

Involvement of Proinflammatory Cytokines IL-1 β and IL-6 in Progression of Human Gastric Carcinoma

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Abstract. Gastric carcinoma occurs in response to chronic inflammation of gastric mucosa infected with *Helicobacter pylori*. It is not known how cytokines affect the growth and progression of gastric carcinoma. **Materials and Methods:** We measured tissue concentrations of the proinflammatory cytokines interleukin (IL)-1 β and IL-6 in gastric carcinoma and investigated the correlation between the levels of these cytokines and clinicopathological features. Biopsy specimens of tumors or adjacent normal mucosa were obtained from 42 Japanese patients with gastric carcinoma. Tissue levels of IL-1 β and IL-6 were measured by enzyme-linked immunosorbent assay. **Results:** IL-1 β levels were significantly higher in the neoplasm than in the corresponding normal mucosa. The IL-6 levels in the neoplasm correlated significantly with the depth of invasion and lymphatic invasion. High levels of IL-1 β and IL-6 were characteristic of non-scirrhous type gastric carcinoma. **Conclusion:** These results suggest that IL-1 β and IL-6 are involved in the growth and progression of human gastric carcinoma.

Gastric carcinomas express a variety of growth factors/cytokines and their receptors that regulate tumor growth and development in an autocrine manner (1). Growth factors/cytokines mediate interactions between tumor cells and host cells, and they function as paracrine factors for endothelial cells and stromal fibroblasts to promote angiogenesis and fibrosis (2, 3). We and others have reported that gastric carcinoma cells secrete various cytokines (4-6), which play central roles in both cellular and immune responses.

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Interleukin (IL)-1 is expressed by a variety of malignant tumor cells (7-9), and diverse effects on the growth of solid tumors have been reported. IL-1 inhibits the growth of melanoma and breast and ovarian carcinomas (9-11), whereas IL-1 stimulates the growth of gastric and colorectal carcinomas (4, 12, 13). There are two IL-1 proteins, IL-1 α and IL-1 β . These proteins bind to the same receptors and have agonistic roles (14). There are no significant differences in the spectrum of activities induced by IL-1 α and IL-1 β in *in vitro* and *in vivo* assays (14).

IL-6 is a multipotent cytokine that has several immunological activities including induction of several acute phase proteins and regulation of proliferation and differentiation of immunocompetent cells (15). IL-6 has also been implicated in the pathogenesis and/or prognosis of several different tumors, including multiple myeloma, lymphoma, ovarian cancer, prostate cancer and renal cell carcinoma (16-20).

Ito *et al.* (4, 5) reported that IL-1 and IL-6 are secreted by gastric carcinoma cells and that they stimulate growth of these cells *in vitro*. However, the relationship between levels of these cytokines in gastric carcinoma tissues and clinicopathological data has not yet been clarified. In the present study, we measured tissue concentrations of IL-1 β and IL-6 in human gastric carcinoma and the correlation with various clinicopathological features.

Materials and Methods

Patients and tumor specimens. This study included 42 randomly selected Japanese patients with primary gastric carcinoma who underwent surgery or endoscopic mucosal resection at Hiroshima University Hospital, Japan between 1996 and 1998. The patients ranged in age from 39 to 83 years (mean 62.5 years). Of these patients, 19 (45.2%) had stage I disease, 9 (21.4%) had stage II disease, 9 (21.4%) had stage III disease and 4 (9.5%) had stage IV disease. Pathological data for the entire study group is given in

Table I. Tissue levels of IL-1 β and IL-6 with respect to clinicopathological features of gastric carcinoma.

Clinicopathological feature ^{a)}		IL-1 β pg/mg-protein	IL-6 pg/mg-protein
Histological type ^{b)}			
Well	(20)	207.4 \pm 146.0	94.0 \pm 54.0
Poor	(22)	183.8 \pm 106.3	43.0 \pm 15.1
Growth pattern ^{c)}			
Non-scirrhus type	(31)	261.5 \pm 115.5 *	90.3 \pm 35.5 *
Scirrhus type	(11)	7.9 \pm 6.2 *	2.3 \pm 2.3 *
Depth of invasion ^{d)}			
m	(7)	24.3 \pm 64.2	4.3 \pm 11.3 *
sm	(9)	43.5 \pm 69.8	74.3 \pm 191.2 *
mp,ss	(26)	321.9 \pm 737.4	87.6 \pm 197.2 *
Lymphatic invasion			
positive	(25)	294.7 \pm 711.4	104.5 \pm 216.6 *
negative	(17)	33.2 \pm 57.7	6.8 \pm 17.6 *
Venous invasion			
positive	(20)	349.1 \pm 790.9	124.3 \pm 240.3
negative	(22)	55.0 \pm 72.1	15.4 \pm 34.6
Lymph node metastasis			
positive	(15)	302.8 \pm 753	147.5 \pm 273.8
negative	(27)	141.2 \pm 477.6	27.1 \pm 33.6

^{a)}According to the criteria of the Japanese Gastric Cancer Association (1998).

