Virchows Arch (1996) 429:377–382
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ORIGINAL ARTICLE

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Expression of adhesion molecules in primary B-cell gastric lymphoma and lymphoid follicles

Received: 17 October 1995 / Accepted: 12 August 1996

Abstract To determine whether primary B-cell gastric lymphoma (GL) is one entity, we examined the expression of three adhesion molecules in the microvasculature of lymphomas. Stromal cells, including vascular endothelial cells, within lymphoid follicles of the gastric mucosa were also investigated. Twenty-two surgical specimens of GL were classified into low-grade malignant lymphoma arising from mucosa-associated lymphoid tissue (low-grade lymphoma, n=9), and high-grade malignant lymphoma with (secondary high-grade lymphoma, n=6) or without (primary high-grade lymphoma, n=7) a low-grade component. The proportion of venules positive for ELAM-1 or VCAM-1 was significantly higher (P<0.001) in primary high-grade lymphoma than in low-grade and secondary high-grade lymphomas. In gastric lymphoid follicles, the stromal cells of the germinal centre (GC) were positive for ICAM-1, ELAM-1, and VCAM-1, but the stromal cells of the marginal zone (MZ) were positive only for ICAM-1. We found two patterns of adhesion molecule expression in gastric lymphoid follicles, the MZ pattern and the GC pattern. Low-grade and secondary high-grade lymphomas, which had the MZ pattern, might be of MZ-cell lineage, but most primary high-grade lymphomas, which had the GC pattern, might be of follicular centre cell lineage.

Key words Gastric lymphoma · Mucosa-associated lymphoid tissue (MALT) · Lymphoid follicle · Adhesion molecule

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Introduction

Since the concept of extranodal malignant lymphomas arising from mucosa-associated lymphoid tissue (MALT) in the stomach, salivary gland, lung, and thyroid was proposed by Isaacson and Wright in 1984 [10], there have been extensive clinical and pathological studies on this type of lymphoma [7, 8, 20]. These studies have given special consideration to low-grade extranodal lymphoma, and have shown that in several features it differs from low-grade nodal lymphoma. Most of the studies have involved the stomach, which is the most common primary site of extranodal lymphoma [2, 5, 9, 11, 13–15, 17, 18, 22, 27]. However, the origin of the tumour cells of low-grade lymphoma and the relationship between low-grade and high-grade lymphomas remain controversial.

The tumour cells of low-grade lymphoma, centrocyte-like (CCL) cells, are generally characterized as CD5-, CD10- and have a characteristic interfollicular infiltration, and a nodular or diffuse pattern remains when follicles are replaced. These findings suggest that the origin is not of follicular centre cells, but of marginal zone cells. One DNA analysis of the *bcl-2* gene suggested that gastrointestinal B-cell lymphomas arising from MALT were not of follicular centre cell lineage, because no rearrangement of the *bcl-2* gene could be detected. In contrast, *bcl-2* gene rearrangement has been demonstrated in 75% of follicular lymphomas [16, 26]. However, CCL cells are not morphologically similar to any cells in the marginal zone, but rather similar to follicular centre cells, and, in some cases, have been characterized as CD5+, CD10-. In some studies of the relationship between low-grade and high-grade lymphomas, the tumour cells in the low-grade and high-grade components have been found to express the same classes of immunoglobulin light chains, suggesting that the high-grade component arises through blastic transformation of the low-grade component [4].

