Lymphocyte-Rich Gastric Cancer: Associations with Epstein-Barr Virus, Microsatellite Instability, Histology, and Survival

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Lymphocyte-rich gastric carcinomas may have a better prognosis than cancers without a pronounced host inflammatory response. Two subsets of gastric cancer—Epstein-Barr virus-positive and microsatellite instability high—have been associated with a lymphocyte-rich phenotype. We assessed relationships between tumor-infiltrating lymphocytes, Epstein-Barr virus status, microsatellite instability status, and cancer-specific survival in 110 resected gastric cancers. Seven patients had Epstein-Barr virus-positive cancer, including 4 (3.7%) of 107 consecutive patients. Tumors from 17 patients (16%) were designated microsatellite instability high on the basis of negative immunohistochemical staining for MLH1; all tumors had intact expression of MSH2 and MSH6. Epstein-Barr virus-positive cancers had increased tumor-infiltrating lymphocytes compared with Epstein-Barr virus-negative cancers (median 450/10 HPF versus 21/10 HPF, $P < .001$). Microsatellite instability–high cancers also had increased tumor-infiltrating lymphocytes compared with non–microsatellite instability–high cancers (median 150/10 HPF *versus* 20/HPF, $P < .001$). Microsatellite instability–high cancers affected older patients and were more likely to be intestinal in the Lauren classification and expanding in the Ming classification. By univariate analysis, decreased risk of death from gastric cancer was significantly associated with low tumor stage, expanding growth pattern, increasing tumor-infiltrating lymphocyte count, and microsatellite instability–high status. High tumor-infiltrating lymphocyte count and microsatellite instability–high status retained statistical significance as favorable prognostic factors after adjustment for tumor stage in multivariate analysis. Tumor-infiltrating lymphocyte count retained statistical significance as a favorable prognostic factor after adjustment for microsatellite instability–high status; but microsatellite instability–high status did not remain a significant independent prognosticator after adjustment for tumor-infiltrating lymphocyte count. The association between microsatellite instability–high cancers and high tumor-infiltrating lymphocyte counts may account for the association of microsatellite instability–high gastric cancers with improved survival.

KEY WORDS: Epstein-Barr virus, Gastric carcinoma, Microsatellite instability, Tumor-infiltrating lymphocytes.

Watanabe *et al.* (1) described a specific type of lymphocyte-rich gastric cancer, termed *gastric carcinoma with lymphoid stroma,* that had a better prognosis than other gastric carcinomas. Subsequent reports, some using the synonyms lymphoepithelioma-like carcinoma or medullary carcinoma of the stomach, confirmed the observation (1–5). Strictly defined, gastric carcinoma with lymphoid stroma shows sharply demarcated margins and non-desmoplastic stroma reminiscent of lymphoid tissue, with small nests of cancer cells uniformly distributed throughout the lymphoid stroma. It is not known whether less stereotypic patterns of lymphocyte infiltration are associated with survival differences.

Two subsets of gastric carcinoma can have large numbers of lymphocytes: Epstein-Barr virus (EBV)–positive cancers (5–12) and cancers with high levels

of microsatellite instability (13–16). EBV can be demonstrated in the tumor cells in 1.8–18% of sporadic gastric carcinomas, and the prevalence is significantly higher (>80%) in gastric carcinoma with lymphoid stroma (4, 5, 7, 10, 17–20). The relationship between EBV status and survival is not clear; it has been suggested that a survival advantage is seen only in those tumors that can be categorized as gastric carcinoma with lymphoid stroma, and that EBV-positivity itself does not confer a more favorable prognosis (4, 5, 17).

Instability involving microsatellite DNA sequences was originally recognized in tumors from patients with hereditary non-polyposis colon carcinoma, but has subsequently been observed in a subset of sporadic tumors from various sites including colon, stomach and endometrium (13, 14, 16, 21–27). High levels of microsatellite instability result from defective function of DNA mismatch repair enzymes, usually hMLH1or hMSH2, rarely hMSH6 (15, 22, 28). The reported prevalence of high microsatellite instability in gastric carcinoma ranges from 7–39%, with apparent geographic variability (15, 16, 23, 27, 29–31). Most studies have suggested improved survival compared with tumors with stable microsatellite sequences or low levels of microsatellite instability (13, 16, 26, 27, 31).

The gold standard method to assess microsatellite instability is PCR analysis of microsatellite loci, with instability at >30% of loci indicating defective DNA mismatch repair. Recent studies in colorectal carcinoma have shown that immunohistochemistry using antibodies directed against DNA mismatch repair enzymes hMLH1 and hMSH2 can be used as a surrogate marker for MSI status. Lack of expression of one of these proteins by immunohistochemistry has essentially perfect specificity for microsatellite instability–high status (32, 33). The sensitivity of immunohistochemistry for detecting the microsatellite instability–high phenotype is also very high, having been reported as 92% in colorectal carcinoma (34). The sensitivity is likely even higher in gastric carcinoma, where the majority of microsatellite instability–high cases result from hypermethylation of the hMLH1 gene promoter, a situation that will always lead to absent expression of the protein (32, 33, 35).

