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Paraffin-immunohistochemical features of lymphocytes in mucosa-associated lymphoid tissue and the regional lymph node in stomach with Helicobacter pylori-associated peptic ulcer

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Summary

Immunological features of cellular components of the mucosa-associated lymphoid tissue (MALT) in a stomach with peptic ulcer and infestation of Helicobacter pylori were examined by means of paraffin-immunohistochemistry in comparison with the cellular components in the regional lymph node. The MALT comprises follicles of B-cells and perifollicular areas that corresponding marginal zone in lymph node. In the perifollicular areas there were many CD3-positive, CD5-positive CD4-positive/CD8-positive T-cells, while only a few T-zone-associated dendritic cells (DCs), which were positive for S100 protein and LN-3 (Ia-like antigen), were seen. T-cells in the perifollicular areas of the MALT would come from the regional lymph node, where T-cell blast formation associating T-zone DCs was recognized. The DCs were thought to be activated, showing positive stain of S100 protein and LN-3 (Ia-like antigen), and nuclear stain of thymidine phosphorylase, and expressing inducible nitric oxide synthase (iNOS). Becasue expression of iNOS was seen strongly in FDCs in small germinal centers and weakly in dendrites of FDCs in large germinal centers in the MALT and in the regional lymph node, the iNOS in FDCs would prepare the microenvironment of B-cell blast formation in the germinal centers. Furthermore, because activated T-zone-associated DCs also had iNOS, nitric oxide produced by the iNOS in lymphatic tissue might be a physiological mediater of B- and T-cell blast formation in the antigen-sensitization rather than that of lipopolysaccharide-antigen sensitization, apoptosis and tissue injury.

Figure 1. MALT and the regional lymph node

The sections with a peptic ulcer show development of MALT above, in and below the mucosal muscular lamina. In the subserosal tissue, a swollen lymph node is recognized.

In the MALT, CD3-positive pan-T-cells are seen around the aggregation of CD79a-positive B-cells. More B-cells are seen also in the stroma of the mucosa than T-cells.

In the lymph node, folliclular hyperplasia comprising many CD79a-positive B-cells and development of paracortical T-zone areas comprising many CD3-positive pan T-cells.

