Intranodal Antitumor Immunocyte Infiltration in Node-negative Gastric Cancers

Sumiya Ishigami,1 Shoji Natsugoe, Shuichi Hokita, Che Xiangming, Kuniaki Aridome, Hiroyuki Iwashige, Koki Tokuda, Akihiro Nakajo, Futoshi Miyazono, and Takashi Aikou
First Department of Surgery, Kagoshima University School of Medicine, Kagoshima 890-8520, Japan

Abstract
The status and role of immunocytes and dendritic cells in regional lymph nodes in patients with gastric cancer are examined in this study. Forty-nine patients with gastric cancer who underwent curative resection were enrolled in the present study. These patients had no lymph node metastases according to a histological examination. The infiltration of natural killer (NK) cells, dendritic cells, and MIB-1-positive immunocytes was investigated. Based on the Japanese Classification of Gastric Carcinoma, regional lymph nodes were divided into three compartments: (a) compartment 1 (lymph node station numbers 1–6); (b) compartment 2 (lymph node station numbers 7–12); and (c) compartment 3 (lymph node station numbers 14 and 16). Dendritic cells and MIB-1-positive immunocytes infiltrated compartment 1 lymph nodes in increased numbers compared with the lymph nodes of compartments 2 or 3 (P < 0.05). Conversely, intranodal NK cell infiltration did not differ significantly among the three compartments. The incidence of intranodal dendritic and MIB-1-positive cell infiltration in patients with submucosal gastric cancer was significantly higher than in patients with tumors that invaded beyond the muscularis propria. The decreased expression of these immunological markers correlated well with recurrent disease, regardless of tumor depth. The immunocyte level is higher in lymph nodes near the primary tumor (compartment 1) than in those that are distant from the tumor (compartments 2 and 3). This pertains to all three markers, i.e., NK, dendritic, and MIB-1-positive cells. Unlike dendritic and MIB-1-positive cells, intratumoral infiltration of NK cells did not correlate well with either lymph node compartment or the depth of tumor invasion. The degree of NK cell infiltration may be directly associated with antitumor effects, especially in compartment 1. A decrease in all three markers is associated with tumor recurrence.

Introduction
Regional lymph nodes play an important role in the immunodefense against cancer cells and are considered the first line of defense against metastasis by the lymphatic route, which is the principle route of metastasis (1, 2). Because regional lymph nodes are frequent sites of metastasis in gastric carcinoma, surgical removal can control distant spread of tumor. Although the question of whether or not lymph node resection reduces the efficacy of the regional immune system cannot be answered at present, the prophylactic removal of lymph nodes that are not affected by metastasis probably affects the immune defenses (3, 4).

The advent of immunological exploration of immunocytes has revealed detailed information about the properties of tumor-infiltrating immunocytes (5–7). Therefore, the relationship between tumor-infiltrating immunocytes and clinicopathological features has been studied. Dendritic cells serve as accessory cells that present antigens to sensitized T cells (5). Because these cells present antigens and stimulate CTLs (8–10), the density of dendritic cells is associated with the local immunity of CTLs (11, 12). NK2 cells are also antitumor effectors that act without recognizing the HLA antigen. NK cells attack tumor cells in a manner different from that of CTLs (13–15). MIB-1 is an antigen that recognizes intracellular substances in proliferating cells and is used to determine the degrees of immunocyte activation and proliferation, especially those of lymphoma cells
MIB-1-positive immunocytes are considered to be stimulated immunocytes after receiving antigenic information. The intranodal infiltration of immunological effectors in gastrointestinal cancer has been demonstrated (18). Here we investigate local intratumoral immunocyte status in regional lymph nodes using intranodal NK cell and dendritic cell infiltration as well as the MIB-1-positive cell LI as markers in patients with node-negative gastric cancer. Furthermore, we examined the relationship between the expression of these immunological effector cells and clinicopathological findings.

Patients and Methods

Patients. A total of 49 patients with node-negative gastric cancer who underwent curative resection with lymphadenectomy in the First Department of Surgery, Kagoshima University Hospital between 1988 and 1998 participated in the current study. The age range of the 33 male and 16 female patients was from 35–88 years (mean age, 48 years). Distal gastrectomy was performed in 26 patients, total gastrectomy was performed in 17 patients, and proximal gastrectomy was performed in 6 patients. All of the patients received no preoperative chemotherapy, underwent curative resection, and had no lymph node metastasis by histological examination. Eight (14%) patients died of recurrent gastric cancer [six with hepatic metastases, one with peritoneal metastases, one with lymph node metastases, and one with cachexia (Table 1)].