In the present study, we focused on this point and dealt with only primary B-cell gastric lymphoma (GL) because these are the most frequent extranodal lymphomas. We

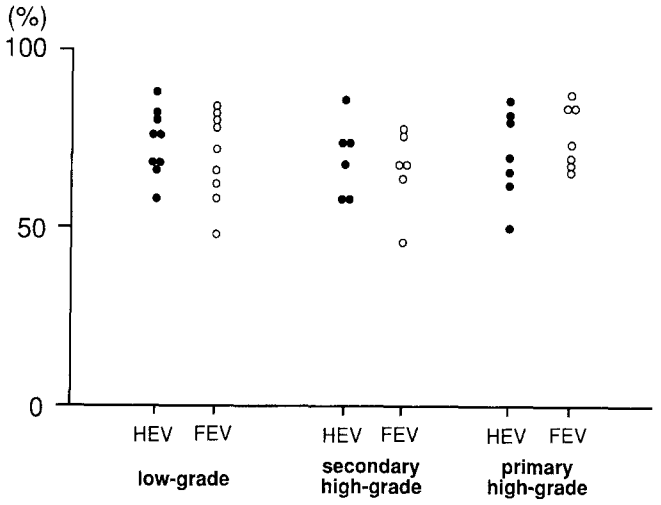

Fig. 1 The proportions of high and flat endothelial venules (HEV and FEV) positive for ICAM-1. There was no significant difference in ICAM-1 expression among the three gastric lymphoma groups. There was no significant difference in ICAM-1 expression between the HEV and FEV in any group

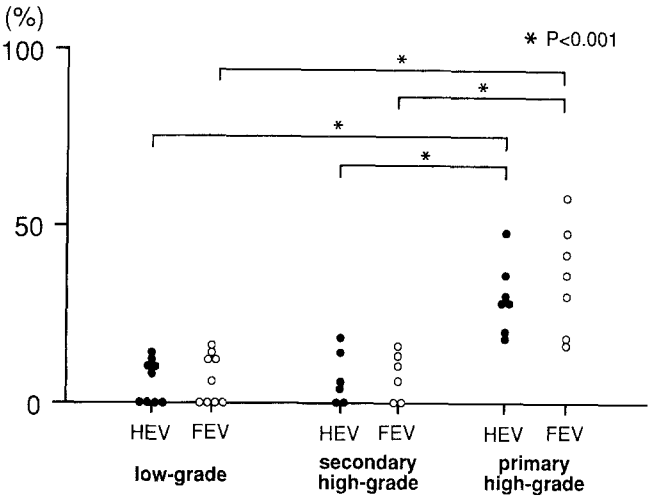

Fig. 2 The proportions of HEV and FEV positive for ELAM-1. The proportion of HEV and FEV positive for ELAM-1 was significantly higher ($P<0.001$) in primary high-grade lymphoma than in low-grade or secondary high-grade lymphomas. There was no significant difference in ELAM-1 expression between HEV and FEV in any group

focused especially on the expression of adhesion molecules on stromal cells (mainly vascular endothelial cells) in lymphomas and lymphoid follicles, because our idea is that the adhesion molecule expression on stromal cells in lymphoma might be similar to that found in the non-neoplastic putative counterpart of the lymphoid follicles. Our aim was to investigate the distribution of adhesion molecule expression in the microvasculature of lymphoma tissues, in which venules are divided into high endothelial venules (HEV) and flat endothelial venules (FEV) [12]. A further aim was to investigate whether the stromal cells (follicular dendritic cells, follicular reticular cells, and vascular endothelial cells) located in the lymphoid follicles of the gastric mucosa and mesenteric lymph nodes express these adhesion molecules.

Materials and methods

Twenty-two consecutive surgical cases of GL, based on the diagnostic criteria of Dawson et al. [6] were collected from 1989 to 1993. The patients had received no prior therapy and suffered from no systemic diseases. Tissue specimens obtained from surgical gastrectomy were submitted for routine examination and were demonstrated histologically to be non-Hodgkin's lymphoma. The B-cell identity was determined by reactivity with L26 (CD20).

All the cases originally classified by the updated Kiel classification [23] were reclassified into three groups according to the concept of MALT-derived low-grade lymphoma. The classification consisted of low-grade B-cell malignant lymphoma arising from MALT (low-grade lymphoma, $n=9$), and high-grade B-cell malignant lymphoma with (secondary high-grade lymphoma, $n=6$) and without (primary high-grade lymphoma, $n=7$) a low-grade component [5]. Burkitt's lymphomas and centroblastic-centrocytic lymphomas were excluded from this study.

