Epstein-Barr virus (EBV) concerns also with gastric lymphomagenesis: An EBER-1 in-situ hybridization analysis

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Epstein-Barr virus (EBV) concerns also with gastric lymphomagenesis:

An EBER-1 in-situ hybridization analysis

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Introduction

It is well known that there is a close relation between gastric mucosa-associated lymphoid tissue (MALT) type lymphoma and infestation of Helicobacter pylori (HP)[1-5)]. Because there are dfferences in the ability to induce inflammatory changes in the gastric mucosa among HP strains, some studies have been started to investigate the differences among HP strains from this point of view. We examined MALT and the regional lymph nodes of the resected stomach with HP-related peptic ulcer and found that lipopolysaccharides (LPSs) of HP bodies induced inducible nitric oxide synthase (iNOS) in their germinal centers (GCs)[6,7)]. We guess that nitric oxide (NO) produced by the iNOS played a role in yielding premalignant/candidate B-cells of MALT type lymphoma cells[7)].

On the other hand, we investigated Chinese gastric malignant lymphoma (gML) and found that there are cases of Chinese gML with giant lymphoma cells (Fig. 1a and b) with cytomegalovirus inclusion disease (CMD) (Fig. 1c) or Epstein-Barr virus (EBV) infection[8)]. Several studies reported also that most of EBV-related gML were of DLBL with[9)] or without immunodiffecient state in Germany[10)], Hong Kong[11-13)] and in Korea[14)], although a few of MALT type were also of EBV-related gML in Japan[15)] and in France[16)]. Further, because there are many EBV-related malignancies in the upper digestive or respiratory tracts, such as epipharyngeal carcinoma[17)], in China, we thought that there is a possibility for EBV to concern with formation

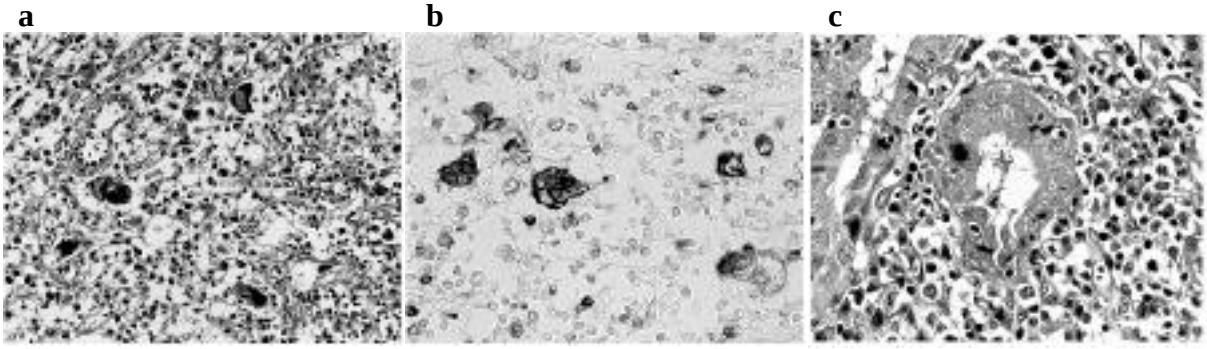

Figure 1. Giant lymphoma cells and cytomegalovirus inclusion disease (CMD) in Chinese gastric lymphomas
a: H.E. stain. A case of DLBL with gaint lymphoma cells.
b: Immunostain of CD79a. The giant lymphoma cells (a) were positive for CD79a.
c: CMD in a case of MALT type. The gland with CMD was encircled by lymphoma cells.

Table 1. EBER-1 in-situ hybridization analysis in Chinese Gastric Lymphomas

	EBV infesction (EBER-1 ISH)			
	Any cells with EBER-1 signals (No. of positive case/cases examined)	Lymphoma cells with EBER-1 signals		
		Nocells	A few cells	Many cells
MALT type lymphoma	4/10	6	4	0
Diffuse Large B-cell lymphoma (DLBL)	5/21	19	2	0
T-cell neoplasm	0/2	0	0	0
Nodal B-cell neoplasm	0/2	0	0	0

of the giant gML cells and CMD in gML in China.

Therefore, this study was performed to found out the EBV-related cases in the Chinese gMLs and how EBV concerned with the pathogenesis of the Chinese gMLs.

