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Serum Interleukin-12 Levels in Patients with Gastric Cancer

SABURO MURAKAMI, KATSUHIKO OKUBO, YOSHITAKA TSUJI, HIDETO SAKATA, SETSUO HAMADA, and RENZO HIRAYAMA

Department of Surgery, Saitama Medical School, 38 Morohongo, Moroyama-machi, Iruma-gun, Saitama 350-0495, Japan

Abstract

Purpose. To evaluate the immunological status of patients with gastric cancer before surgery, we investigated the relationship between serum interleukin-12 (IL-12) levels and clinicopathological factors.

Methods. We measured serum IL-12 levels in 127 patients with gastric cancer and 35 healthy controls, by a sandwich enzyme-linked immunosorbent assay using the Human IL-12 +p40 Immunoassay kit.

Results. The serum IL-12 levels in the patients with gastric cancer were significantly higher than those of the healthy controls ($P < 0.05$). There were no significant differences in disease stage or gross appearance among the cancer groups, but the serum IL-12 levels in patients with T4 disease were significantly lower than those in patients with T1, T2, or T3 ($P < 0.01$). There were no significant differences in serum IL-12 levels between patients with and those without lymph node, liver, or peritoneal metastasis. The serum IL-12 levels in patients with distant metastasis were significantly lower than those in patients without distant metastasis ($P < 0.02$). There were no significant differences in the serum IL-12 levels according to classification by histopathological findings. Analysis with the linear correlation coefficient showed no significant correlation between serum IL-12 and serum carcinoembryonic antigen, carbohydrate antigen (CA) 19-9, CA 72-4, α-fetoprotein, or immunosuppressive acidic protein. However, there was a significant relationship between serum IL-12 levels and soluble IL-2 receptor levels ($r = 0.53$, $P < 0.01$).

Conclusion. Serum IL-12 levels in patients with far-advanced gastric cancer were significantly lower than those in patients with less-advanced gastric cancer. This is because macrophages in patients with far-advanced cancer would be hectic and unable to produce sufficient IL-12.

Key words Interleukin-12 · Soluble interleukin-2 receptor · Gastric cancer · Cytokine

Introduction

Interleukin-12 (IL-12) was originally identified as a natural killer (NK) cell stimulatory factor, being a disulfide-linked heterodimeric cytokine composed of 35 and 40 kDa subunits.[1,2] Secreted principally by antigen-presenting cells (APC), such as macrophages, some B cells, and dendritic cells, IL-12 activates NK cells and T cells to produce interferon-γ (INF-γ), and augments their cytotoxic activity and proliferation. Interleukin-12 was recently found to induce antitumor effects against a variety of tumors in vivo.[3] Furthermore, it is an immunoregulatory cytokine, which may provide an important link between nonspecific immune mechanisms and the development of a specific T cell-mediated immune response. In particular, in influences the development of T helper type 1 (Th1), which produces INF-γ, interleukin-2 (IL-2), tumor necrosis factor-β (TNF-β), and so on.[4,5] A preliminary in vitro study suggested that the administration of IL-12 produced immunomodulatory activity and yielded remarkable antitumor activity.[6–8] Although some investigators have examined the effects of intravenous IL-12 on patients with metastatic renal cell cancer or malignant melanoma, there are very few reports on serum IL-12 levels in cancer-bearing patients. To our knowledge, there is no report describing the serum IL-12 levels in patients with gastric cancer, although some studies have investigated the productivity of IL-12 ex vivo, using peripheral blood mononuclear cells stimulated by certain agents.[9,10] Therefore, we measured the serum IL-12 levels of patients with gastric cancer before

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surgery, to investigate their relationship with clinicopathological factors and evaluate the preoperative immunological status of these patients.

