ABSTRACT

Serum levels of interleukin 6 (IL-6) are correlated with the disease status and prognosis in cancer patients. IL-6 is also an important mediator of experimental cancer cachexia. We investigated the production of IL-6 and IL-6 receptors and expression of IL-6 mRNA by esophageal squamous carcinoma cells using immunohistochemical staining and in situ reverse transcription-PCR. We also measured levels of serum IL-6 using an ELISA in 50 patients with esophageal squamous cell carcinoma (ESCC) to determine the correlation between serum levels of IL-6 and clinicopathological factors. IL-6 mRNA was expressed in the primary tumor. Esophageal squamous carcinoma cells produced both IL-6 and IL-6 receptor. IL-6 concentrations were significantly higher in the primary tumor than in the normal epithelium. The incidences of weight loss, tumor invasion to adjacent organs, and noncurative resection were significantly higher in ESCC patients with serum levels of IL-6 ≥ 7 pg/ml (n = 13, group C) compared with patients with serum levels <7 pg/ml and ≥3 pg/ml (n = 14, group B) and <3 pg/ml (n = 23, group A). Tumor size and C-reactive protein levels were significantly higher and albumin levels were significantly lower in group C. Results suggest that IL-6, which is produced by tumor cells, may be related to various disease parameters as well as to the nutritional status in patients with ESCC.

INTRODUCTION

SCC<sup>2</sup> of the esophagus has a poor prognosis because of its rapid growth and spread and the associated malnutrition due to dysphagia and cachexia (1). Most large investigations of patients with this type of cancer report the 5-year survival rate after surgery or radiation therapy to be below 20% (1-3).

IL-6 is a multipotent cytokine with numerous biological activities (4). It plays an integral role in the induction of B-cell differentiation and IgG secretion (5) stimulates the growth and differentiation of human thymocytes and T cells (6, 7), and enhances the induction of lymphokine-activated killer cells (8) and cytotoxicity by natural killer cell (9). IL-6 is also a potent proinflammatory cytokine. It acts as an endogenous pyogen and induces the expression of acute phase protein genes including the CRP gene (10, 11). IL-6 has been found to be an important mediator of experimental cancer cachexia in the mouse C-26 tumor system (12). IL-6 is produced by a variety of cells, including T cells, B cells, monocytes, fibroblasts, keratinocytes, endothelial cells, astrocytes, bone marrow cells, and mesangial cells (4). Immunohistochemical studies have shown IL-6 immunoreactivity in primary squamous cell carcinomas and in a variety of adenocarcinomas and sarcomas (13). Several human tumor cell lines, including multiple myeloma (14), renal cell carcinoma (15), melanoma (16), lymphoma (17), lung cancer (18), ovarian cancer (19), and cervical carcinomas (20), produce IL-6. The identification of an IL-6→IL-6R autocrine loop in multiple myeloma (14) and renal cell carcinoma (15) suggests that an autocrine mechanism may be involved in oncogenesis. The IL-6 produced by tumors may modulate local immunity around the tumor lesion. Clinically, serum levels of IL-6 are correlated with disease status and prognosis in patients with metastatic renal cell carcinoma (21) and epithelial ovarian cancer (22). Low concentrations of IL-6 can now be detected using commercially available IL-6 kits.

In the present study, we investigated the production of IL-6 and IL-6R and expression of IL-6 mRNA in esophageal cancer cells using immunohistochemical staining and in situ RT-PCR. We also measured serum levels of IL-6 using an ELISA in patients with ESCC to clarify the relationship between serum levels of IL-6 and clinicopathological factors.

