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Gastric carcinoma with lymphoid stroma

Analysis using mucin histochemistry and immunohistochemistry

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Summary. A total of 626 surgically resected gastric carcinomas were reviewed, and 24 cases (3.8%) of "gastric carcinoma with lymphoid stroma" were identified. The tumour cells were consistently arranged in an anastomosing trabecular or alveolar pattern and were densely infiltrated by lymphoid cells. The specimens were studied using mucin histochemistry and the indirect immunoperoxidase method to determine the histochemical properties of this form of gastric carcinoma. The tumour cells were consistently positive for concanavalin A paradoxical staining, class III and almost devoid of acidic mucins, features demonstrating preferential differentiation toward pyloric glands or pseudopyloric glands. Immunohistochemically, positive reactions for Leu M1 and lysozyme, marker substances of (pseudo)pyloric gland cells, were often observed. Carcinoembryonic antigen was positive in focal areas without (pseudo)pyloric glandular patterns. Secretory component was focally positive. HLA-DR was strongly expressed in most cancer cells and 17 tumours (71%) showed positivity for interleukin 1 (IL-1). The lymphoid stroma contained a high percentage of UCHL1-reactive T cells both within and around the cancer cell nests, while SL26-reactive B cells clustered in lymphoid follicles. A considerable number of T-lymphoid cells were also reactive for IL-1. A number of plasma cells with a predominance of IgG-type were distributed around the cancer cell nests. S-100 protein-positive dendritic cells were not identified. We speculate that the prominent lymphoid stroma including intraepithelial lymphocyte-like T cells with IL-1 receptors is possibly induced by IL-1 related mediators released from the HLA-DR-positive gastric cancer cells of the (pseudo)pyloric gland-type.

Key words: Gastric carcinoma – Lymphoid stroma – T lymphocytes – HLA-DR – Interleukin-1 – Mucin histochemistry – Immunohistochemistry

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Introduction

Inflammatory cell infiltration in tumour stroma, particularly lymphocytic, has long been recognized and considered to be a favorable prognostic factor in various neoplasms. Medullary carcinoma with lymphoid infiltration of the breast was recognized by Moore and Foote (1949) as having characteristic histological patterns. A similar type of carcinoma with extensive inflammatory cell infiltrates was first described in the stomach by MacCarty and Mahle (1921). Emphasizing the marked cellularity of these gastric tumours, Steiner et al. (1948) referred to them as "blue cell cancers", and later Hamazaki et al. (1968) termed them "medullary carcinomas with lymphoid infiltration". After detailed histological studies, Watanabe et al. (1976) used the term "gastric carcinoma with lymphoid stroma" and reported an incidence of 4%. Okamura et al. (1983) reported an incidence as 2.7% and emphasized the association of reactive lymphoid hyperplasia in regional lymph nodes. These investigators regarded tumours of this type as a pathological entity distinct from the common variety of gastric carcinomas. A statistically favorable prognosis of patients with gastric carcinoma of this subtype was noted in all of these studies.

The present study was undertaken to establish the phenotypic profile of gastric carcinoma with lymphoid stroma using mucin histochemical and immunohistochemical techniques. The biological significance of the lymphoid stroma is discussed.

Materials and methods

A total of 626 gastric carcinomas removed surgically at Tokai University Hospital during the period from 1983 to 1986 were reviewed microscopically. Twenty-four cases defined as gastric carcinoma with lymphoid stroma were selected on the basis of the histological criteria established by Watanabe et al. (1976) but further modified to include extensive small lymphocytic infiltrates within the cancer cell nests as a criteria. Cases with intramucosal cancer growth alone were excluded from the study due to difficulty in assessing the invasion. Sixteen invasive gas-

tric cancer specimens of common histological types (9 scirrhous, 2 mucinous, 4 adenoplastic, and 1 alpha-fetoprotein producing poorly differentiated carcinoma) were also examined for reference data.

Formalin-fixed and paraffin-embedded gastric specimens containing both benign and malignant tissues were selected for study. Sections of 4 μm in thickness were stained by the following methods:

1. Haematoxylin and eosin (H&E) staining was performed in combination with pretreatment by Victoria blue dye for simultaneous demonstration of the elastic lamina of vessels.
2. Grimelius' silver was employed for argyrophilia.
3. Mucin histochemistry included alcian blue-periodic acid-Schiff (AB-PAS), high iron diamine-alcian blue (HID-AB) and concanavalin A paradoxical staining (CPS), class III. AB-PAS differentiated neutral mucins from acidic mucins while HID-AB was used for the distinction between sialomucin (stained blue) and sulfomucin (stained black). CPS, class III was highly specific to mucins of pyloric glands, pseudopyloric glands, mucous neck cells and Brunner's glands. Detailed staining sequences were described by Katsuyama et al. (1985).
4. Immunohistochemical study was performed with the indirect immunoperoxidase method. Deparaffinized sections were dipped in methanol containing 0.3% hydrogen peroxide for 30 min in order to inactivate endogenous peroxidase. The time of antibody incubation and rinsing was 30 min. Diaminobenzidine coloration and 5% methyl green counterstaining were employed. The primary anti-human antibodies included Dako's rabbit antisera against carcinoembryonic antigen (CEA, 1:1000), lysozyme (1:200), secretory component (SC, 1:200), IgG (1:1000), IgA (1:1000), IgM (1:1000), S-100 protein (1:200) and factor VIII-related antigen (1:100). Anti-CEA was previously absorbed with a perchloric acid extract of human spleen as described previously (Tsutsumi et al. 1984a). Another rabbit antiserum included anti-human interleukin-1 (IL-1, 1:200, Genzyme, Boston, MA, USA) which recognized both alpha and beta forms of IL-1 with a predominance of beta form. Mouse monoclonals such as anti-Leu M1 for neutrophils and epithelial cells (1:100, Becton-Dickinson, Mountain View, CA, USA), anti-leukocyte common antigen (LCA) for total lymphocytes (1:10, Dako, Santa Barbara, CA, USA), UCHL1 for T cells (1:50, Dako), SL26 for B cells (1:500, Kyowa Medex, Tokyo, Japan), and LN-3 for the DR region of human leukocyte antigen (HLA-DR) (1:2, Techniclone International, Santa Ana, CA, USA) were also used. For the specificity of each monoclonal antibody, refer to Sheibani et al. (1986) for anti-Leu M1, Kurtin and Pinkus (1985) for anti-LCA, Norton et al. (1986) for UCHL1, Takami et al. (1985) for SL26, and Okon et al. (1985) for LN-3.

