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Increased Serum Concentrations of Soluble Tumor Necrosis Factor Receptor I in Noncachectic and Cachectic Patients with Advanced Gastric and Colorectal Cancer

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Abstract: The serum levels of soluble tumor necrosis factor receptor I (sTNF-RI) were measured in 74 noncachectic patients including 42 with gastric cancer and 32 with colorectal cancer, as well as in 39 patients with severe cachexia and 15 healthy volunteers. The sTNF-RI levels increased with the advance of disease, being highest in the cachectic patients. The levels were inversely correlated with the serum concentrations of nutritional parameters such as prealbumin, transferrin, retinol binding protein, and the percentages of CD3(+) cells in the peripheral blood lymphocytes, and positively correlated with the serum concentration of immunosuppressive acidic protein (IAP) and soluble interleukin-2 receptors. These findings suggest that sTNF-RI could be an important prognostic factor to predict the advance of gastric and colorectal cancers and deterioration of the patient's nutritional and immune activity.

Key Words: tumor necrosis factor, gastric cancer, colorectal cancer, tumor immunology, macrophage

Introduction

Severe weight loss and debilitating waste of the lean body mass frequently complicates the treatment of patients suffering from malignancy. Cachexia, characterized by the syndrome of weight loss, anorexia, anemia, and weakness, further increases cancer mortality.[1–4] If not reversed, cachexia-associated derangements of homeostasis lead to immunological deficiencies, organ failure, and multiple metabolic abnormalities.

Although the mechanisms underlying cachexia are still not fully understood, it has recently been suggested that the increased production and release of cytokines might be contributing factors.[5] Tumor necrosis factor (TNF), a polypeptide cytokine produced mainly by activated macrophages, has numerous biological functions such as induction of hemorrhagic necrosis of transplanted tumors or cytotoxicity. TNF also plays an important role in endotoxin shock and in the inflammatory, immunoregulatory, proliferative, and antiviral response.[6–8] This potent cytokine initiates its multiple functions by binding to specific, high-affinity receptors and has been implicated as one of the mediators of cancer cachexia. Two distinct TNF receptor subtypes are the cell surface form and the soluble molecules.[9–14] These two soluble TNF receptors, which are thought to represent proteolytic cleavage products of the extracellular domains of membrane-bound TNF receptors of molecular weight 55 kDa (sTNF-RI) and 75 kDa (sTNF-RII), are found in the serum and urine of humans. The smaller molecules of these receptors are present in most cells, particularly in those susceptible to the cytotoxic action of TNF.[9,15] Because of their ability to compete with the cell surface forms of TNF receptor, these soluble forms may function as an inhibitor of TNF bioactivity. The results of comparative in vitro studies on the responses of various cultured cells to the effects of TNF indicate the existence of mechanisms capable of suppressing its function.[16–18]

The present study was undertaken to determine to what extent sTNF-RI is present in cancer patients with severe cachexia.

Subjects and Methods

Patients

The subjects of this study included 77 patients suffering from cancer, as a preoperative sampling group. There were 42 patients with gastric cancer: 18 in stage 1, 6 in stage 2, 10 in stage 3, and 8 in stage 4;[19] and 32 with colorectal cancer: 10 in stage 1, 6 in stage 2, 11 in stage

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3, and 5 in stage 4.[20] The disease was classified as: localized, defined as node-negative without distant metastasis in 38 patients; intermediate, defined as node-positive without distant metastasis in 30 patients; and widespread, defined as node-positive with distant metastasis in 6 patients. As a comparison, 39 patients who had recurrent or metastatic lesions, and had lost more than 5% of their body weight within 3 months or had hypoalbuminemia of less than 3.0 g/dl, were entered as cachectic patients (cachectic group). As a comparative control, 15 healthy volunteers were also studied.

Methods

Sera from peripheral veins were frozen and stored at −20°C until use. The serum concentrations of soluble tumor necrosis factor receptor I (sTNF-RI) were measured by enzyme immunoassay (EIA) R&D Systems, Minneapolis, MN, USA) and those of soluble interleukin-2 receptor (sIL-2R) by EIA (T Cell Diagnostics, Cambridge, MA, USA). As markers for the nutritional status of each patient, prealbumin, transferrin, and retinol binding protein (RBP) were measured by nephelometry, and as a marker for the status of immune activity of each patient, immunosuppressive acidic protein (IAP), blood cell count, and the percentages of CD3(+), CD4(+), CD8(+), CD19(+), and CD57(+) cells were measured by flow cytometry. The serum concentrations of sTNF-RI in these patient groups were compared, and their correlations with other parameters were studied.