^{b)}Well, well-differentiated adenocarcinoma including papillary and tubular adenocarcinoma; poor, poorly-differentiated adenocarcinoma including signet ring cell carcinoma and mucinous carcinoma.

^{c)}Scirrhus type: diffuse infiltration of cancer cells with abundant fibrous stroma; Non-scirrhus type: stroma is scanty (medullary type) or intermediate (intermediate type).

^{d)}m, mucosa; sm, submucosa; mp, muscularis propria; ss, subserosa. Numbers in parentheses are number of cases.

* p <0.05.

Table I. Patients treated preoperatively with cytotoxic drugs were not included in this study. At the time of endoscopy, at least two biopsy specimens were taken from the gastric carcinoma and the surrounding normal mucosa. One specimen was snap-frozen in liquid nitrogen for enzyme-linked immunosorbent assay (ELISA), and the other was placed in formalin and processed for routine histopathology. Tumor stage and histological classification were determined according to the criteria of the Japanese Gastric Cancer Association (21).

ELISA. Tissue specimens were pooled and immediately homogenized in 2.0 ml of phosphate-buffered saline (PBS, pH 7.4). The levels of IL-1 β and IL-6 in homogenates of gastric biopsy specimens were assayed by ELISA as described previously (22) with anti-IL-1 β and anti-IL-6 monoclonal antibodies.

Statistical analysis. The results are reported as mean \pm standard error (SE). Statistical significance was determined by the Mann-Whitney U -test. Survival rates were calculated starting

from the month of surgery. Overall survival curves were drawn according to the Kaplan-Meier method, and differences were analyzed by the log-rank test. The significance level was set at 5% for all analyses.

Results

Tissue concentrations of IL-1 β and IL-6 in gastric carcinoma and corresponding normal mucosa. We first examined IL-1 β and IL-6 protein levels in human gastric carcinoma by ELISA. The levels of IL-1 β protein were more than 50-fold higher in tumors than in normal gastric mucosa (190.7 \pm 86.6 vs. 3.7 \pm 3.7 pg/mg protein; p <0.01). The levels of IL-6 were also higher in most neoplasms than in normal mucosa, but the difference was not statistically significant (Figure 1).

IL-1 β and IL-6 levels and clinicopathological characteristics.

Tissue concentrations of IL-1 β and IL-6 were significantly higher in non-scirrhus type tumors than in scirrhus type tumors. IL-6 levels in the tumors correlated significantly with depth of invasion and lymphatic invasion. However, histological type, venous invasion and lymph node metastasis did not correlate with the levels of IL-1 β or IL-6. The levels of IL-1 β and IL-6 in the background gastric mucosa were not associated with clinicopathological features of gastric carcinoma (data not shown).

Correlation between IL-1 β and IL-6 levels and survival rate.

To determine whether expression of IL-1 β or IL-6 in tumor tissues influences patient outcomes, we selected 40 patients that were followed-up in our hospital and performed a Kaplan-Meier analysis. As shown in Figure 2, there are no significant differences in the survival rates of patients when categorized according to high concentration and low concentration of IL-1 β and of IL-6.

Discussion

Gastric carcinoma cells produce various cytokines, including IL-1, IL-6, IL-8 and monocyte chemoattractant protein (MCP)-1 (3-6). Among them, IL-8 and MCP-1, which are chemokines, play important roles in angiogenesis of gastric carcinoma (2, 6, 23). Gastric carcinoma cells express IL-1, IL-6 and their receptors to form multiple-autocrine loops that regulate the growth of these cells in culture (4, 5). However, the relationship between the levels of these cytokines in tumor tissues and clinicopathological features has not yet been clarified.

We measured tissue concentrations of IL-1 β and IL-6 in gastric carcinoma and corresponding normal mucosa by ELISA. Most neoplastic lesions showed high concentrations of IL-1 β and IL-6. In particular, the levels

