Expression of inducible nitric oxide synthase in epithelial cells, mucosa-associated lymphoid tissue, and regional lymph nodes of the stomach with Helicobacter pylori-associated peptic ulcer

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This study examined expression of inducible nitric oxide synthase (iNOS) by means of antigen-retrieval immunohistochemistry of anti-iNOS antibody in 4 cases of the stomach with Helicobacter pylori (HP)-associated peptic ulcer and in 29 cases of non-neoplastic and neoplastic lymphatic tissue in order to understand pathogenicity of iNOS in HP-associated diseases. 1) Crypt foveolar epithelial cells in the stomach and covering squamous cells in the tonsil expressed iNOS, suggesting that iNOS is expressed as a part of mucosal defence mechanism. 2) Some germinal centers (GCs) in mucosa-associated lymphoid tissue (MALT) and in regional lymph nodes of the stomach expressed iNOS, suggesting that iNOS is expressed in the GCs as a part of immunity against HP infestation, although some GCs expressed iNOS in non-specific lymphadenitis. 3) Including foreign body type multinuclear giant cells in tuberculous lymphadenitis and foamy cells in the mesenteric lymph node, histiocytes expressed iNOS that would be induced in a part of foreign body reaction against bacteria. 4) Thymic epithelial cells expressed iNOS that may concern with apoptosis of thymocytes. 5) A few metastatic mammary carcinoma cells in a lymph node expressed iNOS that may have relation to metastatic activity. 6) GCs in atypical lymph follicles of the early marginal zone B-cell lymphoma in the skin expressed iNOS, suggesting a role of iNOS in lymphomagenesis. Consequently, iNOS, which is expressed in GCs of the lymphatic tissue of the stomach with HP infestation, would produce nitric oxide (NO) that effects on the immunity against HP and prepares microenvironment for MALT type/marginal zone B-cell lymphomagenesis.

Key words: inducible nitric oxide synthase(iNOS), immunohistochemistry, stomach, *Helicobacter pylori* (HP), mucosa-associated lymphoid tissue (MALT), thymus, tonsil, lymph node, carcinoma, MALT type lymphoma

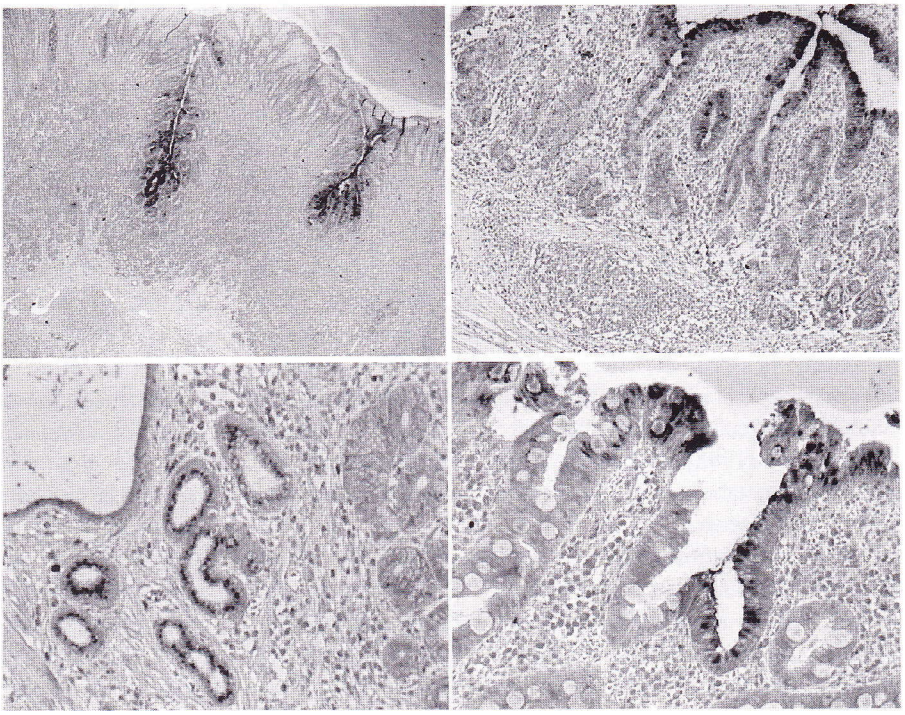

Figure 1. Expression of iNOS in the epthelial cells of the gastric mucosa around the ulcer

Upper left: In the stomach with hyperplastic foveolar epithelial mucosa, foveolar epithelial cells show strong expression of iNOS in the crypts.

