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Immune aspects of intestinal metaplasia of the stomach: an immunohistochemical study

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Summary. Immune characteristics of intestinal metaplasia of the stomach were analyzed by the immunoperoxidase technique in frozen and paraffin-embedded specimens. In fetal and minimally inflamed adult gastric mucosa, secretory component (SC) was absent from epithelial cells. Non-intestinalized gastric mucosa with evident inflammatory changes showed weak SC immunoreactivity at the generative cell zone. Enhanced immunoreactivity of SC with evidence of transepithelial transport of IgA and IgM, but not of IgG, was demonstrated in intestinalized glands of either the complete or incomplete type. The number of inflammatory cells and lymphoid follicles was decreased in intestinalized mucosa when compared with that in non-intestinalized gastritic mucosa; J chain-negative IgG plasma cells and T cells, both of which were fairly abundant in the latter mucosa, were remarkably decreased in the former mucosa, whereas the decrease of J chain-positive IgA or IgM plasma cells was slight or equivocal. In either mucosa, IgA was the most popular immunoglobulin class in plasma cells. IgD plasma cells were very rare. In the germinal centers of lymphoid follicles which were preferentially distributed in non-intestinalized gastritic mucosa, IgM or IgG germinocytes predominated over IgA germinocytes, and a few T cells and NK cells also were present. Intraepithelial lymphoid cells with a T-suppressor phenotype were detected in intestinalized glands. The possibility that intestinal metaplasia is an adaptation to long-standing chronic gastritis is discussed.

Key words: Chronic gastritis – Intestinal metaplasia – Secretory immunity – Immunoperoxidase method

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Several functional aspects of intestinal metaplasia of the stomach have been investigated to date, including the absorption of dye (Suzuki et al. 1973) and fat (Rubin et al. 1967), the appearance of intestinal marker enzymes (Abe et al. 1974; Matsukura et al. 1980), the depletion of blood group substances (Kapadia et al. 1981) and changes in mucin (Matsukura et al. 1980; Nardelli et al. 1983), endocrine cells (Bordi and Ravazzola 1979; Tsutsumi et al. 1983a; 1983b), cell kinetics (Hattori and Fujita 1979; Hashimoto et al.) and immunological status (Brus et al. 1968; Yamagiwa 1977; Ohta et al. 1979; Hasegawa et al. 1980; Wada et al. 1981; Morise 1982; Isaacson 1982; Nagura et al. 1983). Furthermore, intestinal metaplasia has been subdivided into two types, complete and incomplete. The complete type is characterized by the total expression of intestinal marker enzymes, the absence of sulfomucin-containing goblet cells and the presence of Paneth cells as in the mucosa of normal small intestine; the incomplete type is categorized by the partial expression of intestinal marker enzymes, the appearance of sulfomucin in goblet cells and the absence of Paneth cells (Abe et al. 1974; Matsukura et al. 1980). We have recently proposed a new subtyping of intestinal metaplasia based on the degree of pyloric gland involution (Tsutsumi et al. 1983b); this subtyping reflects cell kinetics in the intestinalized mucosa (Tsutsumi et al. 1983b).

Currently, an intimate relationship between intestinal metaplasia and gastric cancer is stressed (Nakamura et al. 1968; Imai et al. 1971; Matsukura et al. 1980; Isaacson 1982; Sugano et al. 1982). On the other hand, approaches from an immunologic standpoint have not clarified the pathophysiological significance of the immune status of the gastric mucosa with intestinal metaplasia. In this article, we present immunohistochemical observations on the local immune response in chronically inflamed gastric mucosa with or without intestinal metaplasia, and show the change of the immune status in intestinal metaplasia.

Materials and methods

Specimens of 3–4 cm length were taken from the antropyloric portion of 16 surgically resected stomachs (cancer, 8 cases; peptic ulcer, 7 cases; adenoma, 1 case; mean age: 55.1). Parts of the tissues were fixed in 10% formalin for 1–7 days and prepared for 4 μm paraffin sections. Each section contained a part of the duodenum. Other parts of the specimens were fixed in 4% buffered paraformaldehyde, pH 7.2, or periodate-lysine-4% paraformaldehyde at 4° C overnight. After fixing, these parts were washed in 0.01 M phosphate-buffered saline (PBS), pH 7.2, containing 10–20% sucrose, embedded in OCT-compound (Lab-Tek Products, Naperville, IL, USA), frozen quickly in dry-ice ethanol, and sectioned at 6 μm thickness on a cryostat.

As "normal" controls, six paraffin-embedded adult antral mucosae showing scanty evidence of inflammation were selected from the file of thousands of gastric biopsy specimens at Tokai University Hospital (age: 28–76; mean 43.2). In addition, antral mucosae from four fetuses at 22, 27 and 37 gestational weeks were also studied.

For the identification of intestinal metaplasia, high iron diamine-Alcian blue (HID-AB) staining after Spicer (1965) was used in addition to haematoxylin and eosin (H&E) staining. Pyloric gland mucin was specifically demonstrated by the paradoxical Concanavalin A staining after Katsuyama and Spicer (1978).