PATIENTS AND METHODS

Patients. We studied 50 patients with SCC of the esophagus admitted to our department between 1992 and 1994 (Tables 1 and 2). None of the patients had inflammatory diseases or had received any treatments, including nutritional support, before admission and biopsy-proven diagnosis of SCC of the esophagus. The location of tumors and distant metastases was determined by barium esophagography; chest radiography; endoscopy of the tracheobronchial tree, pharynx, larynx, and esophagus; computed tomography and MRI of the thorax and abdomen, and radionuclide bone scanning. Tumor resection was performed in 38 (76%) of 50 patients; in 12 patients (24%) in whom distant metastases or invasion to adjacent organs was confirmed by computed tomography and/or MRI, tumors were considered unresectable. Eleven patients were able to swallow liquids only. We evaluated the following physical and pathological factors: weight loss, location, tumor size, macroscopic tumor type, tumor depth, lymph node metastasis, histological type, and pTNM stage (23). In patients with unresectable tumors, the tumor size, macroscopic tumor type, tumor depth, lymph node metastasis, and the pTNM stage were evaluated by radiographic, endoscopic, and MRI findings. Curability and postoperative complications were also evaluated. Informed consent was obtained from all patients.

Serum Sampling. Serum samples were obtained from all patients on the day before any treatment and stored at −80°C until the assay. Serum samples were also obtained from a control population of 25 normal healthy age- and sex-matched volunteers.

Surgical Specimen. Fresh surgical specimens of primary tumors and normal epithelium were collected under sterile conditions from seven patients. The specimens were immediately prepared for analysis of tissue IL-6 levels, immunohistochemical staining, and in situ RT-PCR (see below).

Tumorous and Normal Mucosal Homogenates. The specimens were immediately stored in liquid nitrogen until use. These samples were thawed, quickly weighed, placed in 2 ml PBS, and homogenized for 10 s in a tissue homogenizer. The homogenates were then centrifuged twice at 4°C at 10,000 × g, and aliquots of the supernatants were prepared for the IL-6 assay (pg/g tissue).

IL-6. Serum and supernatant levels of IL-6 were measured using an ELISA according to the manufacturer’s instructions (Human IL-6 Immunoassay kit; Cytoscreen, Biosource Co., Ltd., Camarillo, CA). The limit of detection of the assay was 3 pg/ml, and levels below 3 pg/ml were considered undetectable.

Received 1/4/96; accepted 4/16/96.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom requests for reprints should be addressed. Phone: 81-8342(22)2264; Fax: 81-8342(22)2263

2 The abbreviations used are: SCC, squamous cell carcinoma; IL-6, interleukin 6; CRP, C-reactive protein; RT, reverse transcription; MRI, magnetic resonance imaging; mAb, monoclonal antibody; IL-6R, IL-6 receptor; DEPC, diethyl pyrocarbonate; ESCC, esophageal squamous carcinoma; ESCC, esophageal squamous cell carcinoma.

Masaaki Oka, Kohtaro Yamamoto, Mutsumi Takahashi, Michinori Hakozaiki, Toshihiro Abe, Norio Iizuka, Shoichi Hazama, Katsutoshi Hirazawa, Hiroto Hayashi, Akira Tangoku, Kunitaka Hirose, Tokuhiko Ishihara, and Takashi Suzuki

Department of Surgery II \[M.O., K.Y., T.A., N.I., S.H., K.H., H.H., A.T., T.S.I.\] and Departments of Clinical Laboratories \[M.T.I and Pathology \[I.T./. Yamaguchi University School of Medicine, 1144 Kogushi, Ube, Yamaguchi 755, and Biomedical Research Institute, Kurelia Chemical Industry Co. Ltd., 3-26-2, Hyakunin-cho-. Shinjuku-ku, Tokyo 169 \[M.H., Ku. HJ. Japan\]
cutoff for detection of SCC-related antigen was 2.0 ng/ml.
The cutoff value for the CRP assay was 0.25 mg/dl.

CRP. Serum levels of CRP (mg/dl) were measured with an immunoturbidimetric assay (Iatromate CRP [A]; Yatoron Co., Inc., Tokyo, Japan). The cutoff value for the CRP assay was 0.25 mg/dl.

**SCC-related Antigen.** Serum concentrations of SCC-related antigen were measured with an enzyme immunoassay kit (Dynabot, Tokyo, Japan). The cutoff for detection of SCC-related antigen was 2.0 ng/ml.

