CD3 ζ Expression of Regional Lymph Node and Peripheral Blood Lymphocytes in Gastric Cancer

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Abstract. Background and Aim: Impaired expression of the CD3 ζ chain in T cells associated with T cell anergy has been reported in cancer patients. However, few studies have investigated CD3 ζ expression in regional lymph node lymphocytes (LNL) in cancer patients. This study aims to confirm CD3 ζ expression levels by lymph node and peripheral blood lymphocytes (PBL) in gastric cancer patients and to discuss the clinical implications of intranodal or peripheral blood expression of CD3 ζ in gastric cancer. Patients and Methods: Twenty-two gastric cancer patients were enrolled. Macroscopically non-metastatic compartment 1 LNL (C1LNL), compartment 2 LNL (C2LNL) and PBL were surgically obtained. Two-color flow cytometry was then used to quantify the levels of CD3 ζ expression in C1LNL, C2LNL and PBL. Results: Impaired CD3 ζ expression was confirmed in 9.5% of C1LNL, 8.9% of C2LNL and 4.2% of PBL. There was a significant difference in CD3 ζ expression levels between C1LNL and PBL (p<0.01). CD3 ζ expression in PBL was significantly correlated with depth of invasion but not nodal involvement. Distinct differences between the respective lymph node compartments were not identified. Conclusion: Immunological paralysis following CD3 ζ impairment may occur more frequently in LNL than in PBL in gastric cancer. Identifying such patients during the perioperative period using flow cytometric methods will increase the efficiency of cytokine therapy aiming to normalize CD3 ζ expression levels.

Cytotoxic T cells are known to be one of the major effector cells in tumor immunity following detection of tumor-specific antigen and T cell clone response to autologous tumor cells in vitro (1).

Mizoguchi et al. (2) demonstrated that tumor-bearing mice have an abnormally structured T cell receptor-CD3 complex that lacks the CD3 ζ chain. This phenomenon is observed in various types of cancer and is regarded as a causative factor in immune suppressive conditions in cancer patients (3, 4). CD3 ζ impairment has been detected not only in peripheral blood lymphocytes (PBL), but also in tumor-infiltrating lymphocytes (TIL) in cervical (5), head and neck (6), colorectal (7) and oral cancer (8).

Regional lymph nodes (RLN) are regarded as both mechanical barriers for solid cancers and as sites where local immunocompetent cells are mobilized against tumor cells (9). The RLN are subject to different lymphatic flow circumstances from the respective tumor locations. We previously showed that the degree of infiltration of antitumor effector cells in the lymph nodes was significantly different depending on the lymph node compartments (10). Intranodal lymphocytes in each compartment are affected by the primary tumor and it is speculated that CD3 ζ expression levels may vary in each compartment. In the current study, we attempted to estimate the CD3 ζ expression levels in the respective lymph node compartments and in peripheral blood lymphocytes using flow cytometric analysis (FACS) and to evaluate the clinical implications of CD3 ζ expression in gastric cancer.

Patients and Methods

Patients. A total of 22 gastric cancer patients who underwent gastrectomy with lymph node dissection at the Kagoshima University Hospital, Japan, between 1999 and 2001, were enrolled. The patient age ranged from 45 to 77 years (median: 65); 15 patients were male and 7 were female. Sixteen patients underwent total gastrectomy and the other 6 underwent distal partial gastrectomy. All patients were subjected to D2 lymph node dissection. Of 22 patients, 16 underwent curative resection and 6 underwent non-curative resection (Table I). No patients received chemotherapy preoperatively. Classification of compartment 1 lymph node (C1LN) and compartment 2 lymph node (C2LN) was conducted based on the General Rules of Gastric Cancer by the Japanese Society of Gastric Cancer (11).

Flow cytometric analysis of CD3 ζ expression in regional lymph node and peripheral blood lymphocytes. As representative of C1LN, a single non-metastatic lymph node along the lesser curvature was removed. As representative of C2LN, a single lymph node along the left gastric
artery was extirpated (Figure 1). After removal of fat tissue, the lymph nodes were washed in phosphate-buffered saline (PBS), minced to small pieces and filtered. Enzymatic digestion was not conducted. Extirpated node tissue was diluted with RPMI1640 with 10% FBS. From the minced lymphoid tissue, a minimum of 10^7 lymph node lymphocytes were obtained. Simultaneously, 20 cc of peripheral blood was obtained during surgery. PBL were separated by Ficoll and washed twice in PBS.

In order to measure CD3⁺ expression levels, two-color flow cytometric analysis using anti-CD3⁺- and anti-CD3⁻-antibodies was performed. Briefly, the cells were washed with PBS and fixed with 0.25% paraformaldehyde for 10 min. The cells were then permeabilized for 20 min in 100 μl of saponin solution. A suspension of 10⁶ cells was obtained, washed with PBS and then treated with 3 μl of primary anti-CD3⁺-antibody (Immunotech, USA). The cells were washed twice with PBS and incubated for another 20 min with 5 μl of a 1:100 diluted fluorescent-labeled secondary antibody (DAKO). The cells were then washed and immediately analyzed on a flow cytometer (Epics XL CORTER). The population showing impaired CD3⁺ expression was evaluated as the percentage of CD3⁻⁺ CD3⁺⁻ cells.