Material and method

Materials used in this study were paraffin sections of 35 cases of gML resected in the First University Hospital of China Medical University. Based on paraffin-immunohistochemistry[6], these Chinese gMLs comprised 10 cases of MALT type, 21 cases of diffuse large B-cell lymphoma (DLBL), two cases of T-cell neoplasm, and two cases of nodal B-cell neoplasm.

EBER-1 in-situ hybridization

EBER-1 in-situ hybridization was performed according to the method that was established by Dr. Masayoshi Tokunaga, employing digoxigenin-labeled TOK-1 anti-sense (5'-AGA CAC CGT CCT CAC CAC CCG GGA CTT GTA-3') and TOK-2 sense probes (5'- TCT GTG GCA GGA GTG GTG GGC CCT GAA CAT-3')[18]. After deparaffinized, sections were digested by proteinase K at 37°C for 30 min, were dehydrated, and were dried. Hybridization with TOK-1 and -2 probes at 37°C more than three hours. The hybridized probes were visualized by means of alkaline phosphatase (AlP)-labeled anti-digoxigen antibody and AlP reaction. After nuclear counterstain by methylgreen, sections were dehydrated and mounted in plastic medium.

As a positive control, each one case of EBV-related gastric adenocarcinoma and the nasal NK-cell lymphoma was examined.

Result

A few lymphoma cells showed EBER-1 signals in four of 10 MALT type cases and two of 21 DLBL cases, as indicated in Table 1. But there was no significant difference in the rate of the cases with a few EBER-1 signals-positive lymphoma cells, although the rate of the cases was higher in MALT type (40%) than in DLBL (9.5%). Most of the lymphoma cells showed EBER-1 signals in nuclei (Fig. 2a, b and e) and some of them revealed EBER-1 signals in cytoplasm (Fig. 2c and f). The lymphoma cells with EBER-1 signals in cytoplasm had nuclei of mitotic cells (Fig. 2c and f).

As for giant lymphoma cell formation, the giant lymphoma cells were negative for EBER-1 signals (Fig. 2d).

As for the relation between CMD and EBV infection, the distribution of EBER-1-positive lymphoma cells had no tendency in the areas with and without CMD (Fig. 2e and f).

On the other hand, the rate of cases with EBER-1 positive cells was also higher in MALT type (4/10: 40%) than in DLBL (5/21: 24%) (Table 1).

