CASE REPORT

Thymic Carcinoma Associated with a High Serum Level of Interleukin 6 Diagnosed through the Evaluation for Asymptomatic Elevation of Acute-Phase Reactants

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A case of thymic squamous cell carcinoma producing interleukin-6 (IL-6) is reported. C-reactive protein (CRP), white blood cell (WBC) count, and serum IL-6 had been elevated, and normalized immediately after tumorectomy. IL-6 in the culture supernatant from the tumor was significantly elevated and the expression of IL-6 mRNA was demonstrated in the tumor by a reverse transcriptase polymerase chain reaction method. Immunohistochemical study demonstrated that tumor cells were stained positive with an anti-IL-6 antibody. IL-6 derived from the tumor cells reflected the increase CRP and WBC counts. This case suggested that asymptomatic elevation of acute-phase reactants might be clues for the diagnosis of an IL-6 producing tumor.

(Key words: squamous cell carcinoma, C-reactive protein, fever of unknown origin)

Introduction

Interleukin 6 (IL-6) is responsible for a variety of biological effects related to the activation of immune responses and the acute-phase reaction against infection or inflammation. Although several cytokines including interleukin 1 (IL-1) and tumor necrosis factor (TNF) are implicated in the acute-phase response, a previous study has demonstrated that the synthesis of the acute-phase proteins (e.g. C-reactive protein (CRP)) is mainly induced by IL-6 (1). In addition, IL-6 has emerged as a likely mediator of many systemic effects (fever, increased erythrocyte sedimentation rate (ESR), and alterations in plasma protein composition) observed in patients with cancer. Furthermore, IL-6 may also mediate local effects such as alteration in proliferation of tumor cells (2, 3), increased tumor cell motility, and decreased intercellular adhesions between tumor cells (4, 5). More recently, IL-6 was reported to act as a tumor suppresser (6–8).

We report a patient with IL-6 producing thymic carcinoma which was recognized through examinations for the elevation of acute-phase reactants without an apparent infection. The serum level of IL-6 was markedly elevated and analysis using a reverse transcriptase polymerase chain reaction (RT-PCR) method demonstrated that this tumor produced IL-6. Although IL-6-producing squamous cell carcinomas in other organs have been reported (9–11), this is the first case of an IL-6 producing thymic carcinoma.

Case Report

A 43-year-old Japanese woman was admitted to our hospital because of asymptomatic inflammatory findings (ESR: 39 mm/1 hour, elevation of CRP) and anemia (red blood cell count: 400 x 10^4/μl, hemoglobin: 11.5 g/dl), which were pointed out 3 years before admission. Although many examinations had been undertaken at three hospitals for her inflammatory signs, the cause had remained unknown, and her temperature had not exceeded 37°C.

On admission, her height was 154 cm, body weight: 53 kg, body temperature: 36.5°C, pulse rate: 86/min, and blood pressure: 104/68 mmHg. There were no abnormal findings in the physical examinations. The white blood cell (WBC) count and platelet count had increased to 10,000/μl and 24.4 x 10^4/μl, respectively. Neutrophilia (72%) was observed, and CRP was elevated to 10.1 mg/dl (normal value: less than 0.5 mg/dl). The percentage of α1-globulin in total protein was increased to 3.5%. All evaluations for infection were negative. On chest roentgenogram, there was a projection from the mediastinum at the aortopulmonary window, and chest computed tomography demonstrated that a tumor with a large calcification was located...
Interleukin 6 Producing Thymic Carcinoma

Tumorectomy Irradiation (40 Gy)

IL-6 39.2 35.4 19.8 >4.0 >4.0

(\text{pg/ml})

Figure 2. Serum level of IL-6, CRP, and WBC count were all normalized immediately after the tumorectomy. CRP: C-reactive protein, IL-6: interleukin 6, WBC: white blood cell.

at the left anterior mediastinum adjacent to the aortic arch (Fig. 1). According to retrospective studies, a thymic tumor had appeared about 2 years before, and the growth rate of this tumor was thought to be very low.

To determine the cause of her inflammatory signs, the serum level of several acute inflammatory cytokines; IL-1\(\alpha\), IL-1\(\beta\), IL-6, transforming growth factor-\(\beta\) (TGF-\(\beta\)), and TNF-\(\alpha\) were measured. Serum level of IL-6 was raised to 39.2 pg/ml (normal value; less than 4.0 mg/dl). Nevertheless, other cytokines (IL-1\(\alpha\), TNF-\(\alpha\), TGF-\(\beta\), IFN-\(\gamma\), and IL-3) were not increased.