Immunohistochemical staining was performed by the indirect immunoperoxidase technique after Nakane (1975). Immunoglobulins and secretory component (SC) were detected in paraffin

sections. Rabbit antibodies specific for γ, α, μ and δ chains of human immunoglobulins were purchased from DAKO Immunoglobulins, Copenhagen, Denmark. Rabbit antiserum against SC, which was incubated with 1% normal human serum to absorb a minor anti-α chain contaminant, was obtained from Behringerwerke, Marburg, West Germany. Anti-J chain rabbit serum was kindly provided by Dr. Kunihiko Kobayashi, Department of Pediatrics, Yamaguchi University School of Medicine, Ube, Japan. These antisera at a 1:100 or 1:200 dilution were incubated on the sections at room temperature for 30 min. For negative control purposes, 1:100 diluted nonimmune rabbit serum was used. The second layer reagent was horseradish peroxidase (HRP)-labeled anti-rabbit IgG Fab fragments of goat IgG (prepared in our laboratory), incubation time of which was 30 min.

For the detection of lymphocyte surface antigens, cryostat sections were incubated at 4° C overnight with 1:100 diluted mouse monoclonal anti-human antibodies: anti-Leu 1, anti-Leu 2a, anti-Leu 3a, anti-Leu 4, anti-Leu 7 (Becton-Dickinson & Co, Sunnyvale, CA, USA) and anti-OKIa 1 (Ortho Diagnostic Systems, Raritan, NJ, USA). Leu 1 and Leu 4 are generalized markers for T cells (Ledbetter et al. 1981). Leu 2a and Leu 3a antibodies detect suppressor/cytotoxic T cells and helper/inducer T cells, respectively (Ledbetter et al. 1981). Leu 7 antigen is expressed on natural killer (NK) and killer (K) cells (Abo and Balch 1981). Ia-like antigen detected by anti-OKIa 1 antibody is present on B cells, macrophages and some activated T cells (Beckman et al. 1980). Negative controls were cryostat sections incubated with nonimmune mouse serum at a 1:100 dilution. HRP-labeled anti-mouse polyvalent immunoglobulins $F(ab)'_2$ fragments of rabbit IgG (prepared in our laboratory) were used as a second layer reagent with overnight incubation.

Rinsing of the sections was performed with 0.01 M PBS, pH 7.2, for 30 min in each step. Endogenous peroxidase activity was inactivated both by dipping deparaffinized or frozen sections in 0.5% periodic acid for 10 min and by adding 0.01 M sodium azide in diaminobenzidine (DAB) solution which contained 30 mg% 3,3′-DAB tetrahydrochloride (Wako Pure Chemical Industries, Osaka, Japan) and 0.01 M hydrogen peroxide in 0.05 M Tris-HCl buffer, pH 7.6. Nuclei were counterstained with 1% methyl green, pH 4.0. For the demonstration of different antigens in the same cells, the mirror sectioning technique after Osamura et al. (1980) was used in representative cases.

Results

All 16 gastric mucosae removed at surgery for gastric lesions showed various degrees of chronic inflammation. Seven specimens revealed severe intestinalization, three moderate and four slight. The remaining two specimens lacked intestinal metaplasia. Intestinal metaplasia was characterized by the appearance of goblet cells containing sialomucin or sulfomucin stained blue or black by HID-AB staining, respectively. Some intestinalized glands showed involuting pyloric glands at their bases, which were clearly demonstrated by paradoxical Concanavalin A staining (Fig. 1a). The severity of pyloric gland involution in the intestinalized area was well correlated with the appearance of sulfomucin-containing goblet cells and Paneth cells as reported previously (Tsutsumi et al. 1983b). Thus, sulfomucin was often noted in goblet cells in intestinalized mucosa with remaining pyloric glands (the incomplete type of intestinal metaplasia), while Paneth cells were frequently present in intestinalized glands without remaining pyloric gland cells (the complete type of intestinal metaplasia).

Immunohistochemically, intestinalized glands revealed a strong immunoreactivity of SC consistently in Golgi areas and frequently in apical cytoplasm at the light microscopic level, irrespective of the degree of pyloric gland involution (Fig. 1b). No difference in the staining intensity for SC