Patients and Methods

We examined 127 patients (82 men and 45 women) aged from 36 to 86 years (mean age 64.3 ± 10.4 years), admitted to our hospital for surgical treatment between 1997 and 2001. As a control for normal serum IL-12 concentrations, 35 healthy clinical personnel (mean age 39.6 ± 16.7 years) volunteered. Written informed consent was obtained from all patients. Blood samples were collected before surgery and specimens were stored at −80°C until later analysis. Disease stages were classified according to the Japanese Classification of Gastric Cancer (edition 13).[11]

Serum IL-12 levels were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) using a Human IL-12 +p40 Immunoassay kit (BioSource International, Camarillo, CA, USA) according to the manufacturer's instructions. Briefly, serum samples were reacted with a monoclonal antibody that recognized an epitope of human IL-12. After 2 h incubation and washing, streptavidin-peroxidase conjugated monoclonal antibody directed to a second epitope was added. This bound to the IL-12 captured by the first monoclonal antibody. The color reaction was terminated by a stop solution containing sulfuric acid, and absorbance was measured at 450 nm. In the control group, the bias of age was not recognized statistically in the levels of serum IL-12 (Kruskal-Wallis test). Levels of soluble interleukin-2 receptor (sIL-2R) were measured by an ELISA method using Cell-free Interleukin-2 Receptor kits (Yamanouchi, Tokyo, Japan).

Results are expressed as mean ± standard error of the mean. Data were analyzed with the analysis of variance procedure and the linear correlation coefficient. A P value of less than 0.05 was considered significant.

Results

The serum IL-12 levels in the patients with gastric cancer were significantly higher than those in the healthy controls ($P < 0.05$). There were no significant differences among the cancer groups, according to disease stage and gross appearance, but the serum IL-12 levels in patients with T4 disease were significantly lower than those in patients with T1, T2, or T3 disease ($P < 0.01$) (Table 1). There were no significant differences in serum IL-12 levels between patients with lymph node, liver, or peritoneal metastasis and those without metastasis, but the serum IL-12 levels in patients with distant metastasis were significantly lower than those in patients without distant metastasis ($P < 0.02$) (Table 2). There were no significant differences in serum IL-12 levels among cancer groups classified by histopathological findings such as pathological grade, lymphatic invasion, and venous invasion (Table 3). Analysis with the linear correlation coefficient showed no significant cor-

Table 1. Serum interleukin (IL)-12 levels in healthy subjects and patients with gastric cancer, and those in each cancer group according to disease stage, gross appearance, and tumor depth

	No. of patients	Mean ± SEM (pg/ml)	
Healthy controls	35	49.9 ± 4.9	$P < 0.05$
Gastric cancer (total)	127	63.3 ± 4.2	
Stage			
I	53	60.4 ± 7.7	NS
II	13	64.0 ± 8.2	
III	22	71.9 ± 11.2	
IV	39	62.1 ± 5.8	
Gross appearance			
Type 0	55	66.3 ± 7.7	NS
Type 1	1	28.0	
Type 2	11	50.7 ± 8.4	
Type 3	40	64.1 ± 5.5	
Type 4	22	62.6 ± 10.0	
Tumor depth			
T1	44	64.1 ± 8.6	$P < 0.01$
T2	38	60.0 ± 6.3	
T3	30	74.9 ± 9.0	
T4	15	46.1 ± 4.8	

NS, not significant

Table 2. Serum IL-12 levels in patients with and without lymph node, liver, peritoneum, or distant metastasis

	No. of patients	Mean ± SEM (pg/ml)	
Lymph node metastasis			
Negative	53	63.3 ± 7.7	NS
Positive	74	63.5 ± 4.8	
Liver metastasis			
Negative	112	62.7 ± 4.5	NS
Positive	15	67.5 ± 9.8	
Peritoneal metastasis			
Negative	104	63.7 ± 4.8	NS
Positive	23	61.5 ± 8.8	
Distant metastasis			
Negative	123	64.1 ± 4.3	$P < 0.02$
Positive	4	36.5 ± 6.2	

Table 3. Serum IL-12 levels in each cancer group, classified by histopathological findings

	No. of patients	Mean ± SEM (pg/ml)	
Grade			
Well differentiated	4	84.0 ± 18.4	NS
Moderately differentiated	62	57.6 ± 5.8	
Poorly differentiated	47	69.3 ± 6.6	
Signet	11	52.6 ± 10.1	
Mucinous	3	67.5 ± 12.2	
Lymphatic invasion			
Negative	34	63.3 ± 10.8	NS
Positive	82	65.9 ± 4.6	
Venous invasion			
Negative	47	67.4 ± 8.8	NS
Positive	68	63.2 ± 4.9	

Table 4. Relationship between serum IL-12 levels and various tumor markers (CEA, CA 19-9, CA 72-4, FTP, IAP, and sIL-2R)