Upper right: In the stomach with regenerative hyperplastic foveolar epithelial mucosa, foveolar surface epithelial cells express iNOS. The MALT tissue with germinal center in the mucosa does not express iNOS.

Lower left: Regenerative epithelial cells express iNOS in the supranuclear cytoplasm.

Lower right: Epithelial cells reveal intestinal metaplasia and express iNOS in the surface portion.

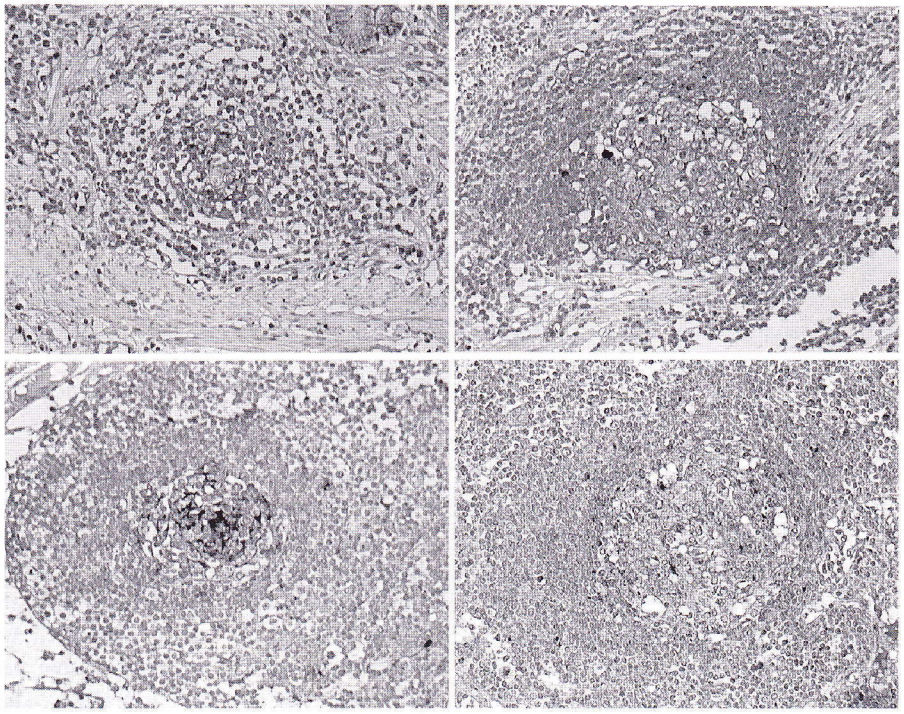

Figure 2. Expression of iNOS in MALT and in the regional lymph nodes of stomach with infestation of Helicobacter pylori

Upper left and right: MALT. In the left figure, obscure ecpression of iNOS is recognized in the germinal center (GC). In the right figure, a few histiocytes express iNOS in the GC.

Lower left and right: Regional lymph node. In the lymph follicle with developing GC of the left figure, meshwork pattern of follicular dendritic cells/germinal center dendritic cells is labeled by the anti-iNOS antibody. In the right figure, only a few histiocytes express iNOS in the developed GC.