We studied clinicopathologic features and outcome in 110 resected gastric carcinomas to better define the relationships between lymphoid infiltrate, histology, EBV status, MSI status and prognosis. For the reasons outlined above, MSI status was assessed using immunohistochemistry for DNA mismatch repair enzymes. The lymphoid infiltrate was quantified as tumor-infiltrating lymphocytes per 10 high-power microscopic fields to better illustrate the association between tumor infiltrating lymphocyte count and outcome in gastric cancer.

MATERIALS AND METHODS

Case Selection

Mayo Clinic files were searched for patients who underwent partial or complete gastric resection between 1990 and 1998. Tumors of the gastric cardia were excluded from this analysis. One hundred seven cases were identified for which histologic material was available. Three additional cases of known EBV-positive gastric carcinoma from the consultation files were added to the series. Clinical information regarding follow-up was obtained by chart review and phone contact with primary physicians. This study was approved by the Mayo Clinic Institutional Review Board, and all patients consented for use of their medical records for research.

Histologic Evaluation

Two pathologists (KLG, TCS) independently analyzed the hematoxylin and eosin-stained slides from each case and assessed histologic grade, Lauren and Ming classifications, gastric carcinoma with lymphoid stroma categorization (according to the criteria of Watanabe *et al.,* 1), and tumor-infiltrating lymphocytes per 10 HPF (Nikon E600; field diameter 0.52 mm). Histologic evaluation was done without prior knowledge of the microsatellite instability status and EBV status of the tumor. Any discrepancy was reviewed to reach consensus. To be included in the tumor-infiltrating lymphocyte count, the lymphocyte had to be clearly identifiable as a lymphocyte, and be in direct contact with tumor cells (Fig. 1). Lymphocytes present in the stroma but not in contact with tumor cells were not counted.
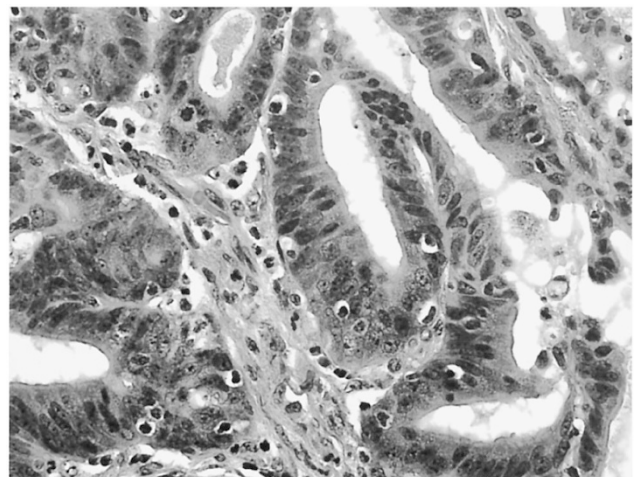

FIGURE 1. Gastric cancer with abundant tumor-infiltrating lymphocytes. Lymphocytes are intimately associated with tumor cells and have a characteristic perinuclear white halo. Stromal lymphocytes are not included in the tumor-infiltrating lymphocyte count (H&E; original magnification, 600×).

In Situ Hybridization for EBV

EBV RNA *in situ* hybridization was performed on paraffin sections from each case. Oligonucleotides complementary to a portion of the EBV early RNAs (EBER1 and EBER2) were applied using the procedure of Chang *et al.* (36). A case was determined to be positive if intranuclear staining was observed in gastric tumor cells. Cases with scattered lymphocytes containing EBV genome were not scored as positive.

Evaluation of Microsatellite Instability Status

Immunohistochemical staining for hMLH1, hMSH2 and hMSH6 was performed as described by Halling and Roche (37). The antibody to hMSH2 (Clone FE11, 0.5 μg/mL, Oncogene Science) is a mouse monoclonal antibody generated with a carboxy-terminal fragment of the hMSH2 protein, whereas the hMLH1 antibody (clone G168–728, 1 μg/mL, Pharmingen, San Diego, CA) is a mouse monoclonal antibody that was prepared with full-length hMLH1 protein. The antibody to hMSH6 was clone 44 (0.5 mg/mL, Transduction Laboratories, Lexington, KY). Lymphocytes and normal epithelium exhibit strong nuclear staining for hMLH1, hMSH2 and hMSH6 and served as positive internal controls.

Statistical Methods

Comparisons of clinicopathologic tumor features were assessed using Wilcoxon rank sum, chi-square, and Fisher's exact tests. All tests were two-sided and P values $< .05$ were considered statistically significant.

The Kaplan-Meier method was used to estimate cancer-specific survival. Duration of follow-up was defined from the date of diagnosis to the date of death or last follow-up. Univariate and multivariate Cox proportional hazards regression models were fit to assess which clinicopathologic features were associated with cancer-specific survival. The relationships between these features and cancer-specific survival were summarized with risk ratios and 95% confidence intervals (95% CI).

