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ORIGINAL ARTICLE

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Immunohistochemical analysis of intercellular adhesion molecule-1 expression in human gastric adenoma and adenocarcinoma

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Abstract In this study, we examined the distribution of intercellular adhesion molecule-1 (ICAM-1) in gastric adenomas and carcinomas immunohistochemically at the light and electron microscopic levels. ICAM-1 was expressed on tumour cells in 12 of 28 gastric carcinomas and in 3 of 11 adenomas but not on most normal gastric epithelial cells. ICAM-1 was localized on luminal sites of neoplastic glands in adenomas and in intestinal-type carcinomas, and rarely on the surface of tumour cells of diffuse carcinomas. Expression of ICAM-1 on the tumour cells was more frequent in intestinal-type than diffuse carcinomas ($P<0.005$). At the ultrastructural level, ICAM-1 was present prominently on the apical membrane and weakly on the lateral surface of the tumour cells of the intestinal-type carcinoma and also localized on the perinuclear membrane and the membrane of the endoplasmic reticulum of cancer cells. There was no significant association between ICAM-1 expression and HLA antigen expression or the number of infiltrating lymphocyte subsets. These results may implicate the synthesis of ICAM-1 by gastric cancer cells, but the expression is infrequent and may not be sufficient for host immune surveillance of the tumour cell.

Key words Intercellular adhesion molecule-1 · Gastric cancer · HLA antigen · Immunohistochemistry · Immunoelectron microscopy

Introduction

Recent studies have disclosed that many kinds of cell adhesion molecules play important roles in the various steps of the cellular immune response. Among the cell adhesion molecules, intercellular adhesion molecule-1 (ICAM-1) and its ligand, lymphocyte function associated antigen-1 (LFA-1), are involved in the initial step of adhesion of cytotoxic T cells (CTL) to target cells, or helper T cells to antigen presenting cells [2]. In tumour immunity, where cellular immunity plays a major role, ICAM-1 is important in T cell-mediated cytotoxicity, and this response to tumour cells in vitro is inhibited by an anti-ICAM-1 monoclonal antibody [2, 27]. Tomita et al. [25] showed that the number of ICAM-1 positive tumour cells in renal cell cancer was significantly correlated with the degree of T cell infiltration. An analysis of ICAM-1 expression on tumour cells will add to our understanding of the mechanism of host immune responses to tumour cells and although there are several reports on the expression of ICAM-1 in various malignant tumours including melanoma [20], lymphoma [8] and carcinomas [3, 13, 23, 25], its precise localization in the component cells of gastric cancer has not been studied. Here, we examined the distribution of ICAM-1 immunohistochemically in gastric adenomas and carcinomas at the light and electron microscopic levels.

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Materials and methods

Tissues were obtained from 28 patients with gastric cancer and 11 patients with gastric adenoma obtained by surgical resection or endoscopic biopsy. Nineteen specimens of gastric tissue obtained from patients with an endoscopically normal stomach were used as normal controls. Informed consent was obtained from each patient. Seventeen of the twenty-eight gastric cancers were intestinal-type, and the other 11 were diffuse carcinomas, according to the Lauren classification of gastric carcinoma [15].

The tissues were fixed in a periodate-lysine-paraformaldehyde fixative [16] and cryostat sections were stained using an indirect peroxidase-labelled antibody method. The following monoclonal antibodies were used as primary antibodies: 84H10 antibody to the 105 kDa epitope of ICAM-1 (Immunotech, Marseille, France); anti-LFA-1 (Nichirei, Tokyo, Japan); anti-HLA-ABC (DAKO, Glostrup, Denmark); anti-HLA-DP, HLA-DQ, HLA-DR, CD4, CD8, and CD57 (Becton-Dickinson, Mountain View, Calif.) and anti-CD28 (Nichirei). After incubation with the primary antibodies, the sections were reacted with horseradish peroxidase (HRPO)-labelled Fab' of rabbit anti-mouse immunoglobulin prepared as described [19], and 3,3'-diaminobenzidine (DAB) containing hydro-

