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ARTICLE

General Features of Gastric Carcinomas and Comparison of HSP70 and NK cell Immunoreactivity with Prognostic Factors

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During the period of 1996-1998 ninety-four gastrectomy specimens with gastric carcinoma referred to Erciyes University, Medical Faculty, Department of Pathology, were examined histopathologically, histochemically and immunohistochemically. General characteristics of gastric carcinomas and prognostic factors were studied. According the Lauren classification, of the 94 cases of gastric carcinomas, 56 were intestinal type, 21 were diffuse type and 17 were mixed type carcinoma. The association rates of Helicobacter pylori, chronic atrophic gastritis and intestinal metaplasia with gastric carcinomas were high. There was strong immunorectivity with HSP70 in 62,5% of the intestinal type carcinomas. This ratios were lower in diffuse and mixed type carcinomas ($p<0.05$). The more tumor size and invasion depth increased, the more HSP70 immunoreactivity was obtained ($p<0.05$). HSP70 immunorectivity was considerably higher in the patients having lymph node metastasis and vascular invasion ($p<0.05$). It was found that the NK cell number was low in the tumor but higher around the tumor in early gastric carcinomas, compared with advanced carcinomas ($p>0.05$). In the tumors larger than 10 cm with vascular invasion, NK cell number was lower around the tumor ($p>0.05$). Defining prognostic factors of gastric carcinomas is of importance to clinicians. It is thought that HSP70 immunoreactivity, besides invasion depth, lymph node metastasis, vascular invasion, tumor size and inflammatory reaction against the tumor, is important in prognosis and associated with advanced stage. (Pathology Oncology Research Vol 8, No 4, 262–269)

Keywords: gastric carcinoma, HSP70, NK-cell, prognostic factors

Introduction

Although the incidence of gastric carcinoma in the United States and some European countries has been steadily decreased over the last five decades, it is still the second most common cause of cancer deaths.[1,2]

The Lauren classification that categorizes gastric carcinoma to the intestinal and diffuse types is important for epidemiological studies.[3] It has been proposed that the intestinal type is caused mainly by environmental agents whereas factors specific to the individual are more important in the diffuse type.[2,4] Although some studies have shown that the frequencies of Helicobacter pylori (H. pylori), chronic atrophic gastritis and intestinal metaplasia (IM) do not differ significantly between the two types, chronic atrophic gastritis-IM-dysplasia-carcinoma is the accepted sequence for the development of intestinal type gastric carcinomas.[3,5] In contrast, the diffuse type that occurs in relatively younger patients, is believed to arise from nonmetaplastic epithelium under the more prominent effect of genetic factors.[3,6]

Heat-shock proteins are ubiquitous molecules which play vital roles in the maintenance of viability in cells injured by noxious stimuli such as heat, ischemia, heavy metals, ethanol, toxins, oxidants, bacteria and viruses; their main function is protection from stressful stimuli.[7,8] Although data on heat-shock proteins in tumors are limit-

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ed, recent studies show that they may play a role in the antitumor immune response.[9]

Natural killer (NK) cells function as a component of innate immunity and serve as the first line of defense against neoplasms and virus-infected cells.[8] In some studies, it has been shown that NK cells that have a spontaneous cytotoxic activity against tumor cells can affect the prognosis of intestinal tumors.[10,11]

Taking these data into consideration, we retrospectively investigated the relationships between general characteristics of gastric adenocarcinomas, heat-shock proteins, natural killer cells and other prognostic factors in gastrectomy specimens.

Patients and Methods

The gastrectomy specimens of 94 patients who underwent surgery for gastric adenocarcinoma between 1996 and 1998 at the Erciyes University Medical School were evaluated. The macroscopic findings were obtained from the pathology reports. The hematoxylin-eosin-stained (HE) slides of all cases were re-evaluated. The histopathological features of the tumor, extension in the gastric wall and lymph node metastases were determined.

The tumors were classified into intestinal, diffuse and mixed types according to the Lauren classification. The mucosa adjacent to the tumor was examined for H. pylori, gastritis, IM and dysplasia. Genta staining was used for H. pylori and staining with PAS-AB (pH: 2.5) and HID-AB (pH: 2.5) for IM typing. After identification, IM was classified into type 1, type 2 and type 3.

The heat-shock protein immunoreactivity and the number of NK cells in the tumor as well as the peritumoral areas were investigated by immunohistochemistry with the avidin-biotin method. Polyclonal rabbit anti-HSP70 antibody (DAKO Corporation,CA, USA) was used at a dilution of 1:700 and anti-human natural killer cell-like antibody (subclass: IgM, kappa, DAKO Corporation, CA, USA) was used at a dilution of 1:150.