Gastric tissue and mesenteric lymph nodes containing lymphoid follicles with germinal centre hyperplasia were obtained surgically from four patients with early gastric cancer.

The fresh specimens from all cases were fixed in periodate–lysine–4% paraformaldehyde (PLP), washed in increasing concentrations of sucrose in phosphate-buffered saline (PBS), and finally

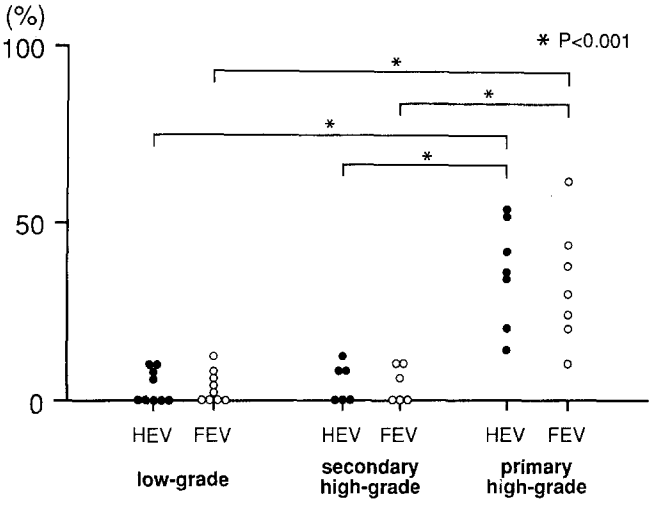

Fig. 3 The proportions of HEV and FEV positive for VCAM-1. The proportion of HEV and FEV positive for VCAM-1 was significantly higher ($P<0.001$) in primary high-grade lymphoma than in low-grade or secondary high-grade lymphomas. There was no significant difference in VCAM-1 expression between HEV and FEV in any group

placed in 20% sucrose in PBS. The fixed specimens were embedded in an OCT compound (Miles Scientific), frozen in dry ice–ethanol, and cut at 5 μm using a cryostat microsome. The serial sections were placed on silan coated slides and air-dried for 30 min.

Leu4 (CD3) and Leu1 (CD5) monoclonal antibodies (MoAb) were purchased from Becton-Dickinson (San Jose, Calif.). CALLA (CD10), L26 (CD20), and JC/70A (CD31) MoAb were from Dako (Glostrup, Denmark). ICAM-1 (CD54), ELAM-1 (CD62E), and VCAM-1 (CD106) MoAb were from Immunotech (Marseille, France).

Cryostat sections for light microscopy were immunostained using the streptavidin–biotin–alkaline phosphatase method. All MoAb were used at concentrations that gave optimal staining results. Sections were thoroughly washed with 0.05 M Tris HCl buffer (pH 7.2)