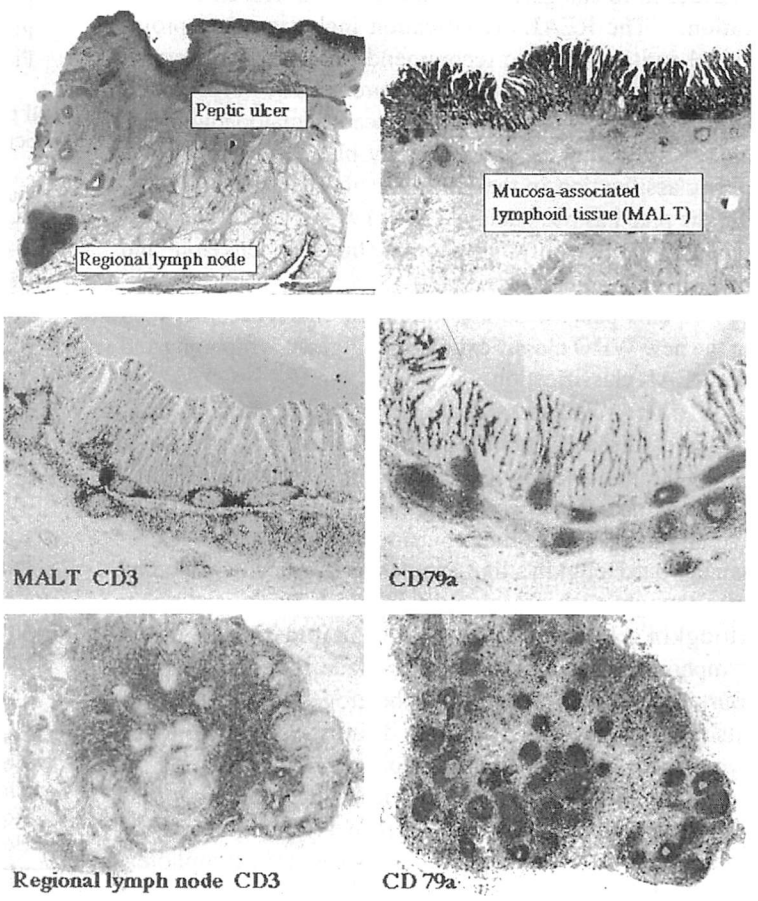

Figure 2. Infestation of H. pylori in the surface mucous coat

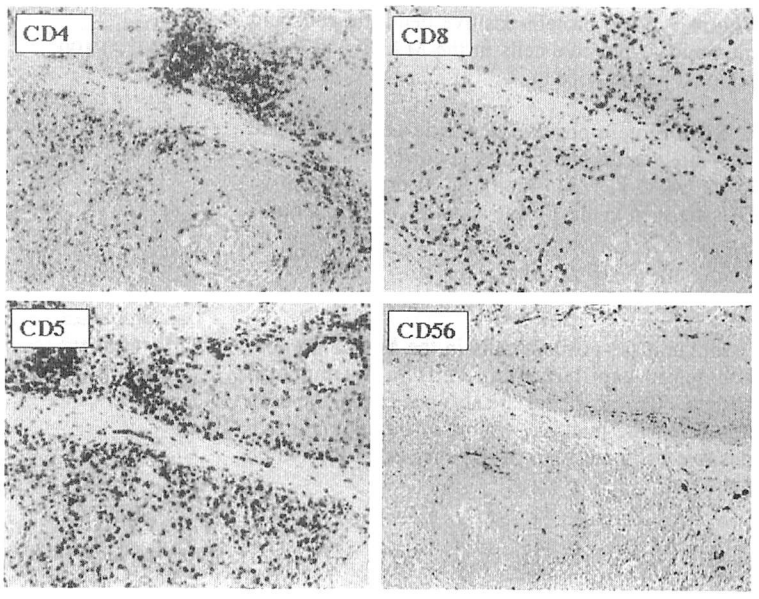

Figure 3. Lymphocytes in the MALT

In the MALT, CD4- and CD8-positive T-cells distribute dominantly in the parafollicular areas (corresponding marginal zone), although CD4-positive cells are seen more numerous than CD8-positive cells. A small number of CD4- and CD8-positive T-cells are seen in the germinal center. Most of CD5-positive cells are T-cells, but CD5-positive or -weakly positive cells are seen in the follicle mantle zone. These cells are CD5-positive B-cells. CD56-positive T/NK-cells are seen rarely.

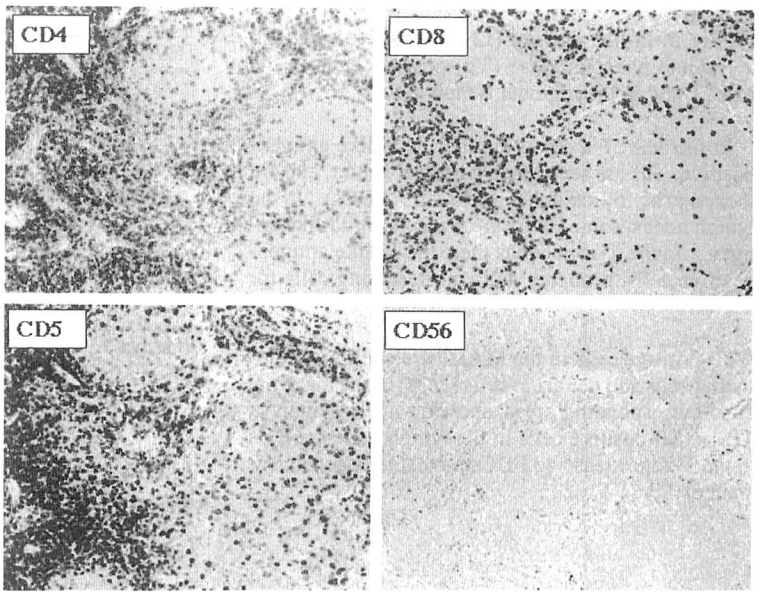

Figure 4. Lymphocytes in the regional lymph node in the subserosa of the stomach

The lymph node shows follicular hyperplasia and developing T-zone areas in the parafollicular areas and in the medulla. CD4- and CD8-positive T-cells are seen in the T-zone areas and some are in follicles, especially in germinal centers. In the mantle zone of lymph follicles CD5-postive or weakly positive cells are CD5-positive B-cells. Most of CD5-positive cells in the T-zone areas are T-cells. A small number of CD56-positive NK-cells are recognized in the sinuses.