The slides were evaluated by light microscopy. In all sections containing the tumor and the adjacent mucosa, HSP 70 staining in the tumor, adjacent mucosa, and metaplastic mucosa were evaluated. Specimens were considered positive only when brown cytoplasmic staining was present at least 5% of the contained epithelial cells (either normal or malignant) unequivocally expressed HSP70. The 5% cutoff was chosen because it conforms to the international European Organization for Research and Treatment of Cancer-Gynaecological Cancer Cooperative Group recommendations. For each tissue section, staining was assessed as negative, weakly positive or only focally positive (low-level expression), or strongly positive (high-level expression) and scored as 0, 1, or 2, respectively (***Figure 1 and 2***). In all sections containing the tumor and the adjacent mucosa, the inflammatory cell infiltration in and around the tumor was examined for cells that stained positively with the anti-NK cell-like antibody. The counting was performed in 20 high power fields with the most prominent staining and the average value was calculated.

The X^2 test, unpaired t-test and one-way analysis of variance were used for statistical analysis.

Results

According to the Lauren classification, 56 patients (59.6%) had intestinal type tumors, 21 (22.3%) had diffuse type and 17 had mixed type (18.1%). Five patients (5.3%) had early gastric cancer; one was of the intestinal type (20%) and 4 of the diffuse type (80%). Mean patient age was 59.6; 57 (60.6%) patients were men and 37(39.4%) were women.

The tumor was limited to the mucosa in 2 cases (2.1%), submucosa in 3 (3.2%), muscular layer in 7 (7.4%) and subserosa in 3 (3.2%). The remaining 79 patients (84.04%) had tumors with serosal invasion; 44 of these patients (44.7%) had peritoneal carcinomatosis.

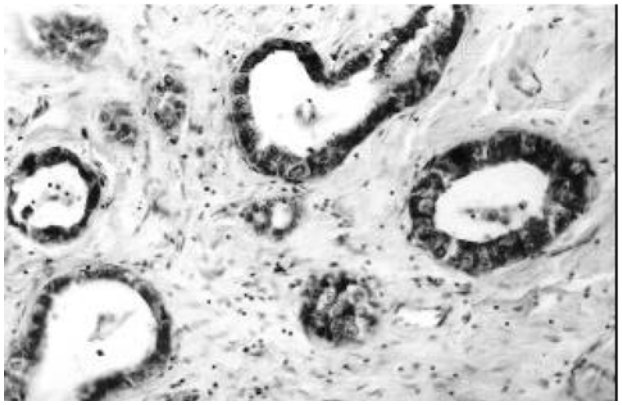

Figure 1. *Strong HSP70 immunoreactivity in the intestinal type adenocarcinoma. X200*

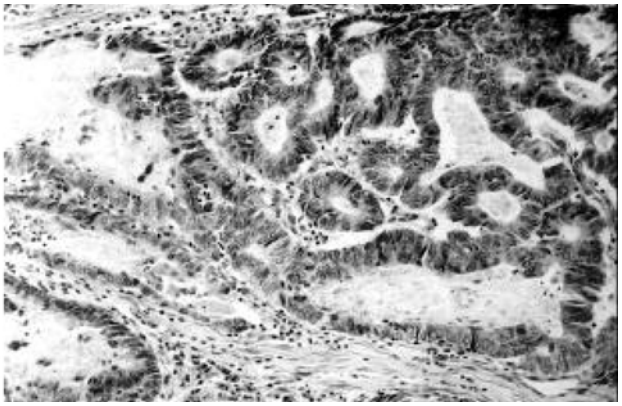

Figure 2. *Weak HSP70 immunoreactivity in the intestinal type adenocarcinoma. X200*

Table 1. **HSP70 immunostaining and clinicopathologic parameters for a series of gastric carcinomas**

