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International academic project analyzing pathogenic factors in Chinese gastric B-cell lymphomas

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We have been studying virus-related malignant diseases in China with Prof. Jia Xin Shan, a member of this project, who was a guest researcher in the Second Department of Pathology, Kagoshima University Faculty of Medicine.

One of our studies was the molecular pathological comparative analysis of a small number of Chinese and Japanese cases of primary gastric B-cell lymphoma[1]. In this study we found the following differences brween Chinese and Japanese cases. 1) Infestation of H. pylori was recognized in smaller number of Chinese cases. 2) DNA amplification in polymerase chain reaction (PCR) of immunoglobulin heavy chain (IgH) gene variable region in the Chinese cases, whereas it was clarified in the result of the PCR of human β-globin (HBG) gene that enough quality of DNA was extracted from paraffin sections. 3) Direct analysis of DNA sequence amplified in the PCR for IgH gene variable region could be performed only in two of Japanese cases. 4) The DNA sequence read suggested that the IgH gene variable region was oriented to endogenous proteins other than rheumatic factor. This study was reported in Japanese with English abstract in the proceedings of International Gonryou Symposium in Tohoku University 1998.

Then, we asked Japanese Administration of Education to support our study of pathogenic factors in Chinese gastric lymphomas from a viewpoint of molecular nature of IgH gene variable region in comparison with Japanese cases. And in December 1998 we got the promise to promote this International academic project.

Here, we reviewed the recent studies of gastric lymphomas in Japan, made a hypothesis of gastric MALT type lymphoma and planed the survey of Chinese gastric lymphomas in comparison with Japanese cases with and without infection of human T-cell leukemia virus type 1 (HTLV-1) that is prevalent in the south part of Japan, especially in Kagoshima.

Recent studies of gastric lymphomas in Japan

Recent studies of the relation between infestation of H. pylori and gastric diseases including mucosa-associated lymphatic tissue (MALT) type gastric B-cell lymphomas in Japan were summarized by Prof. Akagi (Second Department of Pathology, Okayama University) in the symposium[2] of Japanese Division of International Academy of Pathology in Nara 1998. In the infestation of H. pylori, he listed as what injuries the gastric mucosa the followings; factors of H. pylori itself (proteinase, lipase, phospholipase, urease, cytotoxins, neutrophil-activating factor), products of cells (active oxygen, free radicals, cytokines (IL-1, IL-6, IL-8, TNF-α, IFN-γ) and autoimmunity mediated by HLA class II antigen and heat-shock protein that are induced with infection of H. pylori. But he stated that pathogenesis with relation to H. pylori infection of gastric malignant lymphoma has not yet been clarified, although infestation of H. pylori is observed in the stomach with gastric MALT type B-cell lymphoma.

On the other hand, molecular analysis of IgH gene variable region reported by the students of Prof. Mikata (First Department of Pathology, Chiba University) that there were differences in the somatic hypermutation of IgH variable region of lymphoma cells between low and high grade MALT type lymphomas[3]. That in low grade MALT type lymphoma is oriented to endogenous antigen, such as rheumatoid factor, whereas that in high grade MALT type lymphoma is agianst foreign body antigen.

Nowaday, polymerase chain reaction (PCR) analysis of IgH gene variable region has been intorudced in the diagnostic pathology to see clonality of lymphoma cells in B-cell malignant lymphoma instead of immunohistochemical detection of immunoglobulin light chain restriction. But only in more or less 70% of cases applied to this PCR analysis the clonality of B-cell lymphoma cells could be proved. The number of B-cell lymphoma cells in the tissue examined may effect on the PCR result. It is unknown whether the gastric lymphomas without DNA amplification in the PCR of IgH variable region suggest a mutation in the DNA sequence corresponding to the PCR primers or on-going somatic hypermutation in IgH gene. There might be possibility of T-cell lymphomas that present mimicking histology to the MALT type B-cell lymphoma, because we experienced the gastric involvement of angioimmunoblastic lymphadenopathy with dysproteinemia (AILD) type T-cell lymphoma could show many large B-cells resembling those of high grade MALT type B-cell lymphoma.

