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Co-production of Interleukin-1 and Interleukin-6 in Tumor Cell Lines Elaborating Colony-stimulating Factors

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Six carcinoma cell lines elaborating colony-stimulating factors (CSFs) were examined by enzyme-linked immunosorbent assay and Northern blotting to determine whether or not they co-produced interleukin-1α (IL-1α), IL-1β and IL-6. All 6 cell lines were co-producers; IL-1α was produced in all 6, IL-6 in 5 and IL-1β in 3 of them. These results indicate that IL-1 and IL-6 are commonly co-produced in CSF-producing tumors.

Key words: Colony-stimulating factor-producing cell line — Interleukin-1 — Interleukin-6

Colony stimulating factor (CSF)-producing tumors have been well documented.[1)] In recent years, it has been shown that some CSF-producing tumors elaborate multiple cytokines including interleukin-1 (IL-1) or IL-6 in addition to CSF.[2-4)] More recently, we found the co-production of IL-1 and IL-6, as well as granulocyte (G)-CSF, in a lung carcinoma cell line which was established in our laboratory.[5)] Furthermore, we observed fever and a high level of serum C-reactive protein (CRP) in patients with CSF-producing tumors (unpublished results). These observations prompted us to examine whether or not co-production of IL-1 or IL-6 commonly occurs in CSF-producing tumors, using established CSF-producing cell lines.

Human CSF-producing cell lines, T24,[6)] Lu65[7)] and SK-HEP-1[8)] were provided by the Japanese Cancer Research Resources Bank. CHU-2[9)] was kindly provided by Dr. S. Asano (Tokyo University). Thyroid carcinoma cell line, HTC/C3[10)] was generously provided by Dr. T. Enomoto (Kyoto University). A lung carcinoma cell line, KHC287[5)] was established in our laboratory in 1987. Non-CSF-producing tumor cell lines, HeLa, K562 and U937, used as controls, were provided by the Japanese Cancer Research Resources Bank.

Cells from each line were cultured in RPMI-1640 medium (Nissui, Tokyo) supplemented with 10% fetal calf serum (FCS, Hyclone, Logan, UT) at a cell density of 1×10^6/ml for 3 days, after which the conditioned medium was collected. As a positive control, circulating mononuclear cells (5×10^5/ml) from a healthy donor were cultured in RPMI-1640 with 10% FCS and 0.2% phytohemagglutinin-P (Difco, Detroit, MI) for 7 days, and the culture supernatant was collected as the phytohemagglutinin-stimulated leukocyte conditioned medium (PHA-LCM).

Concentrations of IL-1α, IL-1β, IL-6 and granulocyte/macrophage (GM)-CSF in the culture supernatants were measured by using enzyme-linked immunosorbent assay (ELISA) kits purchased from Otsuka Pharm. Co. (Tokyo) (IL-1α, IL-1β) and Genzyme Co. (Boston, MA) (IL-6, GM-CSF). The G-CSF concentrations were measured by ELISA as previously described[11)] in the Assay Technology Research Center of Chugai Pharm. Co. (Tokyo). The concentrations of tumor necrosis factor-α (TNF-α) were measured by using an ELISA kit purchased from Otsuka Pharm. Co.

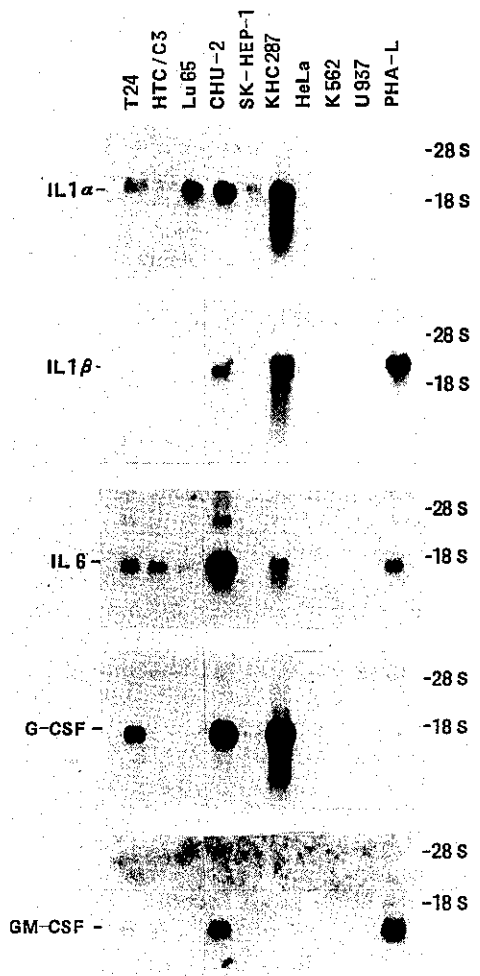
Total cellular RNA was extracted from cells by the acid-phenol method[12)] and Northern blot hybridization was performed according to the standard method described elsewhere.[13)] cDNA probes for IL-1α, IL-6 and G-CSF were kindly provided by Dainippon Pharm. Co. (Osaka), Dr. T. Hirano (Osaka University) and Chugai Pharm. Co., respectively. Oligonucleotide probes for IL-1β and GM-CSF were synthesized by a DNA synthesizer (Applied Biosystem, Tokyo).