	No. of cases (%)				
Parameter	*HSP70 (–)*	*HSP70 (+)*	*HSP70 (++)*	X^2	*p value*
Histopathology					
Intestinal	0 (0)	21 (37.5)	35 (62.5)		
Diffuse	6 (28.6)	12 (57.1)	3 (14.3)	26.06	p<0.05
mixed	1 (5.9)	9 (52.9)	7 (41.2)		
Tumor size					
<5 cm	4 (20.0)	7 (55.0)	9 (45.0)		
5-10 cm	1 (1.6)	31 (50.0)	30 (48.4)	9.64	p<0.05
>10 cm	2 (16.7)	4 (33.3)	6 (50.0)		
Invasion					
Mucosa+/-submucosa	3 (60.0)	1 (20.0)	1 (20.0)		
Muscularis	2 (28.6)	4 (57.1)	1 (14.3)	29.25	p<0.05
Subserosa+serosa	2 (2.5)	37 (45.1)	43 (52.4)		
Blood vessel invasion					
Negative	6 (21.4)	10 (35.7)	12 (42.9)	11.40	p<0.05
Positive	1 (1.5)	32 (48.5)	33 (50.0)		
Lymph node					
Negative	4 (23.5)	5 (29.4)	8 (47.1)	10.14	p<0.05
Positive	2 (2.7)	36 (49.4)	35 (47.9)		
H. pylori					
Negative	2 (6.3)	13 (40.6)	17 (53.1)	0.55	p>0.05
Positive	5 (8.1)	29 (46.8)	28 (45.1)		
Type of IM					
Type 2	2 (22.2)	7 (77.8)	0 (0)	6.70	p<0.05
Type 3	4 (10.2)	17 (43.6)	18 (46.2)		
Sex					
Female	4 (10.8)	13 (35.1)	20 (54.1)	2.66	p>0.05
Male	3 (5.3)	29 (50.9)	25 (43.8)		

The mean number of dissected lymph nodes per patient was 16. Seventy-three patients had lymph node metastasis. The frequency of lymph node metastasis was lower in intestinal type tumors in comparison with diffuse and mixed type tumors.

The degree of intratumoral and peritumoral inflammatory response showed high variability between patients. The degree of infiltration was higher in intestinal type tumors than in diffuse and mixed tumors.

The mucosa adjacent to the carcinoma exhibited chronic superficial gastritis in 9 cases (9.6%) and varying degrees of chronic atrophic gastritis in 85 cases (90.4%). IM was observed in 86 cases (91.5%). In all patients with IM, glands with IM were observed as scattered patches in the normal mucosa. Twenty patients had type 2 IM, 24 had type 3 IM and the remaining patients had various combinations. Type 1 IM accompanied type 2 IM or type 3 IM in 12 patients. Because solitary type 1 IM was not observed, complete IM was not taken into consideration. In cases with more than one type of incomplete metaplasia, type 3 was taken into consideration in view of its high malignant potential. IM was present in 91.5% of the cases. Type 3 was the most common IM type (p<0.05).

Genta staining showed that 65.9 % of the cases were H. pylori positive. There were no statistically significant differences in H. pylori positivity between the three groups.

In 36 cases, the mucosa adjacent to the tumor showed varying grades of dysplasia. High-grade dysplasia was more common than low-grade dysplasia. Dysplastic change (regardless of grade) was observed in 44.7% of intestinal type carcinomas, 47.1% of mixed-type carcinomas and 14.3% of diffuse-type carcinomas.

In the adjacent mucosa weak staining was observed in 64 patients (68.1%), strong staining in 20 patients (21.3%) whereas no staining was observed in 10 patients (10.6). In 48 sections positive for staining, the adjacent mucosa showed IM; among these cases, HSP70 staining was positive in varying degrees in 42 (87.5%). HSP70 staining was more intense in type 3 IM than in type 2 IM. Seven tumors

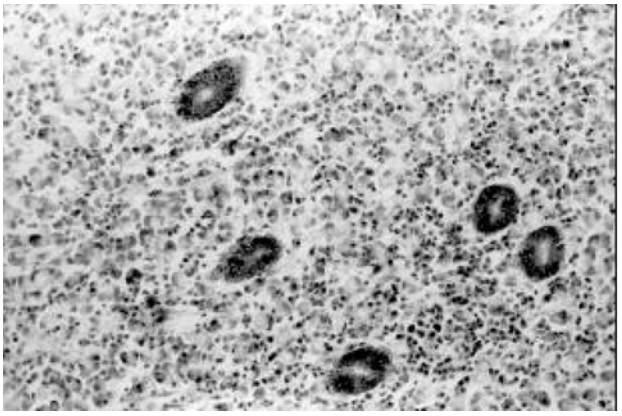

Figure 3. No staining for HSP 70 in signet ring cell carcinoma. Positive staining was observed in the normal glands. X200