Mouse mAbs. A mouse anti-human IL-6 mAb (IgGl class: PM1) and a mouse anti-IL-6R mAb (IgGl class: MH166) were kindly provided by Chugai Pharmaceutical Company (Shizuoka, Japan). These mAbs have been described previously (15, 24, 25). Mouse IgG1 was used as the control mAb.

**Immunohistochemical Staining of IL-6 and IL-6R.** Specimens obtained from resected primary esophageal carcinomas were immediately stored at —80°C or by replacing them with nonimmune, species-specific serum.

**Results**

IL-6 mRNA Expression in Esophageal Carcinoma Cells. IL-6 mRNA expression in the primary tumor and normal mucosa in vivo was determined using in situ RT-PCR (Fig. 1). There was no difference of intensity of β-actin expression (positive control) between the two conditions.

**Tables**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (yr)</td>
<td>60.0 (37-82)</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 46, Female 4</td>
</tr>
<tr>
<td>Degree of dysphagia</td>
<td>None 9, Minimal 9, Mild (can swallow soft food) 21</td>
</tr>
<tr>
<td>Location</td>
<td>Cervical 5, Thoracic 43, Abdominal 2</td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td>&lt;3, 3-4.9, 5-7.9, ≥8</td>
</tr>
<tr>
<td>Tumor type</td>
<td>Tumorous 2, Ulcerative 46, Diffuse 2</td>
</tr>
<tr>
<td>Macroscopic tumor type</td>
<td>Tumorous 2, Ulcerative 46, Diffuse 2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor depth</td>
<td>No invasion to adventitia 18, Invasion to adventitia 22, Invasion to adjacent organs 10</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>Negative 16, Positive 34</td>
</tr>
<tr>
<td>Histological type</td>
<td>Well differentiated 4, Moderately differentiated 23, Poorly differentiated 10, Unknown 12</td>
</tr>
<tr>
<td>Distant metastases</td>
<td>Negative 45, Positive 5</td>
</tr>
<tr>
<td>pTNM stage</td>
<td>0 9, I 3, II 4, III 18, IV 12</td>
</tr>
<tr>
<td>Resectability</td>
<td>Resectable 39, Unresectable 11</td>
</tr>
<tr>
<td>Curability</td>
<td>Curative 19, Noncurative 20</td>
</tr>
</tbody>
</table>
IL-6 AND ESOPHAGEAL SQUAMOUS CELL CARCINOMA

Fig. 1. IL-6 mRNA expression of normal epithelium and primary lesion using in situ RT-PCR. There was no difference in intensity of β-actin expression (positive control) between the normal epithelium and primary lesion. IL-6 mRNA was expressed strongly in esophageal cancer cells. IL-6 mRNA expression was very weak in normal epithelial cells.

β -actin

Normal epithelium
Primary lesion

IL-6 mRNA

Fig. 2. IL-6 (left) and IL-6R (right) immunohistochemical staining. IL-6 immunoreactivity was seen intensely in the cytoplasm of the ESC cells, whereas IL-6R immunoreactivity was seen mainly in the cytoplasmic surface of the ESC cells. IL-6 and IL-6R, ×300.

normal epithelium and primary lesion. IL-6 mRNA was expressed strongly in esophageal cancer cells. IL-6 mRNA expression was very weak in normal epithelial cells.

Immunohistochemical Staining. Fig. 2 shows immunohistochemical staining of IL-6 and IL-6R. IL-6 immunoreactivity was seen intensely in the cytoplasm of the ESC cells. On the other hand, IL-6R immunoreactivity was seen mainly in the cytoplasmic surface of the ESC cells, but, to a lesser extent, the cytoplasm of the tumor cells was also reacted with mAb. Immunolabeling was absent when each antibody was omitted or replaced by nonimmune, species-specific serum.
IL-6 Concentrations in Tumor and Normal Epithelium Homogenates. The concentrations of IL-6 in the primary lesions (3427.1 ± 1172.3 pg/g tissue) were significantly higher than those in the normal epithelium (336.8 ± 125.3 pg/g tissue; P = 0.0223; Fig. 3).