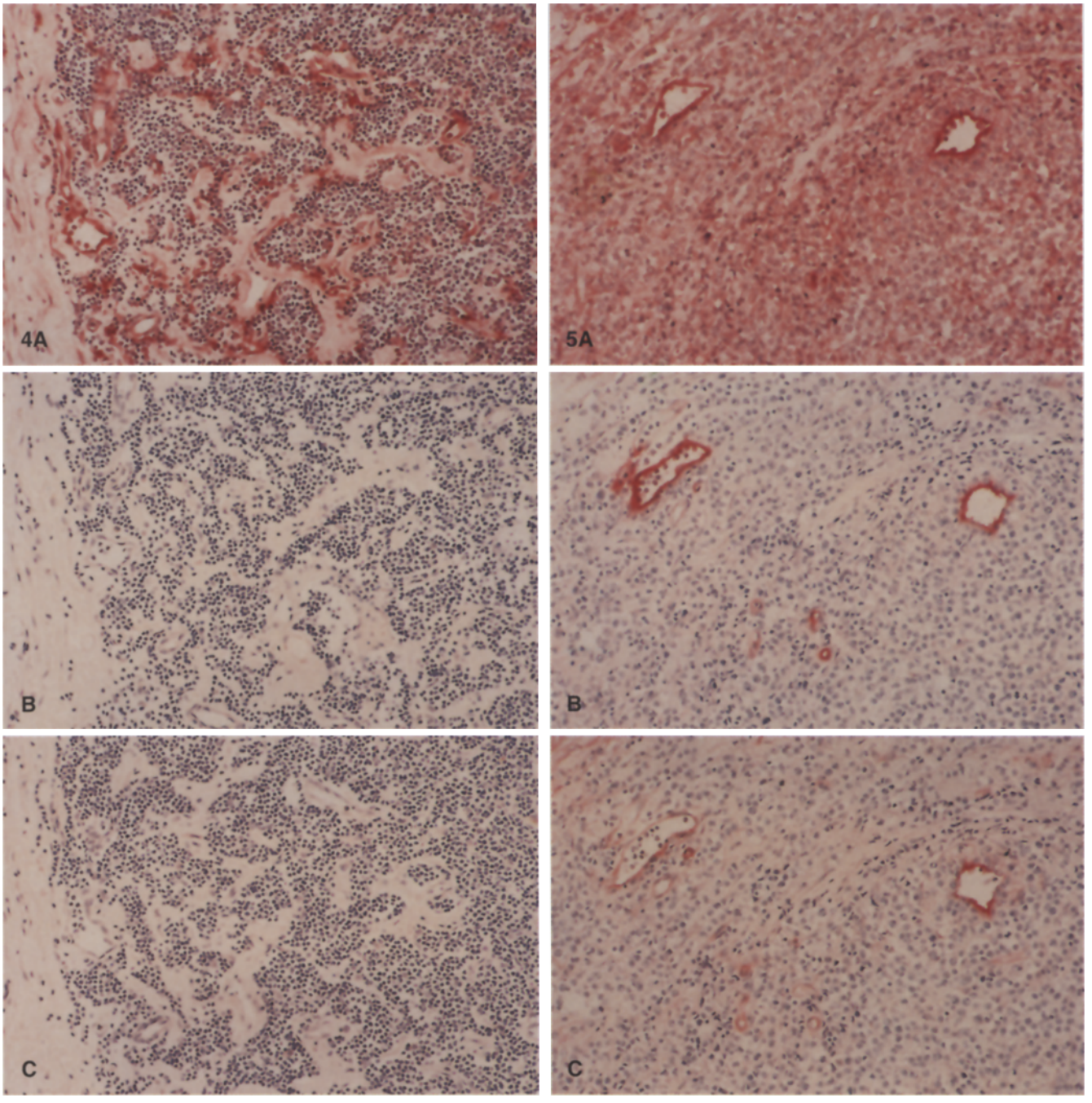

Fig. 4 Expression of **A** ICAM-1, **B** ELAM-1, and **C** VCAM-1 in a low-grade lymphoma. ICAM-1 was expressed in almost all HEV and FEV, but ELAM-1 and VCAM-1 were absent in all HEV and FEV. ×80

Fig. 5 Expression of **A** ICAM-1, **B** ELAM-1, and **C** VCAM-1 in a primary high-grade lymphoma. ICAM-1, ELAM-1, and VCAM-1 were expressed in HEV and FEV. ×160

between steps. Levamisole was used at 24 mg/100 ml to inactivate endogenous alkaline phosphatase. Sections were counterstained with haematoxylin. Appropriate positive and negative controls were included. Sections of gastric tissues with lymphoid hyperplasia were also stained with Giemsa.

The number of venules positive for ICAM-1, ELAM-1, or VCAM-1 among 50 HEV or FEV within each specimen was assessed in five random optical fields at a magnification of ×200. We also assessed the distribution of stromal cells positive for ICAM-1, ELAM-1, or VCAM-1 in the lymphoid follicles of gastric mucosa and mesenteric lymph nodes.