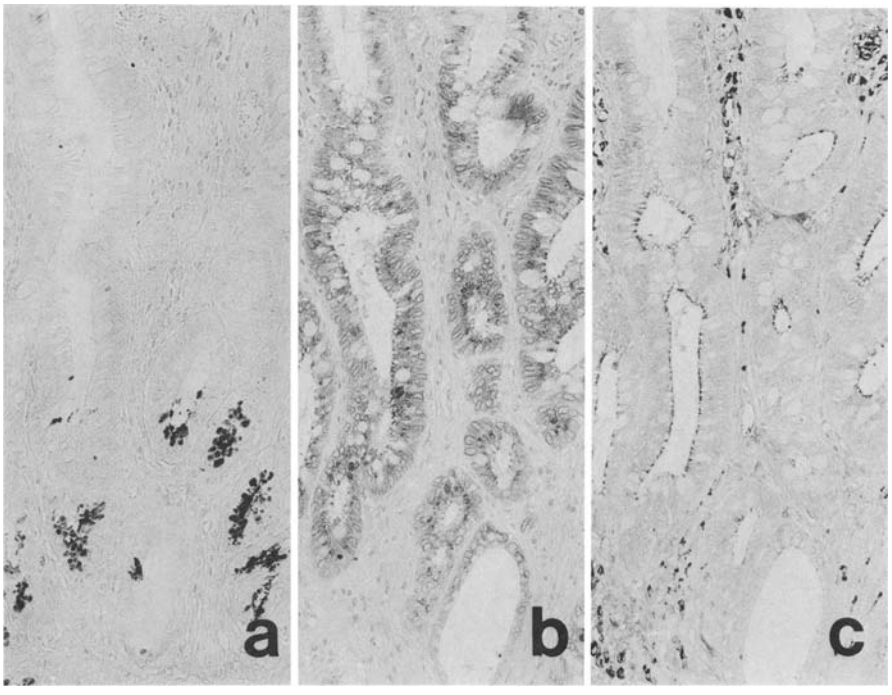

Fig. 1a–c. Incomplete type of intestinal metaplasia with remaining pyloric gland cells. **a** Paradoxical Concanavalin A staining for pyloric gland mucin. **b** and **c** Indirect immunoperoxidase staining for SC and IgA, respectively. ×150. Intestinalized gland cells, regardless of their degree of maturation, show an invariably strong immunoreactivity of SC mainly at the Golgi areas and luminal border with evidence of the transepithelial IgA transport. IgA immunoreactivity is noted at the luminal border of the intestinalized cells as well in plasma cells in the lamina propria

was observed between the complete and incomplete types of intestinal metaplasia. In some intestinalized glands, SC was more strongly stained in the lower part of the glands than in the upper part, as was observed in normal duodenal mucosa; other intestinalized glands showed a strong SC immunoreactivity throughout the mucosa without such a polarity. Goblet cells were invariably negative for SC. The surrounding non-intestinalized areas with various degrees of inflammation showed a weak but consistent SC immunoreactivity mainly in the glandular neck region (around the generative cell zone). The difference in intensity of the SC staining between intestinalized glands and non-intestinalized glands was evident. IgA, J chain and, less consistently, IgM were detected in the intestinalized gland cells mainly in the apical cytoplasm, while IgG was not detected (Fig. 1c). Weak staining for IgA, IgM and J chain also was noted frequently in epithelial cells in non-intestinalized gastritic mucosa. The staining intensity of these secretory immunoglobulins and J chain in the epithelial cells was roughly parallel to that of SC.

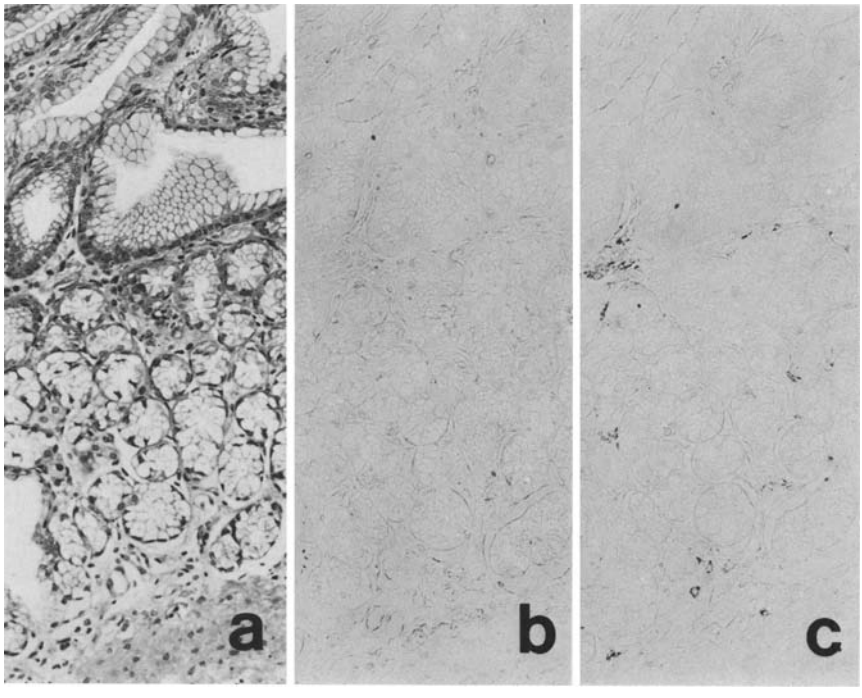

Fig. 2a–c. Minimally inflamed antral mucosa obtained at biopsy from a 35-year-old female. H&E (**a**); indirect immunoperoxidase staining for SC (**b**) and IgA (**c**). ×150. In "normal" gastric mucosa, there are a few IgA plasma cells in the lamina propria, and SC immunoreactivity is absent from epithellial cells

Minimally inflamed adult antral mucosae from control adult samples and fetal antral mucosae showed no staining for SC or IgA in epithelial cells (Fig. 2). Exception included one adult antral mucosa whose neck cells were faintly positive for SC.