Table 1. Cases examined

	No. of cases examined	
Stomach with peptic ulcer		
associating infestation of HP	4	
with Regional lymph node		2
Thymus	2	
Normal		1
Thymoma		1
Lymph node	16	
Non-specific lymphadenitis		3
Subacute lymphadenitis		2
Dermatopathic lymphadenitis		3
Necrotizing lymphadenitis		6
Tuberculosis		1
Whipple dusease-like lymph node		1
Tonsil	8	
Chronic tonsillitis		7
Follicular lymphoma in tonsil		1
MALT type lymphoma in the thyroid	1	
Early phase of marginal zone B-cell lymphoma in the skin	1	
Metastatic mammary carcinoma in the lymph node		1

Introduction

Helicobacter pylori (HP) is regarded as the causative factor for gastric ulcer, gastritis (Marshall & Warren, 1984: Blaser, 1992), gastric cancer (Correa, 1992) and mucosa-associated lymphoid tissue (MALT) type lymphoma (Wotherspoon et al. 1991), although the pathomechanism of such HP-associated diseases has not yet clarified. Recently, Nagata et al. (1998) reported that superoxide produced by HP modulates metabolism of nitric oxide and would induce such HP-related diseases.

In an immunohistochemical analysis of lymphocytes and the other cellular components in MALT and the regional lymph node in a case of stomach with HP-associated peptic ulcer, inducible nitric oxide synthase (iNOS) expressed in some germinal centers (GCs) of the regional lymphoid tissue (Hasui et al. 1999). Because iNOS is well known to be induced in histiocytes under stimulation by lipopolysaccharide (Geller et al. 1993), we thought that the iNOS was induced in follicular dendritic cells (FDCs) and/or germinal center dendritic cells (GCDCs)(Grouard et al. 1996: Dobois et al. 1999) against HP infestation.

This study aimed to see how iNOS is expressed in non-neoplastic and neoplastic lymphatic tissue including that of the stomach with the infestation of HP. Considereing effects of iNOS expressed in the lymphatic tissue, pathogenicity of the iNOS and its product, NO, in MALT type lymphoma is discussed.

Material and method

Four cases of the stomach with HP-associated peptic ulcer were employed for this study. In two of them, regional lymph nodes could be examined. As indicated in Table 1, one case of thymus, one thymoma, 16 lymph nodes, seven tonsils, one metastatic mammary carcinoma in the lymph node, one follicular lymphoma in the tonsil, one MALT type lymphoma in the thyroid, and one early marginal zone B-cell lymphoma in the skin (Hasui et al. 1998) were employed.

After dewaxed, disturbing endogenous peroxidase activity, and hydrated in phosphate buffer saline, sections of these cases were incubated in 0.01M citrate buffer pH 6.0 and were heated in autoclave for 15 min. for antigen retrieval. The sections were reacted with anti-iNOS polyclonal antibody (CALBIOCHEM, Cat. No. 482728) at 4℃ overnight. Reacted antibody was visualized by means of Elite ABC method and DAB H_2O_2 reaction. The sections were dehydrated in graded ethanol and embedded in plastic medium.