Microscopic Examination. The number of extirpated lymph nodes per patient ranged from 10–75, with a median value of 32. Resected lymph nodes were divided into three lymph node compartments according to the Japanese Classification of Gastric Carcinoma (Ref. 19; Fig. 1). A total of 1220 lymph nodes were examined as follows: (a) 156 of lymph node station number 1; (b) 358 of lymph node station number 3; (c) 178 of lymph node station number 4d; (d) 32 of lymph node station number 6; (e) 150 of lymph node station number 7; (f) 106 of lymph node station number 8a; (g) 73 of lymph node station number 9; (h) 49 of lymph node station number 10; (i) 28 of lymph node station number 11; (j) 34 of lymph node station number 12; (k) 13 of lymph node station number 14; and (l) 43 of lymph node station number 16. Lymph node sections were fixed in 10% formalin and embedded in paraffin. Routinely processed and H&E-stained sections were evaluated. Approximately 2000 lymphocytes per sample and field were examined under high-power (×400) magnification.

Dendritic and NK cell infiltration was examined by immunohistochemical staining. Dendritic cells were detected using S-100 protein (DAKO, Denmark) at a dilution of 1:200 (20), and...
Fig. 2  

a, CD57-positive lymphocytes are identified (×200).  
b, diffuse dendritic cell infiltrate is present (×200).  
c, sporadic MIB-1-positive lymphocytes are present (×200).
CD57 (Immunotech, France) was used for detection of NK cells at a dilution of 1:50 (21).

Ki67-positive immunocytes were evaluated by using MIB-1 antibody (Immunotech France; Ref. 22). The antibody was diluted at 1:500 and reacted overnight, and then primary antibodies were visualized using a streptavidin-biotin-peroxidase supersensitive kit (avidin-biotin complex method). A normal prostate gland and nerve tissue were used as positive controls for CD57 and S-100, respectively. The immunohistochemical expression of NK cells, dendritic cells, and MIB-1-positive immunocytes was evaluated by two independent observers (S. I. and S. N.). Ten representative fields were examined, and a total of 2000 lymphocytes (200 lymphocytes/field) were counted under a microscope with using high-power (×400) magnification (20, 21). When evaluating immunocytic infiltration, dendritic cells and MIB-1-positive pericortical and intramedulary immunocytes were excluded because most of these immunocytes were related to B lymphocytes. We also examined the correlation between the degree of infiltration of the three immunocytes and the clinicopathological findings, retrospectively.

**Statistical Evaluation.** Clinicopathological factors were examined using the χ² and t test. A P of less than 0.05 was considered statistically significant.

**Results**

NK cells were distributed homogeneously throughout the lymph nodes, but dendritic cells and MIB-1-positive immunocytes were present mainly in the cortex and paracortical areas (Fig. 2, a–c). The incidence of NK cells, dendritic cells, and MIB-1-positive immunocytes in each compartment is shown in Figs. 3, 4, and 5. Although dendritic cells and MIB-1-positive cells were often found in the germinal center of the primary lymph follicles, they were not counted because they contained mainly activated B lymphocytes. The cell density varied from 1.2–34% (average, 24.0%) for dendritic cells, from 0–4.3% (average, 0.7%) for NK cells, and from 0–32% (average, 4.1%) for MIB-1-positive cells. The mean density of NK cell infiltration in compartments 1, 2, and 3 was 0.5%, 0.4%, and 0.4%, respectively. No significant differences among the three compartments were evident. However, the density of dendritic cell infiltration in compartment 1 was 42%, which was significantly higher than the infiltration in compartments 2 and 3 (P < 0.05). In addition, the percentage of MIB-1-positive cells (6.1%) in compartment 1 was significantly higher than that in compartments 2 (3.3%) and 3 (0.5%; P < 0.01).

The intranodal NK, dendritic, and MIB-1-positive cell values in patients with submucosal cancer were 0.3%, 50%, and 11%, respectively. On the other hand, in patients with tumor invasion beyond the muscularis propria, the values were 0.4%, 28%, and 2.7%, respectively. Thus, dendritic and MIB-1-positive cells differed significantly between submucosal cancer and tumors reaching beyond the muscularis propria (P < 0.05; Table 2).