Statistics

Data are presented as means ± SD. Parametric results were analyzed by Student's *t*-test. The strength and significance of correlations were assessed by Spearman's rank correlation coefficient. Values of less than 0.05 were considered statistically significant.

Results

The serum concentrations of sTNF-RI were 703.3 ± 233.8 pg/ml in the control group, 712.2 ± 271.6 pg/ml in the localized group, 921.3 ± 284.7 pg/ml in the intermediate group, 893.3 ± 177.3 pg/ml in the widespread group, and 1364.8 ± 368.2 pg/ml in the cachectic group, as shown in Fig. 1. The concentrations in the cachexic group were significantly higher than those in the control ($P < 0.01$), localized ($P < 0.01$), and intermediate groups ($P < 0.01$), while those in the intermediate group were higher than those in the control ($P < 0.01$) or localized groups ($P < 0.05$).

The correlations of the concentration of sTNF-RI with nutritional and immunologic parameters were also eveluated. The levels of sTNF-RI were inversely correlated with the nutritional parameters including prealbumin ($r = -0.390$, $P < 0.01$) as shown in Fig. 2, transferrin ($r = -0.383$, $P < 0.01$) as shown in Fig. 3, and retinol binding protein ($r = -0.371$, $P < 0.05$) as shown in Fig. 4. The immunologic parameters that were positively correlated to the concentrations of sTNF-RI were IAP ($r = 0.402$, $P < 0.01$) as shown in Fig. 5, and sIL-2R ($r = 0.709$, $P < 0.01$) as shown in Fig. 6, whereas the percentages of CD3(+) cells were inversely correlated ($r = -0.315$, $P < 0.05$) as shown in Fig. 7. The concentration of sTNF-RI was not correlated with any other parameter tested in this study.

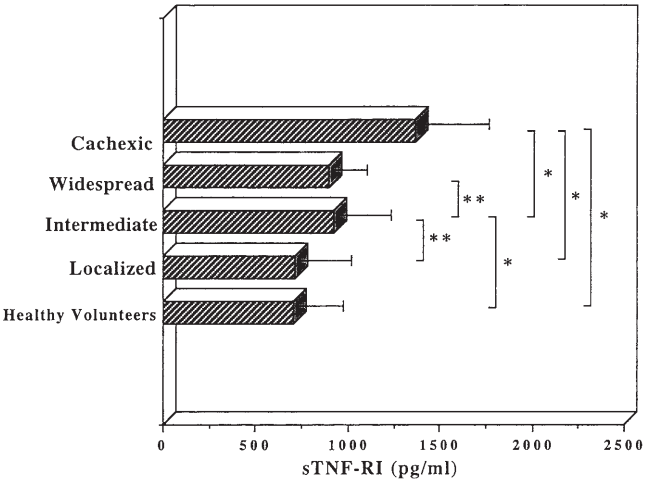

Fig. 1. Serum concentrations of soluble tumor necrosis factor receptor I (*sTNF-RI*) by enzyme immunoassay. The levels increased with the advance of disease, being highest in the cachectic group. *$P < 0.05$; **$P < 0.01$

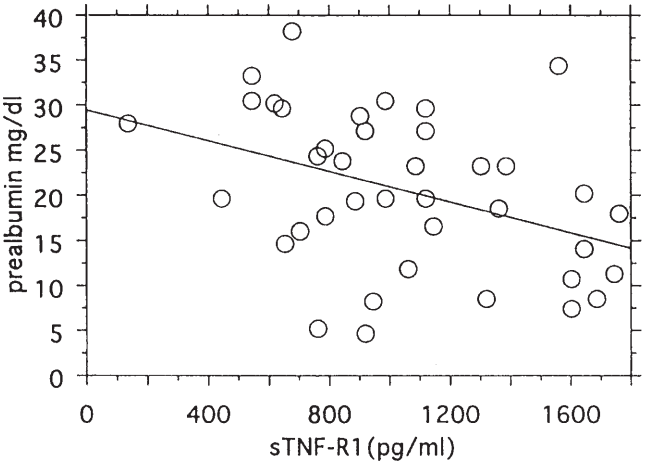

Fig. 2. Correlation between the serum concentrations of sTNF-RI and prealbumin. A reversed correlation was seen ($r = -0.390$, $P < 0.05$)