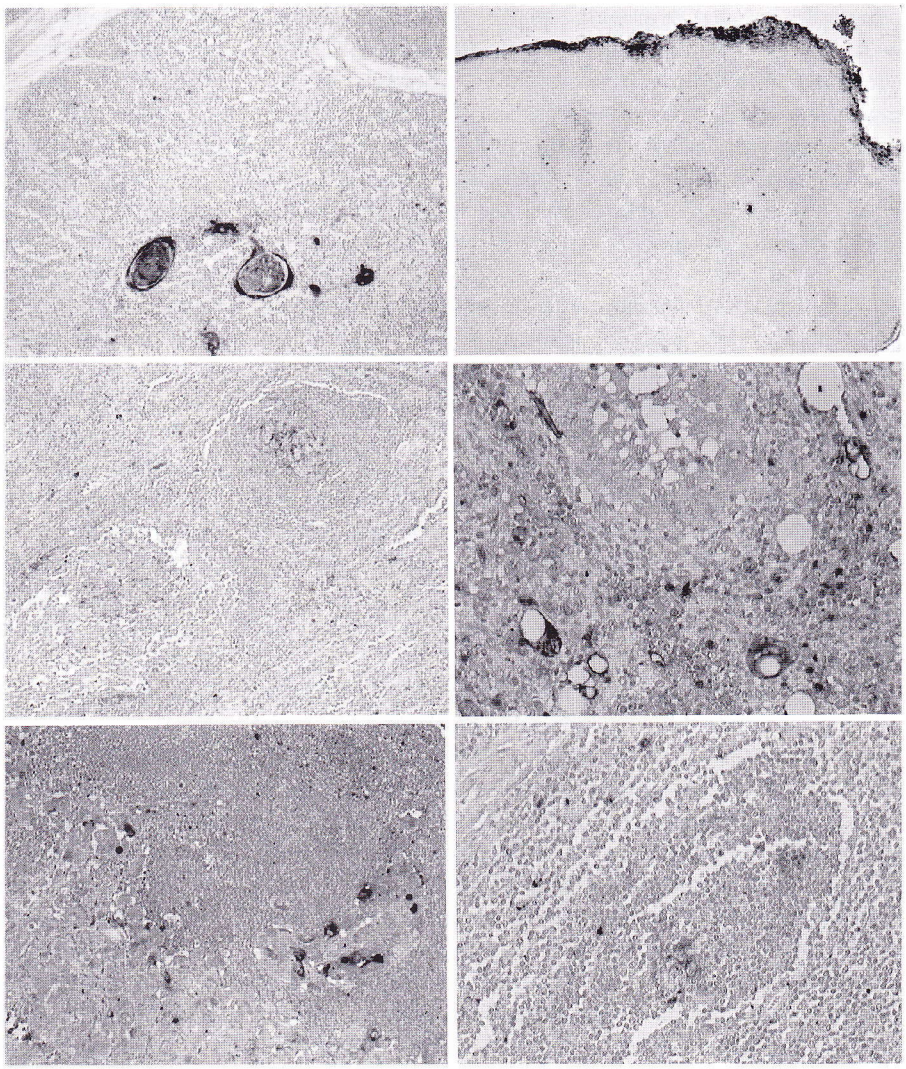

Figure 3. Expression of iNOS in various lymphatic tissue

Upper left: Thymus. Thymic epithelial cells express iNOS. Hassall's corpuscles express iNOS strongly.

Upper right: Tonsil. Squamous cells covering oral tonsil tissue express iNOS. Rarely germinal centers (GCs) of the tonsil express iNOS.

Middle left: Lymph node. In non-specific lymph-adenitis, GCs and histiocytes in paracortex, medulla and sinuses express iNOS.

Middle right: Lymph node. In tuberculous lymphadenitis, epithelioid cells in tuberculomas do not express obviously iNOS, whereas multinuclear giant cells express iNOS.

Lower left: Lymph node. In metastatic mammary carcinoma in a lymph node, a few carcioma cells express iNOS especially in small alveoli.

Lower right: Lymph node. In early phase of marginal zone B-cell lymphoma in the skin, tiny GCs express iNOS in a lymph follicle.

Results

Epithelial cells of the stomach showed various positive stain of the anti-iNOS antibody, as shown in Fig. 1. Epithelial cells in crypts showed cytoplasmic stain of the antibody in a case with the foveolar epithelial hyperplastic mucosa. In the other case with feoveolar epithelial hyperplastic mucosa, the surface epithelial cells showed strong cytoplasmic stain of the antibody. Surface epithelial cells manifesting intestinal metaplasia and regenerative epithelial cells showed also cytoplasmic stain. In the atrophic mucosa the glandular epithelial cells showed weak cytoplasmic stain.

MALT with developed GCs was seen in two cases of the gastric mucosa with foveolar epithelial hyperplasia and with metaplastic and regenerative epithelial cells. As indicated in Fig. 2 (Upper), in small GCs comprising centroblasts, FDCs/GCDCs and their meshwork were labeled weakly by the antibody. In developed or enlarged GCs only a few iNOS-positive histiocytes were seen among centrocytes and centroblasts. In a case of atrophic mucosa aggregated lymphocytes in the deep part of the mucosa showed very weak stain.

The regional lymph nodes in the cases with foveolar epithelial hyperplasia or metaplastic regenerative epithelial cells showed a small number of GCs labeled by the antibody (Fig. 2, Lower). The stain of GCs in the regional lymph node was stronger than that in MALT in spite of the same pattern of the stain.