	Correlation coefficient with IL-12	*P* value
CEA	0.038	0.710
CA 19-9	0.107	0.335
CA 72-4	0.053	0.578
FTP	0.263	0.250
IAP	0.026	0.828
sIL-2R	0.53	0.006

IL, interleukin; CEA, carcinoembryonic antigen; CA, carbohydrate antigen; FTP, α-fetoprotein; IAP, immunosuppressive acidic protein; sIL-2R, soluble interleukin-2 receptor

relation between the serum IL-12 levels and the serum levels of carcinoembryonic antigen, carbohydrate antigen (CA) 19-9, CA 72-4, α-fetoprotein, and immunosuppressive acidic protein (Table 4). However, there was a significant relationship between the serum IL-12 levels and sIL-2R levels ($r = 0.53$, $P < 0.01$; Fig. 1).

Discussion

Interleukin-12 is an immunoregulatory cytokine that triggers the development of a specific T cell-mediated immune response.[12] Interleukin-12 enhances the proliferation, cytokine production, and cytotoxic activity of T lymphocytes and NK cells, with consequent antitumor activity.[7,8,13–16] Recent studies have shown that IL-12 generally promotes the generation of type 1 cells (Th1) and induces the production of IL-2 and INF-γ.[5,6,17–20] Moreover, an in vitro study of peripheral blood leukocytes demonstrated that *Helicobacter pylori* selectively stimulates the induction of IL-12 and Th1 cells, which produce INF-γ.[21] However, there are very few studies on the serum IL-12 levels in cancer-bearing patients, even though they are thought to be necessary for assessing the immunological status. Lissoni et al. reported very high serum IL-12 levels in 8 out of 19 (42%) patients with metastatic renal cell carcinoma, whereas baseline values within the normal range were found in the other 11.[22] Kallio et al. also measured the serum IL-

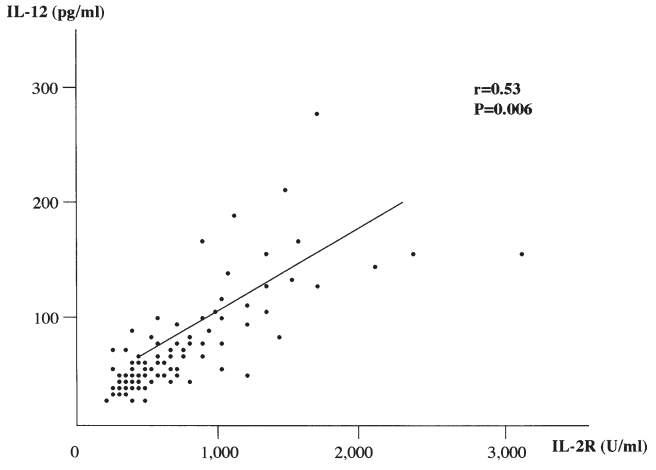

Fig. 1. Correlation of serum interleukin-12 (*IL-12*) levels with soluble interleukin-2 receptor (*sIL-2R*)