Introduction

Hilicobacter pylori (H. pylori) is a causative agent for gastritis, peptic ulcer, gastric cancer and gastric mucosa-associated lymphoid tissue (MALT) type lymphoma[1]. H. pylori-related gastritis and peptic ulcer often associate reactive lymphoid hyperplasia (RLH). The RLH appearing in stomach with gastritis/peptic ulcer is a representative beneign MALT. Gastric MALT type lymphoma would originate in the RLH[2-4]. Recent studies showed that most of gastric lymphomas are of MALT type[1], although it has been reported that malignant lymphomas (ML) other than MALT type can occur in stomach[1].

This paper aimed to see features of the cellular components of the gastric MALT and regional lymph node as the background of the occurrence of ML by employing paraffin-immunohistochemistry.

Material and method

Two sections (Fig.1) of the stomach resected because of perforated peptic ulcer from a Japanese 62 years-old male were employed. The sections included MALT above, in and below the mucosal muscular lamina and the regional lymph node in the subserosal tissue (Fig. 1). In the mucosa without intestinal metapalsia H. pylori are seen in the surface mucous coat (Fig. 2).

Employing the following antibodies; CD3, CD4 and CD8 for T-cells, CD79a for B-cells, CD56 for NK-cells, CD5, S100 protein, muramidase, LN-3 (Ia-like antigen), thymidine phosphorylase (TP) (supplied by Prof. Akiyama S., Kagoshima University Faculty of Medicine) and inducible nitric oxide synthase (iNOS, Calbiochem-Novabiochem Co. Cat. No. 482728, this rabbit polyclonal antibody recognizes iNOS in human, rat and mouse), features of lymphocytes, dendritic cells and the others in the MALT and regional lymph node were examined by means of paraffin-immunohistochemistry with or without antigen-retrieval pretreatment. The reacted antibodies were visualized by means of avidin-biotin complex (ABC) method.

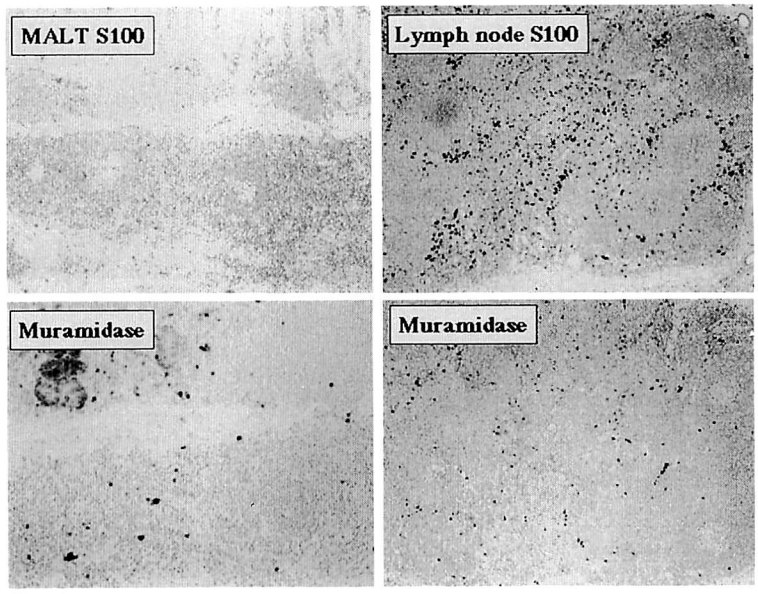

Figure 5. S100 protein-positive dendritic cells and muramidase-positive cells in the MALT and in the regional lymph node

S100 protein-positive dendritic cells (DCs) are seen rarely in the marginal zone-corresponding areas in the MALT, while many S100 protein-positive DCs are seen in the T-zone areas of the lymph node.

Muramidase-positive cells in the MALT are leukocytes in capillaries and cells in the mucosal stroma. Gastric glands are also positive in the deep portion of the mucosa. Most of the muramidase-positive cells are seen in the sinuses in the lymph node.

