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Prognostic value of CD57+ T lymphocytes in the peripheral blood of patients with advanced gastric cancer

Abstract

Background. Natural killer (NK)-like T cells comprising CD56+ T cells and CD57+ T cells belong to a subset of CD1d-independent NKT cells playing an important role in regulating immune responses. Although NK-like T cells are reported to increase in patients with advanced gastric carcinomas, it remains unknown how NK-like T cells are involved in disease progression in gastric cancer patients.

Methods. The proportions of Th1 cells (interferon [IFN]-γ-producing CD4+ T cells), Th2 cells (IL-4-producing CD4+ T cells), and NK-like T cells (CD56+ T cells and CD57+ T cells) in the peripheral blood of 48 gastric cancer patients and 20 healthy controls were measured by two-color flow cytometry analysis and by intracellular cytokine analysis to investigate an association of these immune cells with the survival rate of gastric cancer patients.

Results. Univariate analysis showed that Th1 cells and CD57+ T cells, as well as some clinicopathological factors, significantly influenced the survival rate. CD57-high (≥18%) patients survived for a significantly shorter period after surgery compared to CD57-low patients (P = 0.046; Kaplan-Meier, log-rank test) in the stage III–IV patients, but not in the stage I–II patients. Further, multivariate analysis showed that lymphatic invasion was a statistically significant independent risk factor in all the gastric cancer patients, but the proportion of CD57+ T cells as well as depth of tumor were statistically significant independent risk factors in patients with advanced carcinomas (stage III–IV).

Conclusion. An increased proportion (≥18%) of CD57+ T cells in the peripheral blood of patients with advanced gastric carcinomas could indicate a poor prognosis.

Introduction

Natural killer (NK)-like T cells comprising CD56+ T cells and CD57+ T cells possess characteristics of extrathymic T cells and have immunoregulatory functions similar to mouse NKT cells. Although some earlier studies suggested NK-like T cells to be a counterpart of mouse NKT cells, these cells lacked expression of an invariant T cell receptor (TCR) repertoire, which is an important characteristic of mouse NKT cells. However, more recent studies identified two major subsets of NKT cells. One subset (CD1d-dependent NKT cells) recognizes α-GalCer through CD1d molecules and expresses an invariant Vα24 TCR repertoire, like mouse NKT cells. The other subset (CD1d-independent NKT cells) is activated independently of CD1d and expresses a nonbiased TCRαβ repertoire. Furthermore, some reports have demonstrated that human CD56+ T cells displaying CD1d-independent cytotoxic activities against tumor peptide antigens (MUC-1 and HER-2/NEU) were equivalent to CD1d-independent NKT cells, and suggested that NK-like T cells comprising CD56+ T cells and CD57+ T cells belong to a subset of CD1d-independent NKT cells. However, CD57+ T cells and CD56+ T cells are functionally and ontogenically different populations. Because the CD56 molecule (neural cell adhesion molecule) correlates with cytotoxic T lymphocyte (CTL) effector function, CD56+ T cells have been reported to play the role of cytotoxic T lymphocytes. On the other hand, CD57+ T cells abound in the bone marrow and other organ transplantation, and in patients with acquired immunodeficiency. CD57+ T cells have been shown to mediate immunoregulatory functions rather than CTL effector functions. CD56+ T cells and CD57+ T cells are increased in the peripheral blood and/or among tumor infiltrating lymphocytes (TIL) in patients with gastric and colorectal
carcinomas. However, it remains unknown whether CD56+ T cells and CD57+ T cells are involved in disease progression or affect the prognosis of cancer patients.

The balance of Th1 and Th2 cells, which is regulated by NKT cells, has been shown to be critically important in various immune responses, including the antitumor immune response. Several researchers have reported that Th1-dominant immunity is essential for the induction of strong in vivo antitumor immunity against a variety of tumors. However, Th1-immunity is suppressed while Th2 immunity is dominant in patients with advanced cancer, which possibly prevents the development of tumor immunity in such patients.

We conducted a flow cytometric investigation of peripheral blood lymphocytes and examined postoperative survival rates in 48 gastric cancer patients to further clarify the biological significance of the percentage of CD4+ IFN-γ-producing T cells (Th1 cells), interleukin (IL)-4-producing CD4+ T cells (Th2 cells), and NK-like T cells (CD56+ and CD57+ T cells) in patients with stage I–II (n = 23) and stage III–IV (n = 25) gastric carcinomas.

**Patients, materials, and methods**

**Patients**

Forty-eight patients with gastric cancer operated at Kumamoto University hospital and affiliated hospitals between July 2001 and March 2005 were studied. The patients (34 men and 14 women) ranged in age from 36 to 95 years (mean, 67.8 ± 11.5 years). The gastric cancer patients were classified into 23 stage I–II and 25 stage III–IV patients, according to the unified TNM classification of gastric cancer.

The stage III–IV patients were postoperatively treated with oral Tegafur-Uracil (UFT, 300 mg/m²) and the combi-

mation or affect the prognosis of cancer patients.

**Statistical methods**

The Kruskal-Wallis test was used to compare continuous variables among the three groups. Mann-Whitney’s U-test was used to compare continuous variables between two groups. Cumulative survival rates were determined using the Kaplan-Meier method and the differences between two groups were assessed using the log-rank test. Univariate and multivariate analyses of the risk ratios for the survival rate were studied using Cox proportional-hazards regression analysis. The factors examined included the proportions of CD56+ T cells and CD57+ T cells, and clinicopathological findings (stage, lymph node metastasis, depth of tumor, curability, lymphatic invasion, and vascular invasion). All P values were two-tailed, and P < 0.05 was considered to be significant. The statistical analysis was performed using StatView version 5.0 (SAS Institute, Cary, NC, USA).

**Intracellular cytokine analysis**

Flow cytometry for cytokine production was conducted according to Pölicher et al., with minor modifications. Briefly, heparinized peripheral blood was added to an equal volume of RPMI 1640 and the mixture was incubated for 4 h at 37°C in the presence of 25 μg/ml of phorbol 12-myristate 13-acetate (Sigma, St. Louis, MO, USA), 1 μg/ml of ionomycin (Sigma), and 10 μg/ml of Brefeldin A (Sigma). After two washes with phosphate-buffered saline containing 0.1% bovine serum albumin (0.1% BSA-PBS), the cells were stained for 15 min at room temperature with PerCP-conjugated monoclonal antibody (mAb) to CD4 or CD8 (Becton Dickinson). After a washing with 0.1% BSA-PBS, red blood cells were lysed, and the remaining cells fixed in FACS lysing solution (Becton Dickinson) for 5 min at room temperature. After another two washes with 0.1% BSA-PBS, the cells were incubated for 30 min at room temperature with FITC-conjugated mAb to IFN-γ (Becton Dickinson) and phycoerythrin (PE)-conjugated mAb to IL-4 (Becton Dickinson). Flow cytometry was used to determine the three fluorescent parameters of FITC, PE, and PerCP. Only CD4 T cells were gated by light scattering and CD4 reactivity. More than 5000 gated cells were analyzed both by IFN-γ and IL-4 staining. The proportion of Th1 and Th2 cells was expressed as a percentage of the total number of CD4-positive T cells.

**Results**

Comparison of the proportions of Th1 cells, Th2 cells, the Th2/Th1 ratio, and NKT-like T cells (CD57+ T cells and CD56+ T cells) in the stage III–IV patients with those in the stage I–II patients.

Several reports have shown that Th2 cells (IL-4-producing CD4+ T cells) are predominant or that Th1 cells...