RESULTS

The clinicopathologic features are summarized in Table 1. A total of seven tumors were positive for EBV by *in situ* hybridization (Fig. 2), including 4 of the 107 consecutive cases (3.7%) and the 3 consultation cases added to the series because they were known to be EBV-positive. A comparison of clinicopathologic features by EBV status is shown in Table 2. EBV status was significantly associated with the number of tumor-infiltrating lymphocytes

TABLE 1. Clinicopathologic Features of Gastric Carcinoma Cases

Feature	Mean (SD) Median Range
Age at diagnosis (years)	68.4 (12.4)
	70.5
	38–90
Sex	
Female	38 (34.5)
Male	72 (65.5)
Tumor histologic differentiation	
Well	3 (2.7)
Moderate	32 (29.1)
Poor	75 (68.2)
Primary tumor	
T1	6 (5.5)
T2	19 (17.3)
T3	74 (67.3)
T4	11 (10.0)
Regional lymph nodes	
NX	1 (0.9)
N0	28 (25.5)
N1	50 (45.5)
N2	21 (19.1)
N3	10 (9.1)
Distant metastases	
M0	81 (73.6)
M1	29 (26.4)
Tumor location	
Unknown	25 (22.7)
Body	19 (17.3)
Distal	47 (42.7)
Entire	11 (10.0)
Proximal	8 (7.3)
Lauren classification	
Diffuse	55 (50.0)
Intestinal	51 (46.4)
Mixed	4 (3.6)
Ming classification	
Expanding	43 (39.1)
Infiltrative	67 (60.9)
Gastric carcinoma with lymphoid stroma	
No	104 (94.5)
Yes	6 (5.5)
EBV status	
Negative	103 (93.6)
Positive	7 (6.4)
MLH-1 status	
Negative (microsatellite instability high)	17 (15.5)
Positive (non-microsatellite instability high)	93 (84.5)

per 10 HPF. The median number of tumor-infiltrating lymphocytes per 10 HPF for EBV negative tumors was 21, compared with 450 for EBV positive tumors ($P < .001$).

Six cases were designated as gastric carcinoma with lymphoid stroma (Fig. 3), including 3 of the 107 consecutive cases (2.8%). Three of the patients (42.9%) with EBV positive tumors were designated as gastric carcinoma with lymphoid stroma, compared with 3 patients (2.9%) with EBV negative tumors ($P < .001$). By definition, gastric carcinoma with lymphoid stroma tumors demonstrated a pronounced lymphocytic infiltrate surrounding the cancer cell nests. Tumor-infiltrating lymphocytes in
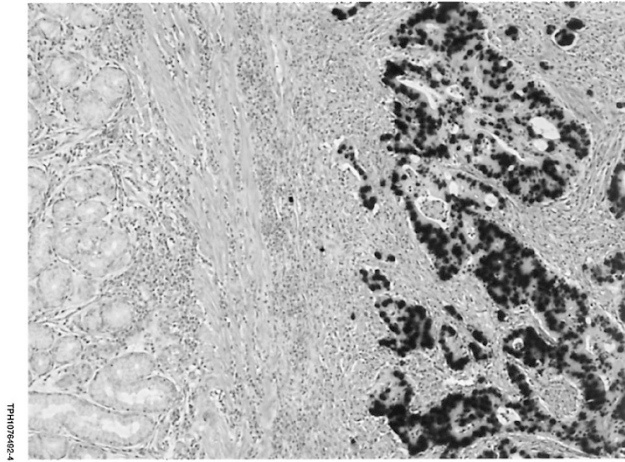
FIGURE 2. EBV RNA detected in gastric cancer nuclei by *in situ* hybridization. The overlying non-neoplastic epithelium lacks staining (*in situ* hybridization for EBER1 and EBER2; original magnification, 100×).

gastric carcinoma with lymphoid stroma ranged from 300 to 800 per 10 HPF, with a mean of 525.

Seventeen of the 110 tumors (16%) showed complete loss of nuclear staining with antibody to hMLH1 and were therefore designated as microsatellite instability high (Fig. 4). None of the cases lacked staining for hMSH2 or hMSH6. A comparison of the clinicopathologic features by hMLH1 status is shown in Table 3. Patients with microsatellite instability–high tumors were significantly older compared with those with non-microsatellite instability–high tumors (median 77 y *versus* 69 y; P = .016). Those with microsatellite instability–high tumors also had significantly higher tumor-infiltrating lymphocytes per 10 HPF compared with patients with non-microsatellite instability–high tumors (median 150 *versus* 20; P < .001). The distribution of tumor-infiltrating lymphocytes in microsatellite instability–high and EBV positive tumors is displayed in Fig. 5. Microsatellite instability high tumors were also more likely to be of lower tumor grade (P = .018), less likely to have distant metastases (P = .039) and more likely to be intestinal type according to the Lauren classification (P = .030) compared with tumors that were non-microsatellite instability high. Lastly, there was a significant association between microsatellite instability status and Ming classification in that most of the microsatellite instability–high tumors (94%) were classified as expanding compared with only 27 (29%) of the non-microsatellite instability–high tumors (P < .001).