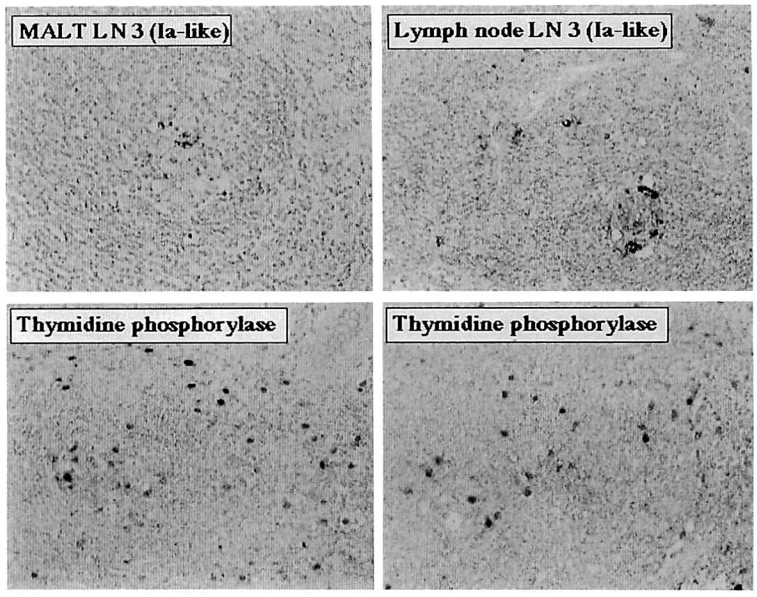

Figure 6. Ia-like antigen (LN-3)-positive cells and thymidine phosphorylase (TP)-positive cells in the MALT and in the regional lymph node

In the MALT a small number of Ia-like antigen-positive cells are seen in the germinal center, and are looked to be follicular dendritic cells (FDCs). In the lymph nodes, some FDCs are positive for Ia-like antigen. In the T-zone areas there are Ia-like antigen-positive cells, probably antigen-presenting cells for T-cells.

TP-positive cells in the MALT are capillary endothelial cells and dendritic cells (DCs) in the marginal zone-corresponding areas, showing nuclear stain of TP. TP-positive cells in the lymph node are DCs in the T-zone areas and FDCs, revealing also nuclear stain of TP.

Result

The MALT included B-cell lymph follciles and T-cell-rich perifollicular areas, which are corresponding to the marginal zone in lymph node (Fig. 1).

The T-cells in the perifollicular areas of the MALT dominated in CD4-positive cells, while relatively many CD8-positive cells were seen (Fig. 3). The T-cells were also positive for CD5. A small number of CD5-positive or -weakly positive cells in the mantle zone of lymph follicles were CD5-positive B-cells. There were rare CD56-positive NK-cells in the MALT.

On the other hand, in the lymph node T-cells and B-cells revealed the same tendency in the distribution (Fig. 4) as in the MALT. But there were some CD56-positive NK-cells in the sinuses.

S100 protein-positive dendritic cells (DCs) were seen rarely in the perifollicular areas of the MALT, when many S100 protein DCs were seen in the T-zone areas of the lymph node (Fig. 5).

Muramidase-positive cells were leukocytes in capillaries in the MALT and in the mucosal stroma. In the lymph node, muramidase-positive macrophage-phagocyte system (MPS) cells were recognized (Fig. 5).

LN-3 (Ia-like antigen)-positive cells were FDCs in germinal centers of the MALT and the lymph node (Fig. 6). In the lymph node DCs in the T-zone areas were positive for LN-3 (Ia-like antigen).

Thymidine phosphorylase (TP)-positive cells in the MALT were capillary endothelial cells and a small number of DCs and FDCs, revealing nuclear stain (Fig. 6). Most TP-positive cells in the lymph node were DCs, revealing nuclear stain. A few FDCs showed nuclear stain of TP.

The cells that revealed strong positive stain of iNOS were FDCs in small germinal centers of the MALT, although B-immunoblast-like centroblasts looked as positive (Fig. 7). In the enlarged germinal centers some FDCs showed dendrites positive for iNOS. In the lymph node FDCs and DCs in the T-zone areas showed positive stain of iNOS. As seen in the MALT, enlarged germinal centers showed decrescent stain of iNOS in the dendrites of FDCs.

