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

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-
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 coloring and 5% methyl green counterstaining were employed. The primary anti-human antibodies included Dako's rabbit antisera against carcinoembrionic 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, Kyoto Medex, Tokyo, Japan), and LN-3 for the DR region of human leukocyte antigen (HLA-DR) (1:2, Technicleone International, Santa Ana, CA, USA) were also used. For the specificity of each monoclonal antibody, refer to Shebani 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.

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...