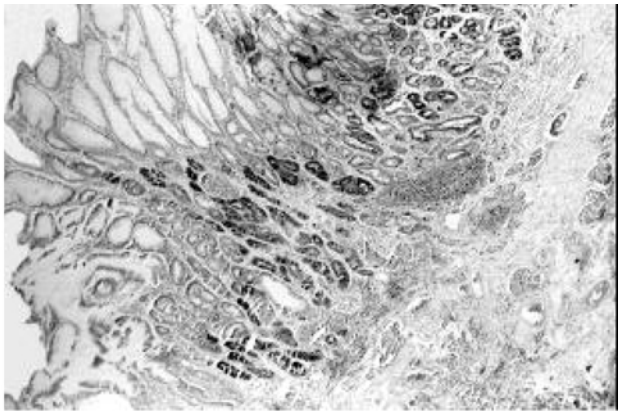

Figure 5. Diffuse staining of the crypts of the basal mucosa adjecent to the tumor with NK cell-like antibody. X40

did not stain positively for HSP70. The other cases showed varying degrees of staining. The relationships between HSP70 immunoreactivity and other features and prognostic factors were evaluated *(Table 1).*

Strong staining for HSP70 was observed in 62.5% of intestinal type carcinomas. This ratio was lower in other types of carcinoma ($p<0.05$) (*Figure 3*). HSP70 staining showed a positive correlation with tumor size ($p<0.05$), depth of invasion ($p<0.05$), vascular invasion ($p<0.05$) and lymph node involvement ($p<0.05$).

The number of NK cells in the intratumoral and peritumoral lymphocytic infiltration were determined separately (*Figure 4*). This was achieved by counting the number of cells that stained with the NK cell-like antibody in 20 high-power fields and taking the average. The NK-cell like antibody stained the basal cells in the normal mucosa and peripheral nerves to varying degrees. However, there was no staining in metaplastic epithelium. In the mucosa distant from the tumor, the epithelial cells were singly and selectively stained whereas diffuse staining was observed in the mucosa adjacent to the tumor (*Figure 5*). In 5 cases, patchy staining of the tumor cells was observed. In 8 cases, the vascular endothelium stained positively with the NK cell-like antibody. The relationships of intratumoral and peritumoral NK cells with HSP70 immunoreactivity, tumor type, age, sex, tumor size, depth of invasion, vascular invasion, lymph node involvement, IM type and presence of H. pylori were evaluated (*Table 2*).

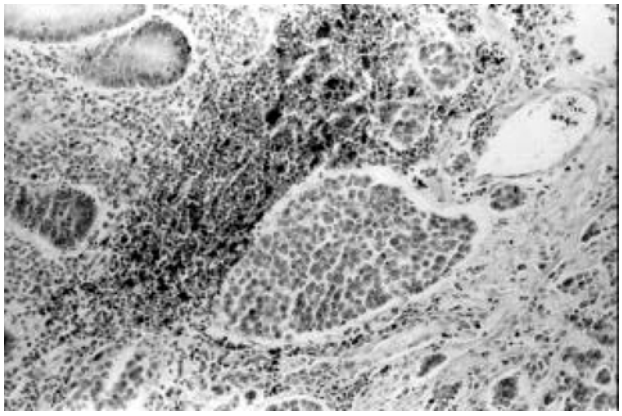

Figure 4. A high number of peritumoral NK cells were seen around area of vascular invasion. X200

Discussion

Although there has been a decrease in the incidence in United States and other countries, the prognosis of gastric carcinoma is still poor.1

The Lauren classification has been used in epidemiological and morphological studies.[12] Although the actual percentages vary, the intestinal type is the most common type.[1,4] In our study, 94 tumors were classified according the Lauren classification: the intestinal type was most common, followed by the diffuse and mixed types.

Tumor infiltrating inflammatory cells reflect the host antitumor immune response better than the peripheral blood lymphocytes.[13] The antitumoral inflammatory response is an important prognostic factor in gastric carcinoma. Regardless of the tumor type, tumors with lymphoid stroma have better prognosis.[1,14,15] Inflammation is more common in intestinal type carcinomas.[1]

The cases in this series had varying degrees of antitumoral inflammatory response. The intratumoral and the peritumoral inflammatory infiltration were evaluated separately. The intratumoral infiltration was more prominent in intestinal type carcinomas than in diffuse type tumors.

Chronic atrophic gastritis is the most important precancerous lesion which shows the same geographic and hereditary distribution as gastric cancer.16 IM and chronic atrophic gastritis are steps in the pathogenesis of the intestinal type cancers that are more closely associated with environmental agents.[1,4,17,18] In our series, regardless

of the histological type, 90.4% of the cases had varying degrees of chronic atrophic gastritis. Remarkably, in addition to the intestinal type carcinomas, the mucosa adjacent to the diffuse and mixed type carcinomas also showed chronic atrophic gastritis.

