemotactic peptides : Influence of calcium ion concentration and cytochalasin B and comparison with stimulation by phorbol myristate asetate. *Blood* 54: 35 - 45, 1979.
- 11) Ikewaki, N., Tamauchi, H., Yamada, A., et al.: A microfilament formation inhibitor, cytochalasin strongly enhances the low-affinity Fc epsilon receptor II (CD23) expression on the human monocyte-like cell line, U937. *J. Clin. Immunol.* 20: 424-433, 2000.

- 12) de Boer, J.H., Hack, C.E., Verhoeven, A.J., et al:  
Chemoattractant and neutrophil degranulation activities related to interleukin-8 in vitreous fluid in uveitis and vitreoretinal disorders. Invest. Ophthalmol. Vis. Sci. 34: 3376 - 3385, 1993.
- 13) Tiemessen, C.T., Martin, D.J.: Cytokine-induced interleukin-8 production is depressed in chronic as opposed to acute human immunodeficiency virus 1 infection of promonocytic cells. Acta. Virol. 44: 193-198, 2000.
- 14) Yakabi, K., Ro, S., Okazaki, R., et al: Water extract of *Helicobacter pylori* stimulates interleukin-8 secretion by a human gastric epithelial cell line (JR-St) through protein tyrosine phosphorylation. J. Gastroenterol. Hepatol. 15: 263 - 270, 2000.

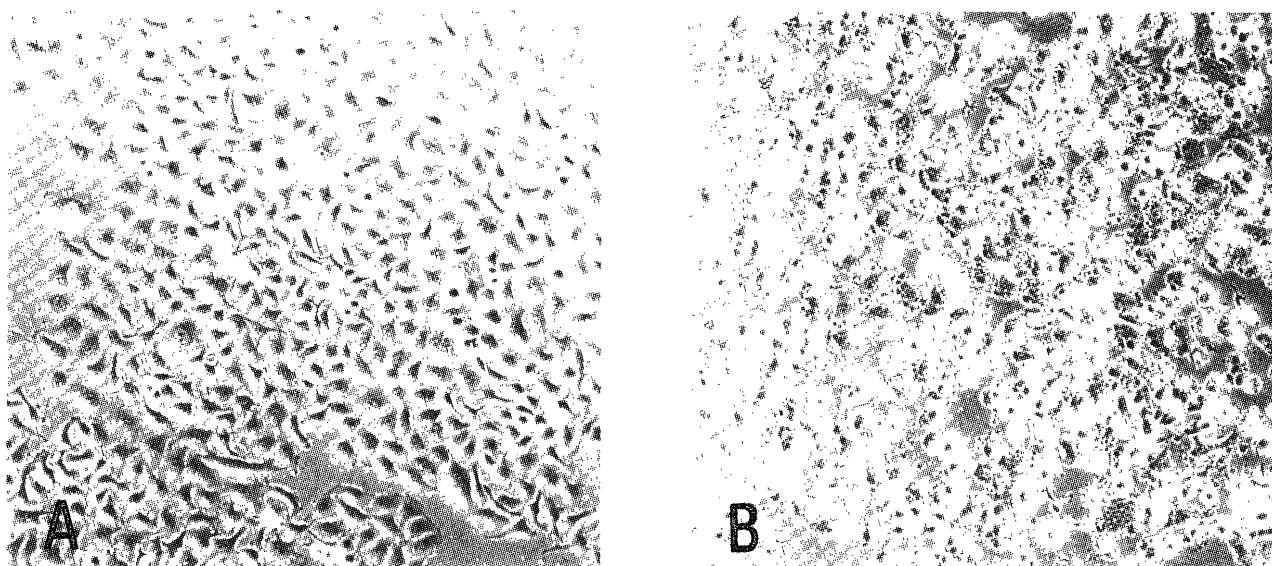


Figure 1. Morphological changes in HeLa cells cultured with CyE.

Morphological changes in HeLa cells cultured with or without CyE (200 ng/ml) for 24 hr were examined using a phase-contrast microscope. (A): without CyE, (B): CyE (200ng/ml).

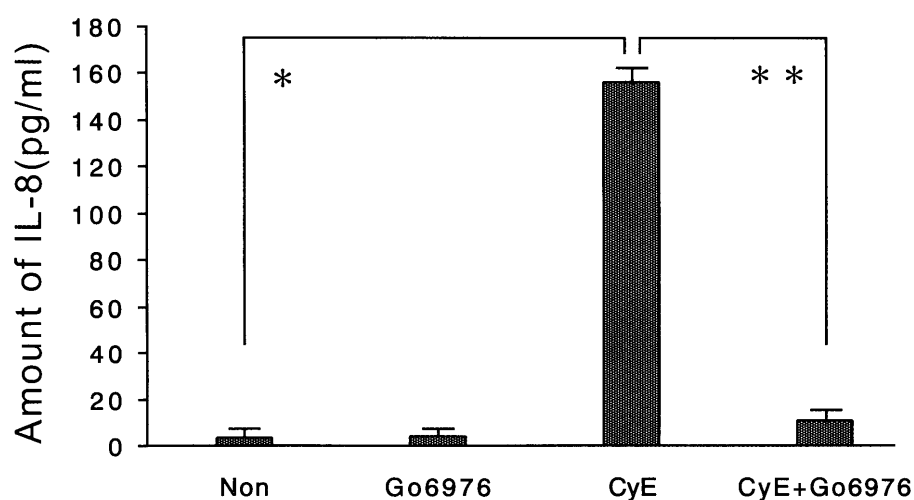


Figure 2. The production of IL-8 by HeLa cells cultured with CyE is mediated by PKC.

The amount of IL-8 in the supernatant from HeLa cells cultured with or without CyE (200 ng/ml), Go6976 (1.3  $\mu$ M) or CyE plus Go6976 (1.3  $\mu$ M) for 24hr was assayed using an EIA-kit. \*Significant difference between the presence and absence of CyE ( $P < 0.01$ ).

\*\*Significant difference between CyE and CyE+Go6976 ( $P < 0.01$ ).

## 細胞骨格粉碎剤（サイトカラシンE）による インターロイキン8（IL-8）の産生

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我々は細胞骨格粉碎剤であるサイトカラシンEがHela細胞 (cervical carcinoma cell line) にインターロイキン8 (IL-8)の産生を誘導することを見い出した。このIL-8の産生は、プロテインキナーゼ C (PKC)の阻害剤であるGo6976で完全に抑制された。この結果は、サイトカラシンEがPKCを活性化することでIL-8産生を誘導することを示すものである。

キーワード : サイトカラシン E, インターロイキン-8 (IL-8), Hela細胞, プロテインキナーゼ C (PKC)