Horseradish peroxidase-labeled anti-rabbit and anti-mouse immunoglobulins were purchased from Dako and used at a dilution of 1:50. For negative control purposes, the primary antibody was replaced by normal rabbit or mouse serum diluted at 1:50.

Results

Among the 626 gastric carcinomas reviewed, 24 cases (3.8%) were identified as gastric carcinoma with lymphoid stroma. Seven tumours were submucosally invading ("sm") carcinomas which represented 7.0% of 100 "sm" carcinomas. The remaining 17 represented 4.5% of 380 advanced carcinomas in the present series. Intramucosal ("m") carcinomas, encountered in 146 cases (23.4%) were excluded from study. The clinicopathological features of the 24 patients are briefly summarized in Table 1.

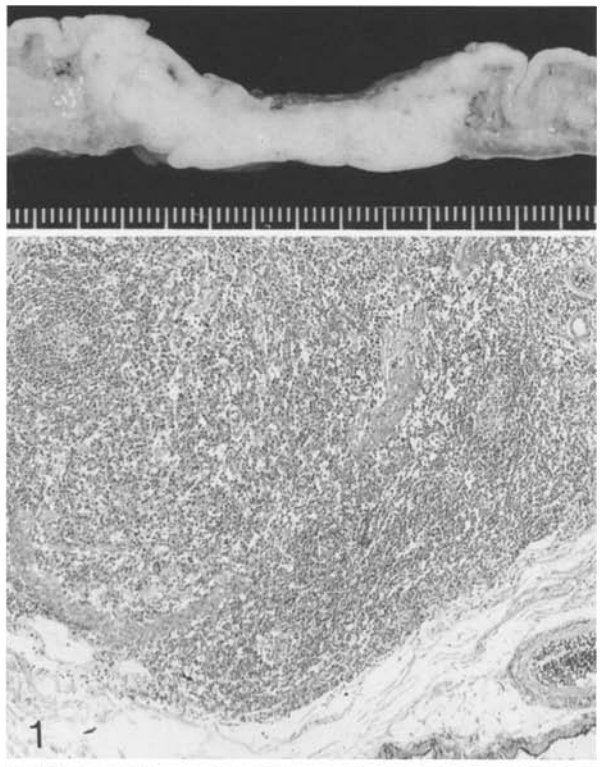
Table 1. Clinical summary of 24 "gastric carcinomas with lymphoid stroma"

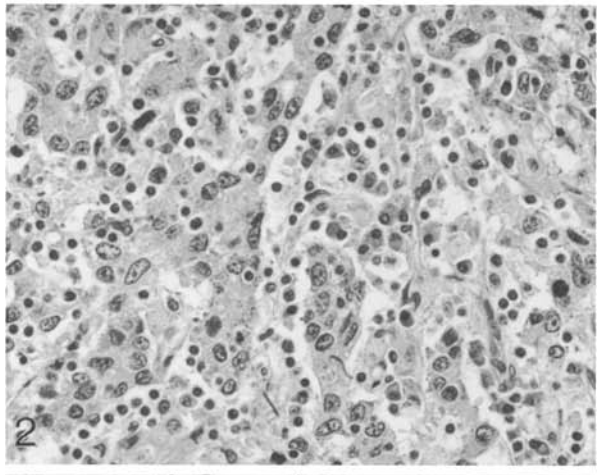
	Early "sm" carcinoma ($n=7$)	Advanced carcinoma ($n=17$)
Age (years old)		
Range	45–72	37–71
Mean	59.9	57.0
Sex		
Male:Female	6:1	13:4
Location		
Cardia :Midportion :Antrum	3:4:0	9:5:3
Macroscopic feature	I:IIc:IIc+III 1:4:2	IIcA:B1:B2:B3 [+] 1:1:7:8
Lymph node metastasis +:−	1:6	8:9
Postoperative course		
Alive:Dead :Unknown	3:1:3*	10:2:5**

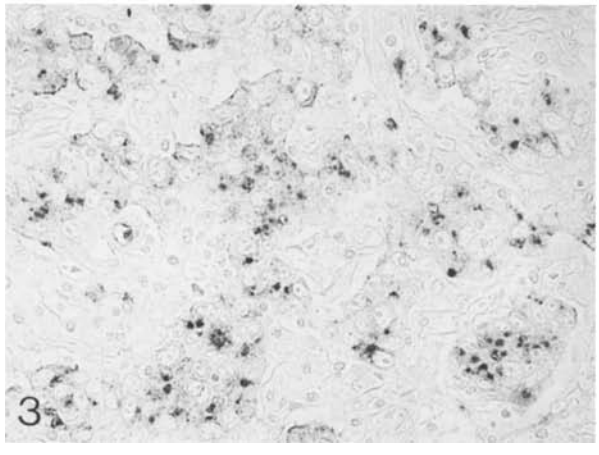
[+] IIcA: IIc advanced-type cancer. "B": Borrmann's classification
* mean follow-up period: 38.6 months
** mean follow-up period: 32.8 months

Grossly, most advanced tumours were categorized as type II or III after Borrmann's classification while "sm" carcinomas often exhibited shallow ulceration (type IIc predominated). Twenty-one cancerous lesions (87.5%) arose in the cardia and body of the stomach. The cut surfaces of the tumour were fleshy and grayish-white (Fig. 1a).