Sixty patients died from gastric cancer. The average (SD) time from diagnosis to death from cancer was 18.4 (16.8) months, ranging from 1 to 70 months. Among the 50 patients who died from other causes or were still alive at last follow-up, the average (SD) time from diagnosis to last follow-up was 37.1 (28.8) months, ranging from 1 to 131 months. The estimated cancer-specific survival rates at 12, 24, 36, and 48 months were 72.1%, 56.5%, 48.9%, and 41.5%, respectively.

Univariate associations between clinicopathologic features and cancer-specific survival are shown in Table 4. The number of tumor-infiltrating lymphocytes per 10 HPF was significantly associated with death from gastric cancer. Because the tumor-infiltrating lymphocyte distribution was severely skewed, this feature was analyzed on the natural logarithmic scale. Each 1-unit increase in the number of tumor-infiltrating lymphocytes per 10 HPF on the natural logarithmic scale (or an increase by a power of 2.72 tumor-infiltrating lymphocytes per 10 HPF on the original scale) decreased the risk of death from gastric cancer by 27% (P = .001). The univariate association of this feature with cancer-specific survival is shown in Figure 6.

Other parameters showing statistically significant association with survival were microsatellite instability status, tumor stage and Ming classification. Patients with microsatellite instability–high tumors were 2.8 times less likely to die from gastric cancer compared with those with non-microsatellite instability–high tumors (P = .021). Patients with stage IV tumors were 11 times more likely to die from gastric cancer compared with those with stage I tumors (P < .001). Patients with Stage IIIa or IIIb tumors were 5 times more likely to die from their disease compared with those with stage I tumors (P = .026). The relationship between tumor stage and cancer-specific survival is shown in Figure 7. Patients with infiltrative tumors according to the Ming classification were three times more likely to die from their disease compared with patients with expanding tumors (P < .001).

Microsatellite instability–high status and tumor-infiltrating lymphocyte count retained a significant association with survival after adjusting for tumor stage (Table 4). Because these two variables were also associated with each other, multivariate analysis was performed to determine their independent effect upon survival (Table 5). A significantly decreased risk of death from gastric cancer with increasing tumor-infiltrating lymphocyte count was apparent even after adjusting for microsatellite instability–high status (P = .013). In contrast, the association between microsatellite instability status and cancer-specific survival was not statistically significant after adjusting for tumor-infiltrating lymphocyte count (P = .125).

DISCUSSION

Lymphocyte-rich gastric carcinoma has been associated with a survival advantage compared with

TABLE 2. Comparison of Clinicopathologic Features by EBV Status

Feature	EBV Status			p-value
	Negative N = 103	Mean (SD) Median Range	Positive N = 7	
Age at diagnosis (years)	68.8 (12.0)		61.1 (16.7)	0.182
	71		65	
	39–90		38–86	
Tumor infiltrating lymphocytes per 10 HPF	79.9 (145.2)		442.9 (181.3)	<0.001
	21		450	
	0–800		250–800	
		N (%)		
Sex				
Female	38 (36.9)		0 (0.0)	0.093
Male	65 (63.1)		7 (100.0)	
Tumor-infiltrating lymphocytes per 10 HPF				
0–5	27 (26.2)		0 (0.0)	<0.001
6–29	29 (26.2)		0 (0.0)	
30–99	25 (24.3)		0 (0.0)	
≥100	24 (23.3)		7 (100.0)	
Tumor histologic differentiation				
Well	3 (2.9)		0 (0.0)	1.00
Moderate	30 (29.1)		2 (28.6)	
Poor	70 (68.0)		5 (71.4)	
Primary tumor				
T1	6 (5.8)		0 (0.0)	0.629
T2	19 (18.5)		0 (0.0)	
T3	68 (66.0)		6 (85.7)	
T4	10 (9.7)		1 (14.3)	
Regional lymph nodes				
NX	1 (1.0)		0 (0.0)	0.642
N0	26 (25.2)		2 (28.6)	
N1	48 (46.6)		2 (28.6)	
N2	19 (18.5)		2 (28.6)	
N3	9 (8.7)		1 (14.3)	
Distant metastases				
M0	75 (72.8)		6 (85.7)	0.673
M1	28 (27.2)		1 (14.3)	
Tumor stage				
Ia	6 (5.8)		0 (0.0)	0.225
Ib	9 (8.7)		0 (0.0)	
II	13 (12.6)		2 (28.6)	
IIIa	32 (31.1)		2 (28.6)	
IIIb	7 (6.8)		2 (28.6)	
IV	36 (35.0)		1 (14.3)	
Tumor location				
Unknown	23 (22.3)		2 (28.6)	0.605
Body	19 (18.5)		0 (0.0)	
Distal	44 (42.7)		3 (42.9)	
Entire	10 (9.7)		1 (14.3)	
Proximal	7 (6.8)		1 (14.3)	
Lauren classification				
Diffuse	50 (48.5)		5 (71.4)	0.074
Intestinal	50 (48.5)		1 (14.3)	
Mixed	3 (2.9)		1 (14.3)	
Ming classification				
Expanding	39 (37.9)		4 (57.1)	0.429
Infiltrative	64 (62.1)		3 (42.9)	
Gastric carcinoma with lymphoid stroma				
No	100 (97.1)		4 (57.1)	<0.001
Yes	3 (2.9)		3 (42.9)	
MLH-1 Status				
Negative (microsatellite instability high)	17 (16.5)		0 (0.0)	0.593
Positive (non-microsatellite instability high)	86 (83.5)		7 (100.0)	