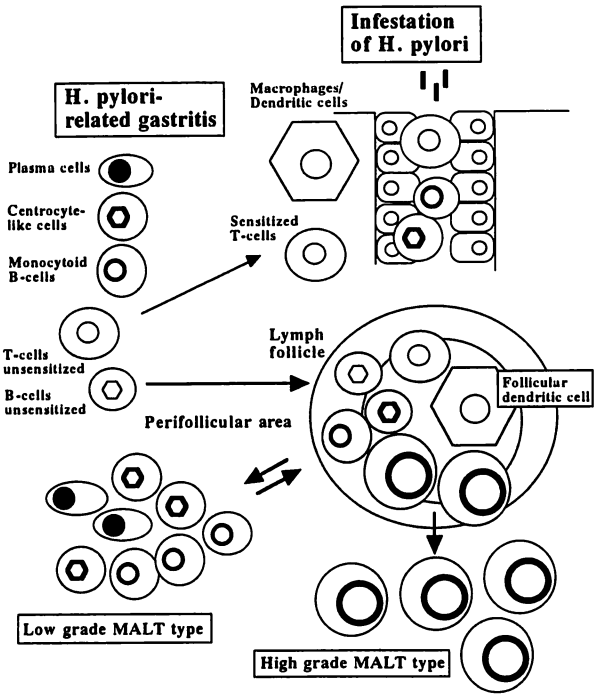
Pathogenesis of MALT type gastric lymphoma

The correlation among the infestation of H. pylori[4], H. pylori-related gastritis, and low and high grade MALT type lymphomas[5] is presented graphically in Fig. 1.

Infestation of H. pylori induces macrophages/histocytes and not-sensitized T-cells in the gastric mucosa. The macrophages/histiocytes, which recognized H. pylori-related antigens, present them to T-cells. The macrophages/histiocytes and the sensitized T-cells stimulate inflammatory cells that injury gastric mucosa with free radicals[6] synthesized by H. pylori and the nitric oxide synthe-

Figure 1. A hypothesis of pathogenesis of gastric MALT type lymphoma under infestation of H. pylori.

The infestation of H. pylori induces macrophages/histocytes and not-sensitized T-cells in the gastric mucosa. The macrophages/ histiocytes recognized H. pylori-related antigens and present them to T-cells. The macrophages/histiocytes and the sensitized T-cells stimulate inflammatory cells that injury gastric mucosa with free radicals synthesized by H. pylori and nitric oxide synthesized by H. pylori and by induced nitric oxide synthetase (iNOS) in the H. pylori-related gastritis. On the other hand, the macrophages/histiocytes and the T-cells produce a large amount of cytokines. The cytokines make B-cells to enter in germinal center of lymph follicle. Abnormal clones of the B-cells appear in germinal center under a large amount of the cytokines and the nitric oxide. Helper T-cells that differentiate from the sensitized T-cells and follicular dendritic cells (FDCs) exist in the germinal center. Neoplastic B-cells appear also in the abnormal clones and pool in the marginal/perifollicular zone of the MALT. The neoplastic B-cells may be those of low-grade MALT type lymphoma. The neoplastic B-cells form germinal center colonization, where FDCs present H. pylori-related antigens and the other antigens. In the microenvironment with a large amount of the nitric oxide that is a strong stimulant to lymphocytes and the other cells, the neoplastic B-cells are oriented to the antigen presented by FDCs. The large neoplastic B-cells appeare in low grade MALT type lymphoma would be those of high grade MALT type lymphoma.



sized by induced nitric oxide synthetase (iNOS) in the macrophages/histiocytes in H. pylori-related gastritis.

On the other hand, the macrophages/histiocytes and the T-cells produce a large amount of cytokines. The cytokines make B-cells to enter in germinal center of lymph follicle. Abnormal B-cell clones appear in germinal center under the large amount of cytokines and the nitric oxide. Helper T-cells that differentiate from the sensitized T-cells and follicular dendritic cells (FDCs) also exist in the germinal center. Neoplastic B-cells that come from the abnormal B-cell clones pool in the marginal/ perifollicular zone. The neoplastic B-cells may be those of low-grade MALT type lymphoma. Histologically, lymphoma cells in low grade MALT type gastric lymphoma comprise centrocyte-like cells, monocytoid cells and plasma cells. The cellular composition varies from that of dominately centrocyte-like cells to that of dominantly plasma cells (gastric immunocytoma and plasmacytoma).