12 levels in 24 patients with renal cell carcinoma, and found higher mean levels in patients with a localized tumor than in those with disseminated disease.[23] Conversely, Lissoni et al. reported that serum IL-12 levels were significantly higher in patients with metastatic renal cell carcinoma and breast cancer than in those with locally contained solid neoplasms.[24] This complete discrepancy cannot be explained and the accumulation of more data is necessary. To our knowledge, no other study on serum IL-12 levels in patients with gastric cancer has been published. Uno et al. reported that phytohemagglutinin-induced production of IL-12 was lower in 174 patients with cancers of various organs, including 81 with gastric cancer, than in healthy controls, by examining peripheral blood mononuclear cells in vitro.[10] In a study of 61 patients with gastrointestinal cancer, including 25 with gastric cancer, by using peripheral blood mononuclear cells stimulated by *Staphylococcus aureus* in vitro, Shibata et al. reported that the production of IL-12 decreased significantly with advancing stages, and was lowest in patients with distant metastases and cachexia.[9] However, these studies were dependent on in vitro data using the culture of peripheral blood mononuclear cells isolated from the cancer-bearing patients. Our study is the first to investigate the levels of IL-12 and their correlation with clinicopathological factors in patients with gastric cancer. We found that the serum IL-12 levels in patients with gastric cancer were significantly higher than those in healthy controls; however, they were significantly lower in patients with far-advanced cancers, such as stage T4 or those with distant metastasis, than in those with less-advanced cancer. According to these data, the productivity of IL-12 by macrophages in patients with gastric cancer could not decrease, except in those with far-advanced or cachectic-stage cancer. In fact, many tumor-infiltrating mononuclear cells were found in some patients, and expression of IL-12 mRNA was recognized in 33% of the gastric cancer tissues.[25] On the other hand, our findings might also indicate the immunological insufficiency of patients with far-advanced cancer or cachexia because the serum IL-12 levels were low in these patients. Interestingly, the serum IL-12 levels in patients with liver metastasis were not as low as those in patients with metastasis in other sites. Kupffer cells as monocytes existing in the liver might react to the cancer cells, and secrete IL-12 in some form. Experimental studies suggest that IL-12 is one of the cytokines that exerts antitumor effects. On the other hand, IL-2 represents one of the most promising components in the immunotherapy of cancer.[26,27] An increased endogenous production of IL-12 has been seen to synergize with IL-2 to induce tumor regression in experimental models.[6,28,29] Unfortunately, serum IL-2 is difficult to measure and it is unstable because of its very short life span of only 10 min. Therefore, serum sIL-2R is often measured as a substitute for IL-2, since it reflects the activity of IL-2 by attaching to its receptor as an autocrine, and it is easy to detect because of its relative stability in the serum.[30] Several investigators have reported recognizing elevated levels of sIL-2R in patients with various malignant disorders,[30–32] and some reports describe the relationship between IL-12 and sIL-2R in other clinical conditions. In acute graft-versus-host disease after allogenetic bone marrow transplantation, the serum IL-12 and sIL-2R levels increased simultaneously[33] and patients with immune arthropathy had increased IL-12 and sIL-2R levels in the synovial fluid.[34] A positive correlation between levels of serum IL-12 and sIL-2R was also recognized in the present study. These data suggest that IL-12 and sIL-2R may be correlated, even though they are produced by cells of different origin; mainly macrophages and T lymphocytes, respectively.

Since preclinical studies have shown that IL-12 has potent immunomodulatory and antitumor effects, clinical trials on the administration of recombinant human IL-12 (rhIL-12) have been conducted in some faculties.[22,35–38] Gollob et al. reported that the long-term administration of rhIL-12 given twice weekly i.v. for 6 weeks to patients with metastatic renal cell cancer or malignant melanoma was tolerated well, stimulated the production of IL-12 costimulatory cytokines and INF-γ, and induced delayed tumor regression.[35] Lissoni et al. gave patients with renal cell carcinoma IL-12 immunotherapy, as 1.25 μg/kg s.c. once a week for 3 consecutive weeks, followed by a 1-week rest period,[19] which helped to stabilize the disease in 20% of patients. They also reported that high serum IL-12 levels indicated a favorable prognosis in response to IL-2 cancer immunotherapy for renal cell carcinoma. A recent study found that IL-2 function was enhanced by IL-12 through a p38

mitogen-activated protein kinase pathway.[20] Ohno et al. reported the biological effects of subcutaneous recombinant human IL-12 in 15 patients with advanced malignancies, including 8 with renal cell carcinoma, 2 with melanoma, 4 with colorectal cancer, and 1 with gastric cancer.[39] They observed a complete response and a partial response in two patients with metastatic renal cell carcinoma after 50 and 300 ng/kg, respectively. However, the clinical trials have not yet shown any effective results of IL-12 administration for patients with gastrointestinal carcinoma, although an accumulation of results from more clinical trials might show the beneficial effects of this treatment for gastrointestinal malignancies. Considering the decreased levels of serum IL-12 in patients with far-advanced gastric cancer in this study, when IL-12 as immunotherapy is clinically approved, it might prove effective for these patients, since their levels of serum IL-12 are so low.

In conclusion, the serum IL-12 levels in patients with far-advanced gastric cancer were significantly lower than those in patients with less-advanced gastric cancer. This can be explained by the fact that in far-advanced cancer, the macrophages would be hectic and unable to produce a sufficient amount of IL-12.

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