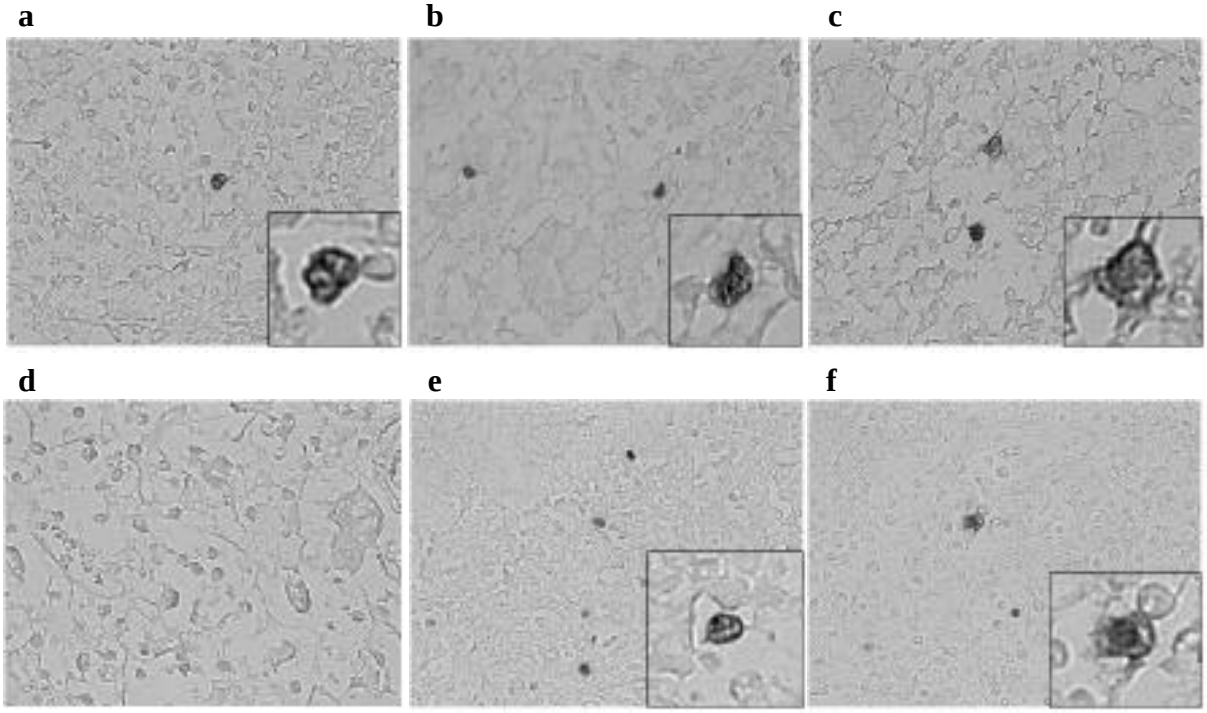

Figure 2. EBER-1 ISH in Chinese gastric lymphomas

a-c: One case of MALT type d: One case of DLBL with giant lymphoma cells

e and f: One case of MALT type with cytomegalovirus inclusion disease (CMD)

The EBV-related gML cases in this study revealed a few lymphoma cells with EBER-1 signals. Most of the lymphoma cells showed EBER-1 signals in nuclei (a, b, e), but a small number of mitotic cells indicated EBER-1 signals in cytoplasm (c, f). In one cases of DLBL with giant lymphoma cells, the giant cells did not show EBER-1 signals. In one case of MALT type with CMD, the distribution and the number of lymphoma cells with EBER-1 were similar to those in the other cases.

Discussion

This investigation did not show any cases, of which most of the lymphoma cells revealed EBER-1 signals in nuclei, although such cases of DLBL[10-14] and MALT type[15, 16] were reported to be EBV-related or positive for EBER-1 signals. One such case of gML with EBER-1 signals was also seen in our cases[8], but the case was not included in the cases examined in this study.

It is well known that EBV infects cells with receptor molecule, CD21[19]. But the lymphoma cells of gML are negative for CD21. EBV would infect lymphoma cells or candidate cells by the other pathway[20]. Once EBV infects lymphoma cells, the cells are expected to proliferate expansively. Probably EBV infection might be secondary phenomenon in the oncogenesis of gML, as suggested previously[15]. However, the rate of cases, of which any cells showed EBER-1 signals, was lower in DLBL (24%) than in MALT type (40%) (Table 1), suggesting that EBV infection would be deleted from the lymphoma tissue in accordance with the progression from MALT type to DLBL.

In this study there were only a few lymphoma cells with EBER-1 signals in the EBV-related cases (Fig. 2), as mentioned previously[15]. But mitotic lymphoma cells revealed EBER-1 signals in cytoplasm (Figs. 2c and f), revealing EBV-related subclones in the lymphoma cells of these cases. Lymphoma cells with EBV genome would delete the EBV genome in their subcloning in vivo. This phenomemon has been reported previously in EBV-related cell line, AKATA cell[21-23]. EBER-1 is one of mRNAs of EBV genome and is looked to enhance several host cell genes, including bcl-2[24] and IL-10 gene[25-27]. In this study it was suggested that there might be many EBV-related cases in the quite early phase of MALT type lymphomagenesis,

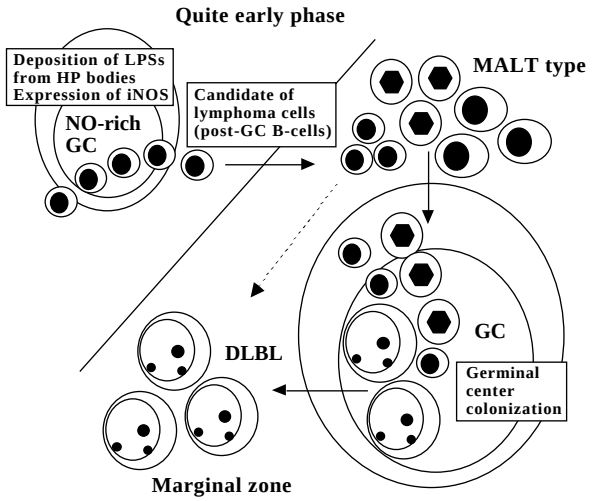

Figure 3. Our hypothesis about the quite early phase in the gastric MALT type lymphomagenesis.