gastric carcinoma without a significant lymphoid infiltrate (1–5). Some have proposed that the inflammatory infiltrate represents an effective host immune response against the tumor cells (1, 4, 10), an idea supported by the presence of increased antigen-presenting cells in lymphocyte-rich tumors of the stomach (10). In other malignancies (colon, breast and lung cancer, and melanoma) tumor-infiltrating lymphocytes have been shown to bear a cytotoxic T-cell phenotype and are linked positively to survival (38–42).

The molecular characteristics of tumors that elicit an enhanced immune response are undefined, but certain subsets of gastric tumors have
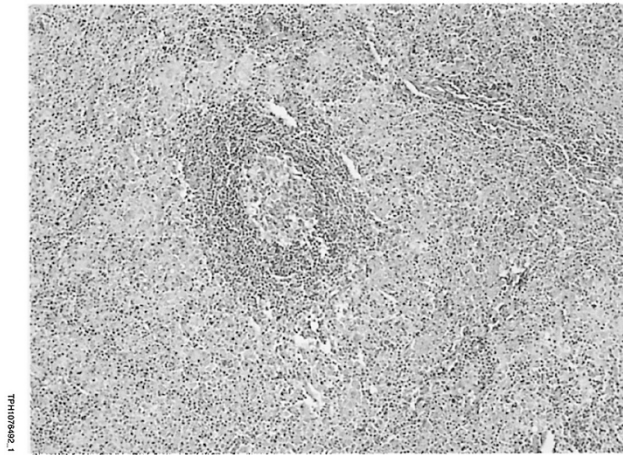
FIGURE 3. Gastric carcinoma with lymphoid stroma. Syncytial nests of tumor cells are distributed in a non-desmoplastic lymphoid stroma. A reactive lymphoid follicle is present in the center of the field (H&E; original magnification, 100×).
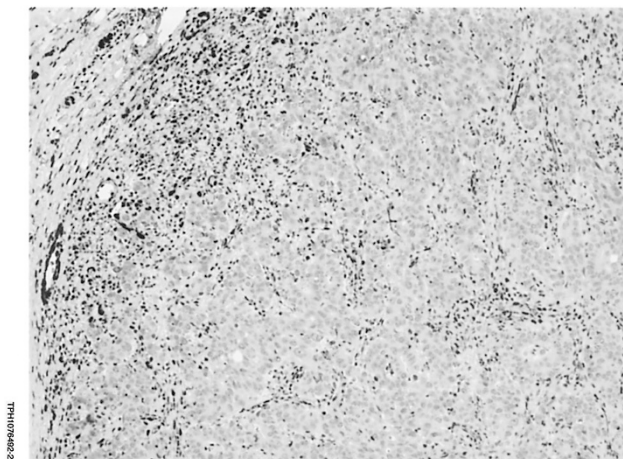

FIGURE 4. Gastric cancer designated as microsatellite instability high on the basis of lack of immunohistochemical staining for the mismatch repair enzyme hMLH-1. Nuclear staining in lymphocytes and stromal cells serves as a positive internal control (original magnification, 100×).

been associated with a pronounced lymphoid infiltrate. These subsets, including EBV-positive tumors and microsatellite instability–high tumors, have themselves been variably associated with a survival advantage (4, 5, 9, 11, 13, 16, 17, 26, 27, 31). It has been unclear whether the survival advantage is related to the lymphoid infiltrate, or whether the EBV or microsatellite instability status themselves serve as independent prognosticators. Our study demonstrates that an increasing tumor-infiltrating lymphocyte count is significantly associated with increasingly improved survival in gastric cancer. EBV positivity and microsatellite instability status (after adjusting for tumor-infiltrating lymphocyte count) were not significantly associated with survival.

The prevalence of EBV-positive and microsatellite instability–high tumors in our study is in agreement with previous reports. Of the 107 consecutive patients, 4 (3.7%) had EBV-positive tumors, which falls within the reported range of 1.8–18% (4, 5, 10, 17–20). Also in agreement with previous studies, EBV positivity was significantly associated with high tumor-infiltrating lymphocyte counts, with all seven cases showing >100 tumor-infiltrating lymphocytes/10HPF. Some reports have suggested that EBV is associated with improved survival in gastric cancer (4). In contrast, Nakamura *et al.* demonstrated that patients with gastric carcinoma with lymphoid stroma have an improved prognosis independent of EBV status, suggesting that the lymphoid infiltrate is the critical variable in terms of survival (5). While we had limited numbers of EBV-positive cases, we did not find an association between EBV positivity and improved prognosis.