Histologically, the tumours were sharply demarcated from the surrounding submucosal tissue by virtue of their dense inflammatory cell infiltrates (Fig. 1b). There were a number of small lymphocytes and plasma cells in the stroma, occasionally forming lymphoid follicles with or without germinal centers. The consistent occurrence of small lymphocytes within the cancer cell nests, "intraepithelial lymphocyte (IEL)"-like lymphoid cells, was noted (Fig. 2). A varying degree of neutrophil infiltration was also observed in 11 tumours (marked in 1, moderate in 2, mild in 4, and none in 4), but histiocytoid cells were infrequently seen. There was almost no desmoplasia. Uniform-sized cancer cells tended to form irregularly anatomosing trabecular or alveolar structures. Glandular structures, generally abortive and small in size, were occasionally found. Argyrophilia was entirely







negative. On occasion, lymphatic and venous tumour invasion was noted and the involved venules were notably obliterated by the inflammatory stroma. Characteristically, no tumour necrosis was encountered. These histological features were present both in the mucosal tumour and the nodal metastasis. A varying degree of intestinal metaplasia was present in the surrounding noncancerous mucosa (severe in 4, moderate in 6, mild in 4, and none in 10).

The histological features of this subtype were quite consistent. However, some minor differences between individual cases were observed. These included foci of papillary adenocarcinoma or signet ring-celled patterns in the mucosa. In some instances, massive lymphoid infiltration obscured the presence of cancer cells, while in others cancer cells relatively predominated.

Tables 2 and 3 summarize results of both mucin histochemistry and immunohistochemistry. Small amounts of PAS-positive neutral mucins were detected in a number of neoplastic cells. HID-AB staining for acidic mucins yielded a fundamentally negative result. The acidic mucins were focally distributed along the apical surface of the gland lumen in 12 tumours (sulfomucin predominated in 8, and sialomucin predominated in 4). CPS, class-III, a specific stain for (pseudo)pyloric gland mucin, invariably showed a positive reaction in the cancer cell cytoplasm (Fig. 3). The cancer cell nests with alveolar structures were stained intensely while the abortive tubuloglandular portions were inclined to be less consistently positive. All of the tumours examined contained cells with pyloric gland-type mucin. More than a half (13) of the tumours were composed of (pseudo)pyloric gland-type cells, seven consisted of a moderate number of reactive cells and four displayed focal reactivity.

In the control gastric cancer group, the detection of CPS, class III mucin and the paucity of

Fig. 1. Representative case of "gastric carcinoma with lymphoid stroma". A well-demarcated invasion margin in the submucosa is noteworthy. The cancer cells are closely intermingled with a number of lymphoid cells, giving a blue-celled appearance. Formation of lymphoid follicles is also noted. × 40

Fig. 2. High magnification of gastric carcinoma with lymphoid stroma. The consistent occurrence of intraepithelial lymphocytes (IEL)-like lymphoid cells within the cancer cell nests is characteristic. × 300

Fig. 3. Concanavalin A paradoxical staining (CPS), class III a specific staining for pyloric gland-type mucin. Many tumour cells exhibit a positive reaction in the cytoplasm. × 300

Table 2. Histochemical data in 24 "gastric carcinomas with lymphoid stroma"

No.	Age/Sex	Cancer cell marker								Lymphoid cell marker							
		CPS III	HID -AB	SC	CEA	Leu M1	Lyso-zyme	LN-3	IL-1	LCA	UCHL1	SL26 *	IL-1	Ig			S-100 protein
														G	A	M	
Early carcinoma ("sm")																	
1	45 M	++	+	+	+	++	+++	+++	−	+++	+++	+	+	+++	+	+	−
2	49 M	+++	−	++	+	++	++	+++	−	+++	+++	++	++	++	+	+	−
3	56 M	+++	+	−	+	+++	++	+	++	+++	+++	+	++	++	+	+	−
4	60 M	++	−	+	++	+++	++	++	+	++	+++	+	+	+	+	+	−
5	67 M	+++	+	+	+	++	++	+++	+	++	+++	+	+	+	+	−	−
6	70 M	++	++	−	+	+++	++	+++	−	+++	+++	++	+	++	+	+	−
7	72 F	+++	−	−	++	+++	+	++	+	+++	+++	++	+	++	+	+	−
Advanced carcinoma																	
8	37 M	+++	−	−	−	+	+	++	++	+++	+++	+	++	++	+	+	−
9	40 M	+++	−	++	+	+++	+	+++	−	+++	+++	+	+	+++	+	+	−
10	44 F	+	−	−	−	+	−	++	−	++	++	−	+	+	+	−	−
11	51 M	+++	−	−	+	+++	++	+++	++	+++	+++	+++	+++	+++	++	+	−
12	53 F	+++	−	−	+	+++	++	+++	++	+++	+++	+	++	+++	+	+	−
13	53 M	+	−	+	+	+++	+	+++	+	+++	+++	+	++	++	+	+	−
14	56 M	+	+	+	++	−	−	+++	++	+++	+++	+	++	+++	+	+	−
15	57 M	+++	+	++	+	++	++	+++	+++	++	++	+	++	+++	+	+	−
16	59 M	++	+	+	+++	−	+++	++	++	++	+++	++	+++	+	+	+	−
17	62 M	+++	+	+	+	++	+++	++	−	+++	+++	++	+	+++	+	+	−
18	63 F	++	+	++	−	+	+	+++	++	++	++	+	++	++	+	+	−
19	63 M	+++	−	−	+	+++	+	+++	+	+++	+++	+	++	+++	+	+	−
20	64 M	++	++	+	+++	+	++	++	−	+++	+++	−	+++	++	+	+	−
21	65 M	++	−	−	+	+	+	++	+	+++	+++	+	+++	++	+	+	−
22	65 M	++	−	++	+++	+++	+++	+++	+++	+++	+++	−	+++	+++	++	+	−
23	66 M	+++	++	++	++	+++	+	++	++	++	+++	+	+++	++	+	+	−
24	71 F	+	+	+	++	−	−	+++	+++	+++	+++	−	+++	+++	+	+	−