The proportions of venules positive for ICAM-1, ELAM-1, or VCAM-1 in the three different GL groups were compared by the Mann-Whitney test. The proportions of HEV and FEV positive for ICAM-1, ELAM-1, or VCAM-1 in the same group were compared by the rank-sum test.

Results

Low-grade lymphoma had the characteristic features of diffuse infiltration of CCL cells, numerous plasma

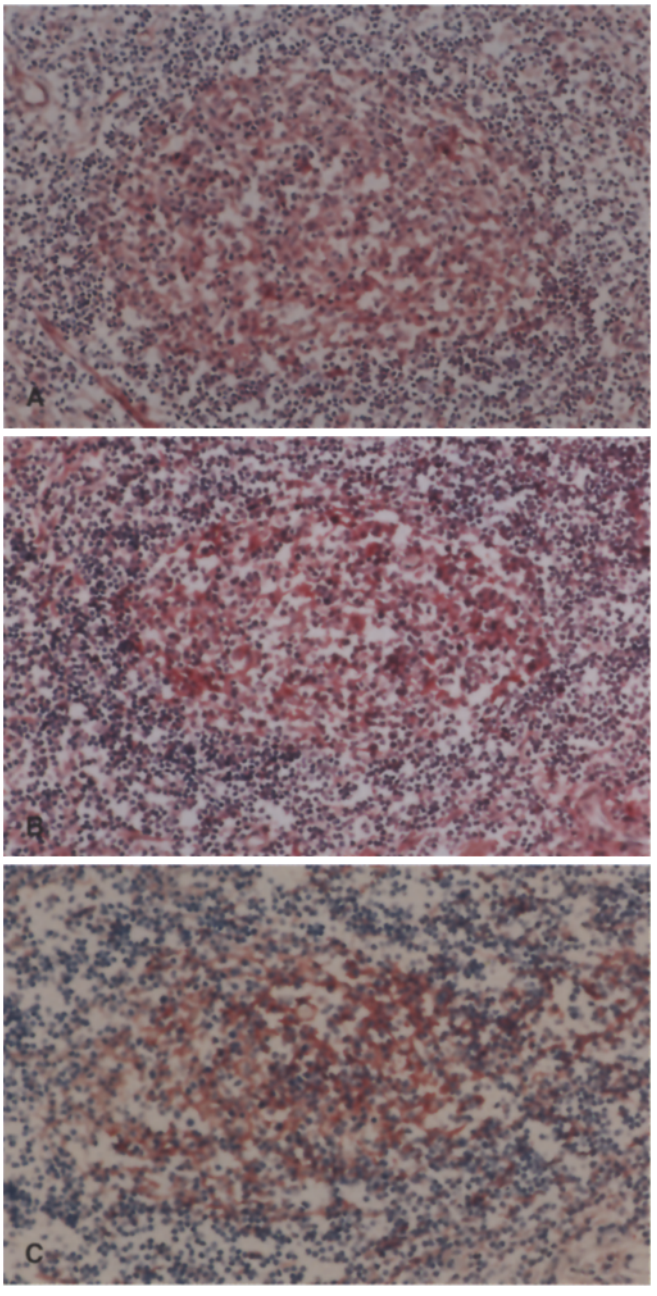

Fig. 6 Expression of **A** ICAM-1, **B** ELAM-1, and **C** VCAM-1 in a lymphoid follicle of the gastric mucosa. The stromal cells in the germinal centre were positive for ICAM-1, ELAM-1, and VCAM-1. The venules in the marginal zone were positive only for ICAM-1. ×135

cells, and scattered immunoblasts. Lymphoepithelial lesions (LEL) caused by tumour cell infiltration of the mucosa were found in all cases, and reactive lymphoid follicles, that is to say residual non-neoplastic follicles in MALT-derived lymphomas, were often recognized. Secondary high-grade lymphoma sometimes had the characteristic features of low-grade lymphoma in that there were LEL and reactive lymphoid follicles, but infiltration of numerous immunoblasts was also detected. Primary high-grade lymphoma did not manifest such low-grade components, and mainly consisted of blastic components.