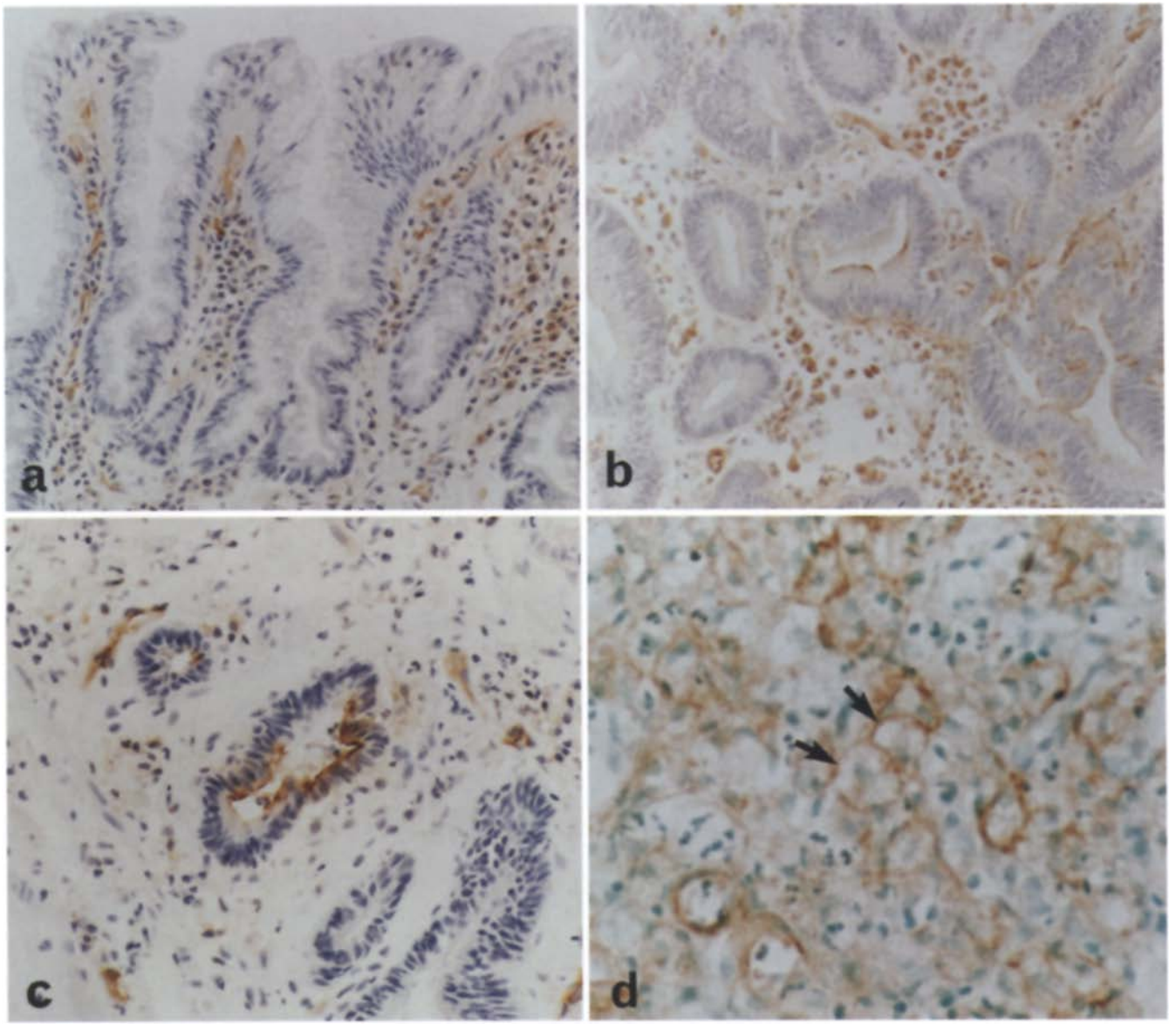

Fig. 1a–d Immunohistochemical localization of intercellular adhesion molecule-1 (ICAM-1) in the gastric mucosa. **a** In normal gastric mucosa, ICAM-1 is expressed on vascular endothelial cells, lymphocytes and fibroblasts in the stroma and is not expressed on epithelial cells (×200). **b** In gastric adenoma, ICAM-1 is expressed on luminal sites of neoplastic glands (×200). **c** In intestinal-type gastric carcinoma, ICAM-1 is expressed on luminal sites of neoplastic glands (×300). **d** In diffuse gastric carcinoma, ICAM-1 is expressed on the cell surfaces of some tumour cells (*arrow*; ×400). Nuclei are counterstained with haematoxylin (**a, b, c**) and methylgreen **d**

gen peroxide (H_2O_2). The stained sections were counterstained with methylgreen or Mayer's haematoxylin, dehydrated and mounted.