For cancer cells markers: +++: More than two thirds of cells are positive. ++: A moderate number (1/10–2/3) of cells are positive. +: Less than one tenth of cells are positive. −: No cells are positive

For lymphoid cell markers other than SL-26: +++: Dense infiltration of cells is observed. ++: A moderate number of cells are scattered. +: Positive cells are sparsely distributed. −: Almost no positive cells are seen

For SL26 (*): indicating the number of SL26-reactive lymphoid follicles (LF). +++: 8 or more LF/low power field (LPF) are seen. ++: 3–7 LF/LPF are seen. +: 2 or less LF/LPF are seen. −: No LF is seen

Table 3. Summary of histochemical analysis using 24 "gastric carcinomas with lymphoid stroma"

Marker	−	+	++	+++[a]	Remarks
Cancer cells					
CPS, class III	0	4	8	12	CPS, class III, a marker of (pseudo)pyloric gland mucin is invariably positive.
HID-AB	12	9	3	0	Acidic mucins predominantly seen in metaplastic goblet cells are solely noted along the gland surface (sialomucin: sulfomucin = 4:8).
SC	9	9	6	0	SC-positive cancer cells are also stained for IgA and less frequently for IgM.
CEA	3	13	5	3	Normal foveolar cells and intestinal metaplastic cells are stained. The CEA-positive cancer area is often in sharp contrast to the CPS III-positive area.
Leu M1	3	5	5	11	Nonneoplastic (pseudo)pyloric glands and granulocytes are strongly positive.
Lysozyme	3	8	9	4	The intramucosal cancer lesions are often positive. Proliferative zone cells, (pseudo)pyloric glands, Paneth cells and granulocytes are also stained.
HLA-DR (LN-3)	0	1	9	14	Consistent expression of HLA-DR is in contrast to a negative result in most gastric carcinomas of common variety.
IL-1	7	6	8	3	The cytoplasm of the cancer cells is often positively stained for IL-1. Control gastric cancers rarely show IL-1 in cancer cells.
Lymphoid cells					
LCA	0	0	7	17	Both T and B cells in the stroma are positive.
T cell (UCHL1)	0	0	3	21	Intraepithelial lymphocyte (IEL)-like lymphoid cells are solely positive for the T cell marker (UCHL1). HLA-DR is negative in IEL-like T cells but presumably positive in some stromal T cells.
B cell (SL26)	4	14	5	1	Lymphoid follicles are main sites of positive staining. HLA-DR is also strongly expressed.
IL-1	0	8	9	7	A considerable percentage of T cells are positive for IL-1. IL-1-positive histiocytic cells are a few in number in the tumour stroma but prominent in the lamina propria mucosae.
IgG	0	4	10	10	Immunoglobulin-positive plasma cells are distributed around the cancer cell nests. IgG plasma cells predominate over IgA and IgM cells. The plasma cells are negative for the markers of T and B cells, HLA-DR and IL-1.
IgA	0	22	2	0	
IgM	2	22	0	0	
S-100 protein	24	0	0	0	Langerhans' type-dendritic cells are hardly seen in the tumour stroma. Autonomic nerve fibers are positive.

[a] See the footnote of Table 2

acidic mucins were not necessarily evident. Cancer cells with (pseudo)pyloric gland phenotypes were usually not predominant.

The results of immunohistochemistry are given in Tables 2 and 3. The cancer cells revealed certain characteristics similar to normal pyloric or pseudopyloric glands. The latter were often identified in atrophic fundic mucosa. In 21 instances, variable but usually extensive positivity for Leu M1 and lysozyme was noted in tumour cells (Fig. 4) as well as in (pseudo)pyloric glands and stromal neutrophils. Leu M1 was only faintly demonstrated in the foveolar cells, and lysozyme was further positive in cells at the proliferative zone as well as in Paneth cells in areas of intestinal metaplasia. Twenty-one tumours demonstrated CEA-positive areas, which were often focally distributed and in sharp contrast to the CPS, class III- or, though less remarkably, lysozyme-positive areas (Fig. 5). CEA was localized in the foveolar cells of the nor-