The eight patients who died of recurrent gastric cancer had infiltration values of 0.07%, 24%, and 3.6% for NK, dendritic, and MIB-1-positive cells in compartment 1. The level of ex-
pression of these immunological markers in patients with recurrent disease was significantly lower than that in patients showing no recurrence (Table 3). When the gastric tumor was confined to the T2 depth of invasion, the amount of dendritic cell and NK cell infiltration differed significantly between patients with and without recurrence (Table 4).

**Discussion**

Clinical and experimental data have indicated that the immunological balance might be disturbed by lymphadenectomy because immunocytes in regional lymph nodes actually function as antitumor effector cells. Some authors have even...
Table 2: NK cell, dendritic cell, and MIB-1-positive lymphocyte infiltration according to the depth of invasion.

<table>
<thead>
<tr>
<th>Depth of Tumor Invasion</th>
<th>Depth of Invasion</th>
<th>sm (n = 16)</th>
<th>mp/ss (n = 33)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK Cell Infiltration</td>
<td></td>
<td>0.3 ± 0.3</td>
<td>0.4 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>Dendritic Cell Infiltration</td>
<td></td>
<td>50 ± 27</td>
<td>28 ± 16</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MIB-1 LI (%)</td>
<td></td>
<td>11 ± 3.1</td>
<td>2.7 ± 3.8</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*sm, submucosa; mp, muscularis propria; ss, subserosa.

*NS, not significant.

Table 3: NK cell, dendritic cell, and MIB-1-positive lymphocyte infiltration in patients with or without recurrence.

<table>
<thead>
<tr>
<th>Patients without recurrence</th>
<th>Patients with recurrence</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK Cell Infiltration</td>
<td></td>
<td>0.3 ± 0.4</td>
</tr>
<tr>
<td>Dendritic Cell Infiltration</td>
<td></td>
<td>46 ± 26</td>
</tr>
<tr>
<td>MIB-1 LI (%)</td>
<td></td>
<td>6.8 ± 5.8</td>
</tr>
</tbody>
</table>

reported that intratumoral infiltration of immunocytes reflects an immunological reaction to cancer (23, 24). However, the relationship between the antitumor effect of the regional lymph nodes against primary tumors and the role that lymphadenectomy plays has not been fully defined. In the present study, we attempted to examine the effect of intranodal infiltration of lymphocytes using three distinctive immunological cellular markers in patients with node-negative gastric cancer.

The presence and clinical significance of lymph node micrometastasis have been elucidated by immunohistochemical and genetic methods (25, 26). Some of our patients probably harbored such metastases in these series. We attempted to study whether or not lymph node micrometastasis can be checked by immunocytes. We report that perigastric nodes contained more infiltrating dendritic and MIB-1-positive cells in compartment 1 than in compartments 2 and 3. This finding is in agreement with the notion that regional lymph nodes serve as a reservoir of dendritic cells that connect the primary tumor to distant lymph nodes (18). Moreover, these findings suggest that intragastric stimulation against tumors is reflected in the lymph nodes of compartment 1 because such stimulation may become weaker as the distance from the primary tumor increases. Similarly, NK, dendritic, and MIB-1-positive cells in each compartment showed similar tendencies.

Unlike dendritic cells and MIB-1-positive cells, intranodal infiltration of NK cells did not correlate well with either lymph node compartment or the depth of tumor invasion. Because the decrease in NK cells was related to tumor recurrence, the degree of NK cell infiltration may be directly associated with antitumor effects, regardless of whether or not there is antigen stimulation. Lymph nodes with intact of compartment 1 possess the strongest antitumor activity. Therefore, in patients with early-stage gastric cancer, the lymph nodes should be preserved.

The incidence of recurrent disease, irrespective of tumor depth, was low in patients with increased dendritic and NK cell infiltration. Moreover, hematogeneous metastasis was the most frequent mode in recurring gastric cancer.

We have attempted to demonstrate the behavior of regional lymph nodes through NK, dendritic, and MIB-1-positive cell markers. Specifically, a decrease in these three markers was associated with tumor recurrence in patients with node-negative gastric cancer. These three markers may also exhibit antitumor effectors in regional lymph nodes.

### References


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