For immunoelectron microscopic study, sections were reacted with the primary antibodies and the HRPO-labelled secondary antibody, post-fixed in 2% glutaraldehyde and incubated sequentially with DAB and DAB containing H_2O_2. The stained sections were osmicated, dehydrated and embedded in Epon-Araldite as described [17]. Ultrathin sections were observed under an electron microscope without additional staining.

Lymphocyte subsets infiltrating into the gastric cancer tissues were examined by counting stained cells in three random microscopic fields of each section at × 200 magnification, and the numbers of positive cells in 1mm^2 were calculated. For statistical analysis, the chi-square test was used.

Results

In the normal gastric mucosa, ICAM-1 was expressed on vascular endothelial cells, lymphocytes and fibroblasts in the stroma and was not expressed on most epithelial cells (Fig. 1a). In 3 of 11 adenomas and 12 of 28 cancers, ICAM-1 was present on the tumour cells as well as the stromal cells. ICAM-1 was localized at the luminal aspect of neoplastic glands in adenomas (Fig. 1b) and intestinal-type carcinomas (Fig. 1c), and rarely on the surface of tumour cells of diffuse carcinomas (Fig. 1d). ICAM-1 expression on the tumour cells was more frequent in intestinal-type than diffuse carcinoma ($P<0.005$, Table 1).

Table 1 Frequency of intercellular adhesion molecule-1 (ICAM-1) expression on epithelial cells in gastric cancers, adenomas and normal mucosa

	Number of cases		
	+	−	
Normal mucosa	1	18	
Adenoma	3	8	
Intestinal type carcinoma	11	6	] $P < 0.005$
Diffuse carcinoma	1	10	

At the ultrastructural level, ICAM-1 was present predominantly on the apical membranes and weakly on the lateral surface of the tumour cells (Fig. 2a) of intestinal-type carcinoma. ICAM-1 was also present on the perinuclear membrane (Fig. 2b) and the membrane of the endoplasmic reticulum (Fig. 2c), suggesting synthesis of ICAM-1 by these cancer cells.

In the stromal tissue, ICAM-1 expression on vascular endothelial cells in the carcinoma and adenoma tissues was more prominent than in the normal mucosa. No significant difference in the stromal expression of ICAM-1 was seen between the ICAM-1 positive and negative cancers.