Histological changes in inflammatory cells in the gastric mucosa were very characteristic. Inflammatory cell infiltration in the lamina propria was far less conspicuous in many of the intestinalized areas in comparison with that in the non-intestinalized gastritic mucosa where lymphoid follicles were preferentially distributed (Fig. 3). The extent of the infiltration was not correlated with the severity of pyloric gland involution in the intestinalized mucosa. Table 1 summarizes the appearance of lymphoid follicles in 16 surgically removed gastric mucosae examined. Lymphoid follicles were found about six times more frequent in non-intestinalized portions of the mucosa with evident inflammation than in intestinalized portions of the mucosa. The mean number of lymphoid follicles per section was decreased in the order non-intestinalized > slightly intestinalized > moderately intestinalized > severely intestinalized stomachs. Even in the severely intestinalized mucosa, lymphoid follicles were fairly frequently distributed in barely remaining non-intestinalized portions of the mucosa. In control "normal"

Table 1. Relationship between lymphoid follicles (*LF*) and intestinal metaplasia (*IM*) in chronically inflamed antral mucosa

Degree of IM	No. of Sections Examined	No. of LF in Gastritic mucosa with or without IM			LF
		non-IM	IM	non-IM/IM	Total
Severe	7	13 (1.9)	14 (2.0)	2 (0.3)	29 (4.1)
Moderate	3	12 (4.0)	2 (0.7)	2 (0.7)	16 (5.3)
Slight	4	36 (9.0)	1 (0.3)	4 (1.0)	41 (10.3)
None	2	34 (17.0)	–	–	34 (17.0)
Total	16	95 (5.9)	17 (1.1)	8 (0.5)	120 (7.5)

non-IM: Portions of gastric mucosa without IM; IM: Portions of gastric mucosa with IM; non-IM/IM: Junctional zones of the above two; ( ): Mean number/section (3–4 cm in length)

samples, there were very few monunuclear infiltrates in the lamina propria, and no lymphoid follicles were seen. Atrophy of the pyloric glands, a consistent finding in evidently inflamed mucosa, was hardly observed in these samples. In fetal stomachs, mononuclear cells were almost entirely absent from the mucosa.

Immunohistochemically, IgA-containing plasma cells predominated over IgM- or IgG-containing plasma cells in surgical specimens showing chronic gastritis with or without intestinalization. IgA plasma cells were most abundant in the upper part (superficial layer) of the gastric mucosa, whereas IgG or IgM plasma cells tended to be distributed in the middle part of the mucosa. IgD plasma cells were very rare or absent anywhere in the specimens examined. In the non-intestinalized mucosa with chronic inflammation, the ratio of IgA:IgM:IgG detected in plasma cells was roughly constant irrespective of degrees of inflammation. The immunohistochemical study confirmed the sparsity of immunoglobulin-containing plasma cells in mimimally inflamed adult antral mucosa: J chain-positive IgA plasma cells were infrequently scattered, IgM plasma cells were rare and IgG plasma cells were practically absent. There were no immunohistochemically detectable plasma cells in fetal antral mucosa. A qualitative change of immunoglobulin-containing plasma cells in the intestinalized mucosa was very characteristic. When compared with the surrounding non-intestinalized mucosa with chronic inflammation, intestinalized mucosa very often showed a dramatic decrease of IgG plasma cells, while the reduction in the number of IgA plasma cells was slight or equivocal (Fig. 4). Plasma cells containing IgM or J chain were slightly decreased or unchanged in gastric mucosa with intestinal metaplasia.

With the mirror sectioning technique, almost all of the plasma cells containing IgA or IgM were positive for J chain, although IgA plasma cells lacking J chain were occasionally recognized (Fig. 5). On the contrary, most, but not all, IgG plasma cells lacked J chain.

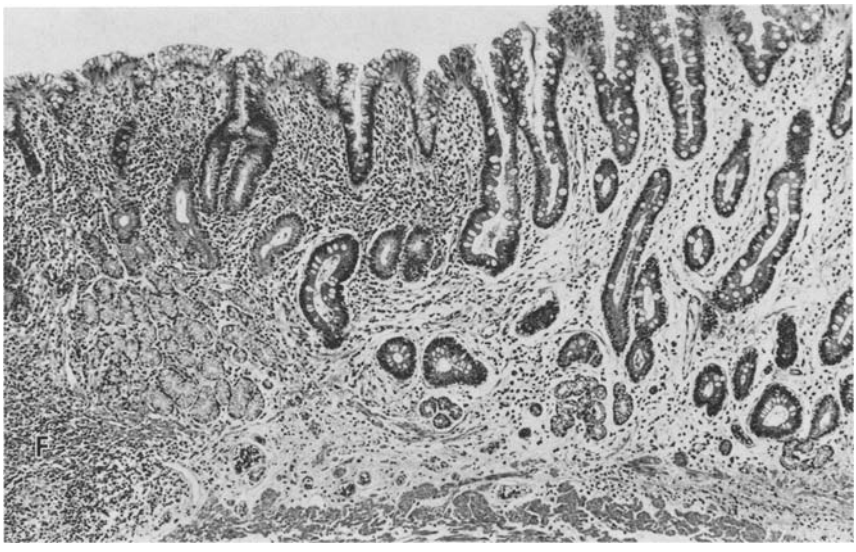

Fig. 3. Antral mucosa with or without intestinal metaplasia. H&E, ×60. Non-intestinalized gastritic mucosa (*left half*) shows severe inflammatory cell infiltration with lymphoid follicle (*F*) formation, while the number of inflammatory cells in the lamina propria is dramatically decreased in intestinalized mucosa (*right half*). The remaining pyloric glands are noted at the base of the intestinalized glands

Table 2. Immunoglobulin Classes in Cells of the Germinal Center Formed in Gastritic Mucosa

No. of Lymphoid Follicles	Immunoglobulin Classes in Germinocytes			
	IgG	IgA	IgM	J chain
120 (100%)	35 (29%)	6 (5%)	59 (49%)	25 (21%)

In the germinal centers of lymphoid follicles, immunoglobulin classes present in the cytoplasm of germinal center cells ranged in the order IgM > IgG > IgA, and J chain-containing cells were obviously fewer than IgM-containing cells (Fig. 6, Table 2). The staining intensity of J chain was often weaker there than in the lamina propria.