Using immunohistochemistry as a marker of microsatellite instability status, 16% of the tumors in our series were microsatellite instability high. The range of microsatellite instability high in gastric cancer from previous reports is 9–39% (15, 16, 23, 27, 29–31). All of our microsatellite instability–high cases showed loss of expression of hMLH1; hMSH2 and hMSH6 expression was preserved in all tumors. We screened for false–negative immunostain results by performing PCR-based microsatellite analysis on four tumors with high tumor-infiltrating lymphocyte counts but positive staining for mismatch repair enzymes; all were microsatellite stable (data not shown).

Gastric adenocarcinomas with high levels of microsatellite instability have characteristic clinicopathologic features, including older age, intestinal histotype according to Lauren's classification, an expanding growth pattern according to Ming's classification, and low pTNM stage including less frequent nodal metastases (13, 14, 16, 22, 23, 27, 30, 31). Most studies, but not all, have also shown increased lymphocytic infiltration compared with tumors with no or low levels (<30% of loci affected) of microsatellite instability (13–16). Our study confirms the association of microsatellite instability–high tumors with older age, an intestinal histotype and expanding growth pattern. Microsatellite instability–high tumors were represented among all pTNM stages, but they had significantly fewer distant metastases. There was also a strong association between microsatellite instability–high status and increased tumor-infiltrating lymphocytes. The mean tumor-infiltrating lymphocyte count was lower than that associated with EBV positivity (150 *versus* 450 tumor-infiltrating lymphocytes/10HPF) but significantly higher than the average for non-microsatellite instability–high tumors (20 tumor-infiltrating lymphocytes/10HPF). As has been suggested by others, it may be that the accumulation of large numbers of mutations in cells with defective DNA mismatch repair alters the antigenicity of the

TABLE 3. Comparison of Clinicopathologic Features by MLH-1 Status

Feature	MLH-1 Status		p-value
	Negative (Microsatellite Instability High) N = 17	Positive (Non-Microsatellite Instability High) N = 93 Mean (SD) Median Range	
Age at diagnosis (years)	74.8 (7.7) 77 58–84	67.2 (12.7) 69 38–90	0.016
Tumor-infiltrating lymphocytes per 10 HPF	224.5 (211.4) 150 0–800	80.8 (154.7) 20 0–800	<0.001
		N (%)	
Sex			
Female	4 (23.5)	34 (36.6)	0.299
Male	13 (76.5)	59 (63.4)	
Tumor-infiltrating lymphocytes per 10 HPF			
0–5	2 (11.8)	25 (26.9)	<0.001
6–29	1 (5.9)	26 (28.0)	
30–99	2 (11.8)	23 (24.7)	
≥100	12 (70.6)	19 (20.4)	
Tumor histologic differentiation			
Well	1 (5.9)	2 (2.2)	0.018
Moderate	9 (52.9)	23 (24.7)	
Poor	7 (41.2)	68 (73.1)	
Primary tumor			
T1	2 (11.8)	4 (4.3)	0.525
T2	2 (11.8)	17 (18.3)	
T3	11 (64.7)	63 (67.7)	
T4	2 (11.8)	9 (9.7)	
Regional lymph nodes			
NX	0 (0.0)	1 (1.1)	0.053
N0	9 (52.9)	19 (20.4)	
N1	7 (41.2)	43 (46.2)	
N2	1 (5.9)	20 (21.5)	
N3	0 (0.0)	10 (10.8)	
Distant metastases			
M0	16 (94.1)	65 (69.9)	0.039
M1	1 (5.9)	28 (30.1)	
Tumor stage			
Ia	2 (11.8)	4 (4.3)	0.188
Ib	2 (11.8)	7 (7.5)	
II	4 (23.5)	11 (11.8)	
IIIa	6 (35.3)	28 (30.1)	
IIIb	0 (0.0)	9 (9.7)	
IV	3 (17.7)	34 (36.6)	
Tumor location			
Unknown	4 (23.5)	21 (22.6)	0.504
Body	4 (23.5)	15 (16.1)	
Distal	7 (41.2)	40 (43.0)	
Entire	0 (0.0)	11 (11.8)	
Proximal	2 (11.8)	6 (6.5)	
Lauren classification			
Diffuse	4 (23.5)	51 (54.8)	0.030
Intestinal	13 (76.5)	38 (40.9)	
Mixed	0 (0.0)	4 (4.3)	
Ming classification			
Expanding	16 (94.1)	27 (29.0)	<0.001
Infiltrative	1 (5.9)	66 (71.0)	
Gastric carcinoma with lymphoid stroma			
No	16 (94.1)	88 (94.6)	1.00
Yes	1 (5.9)	5 (5.4)	
EBV status			
Negative	17 (100.0)	86 (92.5)	0.593
Positive	0 (0.0)	7 (7.5)	

proteins manufactured and in turn leads to an increased immune response to the tumor.