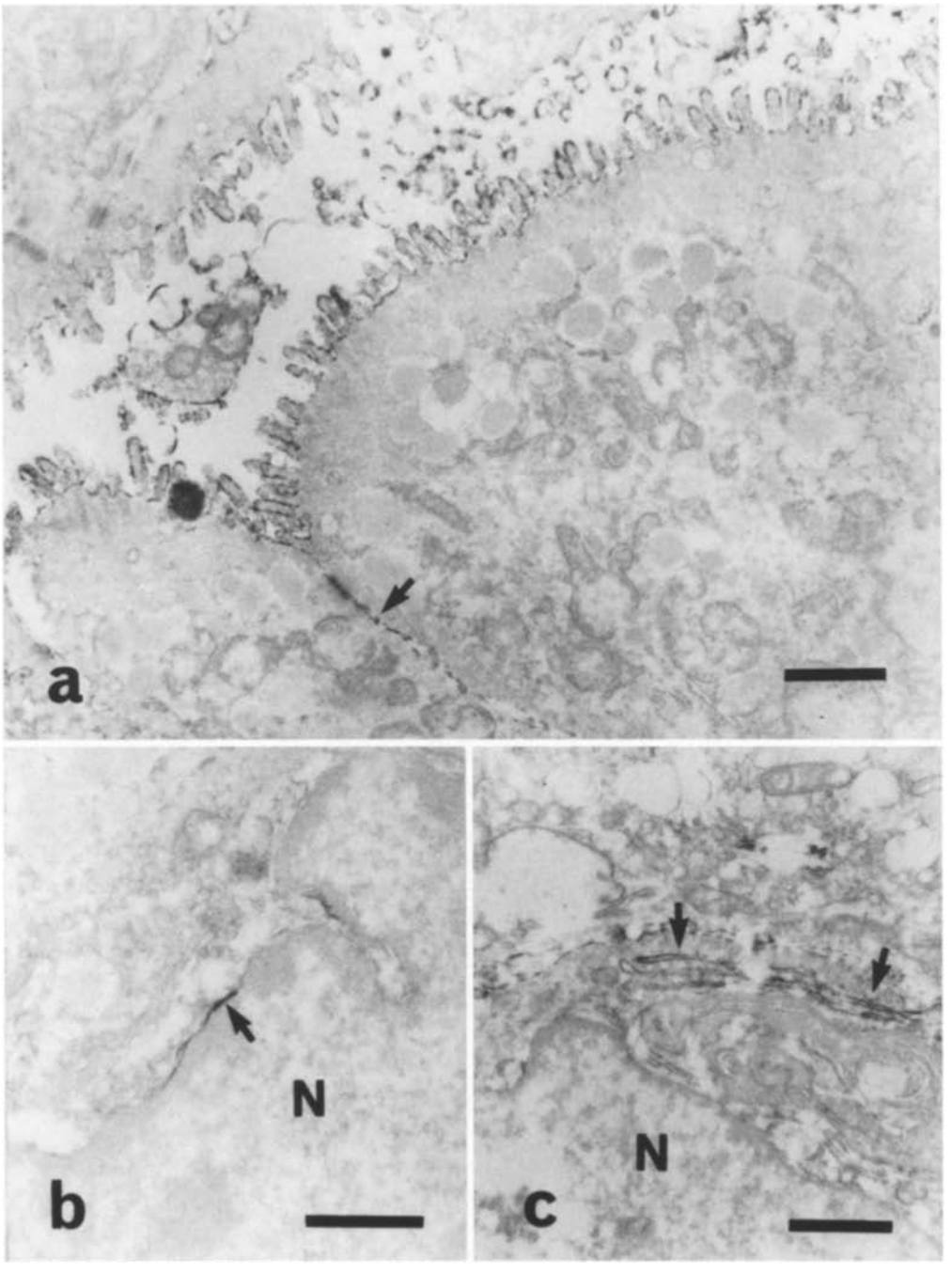

Fig. 2a–c Immunoelectron microscopic localization of ICAM-1 in intestinal-type gastric carcinoma. ICAM-1 is present predominantly on the apical membranes, and weakly on the lateral surface of the tumour cells (*arrow*; **a**). ICAM-1 is also present on the perinuclear membrane (*arrow*; **b**) and on membranes of the endoplasmic reticulum (*arrows*; **c**) of the tumour cells. *Bar* = 1 μm

Table 2 Frequency of HLA-ABC,DP,DQ and DR antigen expression on epithelial cells in ICAM-1 positive and negative gastric cancers. (+ diffuse expression, ± focal expression, – negative expression)

		Number of cases		
		+	±	–
HLA-ABC	ICAM-1 (+)	5	4	0
	ICAM-1 (–)	3	8	4
HLA-DP	ICAM-1 (+)	0	2	8
	ICAM-1 (–)	0	3	10
HLA-DQ	ICAM-1 (+)	0	1	9
	ICAM-1 (–)	0	2	12
HLA-DR	ICAM-1 (+)	0	6	2
	ICAM-1 (–)	1	7	8

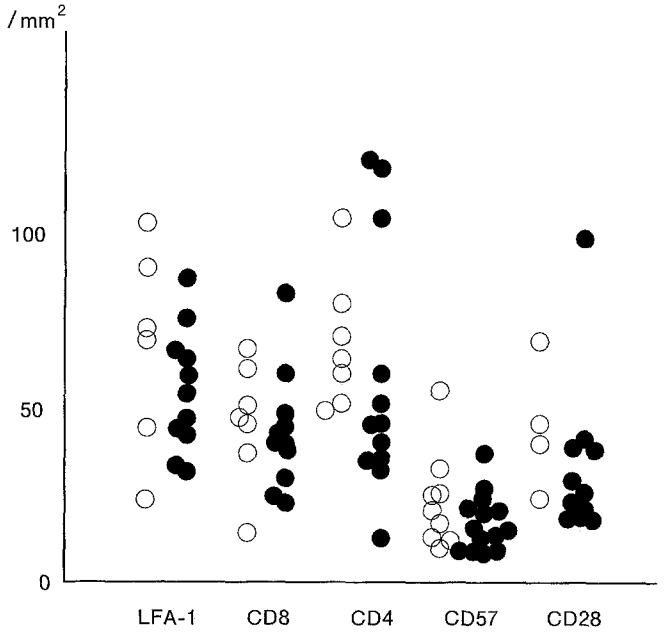

Fig. 3 Relationship between ICAM-1 expression and the degree of lymphocyte infiltration. The number of each lymphocyte subset infiltrating the cancer tissue is shown per mm^2. There is no significant difference in the number of the lymphocyte subsets between ICAM-1 positive (○) and negative (●) specimens

The expression of ICAM-1 was not correlated with expression of HLA class I and II antigens in gastric cancer cells (Table 2), and these molecules were differently distributed when both ICAM-1 and HLA antigens were expressed in cancer cells. There was no significant difference in the numbers of infiltrating lymphocyte subsets between the ICAM-1 positive and negative neoplasms (Fig. 3).