It is unknown where candidate lymphoma cells of gastric MALT type yield. However, the candidate lymphoma cells may yield or have preneoplastic changes in germinal centers (GCs), because the MALT type lymphoma cells are post-GC B-cells. We found abnormal deposition of lipopolysaccharides (LPSs) of HP bodies in the GCs of regional lymph nodes and expression of inducible nitric oxide synthase (iNOS) in the GCs in the MALT and in the regional lymph nodes. Then, we guessed that the candidate cells of MALT type lymphoma yielded in the GCs of the regional lymph nodes, although there is a possibility that hyperplastic GCs, which often associate with MALT type lymphoma in the gastric mucosa, could be the site. But we think that the candidate cells can pass through the hyperplastic GCs after the outcome of obvious MALT type lymphoma.

because the rate of the cases with a few EBER-1 signals-positive lymphoma cells was higher in MALT type (40%) than in DLBL (9.5%). In the quite early phase, proliferation of the lymphoma cells/candidate cells was thought to depend on the EBV infection-effects[24-27].

But, it has not yet clarified where candidate cells of the gastric MALT type lymphoma yield. We thought that the candidate cells might yield in the germinal centers (GCs) of the regional lymph nodes. The GCs were rich in nitric oxide (NO) produced by the inducible nitric oxide synthase (iNOS)[8]. Because NO is a mutagen, the candidate cells can yield in the GCs. And we found atypical nodular hyperplasia of IgM-positive B-cells in the MALT of the stomach with HP-related peptic ulcer, whereas IgM-positive B-cells dominate in the MALT of the stomach with HP-related peptic ulcer. We looked it as a minimum change of post-GC B-cells developing to MALT type lymphoma. On the other hand, it was reported that one case of MALT type gML was negative for EBER-1 in spite of EBER-1 signal-positive cells in the regional lymph nodes[15]. The EBER-1-positive B-cells might be the candiate cells, delete EBV genome, and grow to the MALT type lymphoma. Then, we must seek after the candidate cells of MALT type in the regional lymph nodes.

As for giant lymphoma cell formation in DLBL of Chinese gML, there was no relation between lymphoma cells with EBER-1 signals and the giant lymphoma cells. The giant cells were negative for EBER-1 (Fig. 3). Then, the giant cell formation must be studied further from the other viewpoints, such as the on-going somatic hypermutation of several genes including IgH gene variable region[28] and the cell cycle check point abnormality in G2 or M phase.

As for CMD, around the gastric glands with CMD there were many lymphoma cells, but the lymphoma cells were negative for EBER-1. And the sporadic distribution of lymphoma cells with EBER-1 (Fig. 4) was not different from that in the other cases with EBER-1 signals-positive lymphoma cells.

Nowadays, MALT type gMLs are not resected until it is certified in each case that anti-HP therapy is not effective for the MALT type gML. And the number of resected stomach with HP-related ulcer decreased dramatically after introduction of the anti-HP therapy. But the MALT and the regional lymph nodes of the stomach with HP-related peptic ulcer must be studied more in order to see what the quite early phase of MALT type lymphoma is. And we believe that EBV concerns also with early oncogenesis of MALT type lymphoma in China.

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Summary

This study investigated whether EBV infection concerned with gastric lymphomagenesis by in-situ hybridization (ISH) of EBER-1. Paraffin sections of 35 cases of Chinese gastric lymphoma (gML) were used. These gMLs comprised 10 cases of MALT type lymphoma, 21 cases of diffuse large B-cell lymphoma (DLBL), two cases of T-cell neoplasm, and two cases of nodal B-cell neoplasm. EBER-1 in-situ hybridization (ISH) was performed according to Tokunaga's method. Although a few lymphoma cells showed EBER-1 signals in four (40%) cases of MALT type and in two (9.5%) cases of DLBL, EBER-1 signals were recognized in nuclei in most of the lymphoma cells and intermingling lymphocytes, but mitotic lymphoma cells revealed EBER-1 signals in cytoplasm. A small number of any cells with EBER-1 signals were noted in four cases of MALT type (40%) and in five (24%) cases of DLBL. There was no relation between EBV infection and giant lymphoma cell formation in DLBL or cyctomegalovirus inclusion disease in the mucosa with MALT type. Then, deletion of EBV genome was suggested in accordance with progression from MALT type to DLBL, as reported previously in EBV-related cell line AKATA cell. There were more cases with a few EBER-1 signals-positive lymphoma cells in MALT type than in DLBL, indicating a possibility that there might be an early phase, when many lymphoma cells or their candidate cells showed EBER-1 signals. Further, we must study an early phase of MALT type lymphoma. And we believe that EBV concerns also with early oncogenesis of MALT type gML in China.

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