Gastric carcinomas with a microsatellite instability–high phenotype appear to share certain clinicopathologic features with colorectal carcinomas that display this genetic phenotype. Both tumor types have a tendency to present at low pTNM stage with infrequent nodal metastases and also have a better stage-specific prognosis (16, 43). Histologically, they both are associated with high numbers of
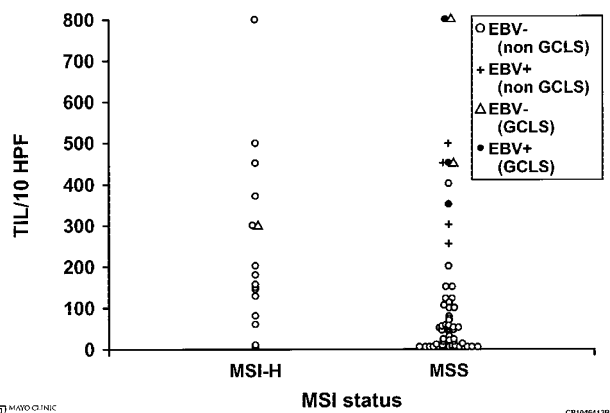
FIGURE 5. Distribution of tumor-infiltrating lymphocyte (TIL) count in microsatellite instability high (MSI-H) *versus* non-microsatellite instability high (non-MSI-H) gastric cancer. Cases that are positive for EBV and/or display a gastric carcinoma with lymphoid stroma (GCLS) phenotype are indicated (see key).

tumor-infiltrating lymphocytes (44, 45). It has been shown that a tumor-infiltrating lymphocyte count of >5/10HPF has a sensitivity of 93% and a specificity of 62% for microsatellite instability–high status in colorectal carcinoma (46). Based on the results of the current study, the presence of tumor-infiltrating lymphocytes in gastric cancer appears to be a less specific and less sensitive marker for microsatellite instability–high status compared with colorectal cancer. Gastric tumors with high tumor-infiltrating lymphocyte counts include both microsatellite instability–high tumors and also EBV-positive tumors, and show a broader range and much higher average number of tumor-infiltrating lymphocytes than colorectal carcinomas.

None of the cases in our series demonstrated concomitant EBV positivity and microsatellite instability–high status. A previous study by Leung *et al.* (14) found two gastric cancers that were both EBV positive and microsatellite instability high in a series of 79 cases (18 EBV positive, 13 microsatellite instability high). The rarity of this finding suggests that EBV and microsatellite instability are unrelated factors involved in distinct etiologic pathways in gastric carcinogenesis, independently contributing to the induction of a complex host immune response. Although EBV-positive and microsatellite instability–high cancers share the feature of increased tumor-infiltrating lymphocytes, the characteristic clinicopathologic features found only in microsatellite instability–high cancers (as outlined above) further illustrate that the two types are distinct subsets of lymphocyte-rich gastric cancer.

Each of the clinicopathologic features studied was compared with cancer-specific survival by univariate analysis. The strongest associations with cancer-specific survival were seen with pTNM

stage, Ming classification, and tumor-infiltrating lymphocyte count. Patients with Stage IV disease had a greater than an 11-fold risk of death compared with those with Stage I disease, and patients with infiltrative tumors had a >3-fold risk of death compared with those with expanding tumors. The risk of death from gastric cancer decreased by 27% with each 1-unit increase in tumor-infiltrating lymphocyte count on the logarithmic scale, or an increase by a power of 2.72 on the original scale. The cancer-specific survival rate at 48 months was 58.8% for patients with tumor-infiltrating lymphocyte counts >100/10HPF, compared with 34.0% for patients with tumor-infiltrating lymphocyte counts less than 100/10HPF. The association of tumor-infiltrating lymphocyte count with a decreased risk of death from gastric cancer retained statistical significance even after adjusting for possible compounding variables, including stage and microsatellite instability status.

Microsatellite instability–high tumors also showed a significant association with survival by univariate analysis, which persisted after adjustment for stage in multivariate analysis. However, when adjusted for tumor-infiltrating lymphocyte count, microsatellite instability–high tumors did not show a significant survival advantage. These results suggest that the survival advantage that has been observed in microsatellite instability–high gastric carcinoma is at least in part, if not entirely, attributable to the association of these tumors with increased tumor-infiltrating lymphocytes. Our series did not contain a large number of microsatellite instability–high tumors with low numbers of tumor-infiltrating lymphocytes (only three cases with tumor-infiltrating lymphocyte counts less than the median), and none of these patients died from cancer. Therefore we cannot exclude the possibility that a survival advantage in microsatellite instability–high tumors was masked by the limited sample size. Collection of additional patients in this category may help to further clarify the relationship between these variables.

EBV-positive and microsatellite instability–high tumors accounted for the majority of tumors classified as gastric carcinoma with lymphoid stroma (67%). Gastric carcinoma with lymphoid stroma demonstrated high numbers of tumor-infiltrating lymphocytes in addition to the characteristic stromal lymphocytic infiltrate. While gastric carcinoma with lymphoid stroma tumors showed a tendency toward markedly improved survival, this did not reach statistical significance, likely because of limited numbers. In light of these findings, gastric carcinoma with lymphoid stroma may represent one end of a spectrum of lymphocyte-infiltrated tumors