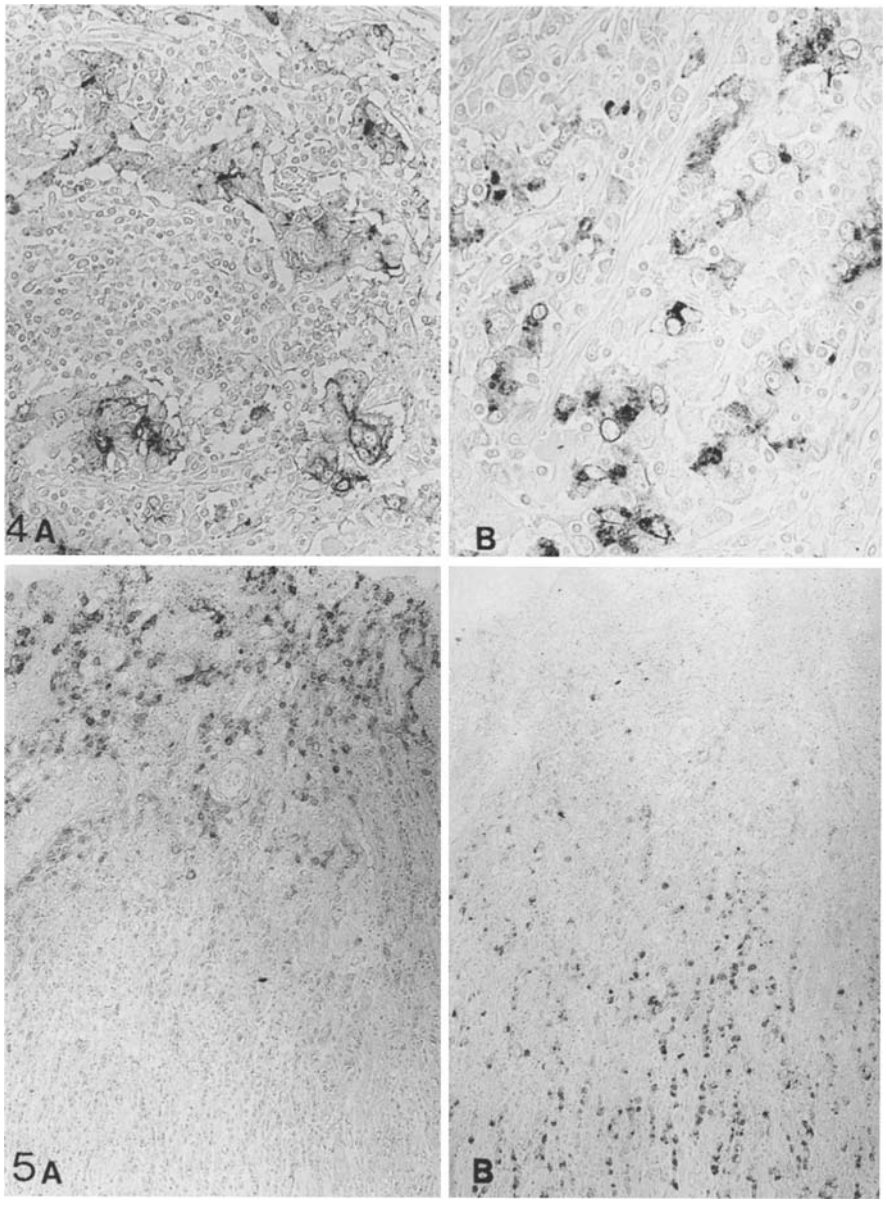
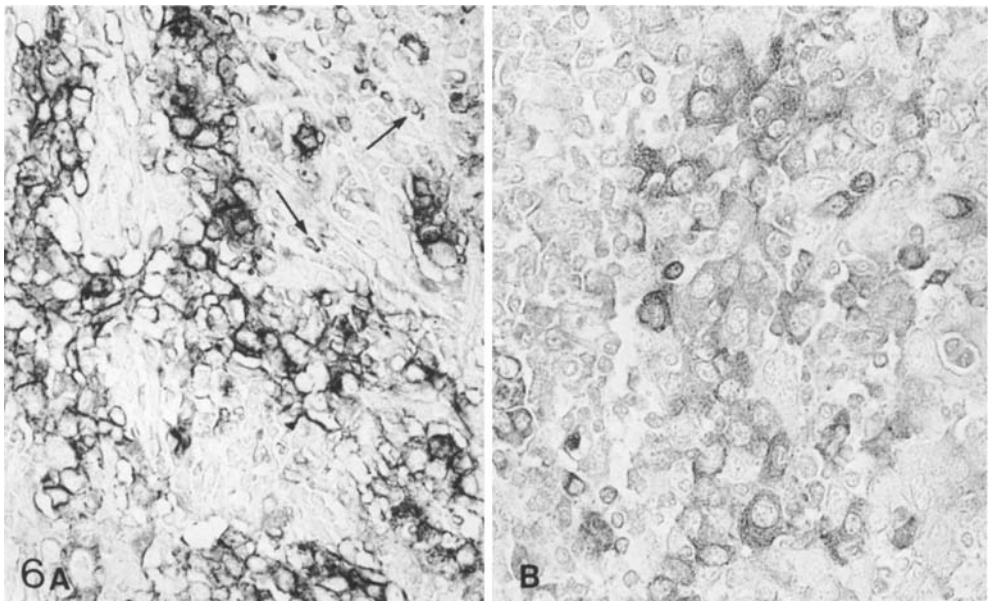


Fig. 4a, b. Immunoperoxidase staining for Leu M1 (**a**) and lysozyme (**b**). The cancer cells are often positive for such markers of (pseudo)pyloric gland cells. (**a**) ×200; (**b**) ×300

Fig. 5a, b. Reciprocal staining pattern of CEA (**a**) and CPS, class III (**b**). CEA immunoreactive cancer cells are located in the uppermost part of the mucosa where CPS, class III is negative, and vice versa. ×75

mal gastric mucosa but not in the (pseudo)pyloric glands. In addition, 15 neoplasms showed a focally positive reaction for SC which was strongly expressed in areas of intestinal metaplasia. The SC-positive cells were also stained consistently with anti-IgA and less frequently with anti-IgM. Furthermore, in every case the cancer cells were decorated with monoclonal antibody LN-3, a probe for HLA-DR. Fourteen tumours (58%) consisted predominantly of HLA-DR-positive cancer cells (Fig. 6a). Portions with abortive glandular structures, however, tended to be poorly stained. HLA-DR was often expressed in pseudopyloric glands, parietal cells and neck cells in the inflamed oxyntic mucosa. The foveolar cells and atrophic pyloric glands in the antral mucosa were occasionally positive for HLA-DR as well. Intestinalized epithelia were usually HLA-DR-negative or only focally positive in the uppermost mucosa. IL-1 immunoreactivity was further demonstrated in the cancer cell cytoplasm in 17 instances (71%) (Fig. 6b). Three tumours were largely composed of IL-1-positive cancer cells, which also demonstrated diffuse HLA-DR immunoreactivity. In contrast, non-neoplastic epithelial components including intestinal metaplastic cells were almost negative for IL-1.