Tumorectomy was performed and histological findings revealed a diagnosis of thymic squamous cell carcinoma. Although the tumor invaded the phrenic nerve, pericardium, and pleura, there was no metastasis to lymph nodes. After tumorectomy, the white blood cell count and CRP were normalized immediately (Fig. 2), and serum level of IL-6 decreased to below the detection limits. Radiation therapy at the dose of 40 Gy was added. Until now (26 months after operation), no relapse has been observed.

To examine the association between thymic carcinoma and the elevation of IL-6, we evaluated whether tumor cells produced IL-6 or not. The resected tumor was treated with trypsin ethylenediaminetetraacetic acid (EDTA), and separated cells were grown in Dulbecco’s modified Eagle’s medium without fetal calf serum in 5% CO\(_2\) humidiated atmosphere at 37°C. After seven days of incubation, the concentration of the cytokines in the culture broth of tumor cells were evaluated. Ribonucleic acid (RNA) was extracted from the tumor by the acid guanidinium-phenol-chloroform method. Complementary deoxyribonucleic acid (cDNA) was synthesized using random hexanucleotide from 100 ng of RNA. Expression of IL-6 in this tumor was evaluated by the polymerase chain reaction (PCR) method using specific primers for the 527 bp mRNA of human IL-6 and quantified with ethidium bromide staining.

IL-6 in the culture supernatant of tumor cells was remarkably elevated to 115,000 pg/ml. In contrast, other inflammatory cytokines (IL-1\(\alpha\), IL-1\(\beta\), TNF-\(\alpha\), TGF-\(\beta\)) were not as elevated. These results suggested that the tumor mainly produced IL-6. The results of the expression of IL-6 mRNA are shown in Fig. 3. The amounts of \(\beta\)-actin were almost identical in three different materials; fibrous capsule of this tumor, thymic carcinoma, and peripheral blood lymphocyte. In contrast, the amplificative products indicating IL-6 were clearly demonstrated in the carcinoma, lymphocytes, and faintly demonstrated in the fibrous capsule. We also stained the resected tumor with polyclonal anti-human IL-6 antibody (Genzyme,
Cambridge, MA) and examined which cells produced IL-6 in this thymic cancer. As a result, the cell surface and cytoplasm of tumor cells were stained with anti-IL-6 antibody. These results indicated that the tumor produced IL-6.

**Discussion**

Interestingly, this case was recognized by the elevation of acute-phase reactants without infection, and thymic carcinoma was discovered through the systemic evaluation. According to the clinical course (Fig. 2), the patient's inflammatory findings seemed to be accompanied with the increase of IL-6. Expression of IL-6 was recognized in the resected tumor at the level of both protein and mRNA.

A previous study has demonstrated that recombinant human (rh) IL-6 increases platelet counts and leukocytes, whereas no change in IL-1β and TNF-α levels is observed (12). In addition, it was also reported that CRP and serum amyloid A protein levels are increased by the administration of rhIL-6 in a dose-dependent manner (12). IL-6 has also emerged as a likely mediator of many of the systemic alterations observed in patients with cancer (fever, increased ESR, and alterations in plasma protein composition). Previous reports have demonstrated that Castleman's disease, multiple myeloma, atrial myxoma, soft tissue tumors including leiomyosarcoma, neurofibrosarcoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, and malignant germ cell tumor can produce IL-6. More recently, immunohistochemical studies on human tumors have revealed that IL-6 is distributed in several human tumors; in the neoplastic elements present in primary squamous cell carcinomas, in adenocarcinomas of mammary, colonic, ovarian, endometrial origin, and metastatic to lymph nodes (5). However, there have been no reports of squamous cell carcinoma in the thymus which produces IL-6.

In the present case, although the tumor had been identified about 2 years before admission, the growth rate was very slow. Previously, IL-6 has been implicated to act as an autocrine and/or paracrine growth factor for various human tumors, including multiple myeloma (2), renal cancer (3), and AIDS-associated Kaposi's sarcoma (13). However, more recently, it has been reported that IL-6 can exert potent anti-tumor effects on lung cancer (6, 8) and melanoma (7). In this case, although the relationship of the tumor growth and the production of IL-6 was not clear, it could be speculated that the slow growth rate of this tumor was caused by the anti-tumor effects of IL-6 produced by the tumor.

In conclusion, this case suggested that asymptomatic elevation of acute-phase reactants might be clues to diagnose a IL-6 producing tumor.

**References**