With regard to T cells detected by monoclonal antibodies, non-intestinalized mucosa with chronic inflammation showed a large number of Leu 1- and Leu 4-positive T cells, and the total number of T cells was decreased in the intestinalized area (Fig. 7a). In the mucosa with rather mild inflammation, T cells were distributed mainly at the glandular neck region. In the lamina propria, there were more Leu 3a-positive cells than Leu 2a-positive

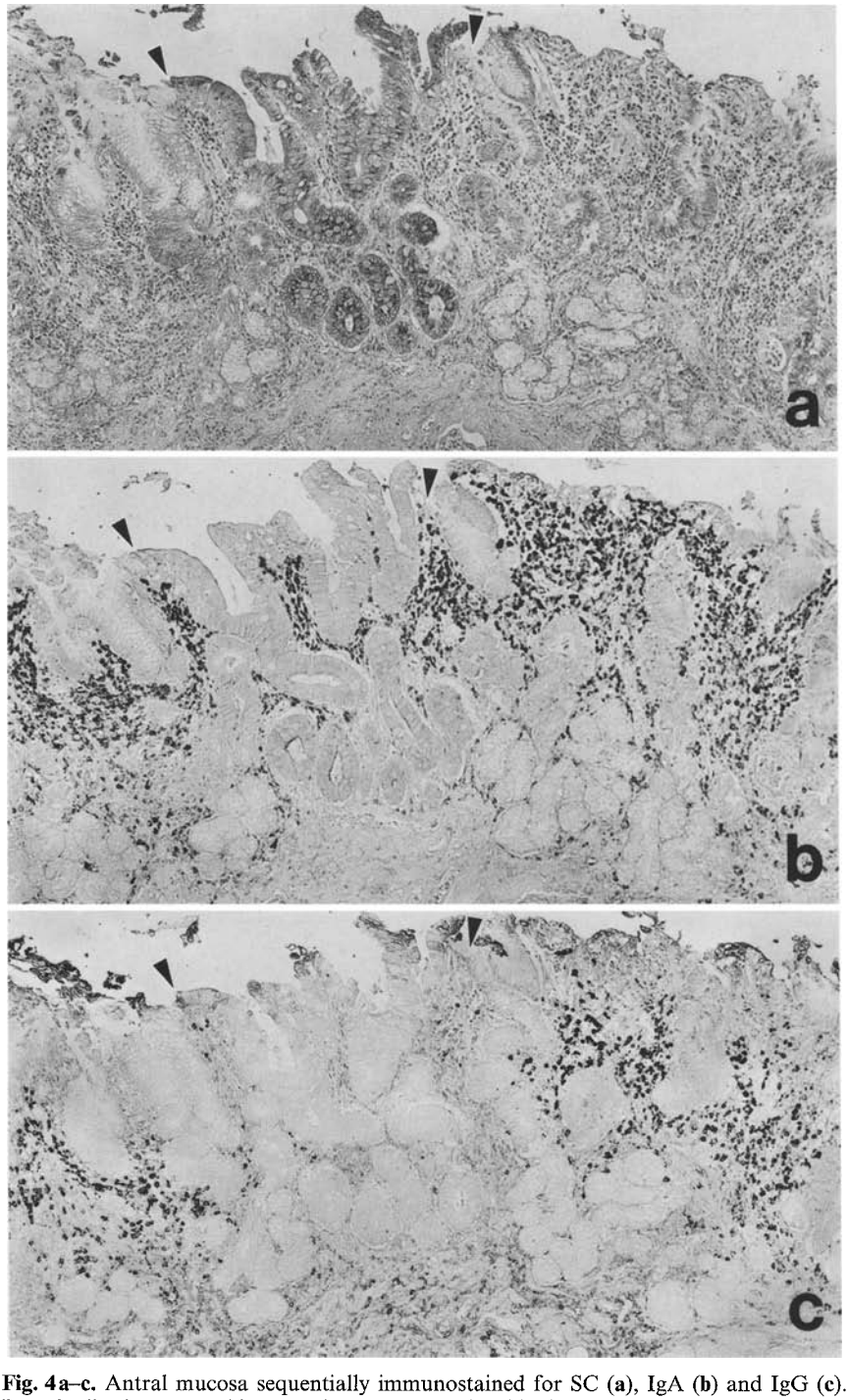

Fig. 4a–c. Antral mucosa sequentially immunostained for SC (**a**), IgA (**b**) and IgG (**c**). ×75. Intestinalized mucosa (the area between arrowheads) shows enhanced immunoreactivity of SC in the epithelial cells. IgA plasma cells are almost evenly distributed, whereas IgG plasma cells are seen exclusively in the non-intestinalized mucosa. IgA cells are most plentiful in the upper part of the mucosa, and IgG cells are mainly observed in the middle part