Discussion

In this study, we have examined the distribution of ICAM-1 in gastric carcinomas and adenomas immunohistochemically, and found that ICAM-1 was expressed on the tumour cell surface in 3 of 11 gastric adenomas and 12 of 28 carcinomas. ICAM-1 was more frequently expressed on gastric cancer cells in intestinal-type than in diffuse carcinomas. Although there have been several studies on the expression of ICAM-1 in various malignant tumours, its precise distribution on the tumour cells of gastric cancer has not yet been examined. By means of immunoelectron microscopy, we have found ICAM-1 present predominantly on the apical membranes and weakly on the lateral surface of the tumour cells. We also localized ICAM-1 in the protein-synthetic organelle – the perinuclear membrane and the membrane of the endoplasmic reticulum. This suggests to us synthesis of ICAM-1 by gastric cancer cells.

Several studies indicate high expression levels of ICAM-1 in inflammatory diseases and tumours. Zheng et al. [29] have reported that ICAM-1 is expressed on thyroid epithelial cells in Graves' diseases as well as in Hashimoto's thyroiditis, and Hansen et al. [7] showed 17 of 21 primary malignant melanomas were ICAM-1 positive. In contrast, Johnson [10] and Natali et al. [20] found relatively low incidence of ICAM-1 expression in various tumours except for melanomas and squamous cell carcinomas. We observed ICAM-1 expression in 12 of 28 (42.8%) cases of gastric cancer; the incidence is consistent with findings of work using flow-cytometric analysis [13], but ICAM-1 was expressed very focally on gastric cancer cells in each of our ICAM-1-positive cases. In addition, although our immunoelectron microscopic findings may suggest the synthesis of ICAM-1 by gastric cancer cells, its expression was mostly confined to the luminal sites of the gland where immune cells were not accessible. In the ten specimens of diffuse gastric carcinoma, the cancer cells of only one specimen were positive for ICAM-1. The inadequate expression of ICAM-1 in gastric cancer cells may contribute to their escape from host immune surveillance and allow growth of the tumours without rejection. In addition, coexpression of ICAM-1 and HLA-DR has been shown to be important in effective antigen presentation in the immune response [1], but we found no correlation of the distribution and frequency of expression between ICAM-1 and HLA antigens in gastric cancer. This finding might explain an modeguate induction of host immune response against gastric cancer cells.

Although the simultaneous expression of ICAM-1 and HLA antigens has been reported in some inflammatory diseases [4–6, 28], the unrelated expression of HLA-DR antigen to ICAM-1, as shown in our study, has been reported in colon and breast carcinoma [10] and in *Helicobactor pylori*-associated gastritis [22]. Expression of these molecules is regulated by various cytokines, but the cytokines which induce ICAM-1 expression are different from those inducing HLA antigens [18, 28]. Moreover, susceptibility to cytokines differs among various cell types [11, 14].

As stated above, ICAM-1 expression is induced by cytokines, such as interleukin-1, tumour necrosis factor-α, interferon-γ [9, 14, 18, 24, 27], and its expression on gastric cancer cells observed here could be induced by these cytokines, produced by tumour-infiltrating inflammatory cells. Indeed, some reports have shown that tumours ex-

pressing ICAM-1 had a high degree of lymphocytic infiltration [12, 25]. In our study, however, there was no correlation between ICAM-1 expression and the degree of lymphocytic infiltration of each subset, which is in accordance with a report on metastatic melanomas described by Natali et al. [20]. Our findings support the hypothesis that regulatory pathways responsible for ICAM-1 expression are related to changes in cellular differentiation and genetic alterations in the events leading to the development of gastric cancer. This has been demonstrated by analogy in the observation of overexpression of carcinoembryonic antigen during colon tumourigenesis [21].

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Note added in proof
An immunohistochemical study on expression of several adhesion molecules including ICAM-1 in human gastric carcinoma [26] has been published since the submission of this paper.