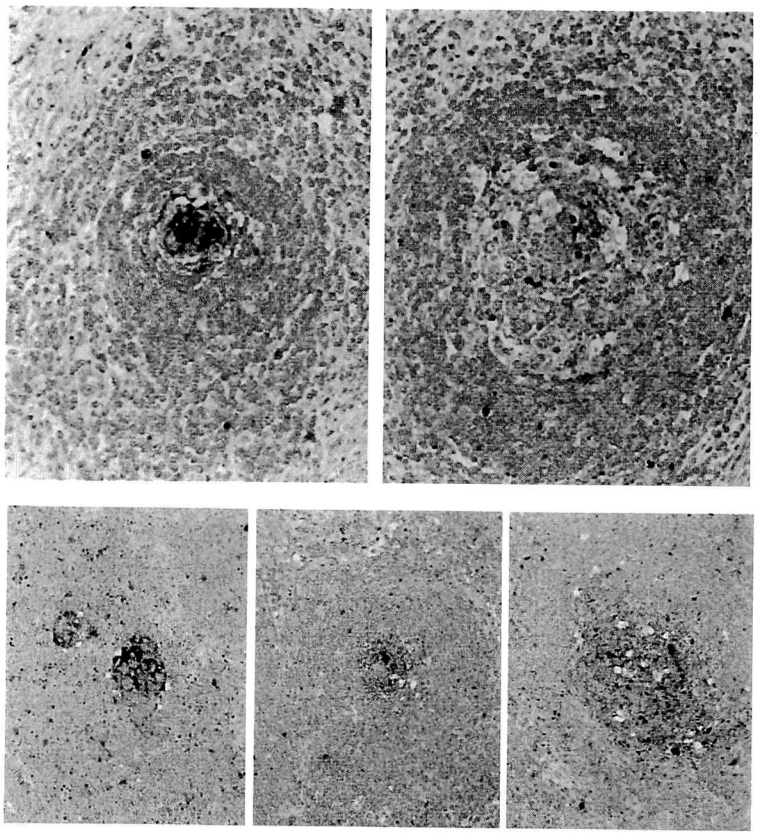

Figure 7. Inducible nitric oxide synthase (iNOS) in the MALT and in the regional lymph node

In the MALT (Above 2 figures) FDCs expressed a large amount of iNOS in the samll germinal center, seen as immunoblast-like centroblasts are positive. In the larger germinal center dendrites of FDCs express a small amount of iNOS. A small number of iNOS-positive cells are seen in the parafollicular areas.

In the lymph node (Below 3 figures), FDCs express strongly iNOS in small germinal centers. Acording to the largeness of the germinal centers, dendrites of FDCs indicate decrescent expression of iNOS, as in the MALT. Some large germinal centers are negative for iNOS.

Discussion

The MALT in the stomach with peptic ulcer and infestation of H. pylori comprises B lymphocytes that would come from extragastric areas and form lymph follcles associating perifollicular areas, because CD5-positive B-cells were recognized in the mantle zone of the lymph follicles. The perifollicular areas in the MALT was different in distribution of S100 protein-positive, LN-3 (Ia-like antigen)-positive, TP-positive and iNOS-positive DCs from the T-zone areas in the regional lymph node. But in the lymph follicles almost the same features of FDCs were seen in the germinal centers of the MALT and in the regional lymph node.

Because the DCs positive for S100 protein and LN-3 (Ia-like antigen) are foreign antigen-presenting cells to T-cells, rare DCs in the perifollicular areas of the MALT suggested that T-cells in the perifollicular areas of the MALT would come from the regional lymph node and the other areas, where the T-cells would be sensitized in the T-zone and its corresponding areas. The DCs in the T-zone areas showed nuclear stain of TP, suggesting active metabolism of thymidine[5].

The iNOS-strongly positive FDCs in small germinal centers and the decrescent iNOS-positive stain in FDCs in large germinal centers in the MALT and in the lymph node suggested that the iNOS in the FDCs prepares the microenvironment of lymphocytic blast formation in the germinal centers rather than the iNOS induced in lipopolysccharide-antigen sensitization in the germinal centers. Furthermore, the iNOS-positive DCs in the T-zone areas of the lymph node suggested that the iNOS plays a role to prepare blast formation of T-cells and B-cells in the antigen-sensitization. Probably, nitric oxide (NO) produced by the iNOS in the microenvironment can be a stimulant for T- and B-cell proliferation, although iNOS is induced in various cells in endotoxin shock[6], appeares in anti-tumor macrophages in an experiment[7] and in apoptosis of the antigen-specific T-cells[8].

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