Statistical analysis. Significant differences in categorical variables were determined using the paired t-test. All p values were based on two-sided testing and values less than 0.05 were considered significant.

Results

Using two-color FACS, lymphocytes were separated into 4 categories based on CD3⁺ and CD3⁻ expression (Table II). The percentage of CD3⁺⁺ CD3⁻⁻ lymphocytes ranged from 2.2% to 16% (average: 4.2%) in PBL, 5.5% to 23% (average: 9.5%) in C1LNL and 3.5% to 22% (average: 8.9%) in C2LNL.

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<th>Table II. CD3⁺, CD3⁻ expression in PBL, C1LNL and C2LNL.</th>
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<td>PBL(%)</td>
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*p<0.05

The population of CD3⁺⁺ CD3⁻⁻ lymphocytes among C1LNL and C2LNL was significantly higher than that of PBL (p<0.05) (Figure 2). CD3⁻⁻ expression in C1LNL tended to be higher than that in C2LNL, but no significant differences were observed. Node-positive or advanced gastric cancer patients tended to have more CD3⁻⁻ impaired lymphocytes among PBL, C1LNL and C2LNL, but no statistically significant differences were observed (Figure 3 and Figure 4). According to the nodal involvement, node-negative gastric cancer patients had fewer CD3⁻⁻ impaired cells in PBL than node-positive gastric cancer patients (p<0.05) (Figure 4).

Discussion

In the present study, 4.2% (from 0.2% to 17%) of PBL were lacking CD3⁺. However, we were unable to examine CD3⁺ expression in a healthy donor sample. Kim (12) showed that CD3⁺ expression in PBL was preserved in almost all healthy donors, whereas tumor-bearing hosts had significantly higher levels of CD3⁺ impairment. Our study showed a significant difference in CD3⁺ expression in PBL between node-negative and node-positive gastric cancer. This observation is similar to the results in other studies (13) and may suggest that CD3⁺ expression in PBL depends on tumor progression. We previously reported that CD3⁺ expression in TIL was an independent prognostic marker for gastric cancer (14) and, thus, CD3⁺ expression of PBL may also be affected not by only tumor progression, but also tumor aggressiveness. Few studies have reported data on CD3⁻⁻ expression in LNL. Our data showed that nodal involvement status did not affect CD3⁻⁻ expression in LNL, which agreed with previous studies (5). However, patients' samples may not be enough to evaluate the relationship between nodal involvement and CD3⁻⁻ expression.

We initially examined CD3⁻⁻ expression by LNL in each lymph node compartment. Anatomically C1LN is closer to the primary tumor than C2LN. Because of a high occurrence of lymph node metastasis, C1LN is completely dissected in standard gastric cancer surgery. We speculated that C1LN might be more directly affected by the primary tumor than C2LN is. However, there was no significant difference in CD3⁻⁻ expression in C1LNL compared to C2LNL.

The population of CD3⁺⁺ CD3⁻⁻ lymphocytes among C1LNL and C2LNL was significantly higher than that of PBL (p<0.05) (Figure 2). CD3⁻⁻ expression in C1LNL tended to be higher than that in C2LNL, but no significant differences were observed. Node-positive or advanced gastric cancer patients tended to have more CD3⁻⁻ impaired lymphocytes among PBL, C1LNL and C2LNL, but no statistically significant differences were observed (Figure 3 and Figure 4). According to the nodal involvement, node-negative gastric cancer patients had fewer CD3⁻⁻ impaired cells in PBL than node-positive gastric cancer patients (p<0.05) (Figure 4).
CD3 expression between C1LNL and C2LNL. Because our study included only advanced gastric cancer patients, further study is required in order to clarify the differences between the two lymph node compartments in early-stage cancer.

Gruijl's immunohistochemical study of cervical carcinoma documented that CD3ζ impairment in TIL was more frequent than in nodal lymphocytes (5). In the current study, we showed significant differences between LNL and PBL. From these findings, such lymphocytes in close proximity to the primary tumor may result in an impaired cell-mediated anti-tumor response. Detecting the CD3ζ status of lymph node or peripheral blood lymphocytes may be a good immunological marker of T cell anergy. On the other hand, CD3ζ expression of regional LNL could reflect the local immunological anergy for cancer cells as well as for TIL. It is also noteworthy that vaccine (15) or cytokine addition (16) recovered CD3ζ expression in cancer patients.

In conclusion, we identified a significantly higher level of CD3ζ impairment in LNL than was observed in PBL. In patients with severe CD3ζ impairment in PBL, it may be necessary to normalize the CD3ζ condition to prevent tumor invasion.

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References


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