Tumour cells from all the cases were reactive with L26 (CD20) but not with Leu4 (CD3). They were not reactive with Leu1 (CD5) or CALLA (CD10). The microvasculature of the lymphomas was morphologically separated into HEV or FEV. JC/70A (CD31) was clearly identified in both types of venule.

The proportion of HEV or FEV positive for each adhesion molecule was not significantly different between the marginal and central areas of the lymphomas. We therefore discuss the findings for the tumour as a whole. ICAM-1 expression was much increased in the HEV in all three groups. The range (median value) of HEV positive for ICAM-1 was 58–88% (76%) in low-grade lymphoma, 58–86% (71%) in secondary high-grade lymphoma, and 50–86% (70%) in primary high-grade lymphoma. There was no significant difference among the three groups (Figs. 1, 4, 5). ELAM-1 was moderately or strongly expressed in some HEV. The range (median value) of HEV positive for ELAM-1 was 0–14% (8%) in low-grade lymphoma, 0–18% (5%) in secondary high-grade lymphoma, and 18–48% (28%) in primary high-grade lymphoma. The proportion was significantly higher ($P<0.001$) in primary high-grade lymphoma than in low-grade or secondary high-grade lymphomas (Figs. 2, 4, 5). VCAM-1 was absent or faintly expressed in low-grade and secondary high-grade lymphomas, but was moderately or strongly expressed in primary high-grade lymphoma. The range (median value) of HEV positive for VCAM-1 was 0–10% (0%) in low-grade lymphoma, 0–12% (4%) in secondary high-grade lymphoma, and 14–54% (36%) in primary high-grade lymphoma. The proportion was significantly higher ($P<0.001$) in primary high-grade lymphoma than in the other two groups (Figs. 3–5). There was no significant difference in the expression of adhesion molecules between the HEV and FEV in each group.

In normal gastric tissue and the areas surrounding the lymphomas, ICAM-1 was moderately or strongly expressed, but ELAM-1 and VCAM-1 were absent from each type of venule.

All gastric tissues showed *Helicobacter pylori*-positive gastritis, confirmed by the Giemsa stain. In the lymphoid follicles of the gastric mucosa, the stromal cells of the germinal centre (GC), which consisted of follicular dendritic cells, follicular reticular cells, and vascular endothelial cells, were positive for ICAM-1, ELAM-1, and VCAM-1. The stromal cells of the marginal zone (MZ), which mainly consisted of vascular endothelial cells, were positive only for ICAM-1 (Fig. 6).

In the lymphoid follicles of mesenteric lymph nodes, the stromal cells of the GC were positive for ICAM-1 and VCAM-1, but not for ELAM-1. The stromal cells of the MZ were positive only for ICAM-1. ELAM-1 was absent in the lymphoid follicles of the mesenteric lymph nodes.

Discussion

We used immunohistochemistry to help determine whether GL is a single entity, by examining the distribution of stromal cells, including vascular endothelial cells positive for ICAM-1, ELAM-1, or VCAM-1 in lymphomas and lymphoid follicles. We found two patterns of adhesion molecule expression in gastric lymphoid follicles, the GC pattern and the MZ pattern. Low-grade and secondary high-grade lymphomas demonstrated the MZ pattern, but most primary high-grade lymphomas had the GC pattern. Some cases of primary high-grade lymphomas had the MZ pattern. Our interpretation of these findings is that low-grade, secondary high-grade, and in some cases primary high-grade lymphomas arise in the MZ, and that the high-grade components of secondary or primary high-grade lymphomas might arise through the blastic transformation of a pre-existing low-grade lymphoma. Alternatively, most primary high-grade lymphomas might arise in the GC, as they have different features from the other lymphomas. Thus, there might be two entities in GL.