Thymic epithelial cells and Hassall's corpuscles were positive for the antibody (Fig. 3 Upper left). In thymoma, a small number of epithelial cells among the thymoma cells were positive.

In the all cases of the tonsil except tonsillar follicular lymphoma, covering squamous cells were positive for the antibody (Fig. 3 Upper right). In an involuting lymph follicle of one case, there were a small number of cells labeled by the antibody. In an other case there were many GCs labeled by the antibody.

In lymph nodes, only three cases of the lymph nodes with non-specific lymphadenitis showed GCs labeled by the antibody (Fig. 3 Middle left), whereas there were histiocytes labeled by the antibody in the interfollicular areas and in the medulla of the most lymph nodes. In cases of necrotizing

lymphadenitis, the aggregated histiocytic reticulum cells were negative for the antibody. In tuberculous lymphadenitis, epithelioid cells were almost negative, whereas multinuclear giant cells in the areas among the tuberculomas were strongly positive for the antibody (Fig. 3 Middle right). In one mesenteric lymph node with many foamy cells in sinuses, resembling Whipple disease, the foamy cells showed strong stain of the antibody, and a small number of GCs were labeled by the antibody.

A few metastatic mammary carcinoma cells showed cytoplasmic stain of the antibody and tend to be in small alveoli (Fig. 3 Lower left). One carcinoma cell, which presented a figure of single cell metastasis, revealed strong cytoplasmic stain.

In the case of early marginal zone B-cell lymphoma in the skin, a few GCs in a lymph follicle with atypical lymphocytes were labeled by the antibody. In the follicular lymphoma in the tonsil and MALT type lymphoma in the thyroid, there are no cells positive for the antibody.

Discussion

Expressed iNOS produces nitric oxide (NO). Effect of NO on cell function is well explained in a case of NO produced by endothelial NO synthase. NO produced in endothelial cells osmoses easily to blood vessel smooth muscle cells and relaxes them, when the endothelial cells recieve an acetylcholin signal from nerve cells (Moncada et al. 1991: Lowenstein & Snyder, 1992: Bredt & Snyder, 1992). NO activates cyclic GMP to change electric polarization of cell membrane by disturbing sodium (Na) channels and relaxes smooth muscle cells (Fig. 4). The changed electric polarization in cell membrane would disturb two of three systems transducting cell membrane-receptor signals, Na channel system and G protein co-operated system (Hirai, 1994). The residual system transducting cell membrane-receptor signals is tyrosine-kinase or -phosphorylase system, which yields a signal to proliferative signal transduction system. Besides the effect of NO on cyclic GMP, NO injuries cells and tissue, and plays as a mutagen to DNA. Cells that can survive in a NO-rich environment might include neoplastic cells, because

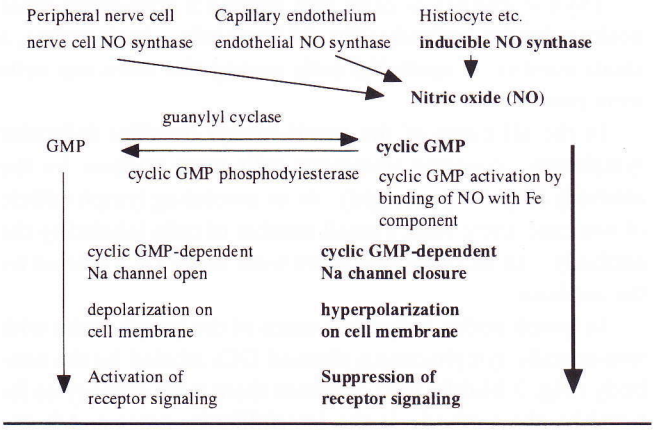

Figure 4. Nitric oxide suppresses receptor signaling in cells

DNA damage induced by NO makes most cells to fall in apoptosis. Although NO can be catalized easily in H_2O, constant production of NO by NO synthase would prepare such NO-rich environment. Oncogenesis and metastatic activity of esophageal squamous cell carcinoma, malignant melanoma and breast cancer are thought to have relation to the expression of iNOS (Duenas Gonzalez et al. 1997: Tanaka eta al. 1999: Tschugguel et al. 1999), as the iNOS was detected in metastatic mammary carcinoma cells in lymph node in this study (Fig. 3 Lower left).