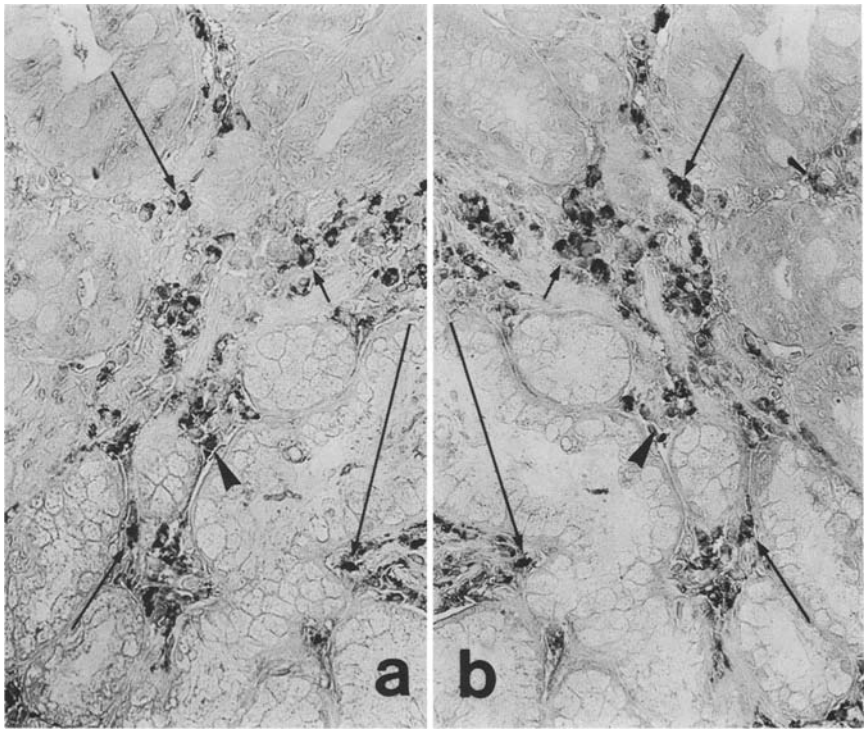

Fig. 5a, b. A pair of mirror sections of antral mucosa immunostained for IgA (**a**) and J chain (**b**). ×385. Most IgA plasma cells simultaneously contain J chain in their cytoplasm (*arrows*). There are a few IgA cells lacking J chain (*arrowheads*)

cells. In the intraepithelial spaces, lymphoid cells positive for Leu 1, Leu 4 or Leu 2a were scattered, but Leu 3a-positive cells were very sparse (Fig. 7b). These intraepithelial lymphocytes varied considerably in number from place to place and were not strictly confined to the intestinalized glands.

Anti-Leu 7 antibody against NK and K cells detected rare intraepithelial lymphoid cells and a small number of lymphoid cells in the lamina propria. More Leu 7-positive cells were present in non-intestinalized mucosa than in intestinalized mucosa. In addition, anti-Leu 7 antibody also stained, as a result of cross-reaction of the antibody, non-lymphoid cell components such as endocrine-like cells and peripheral nerve fibers (Shioda et al. 1984).

Some germinal center lymphoid cells were positive for Leu 1, Leu 2a, Leu 3a, Leu 4, or Leu 7, while most of them and macrophages were positively stained with anti-OKIa 1 antibody.

All of the specimens incubated with nonimmune rabbit or mouse serum were negative, and no endogenous peroxidase activity remained in the specimens after treatments with periodic acid and sodium azide.