Constant properties of stromal lymphoid cells were also evident. Small lymphocytes were largely



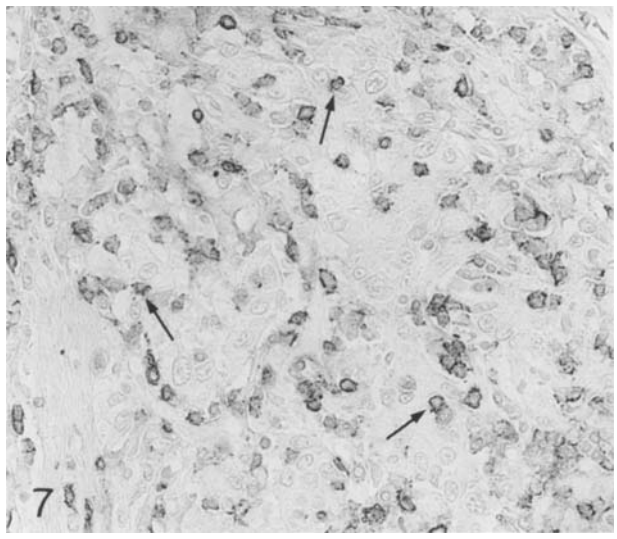


Fig. 6a, b. Immunoperoxidase staining for HLA-DR (**a**) and interleukin-1 (**b**). Strongly positive reaction of the cancer cells was observed with monoclonal antibody LN-3. Isolated HLA-DR-positive cells, possibly some "activated" T cells, are also seen around the cancer cell nests (*arrows*). A considerable percentage of the tumour cells and infiltrating lymphoid cells are positively stained for IL-1. × 300

Fig. 7. Immunoperoxidase staining for T cells with monoclonal antibody UCHL1. Numbers of IEL-like T cells are shown among the cancer cells (*arrows*). T cells are also distributed around the cancer cell nests. × 300

positive for a T cell marker (UCHL1). IEL-like T cells were easily detected among the cancer cells and a good number of T cells were also identified around the cancer cell nests (Fig. 7). Neutrophils were not stained with UCHL1 under the conditions examined, but showed a clear staining for Leu M1 and lysozyme. HLA-DR was negative in the IEL-like T cells. B cell marker (SL26)-positive lymphocytes mainly clustered in lymphoid follicles, in which a few T cells were distributed (Fig. 8). The lymphoid follicles were usually not so plentiful. Anti-LCA monoclonal recognized both T and B cells, but the number of UCHL1-reactive cells was occasionally more than that of LCA-positive cells. The lymphocytes in lymphoid follicles, irrespective of the presence or absence of germinal centers, were further clearly positive for HLA-DR. The detection of SL26-reactive B cells dispersed in the stroma was very exceptional, but non-clustered small lymphoid cells with immunoreactive HLA-DR (probably some of the "activated" T cells) were discernible (Fig. 6a). Scattered stellate-shaped cells ("interstitial cells") in the noncancerous tissue as well as factor VIII-related antigen-positive endothelial cells were also strongly stained for HLA-DR. High endothelial venules as defined by Duijvestijn et al. (1988) expressing factor VIII-related antigen were occasionally distributed a-