The expression of iNOS was reported in various cells under a strong stimulation (Sato et al, 1995). Most of such cells would fall in apoptosis.

The expression of iNOS in thymic epithelial cells was thought to play a role in apoptosis of thymocytes.

In the stomach with HP-associated peptic ulcer and in the tonsil, Epithelial cells that covered the mucosa expressed iNOS, as Tanaka et al reported (1999). In the stomach, the distribution of epithelial cell expressing iNOS changed in accordance with the mucosal response under HP infestation (Goldstone et al. 1996: Yabuki et al. 1997). Crypt and surface foveolar epithelial cells expressed iNOS in the hyperplastic foveolar epithelial mucosa, as shown in Fig. 1. As tonsillar squamous cells would express iNOS as a part of mucosal defence mechanism against bacterial infestation in the oral cavity, the gastric foveolar epithelial cells do in a defence mechanism against HP infestation. In the follciular lymphoma in the tonsil, the covering squamous cells lost the expression of iNOS, suggesting that the mucosal defence mechanism would be lost in lymphomagesis in the tonsil. It must be studied whether the gastric mucosal defence mechanism is lost in gastric MALT type lymphomagenesis.

Histiocytes in the paracortex and in the medulla of the lymph nodes, foamy cells in the mesenteric lymph node, and multinuclear giant cells in tuberculous lymphadenitis expressed iNOS, suggested that iNOS is induced in foreign body reaction against any bacteria. On the contrary, epithelioid cells in tuberculomas did not express iNOS, suggesting that mycobacterial infestation is maintained in such epithelioid cells not-expressing iNOS.

FDCs/GCDCs in the MALT and the regional lymph nodes of the stomach expressed iNOS, when the mucosa showed foveolar epithelial hyperplasia, intestinal metaplasia and regenerative glands. Besides, GCs expressed iNOS only in one case of tonsil, as reported by Li (1999), three lymph nodes with non-specific lymphadenitis, and one whipple's disease-like mesenteric lymph node with many foamy cells in sinus. Because iNOS is well known to be induced on histiocytes under stimulation by lipopolysaccharide (LPS) (Geller et al. 1993), LPS in bacterial body, products of bacteria, and products under bacterial infestation would induce iNOS on FDCs/GCDCs. Because HP includes LPS, such as Lewis X and Y(Appelmelk et al. 1998), the LPS would be trapped by FDCs/GCDCs and induce iNOS in them. The iNOS expression in GCs was thought to be a peculiar phenomenon rather than common.

As mentioned above, NO disturbs two of three systems

transducting cell membrane-receptor signals. Probably in GCs with high NO concentration induced by the iNOS, B-lymphocytes can not receive enough second signal in the processes of antigen presentation and fail to produce antibodies specific for the presented antigen. Therefore, the B-cell immunity against HP may be incomplete. On the other hand, NO synthesized in FDCs/GCDCs might be a stimulus to the proliferation of B-cells (Lowenstein et al. 1992: Hasui et al. 1999). In early marginal zone B-cell lymphoma in the skin, there were GCs expressing iNOS in a lymph follicle. But in MALT type lymphoma in the thyroid and follicular lymphoma in the tonsil, there were no cells expressing iNOS among lymphoma cells. NO produced by the iNOS in GCs might play a role in early phase of lymphomagenesis of marginal zone B-cell lymphoma, stimulating proliferationa of B-cells and adding mutation on B-cells in GCs.

Consequently, it was suggested that expression of iNOS in GCs in lymphatic tissue of the stomach with HP-associated peptic ulcer has relation with infestation of HP, modifies immunity against HP and prepares the microenvironment for MALT type lymphomagenesis, although expression of iNOS must be examined in early MALT type lymphoma.

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