Table I shows the concentrations of cytokines in the culture supernatants of the cell lines examined, as determined by ELISA. Either G-CSF or GM-CSF, or both were detected in the culture supernatants of all CSF-producing cell lines examined except for Lu65. IL-1α was detected in the conditioned medium of all 6 cell lines, and IL-6 in all except SK-HEP-1. IL-1β was detected in 3 of 6 CSF-producing cell lines. On the other hand, none of the 5 cytokines was found in the culture supernatants of control cell lines, HeLa, K562 and U937. TNF-α concentration measured by ELISA was 95.1 pg/ml in the

Table I. Concentration of IL-1α, IL-1β, IL-6, G-CSF and GM-CSF in the Culture Supernatants of 6 CSF-producing Cell Lines

Cell line	IL-1α (pg/ml)	IL-1β (pg/ml)	IL-6 (ng/ml)	G-CSF (ng/ml)	GM-CSF (ng/ml)
T24	300	13	2.0	16.7	0.3
HTC/C3	6	<10	1.5	<0.1	1.3
Lu65	11	<10	1.1	<0.1	<0.1
CHU-2	266	62	14.0	55.4	1.5
SK-HEP-1	4	<10	<0.5	0.3	<0.1
KHC287	100	116	7.0	11.6	<0.1
HeLa	<3	<10	<0.5	<0.1	<0.1
K562	<3	<10	<0.5	<0.1	<0.1
U937	<3	<10	<0.5	<0.1	<0.1
PHA-LCM	79	70	6.0	0.4	0.9
Medium alone	<3	<10	<0.5	<0.1	<0.1

The concentration of each cytokine was measured by ELISA. The lower limits of detection with ELISA kits for IL-1α, IL-1β, IL-6, G-CSF and GM-CSF were 3.0 pg/ml, 10.0 pg/ml, 0.5 ng/ml, 0.1 ng/ml and 0.1 ng/ml, respectively. HeLa, K562 and U937 were examined as controls. PHA-LCM: phytohemagglutinin-stimulated leukocyte conditioned medium.



culture supernatant of CHU-2, but below the minimum detectable level (7.0 pg/ml) in the remaining 5 CSF-producing cell lines.

Fig. 1 shows the Northern blot analysis of IL-1α, IL-1β, IL-6, G-CSF and GM-CSF in the 6 CSF-producing and 3 control cell lines. The mRNA expression of these 5 cytokine genes in each cell line was identical to the results obtained by ELISA.

In this study, we demonstrated co-production of IL-1α in all 6 CSF-producing cell lines examined. IL-6 was also co-produced in 5 of these cell lines. Recently, co-production of IL-1[2,3] or both IL-1 and IL-6[4] has been described in 3 CSF-producing cell lines. Taken together, co-production of IL-1 or IL-6, especially IL-1α, may be universal in the CSF-producing tumor cell lines. This is the first report describing the linkage of CSF, IL-1 and IL-6 production in a large number of tumor cell lines. Furthermore, high frequencies of fever and elevated CRP in patients with CSF-producing tumors (unpublished results) suggest that the co-production of IL-1 and IL-6 occurs not only in established CSF-producing cell lines but also in CSF-producing tumors *in vivo*.

Fig. 1. Northern blot analysis of IL-1α, IL-1β, IL-6, G-CSF and GM-CSF in 6 CSF-producing cell lines, 3 control cell lines and phytohemagglutinin-stimulated human leukocytes (PHA-L). Total RNA was extracted from each cell line, and a 10 μg aliquot was applied to each lane. The blotted membrane was then serially hybridized with each probe.

In this study, we were unable to demonstrate CSF production in Lu65 cell line, which was reported to be a CSF-producer.[7] Presumably, this is because the cells have lost the ability to produce the CSF after multiple subcultures.

IL-1 stimulates the production of IL-6 and CSFs in normal fibroblasts, macrophages and bone marrow stromal cells.[14] In this regard, it would be interesting to know whether or not IL-1 gene expression is the trigger causing subsequent gene expression of IL-6 and CSFs even in the CSF-producing tumor cell lines. We are currently examining the effect of anti-IL-1α antibody on the IL-6 and G-CSF production in G-CSF-producing cell lines. Further studies are needed to clarify the mechanism of co-production of IL-1 and IL-6 in the CSF-producing tumors at the molecular level.

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