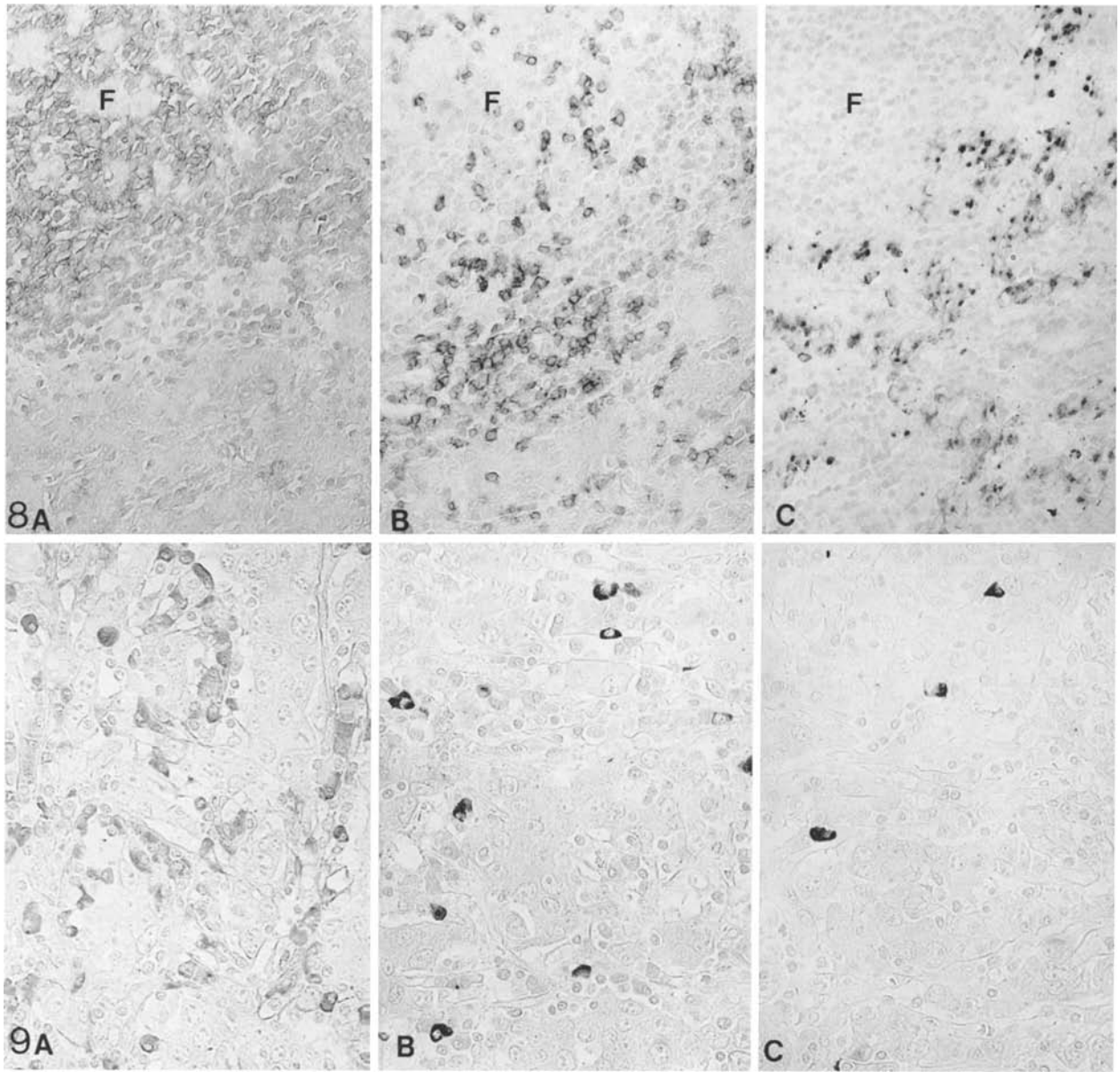


Fig. 8a–c. Adjacent sections immunostained for B cells with SL26 (**a**), and for T cells with UCHL1 (**b**). (**c**) CPS, class III. Lymphoid follicle (F) is mainly composed of B cells. T cells are scattered among CPS, class III-positive tumour cells. A few T cells are also noted in the lymphoid follicle. × 240

Fig. 9a–c. Immunoperoxidase staining for IgG (**a**), IgA (**b**) and IgM (**c**). IgG-positive plasma cells outnumber IgA- and IgM-positive cells. × 300

round the lymphoid follicles. In the tumour stroma, S-100 protein-positive dendritic cells were not identified and only a few histiocytic cells were positive for lysozyme. The tissue of every tumour exhibited varying numbers of IL-1-positive mononuclear cells both within and around the cancer cell nests, irrespective of IL-1 positivity in the tumour cells (Fig. 6b). The lymphoid follicles were negative for IL-1. Although IL-1 was strongly expressed in a few histiocytic cells and intravascular monocytic cells, the distribution and morphology indicated that a considerable proportion of the IL-1-positive mononuclear cells were T cells. More lymphoid cells were positive for IL-1 in advanced tumours than in early "sm" tumours. In non-neoplastic mucosa, IELs and stromal mononuclear cells were also clearly positive for IL-1. The IL-1-positive intramucosal histiocytic cells were more

prominent than those in the tumour stroma. Endothelial cells were also darkly stained for IL-1. A number of immunoglobulin-bearing plasma cells were demonstrated around the cancer cell nests. IgG-positive plasma cells outnumbered cells positive for IgA and IgM (Fig. 9). The plasma cells lacked reactivity against anti-LCA, SL26, LN-3 and anti-IL-1.

Control gastric carcinomas of common types showed prominent CEA expression and a varying degree of positivity for Leu M1, lysozyme and SC. HLA-DR was hardly detected except for two intramucosal lesions of scirrhous carcinoma. The demonstration of IL-1 immunoreactivity in cancer cells was also exceptional in the control group: some infiltrating cancer cells in one of 9 scirrhous tumours showed cytoplasmic IL-1 staining and submucosal cancer cells in one of 4 adenoplastic tumours displayed a supranuclear staining pattern. Stromal lymphoid cell infiltrates were inconspicuous in the control group. A few lymphoid follicles were occasionally scattered in or around the tumour tissue. Only the IL-1-positive scirrhous tumour showed rather dense IL-1-positive T cell infiltrates in the stroma, but IEL-like T cells were seldom seen. A small number of IEL-like T cells and S-100 protein-positive dendritic cells were observed in adenoplastic carcinomas, which also revealed a mild to moderate degree of neutrophilic infiltration.

All sections incubated with normal animal serum failed to stain.

Discussion

The twenty-four tumours were essentially consistent with gastric carcinomas with lymphoid stroma as defined by Watanabe et al. (1976). They demonstrated consistent histological and histochemical features justifying their categorization as a specific subtype of gastric cancer. The cancer cells displayed a distinct tendency of differentiation toward (pseudo)pyloric glands; they produced pyloric gland-specific neutral mucins and often gave a positive immunoreaction to Leu M1 and lysozyme. Moreover, the invariable expression of HLA-DR was worthy of note. HLA-DR was detected in non-neoplastic gastric epithelium including gastric glands in mucosa with chronic gastritis, as reported by Spencer et al. (1986). CEA, which is present in normal foveolar cells and absent from pyloric glands (Tsutsumi et al. 1984a; Tsutsumi 1988), was expressed only in a small number of tumour cells in areas of (pseudo)pyloric gland differentiation. A reciprocal staining pattern was often noted between CEA and CPS, class III or, less consistently, lysozyme. Similar phenomena were shown by focal expression of SC in the cancer tissue, predominantly differentiating toward (pseudo)pyloric glands, in which SC was normally undetectable (Tsutsumi et al. 1984b). Multidirectional differentiation has been shown in gastric carcinomas, and the consistency of the histochemical phenotypes in this variety of gastric carcinoma becomes remarkable when one compares it with the wide variety of histochemical patterns seen in gastric carcinomas of common types (Tsutsumi 1988).