TABLE 4. Univariate Associations with Death from Gastric Cancer

Feature	Risk Ratio (95% C.I.)	p-Value
Age at diagnosis	1.02 (0.83, 1.25)[1]	0.848
Sex		
Female	1.0 (ref)	
Male	0.83 (0.49, 1.42)	0.504
Tumor-infiltrating lymphocytes per 10HPF [stage adjusted]	0.79 (0.68, 0.91)[2]	0.001
	[0.81 (0.70–0.95)][2]	[0.008]
Tumor histologic differentiation		
Well or moderate	1.0 (ref)	
Poor	1.53 (0.85, 2.75)	0.158
Primary tumor		
T1	1.0 (ref)	
T2	2.36 (0.27, 20.34)	0.434
T3	6.30 (0.86, 46.01)	0.070
T4	9.03 (1.14, 71.44)	0.037
Regional lymph nodes		
Nx and N0	1.0 (ref)	
N1	2.16 (1.01, 4.61)	0.047
N2	3.20 (1.39, 7.37)	0.006
N3	5.66 (2.16, 14.86)	<0.001
Distant metastases		
M0	1.0 (ref)	
M1	3.34 (1.97, 5.66)	<0.001
AJCC tumor stage		
I	1.0 (ref)	
II	2.98 (0.58, 15.43)	0.192
III	5.21 (1.22, 22.29)	0.026
IV	11.26 (2.69, 47.22)	<0.001
Lauren classification		
Intestinal and mixed	1.0 (ref)	
Diffuse	1.30 (0.77, 2.19)	0.335
Ming classification		
Expanding	1.0 (ref)	
Infiltrative	3.20 (1.76, 5.83)	<0.001
Gastric carcinoma with lymphoid stroma		
No	1.0 (ref)	
Yes	0.24 (0.03, 1.76)	0.161
EBV status		
Negative	1.0 (ref)	
Positive	1.44 (0.52, 4.02)	0.482
MLH-1 status		
Positive (non-microsatellite instability high)	1.0 (ref)	
Negative (microsatellite instability high) [stage adjusted]	0.36 (0.13, 0.84)	0.021
	[0.36 (0.14–0.91)]	[0.032]

[1] Risk ratio represents a 10-year increase in age.

[2] Risk ratio represents a 1-unit increase in tumor-infiltrating lymphocytes on the natural logarithmic scale.

that show improved survival. In an analogous fashion, lymphoepithelioma-like carcinomas in other anatomic sites may represent the extreme end of a spectrum of tumors that show a host immune response, with the factors contributing to the induction of this response dependent on the anatomic site as well as geographic factors.

This study has demonstrated that an increasing tumor-infiltrating lymphocyte count in gastric carcinoma is significantly associated with an increasingly improved survival. EBV positivity and the microsatellite instability–high phenotype account for significant, but clinicopathologically distinct, subsets of lymphocyte-rich gastric cancer; however, these factors are not themselves independent prognosticators. Identification of molecular alterations common to these subsets of tumors that lead to the development of an effective host immune response may provide insight into gastric tumorigenesis and possibilities for future therapeutic strategies.
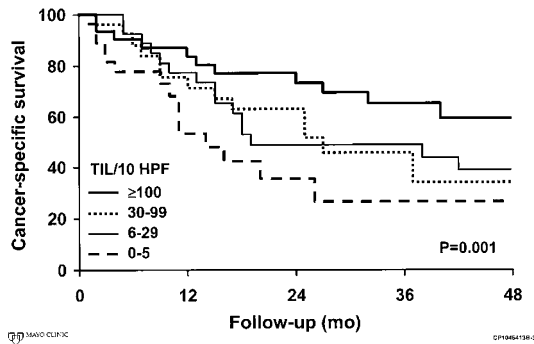

FIGURE 6. Kaplan-Meier survival curve showing survival differences between groups of gastric cancer cases categorized by tumor-infiltrating lymphocyte (TIL) count.
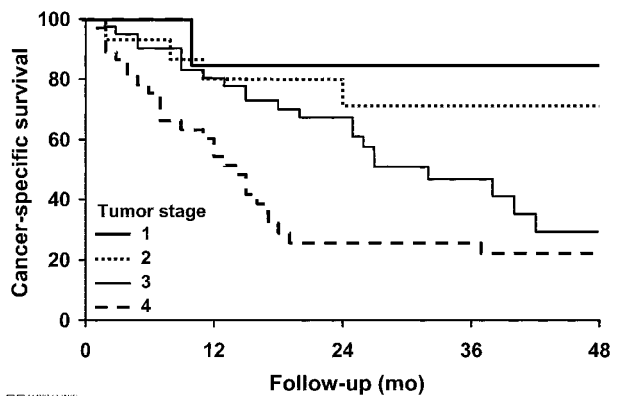
FIGURE 7. Kaplan-Meier survival curve showing cancer-specific survival by stage for 110 gastric cancer cases.

TABLE 5. Multivariate Associations with Death from Gastric Cancer

Feature	Risk Ratio (95% C.I.)	p-Value
Log of tumor-infiltrating lymphocytes	0.82 (0.71, 0.96)[1]	0.013
MLH-1 negative (microsatellite instability high)	0.47 (1.23, 0.18)	0.125

[1] Risk ratio represents a 1-unit increase in tumor-infiltrating lymphocytes on the natural logarithmic scale.

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