IM is frequently associated with gastric cancer, gastric ulcer, duodenal ulcer and atrophic gastritis.[19,20] During the regeneration process, the epithelium exhibiting IM either differentiates into normal gastric epithelium or persists.21 In our study, we divided IM into three categories according to the Filipe classification.[22] Type 1 (complete IM) is frequently associated with benign conditions. On the other hand, type 3 (incomplete IM) that is characterized by columnar cells producing sulphomucin, is associated with malignant lesions and especially intestinal type gastric carcinomas.[23] Morphologically, type 3 IM has features that are compatible with dysplasia i.e. the loss of cellular and structural differentiation is marked. Some biological properties may even resemble carcinomas.[17,23,24] In our study type 3 was the most frequent type ($p<0.05$). The coexistence of intestinal type carcinomas and type 3 IM in 75% of the cases is in accordance with the literature.[17,23] The relatively lower but still important frequency of association with other tumor types (70.6% and 57.1% in the diffuse and mixt types respectively) suggests that IM may precede these also. However, our area is a high-risk region for gastric cancer. In such regions, the risk of IM in the normal population without cancer is also increased. Therefore, the frequency of IM in the 'normal' population should be established before reaching a definite conclusion.

The ubiquitous pathogen H. pylori is significantly associated with chronic gastritis, duodenal and gastric ulcer, gastric carcinoma and gastric lymphoma.[25-28] Many studies have shown that H. pylori infection, which coexists with gastric carcinomas, shows no association with patient age, tumor type and location.[25,27,29] However, H. pylori probably acts through different mechanisms in the pathogenesis of intestinal type and diffuse type tumors. It has been proposed that in intestinal type carcinomas, it triggers the chronic atrophic gastritis-IM-dysplasia-carcinoma sequence whereas in diffuse type carcinomas, it plays a role by producing oxygen radicals that cause DNA dam-

Table 2. **NK cell counts in the tumor and around the tumor and relationship with clinicopathologic parameters**

	NK cell counts x ± SD								
Parameter	*In the tumor*	*around the tumor*	*n*	F_1	F_2	t_1	t_2	p_1	p_2
Histopathology									
Intestinal	7.4 ± 7.8	8.2 ± 6.3	56						
Diffuse	5.2 ± 4.1	9.3 ± 7.2	21	0.94	0.20			>0.05	>0.05
mixed	6.2 ± 4.4	8.4 ± 9.4	17						
Tumor size									
<5 cm	5.7 ± 4.2	8.8 ± 5.1	20						
5-10 cm	7.1 ± 7.2	8.9 ± 7.9	62	0.46	0.81			>0.05	>0.05
>10 cm	6.0 ± 6.4	6.1 ± 3.8	12						
Invasion									
Mucosa+/-submucosa	2.8 ± 1.8	10.8 ± 4.7	5						
Muscularis	8.4 ± 10.4	10.9 ± 12.4	11	1.24	1.12			>0.05	>0.05
Subserosa+serosa	6.7 ± 6.0	8.0 ± 6.0	78						
Blood vessel invasion									
Negative	5.6 ± 5.4	10.5 ± 8.5	29	1.12	1.87			>0.05	>0.05
Positive	7.2 ± 7.0	7.6 ± 6.1	65						
Lymph node									
Negative	5.4 ± 7.9	8.5 ± 8.6	17	1.00	0.07			>0.05	>0.05
Positive	7.2 ± 6.4	8.7 ± 6.8	72						
Type of IM									
Type 2	7.1 ± 7.1	7.9 ± 4.7	20						
Type 3	6.8 ± 6.7	8.7 ± 7.4	66	0.26	0.14			>0.05	>0.05
No IM	5.1 ± 3.4	7.9 ± 9.2	8						
HSP70 immunoreactivity									
Negative	3.7 ± 2.5	8.3 ± 3.7	7						
Mild	7.2 ± 6.8	8.9 ± 8.1	43	0.84	0.14			>0.05	>0.05
Severe	6.7 ± 6.7	8.1 ± 6.3	44						

age.[30] In our study, examination of the Genta-stained sections revealed H. pylori in the mucosa adjacent to the tumor in 65.9% of the cases. The frequency of H. pylori infection did not differ between different tumor types. This finding was in accordance with the reports that show that H. pylori is associated with all types of carcinoma.