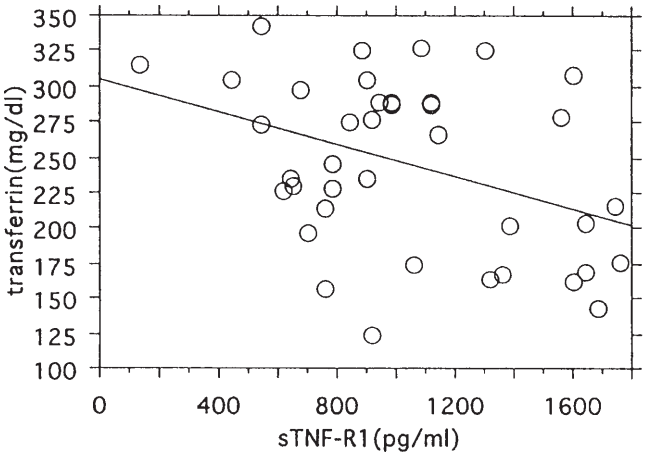

Fig. 3. Correlation between the serum concentrations of sTNF-RI and transferrin. A reversed correlation was seen ($r = -0.383$, $P < 0.05$)

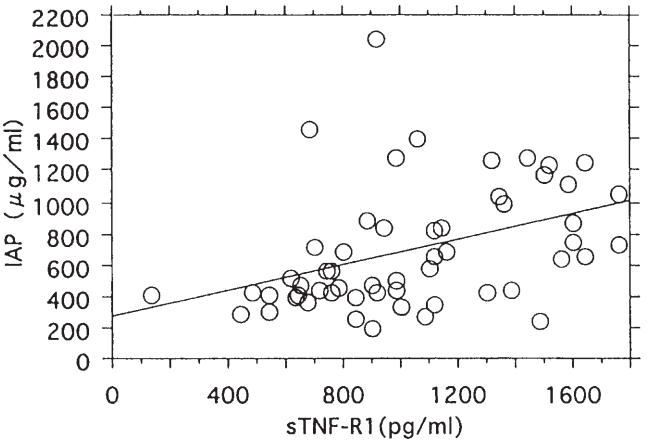

Fig. 5. Correlation between serum concentrations of sTNF-RI and immunosuppressive acidic protein (*IAP*). A significant correlation was seen ($r = 0.402$, $P < 0.01$)

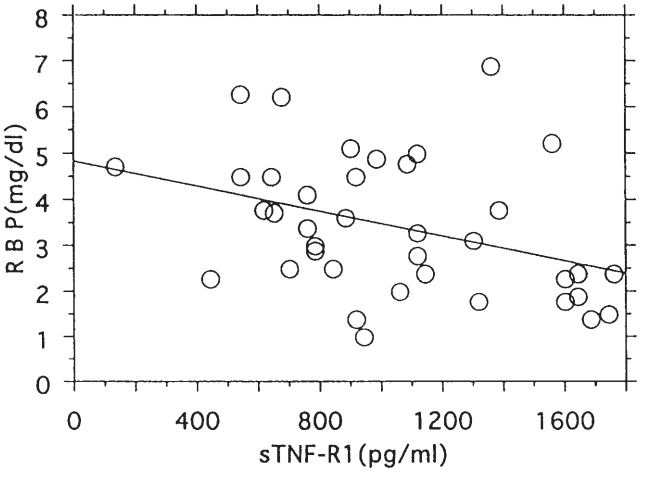

Fig. 4. Correlation between the serum concentrations of sTNF-RI and retinol binding protein (*RBP*). A reversed correlation was seen ($r = -0.371$, $P < 0.05$)