Because these lymphoma cells are of post-germinal center B-cells, occurence of these lymphoma cells is thought to be under the effects of FDCs. The IgH gene variable region of lymphoma cells in MALT type gastric lymphoma would reflect the antigen presentation by FDCs and nature of antigenic microenvironment of the gastric mucosa. The IgH gene variable region oriented to the endogenous antigens in low grade MALT type lymphoma suggests disordered presentation of endogenous antigens by FDCs. The high grade MALT type lymphoma cells come from the low grade MAL type lymphoma cells in the germinal center colonization. Although it is unknown whether once oriented post-germinal center B-cells could be re-oriented in the germinal center, the high grade MALT type lymphoma cells would recieve the presentation of foreign antigen by the FDCs in the germinal center.

In the microenvironment with a large amount of cytokines and nitric oxide, T-cells might be disordered. Considering the blast formation of B-cells among neoplastic T-cells in the AILD type T-cell lymphoma, the other kind of the pathogenesis of high grade MALT type lymphoma may be suggested. In the mixed proliferation of T-cells and B-cells in H. pylori-related gastritis high grade MALT type B-cell lymphoma with many intermingling T-cells might occur. Such T-cell rich B-cell lymphoma can be seen in our collected series of gastric B-cell lymphomas. Metachronous occurrence of T-cell rich gastric B-cell lymphoma was seen in a case of nodal adult T-cell leukemia/lymphoma (ATLL)[7]. Stimulation of B-cells by T-cells and the other microenvironmental factors such as the nitric oxide, Epstein-Barr virus (EBV) in an immunocompromized host[8] and HTLV-1 in a HTLV-1 carrier make a chance for B-cells to be neoplastic. On the other hand, in this mixed proliferation T-cells can be neoplastic. If there is MATL type T-cell lymphoma, the MALT type T-cell lymphoma would associate B-cell proliferation as AILD type T-cell lymphoma does.

Study design for the survey of gastric lymphomas

Considering the above-mentioned pathogenesis of MALT type gastric lymphoma, comparative study of Chinese and Japanese gastric lymphomas needs 1) evaluation of H. pylori infestation in the gastric mucosa with the lymphoma, 2) estimation of an amount of the nitric oxide in the gastric mucosa by means of immunohistochemical detection of iNOS, 3) examination of nature of FDCs in the germinal centers with and without germinal center colonization of low grade MALT type B-cell lymphoma, 4) understanding the state of somatic hypermutation of IgH gene variable region in low and high grade MALT type B-cell lymphoma cells by means of PCR analysis, and 5) reading DNA sequence of the IgH gene variable region for understanding the antigenic structure in a lever of DNA se-

quence.

The dissociation of the H. pylori infestation and the distribution of the iNOS-positive cells will suggest pathogenic factors other than the H. pylori infestation in Chinese MALT type lymphomas.

The relation between the distribution of the iNOS-positive cells and the results in the PCR analysis of IgH gene variable region will inform the degree of somatic hyper mutation and on-going somatic mutation in the IgH gene variable region of the MALT type B-cell lymphomas.

Additonally, a survey of the infection of EBV and HTLV-1 will give information about the factors that concerns with disordered functions of the FDCs and the other antigen-presenting cells.

Furthermore, if there will be a chance to examine T-cell receptor (TCR) γ-chain gene variable region of the primary gastric T-cell lymphomas, it will inform whether there is a MATL type T-cell lymphoma or not.

This study design will clarify the pathogenic factors in Chinese gastric lymphomas in comparison with Japanese cases.

Symposium of gastric lymphomas in China

In order to success in the project study in China, we plan symposium of gastric lymphoma in China from a viewpoint of the PCR analysis and DNA sequencing of the IgH gene variable region of gastric lymphoma cells, inviting Japanese and Chinese speakers.

In a part of the symposium, we will understand how Chinese gastric lymphoma is from viewpoints of clinical diagnosis, indication of the gastrectomy in cases with gastric lymphoma and clinicopathological features.

The other part of the symposium concerns with technical aspect of extracting DNA from paraffin sections and with practice of the study of gastric lymphomas employing the molecular analysis of IgH gene variable region. The analysis of a small piece of gastric mucosa biopsied will be needed in some cases. In order to understand the way of direct DNA sequencing of PCR product, technical report will be needed. Understanding somatic hypermutation and on-going somatic mutation in IgH gene variable region of B-cell lymphoma cells is quite important to perform this project. Homology analysis of IgH gene variable region will inform the antigenic structure presented in the oncogenesis of the B-cell lymphoma.

At first, members of this project and the co-operated reseachers are necessary to understand the plan of the symposium. We wish that the project study will start with ease after the symposium.

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