Heat-shock proteins are produced in response to environmental changes such as heat, ischemia, heavy metals, ethanol, toxins, oxidants, various bacterial and viral infections.[7] Their main function is protection of the body against stress.[7] Recently, it has been reported that heat-shock proteins play an important role in the antitumor immune response. Members of the HSP70 group were found to be expressed on the cell surface by some tumors under physiological conditions and after heat stress; they serve as immunogenic determinants for some effector cells. It has been proposed that HSP70-expressing tumor cells are more susceptible to lysis by IL-2-stimulated NK cells.[31] We performed staining with the HSP70 antibody using the standard immunohistochemical method to investigate the relationships between HSP70 staining intensity, general characteristics of gastric carcinomas, prognostic factors and the number of intratumoral and peritumoral NK cells. Increased HSP70 expression has been reported in atherosclerosis, ischemic diseases, neurodegenerative diseases, alcoholic liver disease, viral infections and some autoimmune disorders.[7] However, immunohistochemical studies on HSP70 in tumors are limited. But some recent reports have revealed that HSP70 is a good prognostic factor in esophageal squamous cell carcinoma and cervical intraepithelial neoplasia and cervical cancer.[32,33] However in primary tumors from patients with node-negative breast cancer HSP70 expression was found useful in identifying patients at high risk for disease recurrence.[34] Future studies should be performed to determine if detection of HSP70 by immunohistochemistry can be used to predict clinical outcome and to better understand the relationships between HSP70 and the effects of various treatment modalities.

The HSP70 immunoreactivity was evaluated separately in the tumor cells, adjacent mucosa, mucosa with IM, smooth muscle cells and fibroblasts and grade as weak or strong. Varying degrees of staining were observed in 92.5% of the tumors. The frequency of strong staining in intestinal type carcinomas was 62.5% that was significantly higher than the frequencies in the diffuse and mixed types. The difference may reflect that intestinal type carcinomas that are more related to environmental agents respond to stress better. If the atrophic mucosa-IM –dysplasia-carcinoma sequence for carcinogenesis is correct, the duration of intestinal carcinoma development should be longer in comparison with the diffuse type carcinomas. That the intestinal type carcinoma is observed in older patients supports this possibility. From this viewpoint, it may be speculated that both the tumor cells and the normal cells in intestinal type carcinomas have a longer period for a HSP70 response. The more intense HSP70 staining in intestinal type carcinomas may show that the prognosis can be affected by multiple mechanisms.

The IM areas also showed varying degrees of staining. Tumors with type 3 IM showed more intense staining than tumors with type 2 IM. This result may reflect that the etiopathogenetic relationship is stronger in type 3 IM and carcinomas.

HSP70 staining showed a positive correlation with depth of tumor invasion and tumor size. Also, tumors with vascular invasion and lymph node metastasis showed significantly stronger staining. At first glance, these findings may appear to contradict the statements in the previous paragraph on the relationship between prognosis and HSP70 immunoreactivity. However, one point that should be kept in mind is that most of our patients had advanced carcinomas. The longer the body combats the tumor, the higher is the expected level of HSP70 which acts as a stress protein and participates in antitumor immunity.

Comparison of the HSP70 staining intensity with the number of intratumoral NK cells, showed that tumors without HSP70 immunoreactivity had lower numbers of NK cells in comparison with the two other groups. This result may be in accordance with the finding that tumor cells expressing HSP70 become more susceptible to NK cell-mediated lysis. Although only the number but not the function of NK cells was investigated in this report, the positive correlation between HSP70 immunoreactivity and NK cell number requires further attention.

The antitumor immune response has been extensively investigated.[14] Tumor infiltrating lymphocytes are believed to be an important determinant of the response of the host to the tumor.[35] NK cells are large granular lymphocytes that can lyse various neoplastic and nonneoplastic cells. These characteristics have attracted attention in tumor immunotherapy. In response to various cytokines including interferons, the number and functional activity of NK cells increase.[36-38] In several studies, NK cells which have spontaneous cytotoxic activity against tumor cells were found to be associated with prognosis in various intestinal cancers.[10,11] In this study, we determined the average number of cells staining with the NK cell-like antibody in the peri- and intratumoral inflammatory cells. There was a statistically significant correlation between the degree of inflammatory infiltration and the number of NK-like cells in the infiltrate. We found no statistically significant association between the number of intratumoral and peritumoral NK cells and histological tumor type, size, depth of invasion, lymph node metastasis, vascular invasion, age, sex, IM type and H. pylori presence. In intestinal type carcinomas , the number of NK cells in the tumor were significantly higher than in other types. This tumor type has a relatively better prognosis.[1,14] The higher

number of NK cells in intestinal type carcinomas is in accordance with the previous findings that NK cells play a role in the prognosis of many gastrointestinal cancers.[11] However, the degree of intratumoral inflammatory infiltration in intestinal type carcinomas is higher than in diffuse type cancers.[1] This may account for the comparatively higher numbers of NK cells. In order to determine its role in prognosis definitely, prospective follow up and its interaction with other factors are required.

Although it was not statistically significant the number of NK cells was lower in tumors larger than 10 cm. Survival decreases significantly with increasing tumor size. The low number of NK cells in large tumors is in accordance with this finding. However, whether the low number of NK cells is a cause or result of large tumor size is unknown.