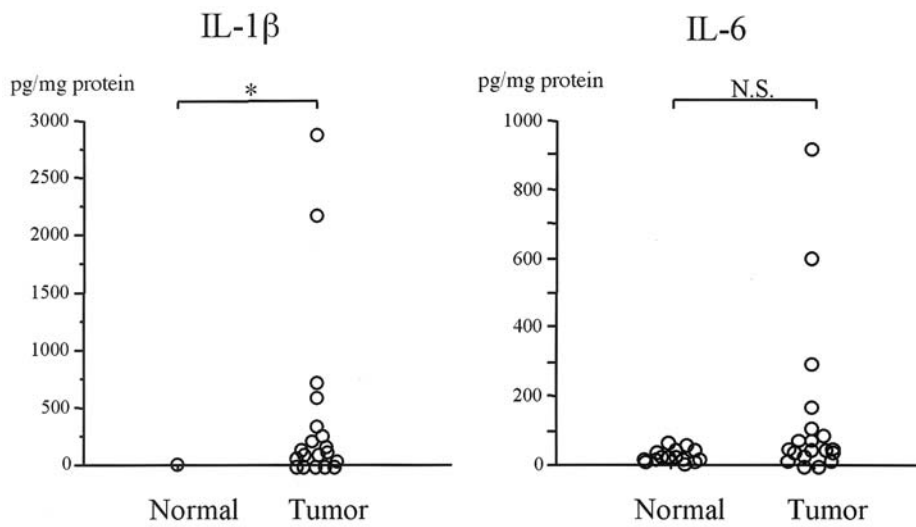


Figure 1. The levels of IL-1β but not IL-6 is significantly higher in carcinoma tissue than in normal corresponding gastric mucosa.

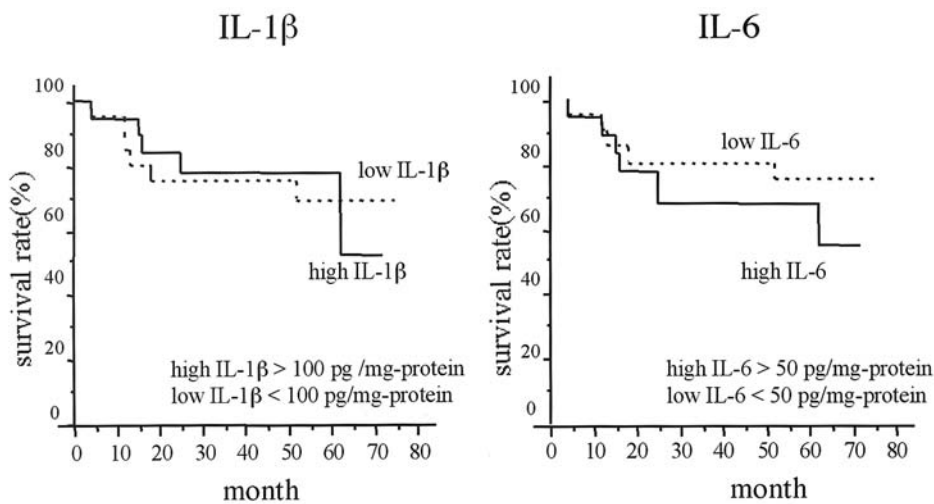


Figure 2. Overall survival of gastric carcinoma patients after therapy. There was no significant difference in survival between the high and low concentration groups for IL-1β and IL-6.

of IL-1β were significantly higher in tumor than in corresponding normal mucosa. Moreover, the levels of IL-6 correlated with the depth of invasion and lymphatic invasion. Previous studies revealed that circulating levels of IL-1β and IL-6 in serum of patients with various malignancies are higher than those in non-cancer patients and correlate with disease status (17, 18, 24-26). Therefore, cytokines derived from tumor tissue may enter the circulation and influence the serum levels of cytokines in patients.

Our study indicated that the tissue concentrations of IL-1β and IL-6 are significantly higher in solid growing non-scirrhus type tumors than in diffuse infiltrating scirrhus type tumors. Non-scirrhus type tumors express high levels of angiogenic factors VEGF and IL-8 and show greater angiogenesis than scirrhus type tumors (2, 27). IL-1β is reported to increase the expression of VEGF and IL-8 mRNAs in human gastrointestinal carcinoma (2, 28). Voronov *et al.* (29) reported that tumor angiogenesis and invasiveness are inhibited in IL-1β knockout mice. IL-6 also

induces expression of VEGF mRNA in various carcinoma cell lines (30). Therefore, IL-1 β and IL-6 may be involved in angiogenesis in solid growing non-scirrhus type gastric carcinoma *via* modulation of VEGF and IL-8. We also determined the survival rate of patients with gastric carcinoma. Although there was a tendency toward shorter survival in patients expressing a high level of IL-6, the correlation was not statistically significant (Figure 2). Because IL-1 β and IL-6 are multifunctional cytokines, other mechanisms, such as inflammatory immune responses, may influence prognosis.

In conclusion, we showed that gastric carcinoma tissues contain high levels of the proinflammatory cytokines IL-1 β and IL-6 and that the levels of these cytokines are associated with the growth pattern of the tumor. Because *H. pylori* infection induces the expression of many cytokines, including IL-1, IL-6 and IL-8, by gastric mucosa and carcinoma cells (31-33), it is of great interest that suppression of proinflammatory cytokines by *H. pylori* eradication may affect the progression of gastric carcinoma.

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