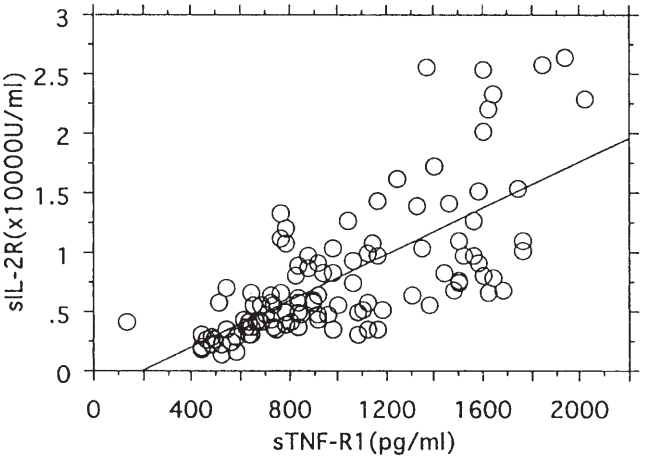

Fig. 6. Correlation between the serum concentrations of sTNF-RI and soluble interleukin 2 receptor (*sIL-2R*). A significant correlation was seen ($r = 0.709$, $P < 0.0001$)

Discussion

It is well known that neoplastic disease has a profound influence on immunological function. A growing tumor burden has been associated with decreasing immunocompetence manifesting the development of lymphocytopenia and disturbances in the network of cells for immune regulation. To understand the mechanisms of these phenomena, several circulating factors including the soluble forms of receptors for cytokines such as sTNF-RI and sIL-2R have been studied.[21–23]

In the present study, we observed that the levels of sTNF-RI in the patients with gastric and colorectal cancers increased significantly, correlating with the advance of disease. We also found that the serum concentrations of sTNF-RI correlated inversely with nutritional parameters such as prealbumin, transferrin, and RBP, and with the percentages of CD3(+) cells, whereas it correlated positively with sIL-2R and IAP.

sIL-2R has been reported to be increased in the serum of patients with hematological disorders, autoimmune diseases, viral infections, and other diseases, and is recognized as a good marker for the activation of T lymphocytes.[24,25] It is believed that sTNF-RI is produced by activated macrophages, and in this study it showed a significant correlation with the levels of sIL-2R. Thus, there seems to be an activated condition of cell-mediated immunity in cachectic patients, in accordance

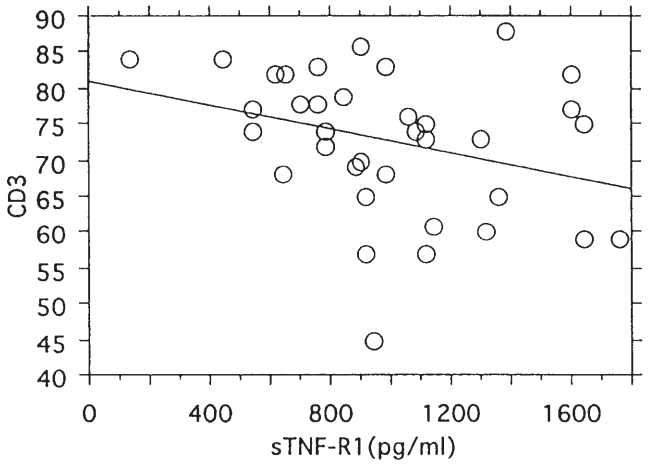

Fig. 7. Correlation between the serum concentrations of sTNF-RI and the percentages of CD3(+) cells in peripheral blood lymphocytes. A reversed correlation was seen ($r = -0.315$, $P < 0.05$)

with the findings of Denz et al.[26] TNF has been shown to be involved in the development of cachexia and possibly activates the macophages in these patients. However, sTNF-RI may block this TNF action.[27–31] The possible existence of a feedback mechanism could explain these phenomena.

Of the cytokines reported to be involved in cachexia, TNF upregulates the expression of TNF receptors and the release of their soluble forms, while interferon-γ is known to activate macrophages.[9,28] These two cytokines are the strongest candidates for activating macrophages in cachectic patients. They have also been reported to exert similar effects on nutrition, as demonstrated in the present study as well as in other recent studies on cachexia.[32–34] Although various cytokines are believed to be involved in the development of cachexia in cancer-bearing hosts, this has not yet been fully clarified. To improve the quality and duration of life of patients with advanced cancer, further investigations must be conducted to promote a better understanding of the mechanisms of cachexia and establish a strategy against it.

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