The number of NK cells in early gastric cancers were lower than in more advanced tumors. On the other hand, the NK cell count was significantly lower in tumors with serosal involvement. In other words, there was a relationship between the number of peritumoral NK cells and the depth of invasion. However, in early gastric cancers, both the degree of intratumoral inflammatory cell infiltration and the number of NK cells were lower than in advanced tumors. This may be due to the fact that the antitumor immune response has not found time to reach the intratumoral areas. It has been reported that in early gastric cancers, the intratumoral inflammatory cell infiltration does not affect the prognosis.[1] Probably an inflammatory response develops around the tumor at an earlier stage and serves as a barrier that prevents dissemination.

Vascular invasion is a known poor prognostic factor in gastric cancers.[1] Although the trend did not reach statistical significance, the number of peritumoral NK cells was higher in cases without vascular invasion. Cases with vascular invasion and lymph node metastasis had higher numbers of intratumoral NK cells than the other cases.

In a prospective study on colorectal carcinoma, the higher the number of NK cells in the tumor, the better was the prognosis.[6] In our study, if the tumor type is not taken into consideration, the number of peritumoral NK cells was more closely associated with other prognostic factors.

In our study, the epithelia of the basal layer glands in the mucosa adjacent to the tumor also stained with the NK cell-like antibody. In the mucosa away from the tumor, cells were stained selectively and singly whereas in the mucosa adjacent to the tumor, staining was diffuse. The majority of the CD57-positive cells give a positive reaction to the NK cell-like antibody. In some studies, neuroendocrine cells were found to interact with the CD57 antibody.[6] The singly stained cells in our study were chromogranin positive neuroendocrine cells. Diffuse staining of the adjacent mucosal glands may suggest that some epithelial cells assume NK cell function.

Determination of prognostic factors in gastric carcinoma is very important for the clinicians. In addition to the known prognostic factors such as depth of invasion, lymph node metastasis, vascular invasion, tumor size and antitumor inflammatory response, factors such as HSP70 immunoreactivity should also be taken into consideration. We believe that our study will be a basis for future prospective studies using molecular biological methods.

References

1. *Davessar K, Pezzullo JC, Kessimian N, et al:* Gastric adenocarcinoma: prognostic significance of several pathologic parameters and histologic classifications. Hum Pathol 21:325-332, 1990
2. *Grabiec J, Owen DA:* Carcinoma of the stomach in young persons. Cancer 56:388-390, 1985
3. *Lauren PA, Nevalainen TJ:* Epidemiology of intestinal and diffuse types of gastric carcinoma. Cancer 71:2926-2933, 1993
4. *Lundergardh G, Lindgren A, Rohul A, et al:* Intestinal and diffuse types of gastric cancer: secular trends in Sweden since 1951. Br J Cancer 64:1182-1186, 1991
5. *Antonioli DA, Goldman H.* Changes in the location and type of gastric adenocarcinoma Cancer 50:775-781, 1982
6. *Zanghieri G, Di Gregorio C, Sacchetti C, et al.* Familial occurence of gastric cancer in the 2-year experience of a population-based registry. Cancer 66:2047-2051, 1990
7. *Lu XP, Omar RA, Chang WWL.* Immunocytochemical expression of the 70 kD heat-shock protein in ischaemic bowel disease. J Pathol 179:409-413, 1996
8. *Crawford JM.* The Gastrointestinal Tract. In: Cotran R, Kumar V, Robbins S (eds), Pathologic Basis of Disease (5th ed). WB Saunders Company, Philadelphia 1994, pp 21, 779-783.
9. *Robert J, Menoret A, Basu S, et al:* Phylogenetic conservation of the molecular and immunological properties of the chaperones gp96 and hsp70. Eur J Immunol 31:186-195, 2001
10. *Monson JRT, Ramsden CW, Giles GR, Brennan TG.* Lymphokin activated killer (LAK) cells in patients with gastrointestinal cancer. Gut 28:1420-1425, 1987
11. *Coca S, Perez-Piqueras J, Martinez D, et al.* The prognostic significance of intratumoral natural killer cells in patients with colorectal carcinoma. Cancer 79:2320-2328, 1997
12. *Lauren P:* The two histological main types of gastric carcinoma: diffuse and so called intestinal-type carcinoma. Acta Pathol Microbiol Immunol Scand [B] 64:31-49, 1965
13. *Holmes EC:* Immunology of tumor infiltrating lymphocytes. Ann Surg 201:158-163, 1985
14. *Nakamura K, Ueyama T, Yao T, et al:* Pathology and prognosis of gastric carcinoma. Cancer 70:1030-1037, 1992
15. *Setala LP, Kosma VM, Marin S, et al:* Prognostic factors in gastric cancer: the value of vascular invasion, mitotic rate and lymphoplasmocytic infiltration. Br J Cancer 74:766-772, 1996
16. *Rotterdam H:* Stomach. In: Silverberg SG (ed), Biopsy Diagnosis of the Digestive Tract (2nd ed). Raven Press, New York pp 188-214, 1993
17. *Sipponen P, Kekki M, Siurala M:* Atrophic chronic gastritis and intestinal metaplasia in gastric carcinoma. Cancer 52:1062-1068, 1983
18. *Iida F, Kusama J:* Gastric carcinoma and intestinal metaplasia. Cancer 50:2854-2858, 1982
19. *Huang CB, Xu J, Huang JF, Meng XY:* Sulphomucin colonic type intestinal metaplasia and carcinoma in the stomach. Cancer 57:1370-1375, 1986