The stromal cells of hyperplastic GC in gastric lymphoid follicles, which are the active areas of lymphoid follicles, were positive for ICAM-1, ELAM-1, and VCAM-1. However, the stromal cells of the MZ, which mainly consisted of vascular endothelial cells, were positive only for ICAM-1. Generally, ICAM-1 is constitutively expressed on endothelial cells. Its expression can be induced by interferon-γ or tumour necrosis factor-α (TNF-α). ELAM-1 and VCAM-1 are absent in normal gastric tissue, while ELAM-1 is strongly expressed in inflammatory sites and VCAM-1 is expressed on activated endothelial cells. ELAM-1 expression can be induced by interleukin-1 (IL-1) or TNF-α, and VCAM-1 expression can be induced by IL-1, IL-4 or TNF-α. Volpes et al. [25] reported that ELAM-1 and VCAM-1 are strongly expressed on sinusoidal lining cells in acute and chronic liver inflammation and might play a crucial role in the recruitment of lymphocytes. The GC has stromal cells characterized as ELAM-1+ VCAM-1+ when the lymphoid follicles are undergoing acute or chronic stimulation.

As for the difference of immunophenotype on B-cells between in the GC and in the MZ, B-cells in the GC are generally characterized as CD5-, CD10+ and express IgM, IgA, or IgG. However, B-cells in the MZ are characterized as CD5-, CD10- and express IgM, or IgA. Through blastic transformation, the tumour cells of high-grade lymphomas might have a different immunophenotype from B-cells found in the non-neoplastic putative counterpart of the lymphoid follicles.

However, the stromal cells within a lymphoma should retain the characteristic features of the area where the lymphoma arises, as suggested by the observation that tumour cells manifest the same features (CD5-, CD10-) as lymphocytes in the areas where they presumably arise. Even if these features are complicated by other factors, their pathological significance should be preserved. The speculation that the origin of low-grade lymphoma might be of MZ-cell lineage corresponds not only to the fact of their characteristic interfollicular infiltration, but also to the findings of DNA analysis of the *bcl-2* gene [16, 26].

There was no significant difference in the proportion of HEV and FEV positive for each adhesion molecule. HEV are generally the venules through which lymphocytes easily migrate. They have a rough surface and their basement membranes are split or show a thick electron-dense layer in place of the normal basement membrane, with a loose connection between endothelial cells [12, 21, 24]. All of these factors affect the migration of lymphocytes as well as the expression of adhesion molecules.

The stromal cells of the GC were positive for ICAM-1 and VCAM-1, but not for ELAM-1, in the lymphoid follicles of mesenteric lymph nodes. The stromal cells of the MZ were positive only for ICAM-1. A study analyzing soluble adhesion molecules in the circulation has also suggested that the concentrations of ICAM-1 and VCAM-1 are elevated, but the concentration of ELAM-1 is not elevated, in patients with non-Hodgkin's lymphoma and Hodgkin's disease, compared with normal controls [1]. In other hyperplastic lymph nodes, such as cervical, axillary, and intestinal nodes, ICAM-1 and VCAM-1 are expressed, but ELAM-1 is absent in the GC [19]. The stromal cells in lymphoid follicles of mesenteric lymph nodes had different immunohistochemical features from those of the gastric mucosa. Hence, nodal lymphomas might have different features from extranodal lymphomas.

Our findings suggest that low-grade and secondary high-grade lymphomas are of MZ-cell lineage, but that most primary high-grade lymphomas are of follicular centre cell lineage, with some cases originating from the MZ-cell lineage. Further studies on the biological characterization of tumour cells could confirm our conclusion.

Acknowledgement We thank Professor Hidehiko Saito, First Department of Internal Medicine, Nagoya University School of Medicine, for his helpful guidance and critical reading of this manuscript.

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