Serum Levels of IL-6 in Patients with Esophageal Carcinoma. Serum concentrations of IL-6 were detectable in 27 (54%) of 50 patients with ESCC (minimum, 3.07 pg/ml; maximum, 32.6 pg/ml), but in only 3 (12%) of 25 healthy volunteers (minimal, 3.1 pg/ml; maximum, 4.2 pg/ml; P = 0.00256). SCC positivity (>2.0 ng/ml) was detected in 14 patients (28%). The rate of IL-6 positivity was significantly higher than the rate of SCC positivity in patients with esophageal cancer (x^2 = 6.98635, P < 0.01).

ESCC patients were classified into three groups based on their serum levels of IL-6: <3.0 pg/ml (undetectable, group A, n = 23), ≥3.0 but <7.0 pg/ml (group B, n = 14), and ≥7.0 pg/ml (group C, n = 13). The incidence of weight loss (>3 kg in the previous 6 months) was significantly higher in group C than in groups A and B (x^2 = 6.3360, P = 0.042; Table 3) (11 patients who were able to swallow liquid meals only or were unable to swallow at all were excluded from analysis). The incidence of invasion to adjacent organs was significantly higher in group C than in group A (Fisher, P = 0.044; Table 3). The incidence of curative resection was significantly higher in group C than in group A (Fisher, P = 0.033; Table 3). The cumulative 2-year survival rate for patients in groups A, B, and C who underwent tumor resection was 39.4%, 59.0%, and 6.2% (Fisher, P = 0.044; Table 3). The cumulative 2-year survival rate for patients in groups A, B, and C who underwent tumor resection was 39.4%, 59.0%, and 6.2% (Fisher, P = 0.044; Table 3).

DISCUSSION

Esophageal squamous carcinoma cells produced IL-6 and expressed IL-6R. Serum levels of IL-6 were significantly higher in patients with ESCC than in healthy controls and were correlated with various disease parameters (including tumor size and tumor depth) and curability. The 2-year survival rate for patients in groups A and B was significantly greater than that for patients in group C. However, this difference should be due to the incidence of curative resection between groups. The serum levels of IL-6 were also correlated with the nutritional status as determined by evaluation of weight loss and the serum level of albumin.

IL-6 concentrations in primary tumors were 10 times greater than those in normal epithelium in the present study. IL-6 enhances the induction of lymphokine-activated killer cells (8) and cytotoxicity by natural killer cells (9). In contrast, it would be interesting to investigate the cytotoxic function of tumor-infiltrating lymphocytes which could be blocked by very high local concentrations of IL-6 at tumor sites (26). Thus, it is possible that increased production of IL-6 by esophageal cancer cells may contribute to the escape of tumor cells from immune surveillance.

The autocrine hypothesis proposes that a cell produces a growth factor that interacts with specific membrane receptors on its own surface to induce various effects such as proliferation (27). There-
fore, the simultaneous production of IL-6 and IL-6R suggests that IL-6 acts in an autocrine manner. The existence of an IL-6–IL-6R autocrine loop in multiple myeloma (14) and renal cell carcinoma (15) suggests that an autocrine mechanism may be involved in oncogenesis. Immunohistochemical analysis showed simultaneous production of IL-6 and IL-6R in an autocrine mechanism may be involved in oncogenesis. Immunohistochemical loop in multiple myeloma (14) and renal cell carcinoma (15) suggests that cancerous epithelial cells with autocrine-activated killer cells. Cancer Immunol. Immunother. 31: 49–52, 1990.


Relationship between Serum Levels of Interleukin 6, Various Disease Parameters, and Malnutrition in Patients with Esophageal Squamous Cell Carcinoma


<table>
<thead>
<tr>
<th>Updated version</th>
<th>Access the most recent version of this article at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><a href="http://cancerres.aacrjournals.org/content/56/12/2776">http://cancerres.aacrjournals.org/content/56/12/2776</a></td>
</tr>
</tbody>
</table>

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.