20. *Hattori T:* Devolepment of adenocarcinomas in the stomachs. Cancer 57:1528-1534, 1986
21. *Oohara T, Tohma H, Aono G, et al:* Intestinal metaplasia of the regenerative epithelia in 549 gastric ulcers. Hum Pathol 14:1066-1071, 1983
22. *Filipe MI, Potet F, Bogomoletz WV, et al.* Incomplete sulphomucin secreting intestinal metaplasia for gastric cancer. Gut 6:1319-1326, 1985
23. *Tanaka F, Yamaguchi K, Urano Y:* Statistical analysis of the surgical materials of stomach. Cancer 59:1978-1982, 1987
24. *Murayama H, Kikuchi M, Enjoji M, et al:* Changes in gastric mucosa that antedate gastric carcinoma. Cancer 66:2017-2026, 1990
25. *Karnes WE, Samloff IM, Sivrala M, Kekki M:* Positive antibody and negative tissue staining for Helicobacter pylori in subjects with atrophic body gastritis. Gastroenterology 101:167-174, 1991
26. *Go MF:* What are the host factors that place an individual at risk for Helicobacter pylori-associated disease? Gastroenterology 113: 15-20, 1997
27. *Shibata T, Imoto I, Ohuchi Y, et al:* Helicobacter pylori infection in patients with gastric carcinoma in biopsy and surgical resection specimens. Cancer 77:1044-1049, 1996
28. *Lee PM, Jang JJ, Kim JS, et al:* Association of Helicobacter pylori infection with gastric adenocarcinoma. Jpn J Cancer Res 89:597-603, 1998
29. *Wee A, Kang JY, Teh M:* Helicobacter pylori and gastric cancer: correlation with gastritis, intestinal metaplasia, and tumour histology. Gut 33:1029-1032, 1992
30. *Asaka M, Takeda H, Sugiyama T, Kato M:* What role does Helicobacter pylori play in gastric cancer? Gastroenterology 113:56-60, 1997
31. *Multhoff G, Botzler C, Jennen L, et al.* Heat-shock protein 72 on tumor cells. J Immunol 158:4341-4350, 1997
32. *Kawanishi K, Shiozaki H, Doki Y, et al:* Prognostic significance of heat shock proteins 27 and 70 in patients with squamous cell carcinoma of the esophagus. Cancer 85: 1649-1657, 1999
33. *Park CS, Joo IS, Song SY, et al:* An immunohistochemical analysis of heat shock protein 70, p 53 and estrogen status in carcinoma of the uterine cervix. Gynecol Oncol 74: 53-60, 1999
34. *Ciocca DR, Clark GM, Tandon AK, et al:* Heat shock protein hsp70 in patient with axillary lymph node-negative breast cancer: prognostic implications. J Nat Cancer Ins 85:570-574, 1996
35. *Chin Y, Janseens J, Vandepitte J, et al.* Phenotypic analysis of tumor-infiltrating lymphocytes from human breast cancer. Anticancer Res 12:1463-1466, 1992
36. *Brittenden J, Heys SD, Ross J, Eremin O:* Natural killer cells and cancer. Cancer 77:1226-1243, 1996
37. *Toliohu Th, Stravoravdi P, Polyzonis M, Vakalikos J:* Natural killer cell activation after interferon administration in patients with metastatic renal cell carcinoma. Eur Urol 29:252-256, 1996
38. *Karimine N, Arinaga S, Inove H, et al:* Lymphokine-activated killer cell function of peripheral blood mononuclear cells, spleen cells and regional lymph node cells in gastric cancer patients. Clin Exp Immunol 96:484-490, 1994