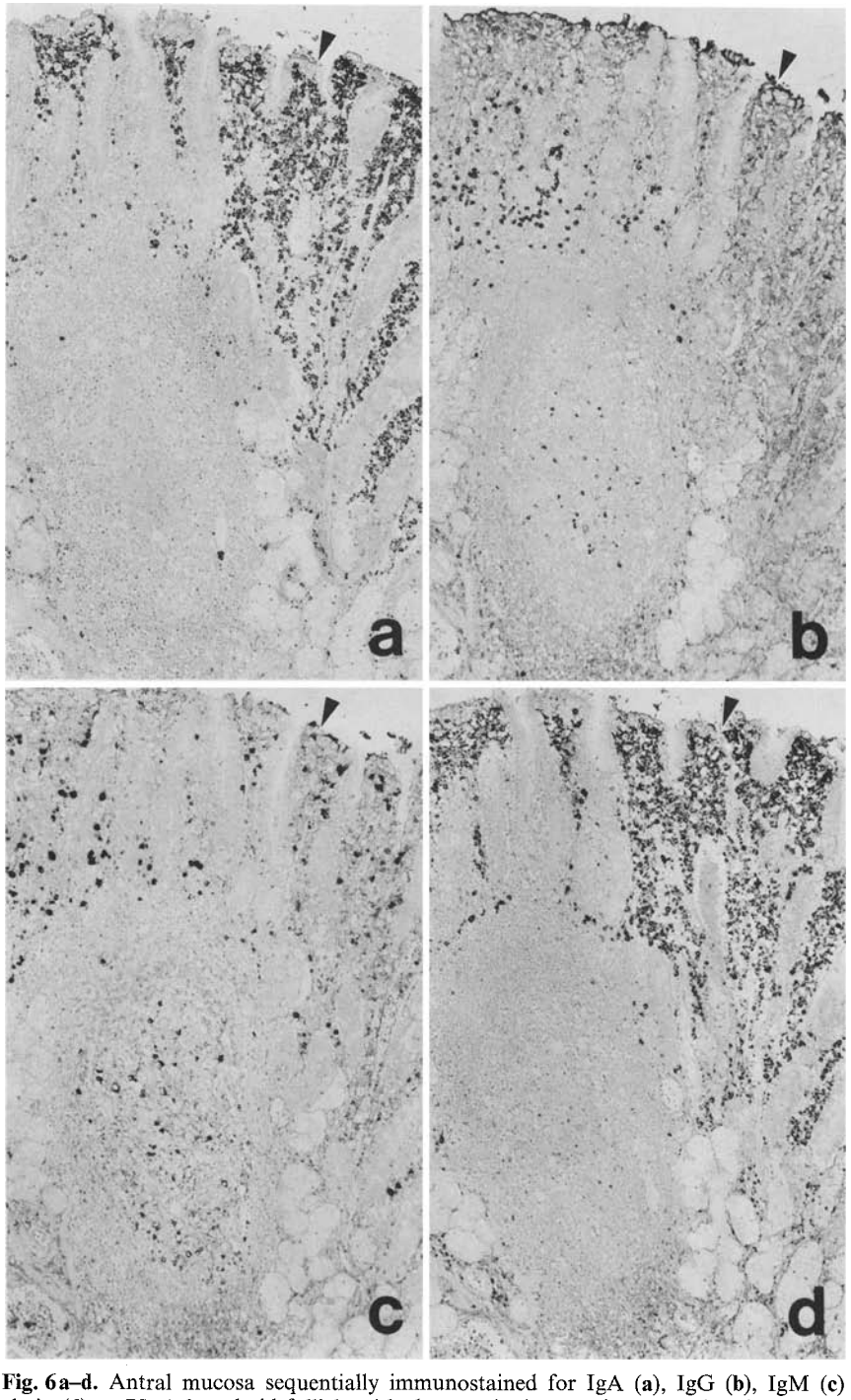

Fig. 6a–d. Antral mucosa sequentially immunostained for IgA (**a**), IgG (**b**), IgM (**c**) and J chain (**d**). ×75. A lymphoid follicle with the germinal center is present just beneath the non-intestinalized part of the mucosa (*left side of arrowhead*), which is adjacent to the intestinalized mucosa (*right side of arrowhead*). In the lamina propria, IgA cells, J chain-containing cells and less IgM cells are widely distributed regardless of intestinalization, but IgG cells are confined to the non-intestinalized mucosa. In the germinal center, there are a modest number of IgG- or IgM-positive cells but no IgA-positive cells. Note a weak staining for J chain in the germinal center in contrast with a strong staining for IgM

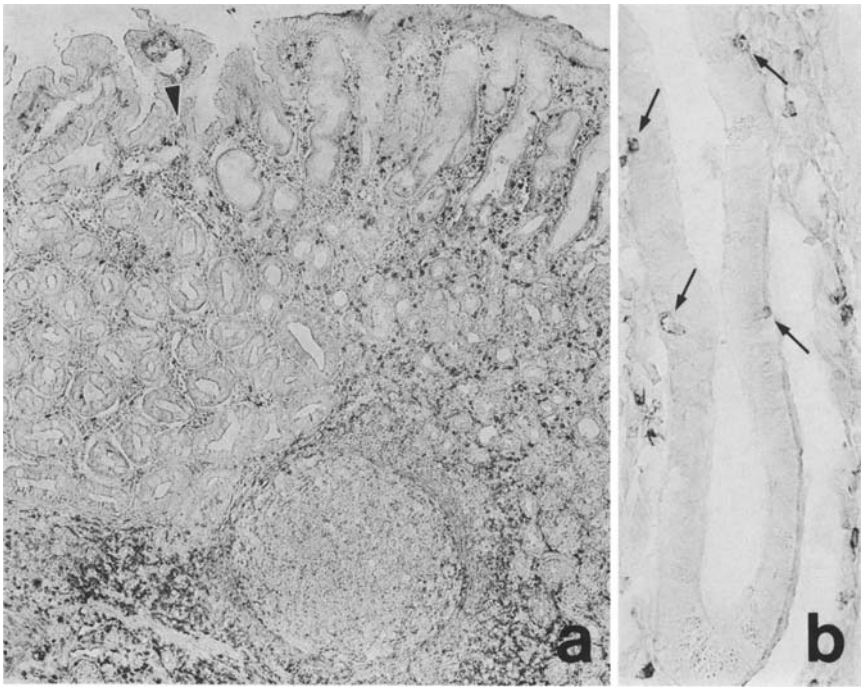

Fig. 7a, b. T cells and their subsets in antral mucosa with intestinal metaplasia. Indirect immunoperoxidase staining for Leu 1 (**a**) and Leu 2a (**b**) with frozen sections. (**a**): ×60; (**b**): ×300. (**a**): The number of Leu 1-positive T cells, which are especially plentiful around the lymphoid follicle in the non-intestinalized area, is evidently decreased in the intestinalized area (*left side of arrowhead*). (**b**): A few intraepithelial T lymphocytes positive for Leu 2a are scattered in the intestinalized gland (*arrows*). Leu 3a-positive cells are absent from this gland

Discussion

Intestinal metaplasia of the stomach, a common abnormality in Japan (Imai et al. 1971; Sugano et al. 1983), has been investigated as a potential precursor of gastric carcinoma (Nakamura et al. 1968). Many of the recent works on various aspects of intestinal metaplasia have been based on this view. Moskowicz (1924) suggested long ago that intestinal metaplasia results from gastric epithelial regeneration, a notion which persists today. As for the immune status in this lesion, opposing views have been presented: diminished activity (Yamagiwa 1977; Wada et al. 1981; Morise 1982) versus enhanced activity (Ohta et al. 1979; Hasegawa et al. 1980; Isaacson 1982).