The extensive and diffuse small lymphocytic infiltrates which predominated in the tumour were of T cell origin, whereas the lymphoid follicles were mainly composed of B cells. The dispersed plasma cells were predominantly IgG in type. Histiocytic cells, which have previously been reported to correlate with tumour spread in gastric cancer (Heidl et al. 1987), were infrequently noted in the tumour stroma. The virtual absence of S-100 protein-positive Langerhans' type dendritic cells failed to support the previous report of this cell type as an indicator of prognosis in certain stages of gastric cancer (Tsujitani et al. 1987). The occurrence of IEL-like T cells inside the cancer cell nests without associated tissue necrosis was worthy of notice. The roles of IELs in the gastrointestinal mucosa are well known and characterized (Douglas and Weetman 1975). Virtually all of the IELs are categorized as T cells predominantly with a suppressor phenotype (Cerf-Bensussan et al. 1983). Cerf-Bensussan et al. (1984) have recently demonstrated that isolated rat IELs secrete a factor (probably γ interferon) capable of inducing Ia antigen (HLA-DR in man) expression by an intestinal epithelial cell line, IEC 17. It has been shown that the number of IELs is increased in inflammatory bowel diseases (Hirata et al. 1986), in which epithelial cells converted to express HLA-DR (McDonald and Jewell 1987). These data suggest that IELs, which have been shown to be negative for HLA-DR (Nakamura et al. 1988), are involved in modulating some epithelial cell functions. The expression of HLA-DR by (pseudo)pyloric gland-type cancer cells in this particular entity may be related to the occurrence of HLA-DR-negative IEL-like T cells within the cancer cell nests.

However, the existence of epithelial cells is thought to be an indispensable factor for homing of lymphocytes which play significant roles in local secretory immunity in the normal gut (Nagura and Sumi 1987): some humoral factors produced by the epithelial cells may cause the lymphocytic accumulation. High endothelial venules (Duijivestijn et al. 1988), occasionally discerned in the cancer

stroma, may be the re-circulation point for T cells. It has been elucidated that in the small intestine, Ia-antigen-positive epithelial cells function as antigen-presenting cells for IELs (Bland and Warren 1986).

The immunohistochemical demonstration of IL-1, which is an important regulator of inflammation and tissue regeneration and is secreted by activated macrophages (Schultz 1987), in a considerable percentage of the HLA-DR-positive cancer cells and of T cells, including IELs, is suggestive of interactions between these two major components in this gastric cancer subtype. IL-1-positivity in T cells probably reflects the presence of IL-1 receptors, which are expressed on activated T cells (Schultz 1987). There is a possibility of either production of IL-1 or expression of IL-1 receptors by the cancer cells. So far, the presence of immunoreactive IL-1 in the HLA-DR-positive epithelial cells has been demonstrated in the inflammatory lesions (Kyogoku et al. 1987; Nagura, personal communication) while the presence of receptors for IL-1 has also been shown not only on mononuclear cell lines but also on epithelial cell lines (Dower and Urdal 1987). We hypothesize here that the specialized HLA-DR-positive cancer cells produce IL-1 or immunologically IL-1 related mediators for colonization of T cells.

In the gastric mucosa, chronic inflammatory processes are associated with infiltrates in both the lamina propria and epithelial cells (Isaacson 1982; Tsutsumi et al. 1984b; Tsutsumi 1986). IgG plasma cells are preferentially distributed in the inflamed nonmetaplastic gastric mucosa, while normal or intestinalized mucosa contains abundant IgA plasma cells but few IgG type (Tsutsumi et al. 1984b; Tsutsumi 1986). Lymphoid follicles are often formed in the lower part of the nonmetaplastic mucosa, especially in areas of pyloric or pseudopyloric glandular differentiation. Immunohistochemically, SC becomes weakly positive in epithelial cells at the proliferative zone. HLA-DR was reported to be expressed by gastric epithelial cells in chronic gastritis (Spencer et al. 1986), as has been confirmed in the present study.

Epithelial-stromal interactions (van den Hooff 1984; Fukamachi 1986) make it possible that the inflammatory cell infiltrate in this subset of gastric cancer may be due to immunological mechanisms similar to those observed in the benign mucosa with nonmetaplastic gastritis. The neoplastic cells predominantly differentiate toward (pseudo)pyloric glands with HLA-DR expression. Direct effects of the functioning cancer cells may induce the lymphoid stroma, consisting of IEL-like T cells, scattered stromal T cells, B cell-dominant lymphoid follicles, and plasma cells mainly of the IgG-type. We suppose that the lymphoid cell reaction itself is not necessarily indicative of anti-tumour immunity. The absence of both tumour necrosis and tumour regression, the expression of consistent histological and histochemical properties by the cancer cells and the close association of lymphocytes even in metastatic lesions may support the above assumption. Similar correlations between tumour cell morphology and inflammatory infiltrates has been seen in granulocyte-colony stimulating factor-producing "anaplastic" carcinomas with marked neutrophilic infiltration (Tamaoki 1985). The favorable prognosis of patients may be reflected by innate characteristics of low-grade malignant neoplastic cells in this special entity.

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