In this article, we have analyzed histologically and immunohistochemically the local immune responses in gastritic mucosa that contained or did not contain intestinal metaplasia. Our findings strongly suggest that the SC-mediated secretory immunoglobulin transport mechanism is effective in the intestinalized gastric mucosa (Ohta et al. 1979; Hasegawa et al. 1980; Isaacson 1982; Nagura et al. 1983), as it is in the normal intestine (Brown et al. 1976). It has been reported that amounts of secretory IgA in gastric

juice increase as atrophy or intestinalization of the gastric mucosa progresses (Wada et al. 1981). Given hyperacidity in the normal stomach, in contrast, secretory immunity would not be a basic function of this organ (Isaacson 1982). In fact, we failed to demonstrate SC or IgA in minimally inflamed antral epithelium. The failure of detection of SC in fetal stomachs could support the absence of this antigen, a key substance for secretory immunity, in the normal stomach. When chronic inflammatory changes occur, cells at the generative cell zone begin to produce SC, though in a small amount (Ohta et al. 1979; Hasegawa et al. 1980; Wada et al. 1981; Isaacson 1982). In such non-intestinalized mucosa presumably with a limited capacity for SC production and less effective transepithelial secretory immunoglobulin transport, an excess of persistent antigenic stimuli from the gastric lumen may have induced the infiltration of J chain-negative IgG plasma cells and T cells and the formation of lymphoid follicles together with the infiltration of J chain-positive IgA and fewer IgM plasma cells. Previously, the evident decrease of inflammatory cells in areas with intestinal metaplasia has been described (Brus et al. 1968; Ohta et al. 1979; Morise 1982), and the preferential distribution of lymphoid follicles in non-intestinalized gastritic mucosa has also been reported (Yamagiwa 1977). The origin of IgG plasma cells in non-intestinalized gastritic mucosa should be attributed to the newly formed lymphoid follicles, since germinal center cells contained more IgG than IgA in the cytoplasm. In fact, it has been clarified that there are sparse IgG plasma cells in normal gastrointestinal mucosa (Taylor 1972). The sparsity of IgD plasma cells in the gastric mucosa with or without intestinalization is an identical finding to the normal intestine (Brandtzaeg et al. 1979). The presence of a few T cells and NK and K cells in the germinal center is comparable with the findings in lymph nodes (Si and Whiteside 1983; Si et al. 1983) and solitary lymphoid follicles in the gut (Shioda et al. 1983). It can be further suggested that once intestinal metaplasia has occurred, the effective clearance of antigens results in a dramatic decrease of plasma cells containing IgG which cannot be transported across epithelium, T cells and lymphoid follicles. Intraepithelial lymphocytes may function as a local defense in the intestinalized mucosa as in the normal intestine (Selby et al. 1981). It is worthy of note that the SC production with secretory immunoglobulin transport seems to be already accomplished in the incomplete type of intestinal metaplasia where expressions of other intestinal phenotypes are not fully developed (Abe et al. 1974; Hattori and Fujita 1979; Matsukura et al. 1980).

From the immunologic point of view, intestinal metaplasia showing an enhanced local immunity could be a positive and purposeful host reaction or adaptation against persistent inflammatory processes in the gastric mucosa. Recently, we have found a similar purposeful phenomenon in intestinal metaplasia of the stomach during observations on endocrine cells (Tsutsumi et al. 1983a; and 1983b). A preferential increase of a limited kind of gut-type endocrine cells including glicentin-containing cells and a drastic decrease of proper gastric-type endocrine cells are characteristic of intestinal metaplasia (Tsutsumi et al. 1983a, 1983b). Glicentin is known to be both a suppres-

sant of gastric acid secretion (Bataille et al. 1981) and a "growth hormone to the gut" (Bloom and Polak 1981), so that the importance of glicentin for the maintenance of intestinalized glands in the stomach should be stressed (Tsutsumi et al. 1983a and b).

Intestinal metaplasia is known to progress slowly with age (Imai et al. 1971; Sugano et al. 1982), and long-standing chronic inflammation should precede for the induction of heterotopic appearance of the intestinal-type epithelium (Glass and Pitchumoni 1975). In this regard, it is worthy of note that a good and long-lasting response in the secretory immune system requires the continuous local persistence of the antigen (Ogra and Karzon 1970); generally, it is difficult to obtain a significant memory in secretory immunity (Brandtzaeg et al. 1979). There should be further investigation of the possible relationship between the alteration of involved gastric mucosa to an intestinalized one and the acquisition of local immune memory in the stomach.

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