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HELICOBACTER PYLORI AND GASTRIC DISEASES

HIDEMI GOTO

Department of Gastroenterology
Nagoya University Graduate School of Medicine

ABSTRACT

Helicobacter pylori (*H. pylori*) infection is a pathogenic agent of gastric diseases, but their mechanisms are unclear. Effects of ammonia, tumor necrosis factor (TNF), and anti-Lewis autoantibodies induced after *H. pylori* infection on the development of gastric diseases were investigated. Ammonia disturbed the collagen metabolism in the ulcer base. Soluble TNF receptors regulate the action of TNF. The involvement of anti-Lewis autoantibodies in the development of peptic ulcer might be unlikely. Moreover, *H. pylori*-specific IgA in gastric juice and TNFα gene polymorphism in persons infected with *H. pylori* were studied. According to *H. pylori*-specific IgA titer in gastric juice, persons were divided into two histologically and endoscopically different states of disease. *TNFA -857* single nucleotide polymorphism (SNP) may be associated with rugal hyperplastic gastritis and gastric carcinomas without severe atrophy. However, complete elucidation of pathogenic mechanisms of *H. pylori*-induced gastric diseases requires further research.

Key Words: ammonia, IgA, Lewis antigen, tumor necrosis factor, polymorphism, gastric disease

Ammonia produced by *Helicobacter pylori*

The effect of ammonia, which is produced by the urease activity of *Helicobacter pylori* (*H. pylori*), on normal gastric mucosa, has been well documented[1-5]. Although a few papers refer to the effects of ammonia on gastric ulcer healing, those on the collagen metabolism in the ulcer base remain totally unclear. Therefore, we investigated the effect of ammonia on the collagen metabolism in the granulation tissue of acetic acid-induced gastric ulcers in rats. Concentration of type III collagen, an embryonic and immature type collagen which appears in early phase of inflammation[6], were also studied.

In patients infected with *H. pylori*, ammonia concentrations in gastric juice are reported to be 0.01–0.02%. Although the ammonia concentrations of 0.1% used in our study is higher than this value, orally administered ammonia is diluted in gastric juice. Furthermore, the acutual ammonia concentration near the gastric epithelium can be higher than in gastric juice, because *H. pylori* colonizes in close contact with epithelium beneath the thick mucus layer and produces abundant ammonia, which spreads and is diluted in gastric juice. Intragastric ammonia did not affect intragastric acidity. Orally administered ammonia had no effects on serum gastrin. We found that healing of gastric ulcers was achieved under the influence of ammonia, although healing was delayed in earlier phase by intragastrically administered ammonia. We demonstrated that chronic ammonia administration increased the hydroxyproline concentration in the ulcer

Address correspondence to: Hidemi Goto, MD, PhD.
Department of Gastroenterology, Nagoya University Graduate School of Medicine
65 Tsuruma-cho, Sowa-ku, Nagoya, 466-8550, Japan
TEL: (81) 52-744-2164 FAX: (81) 52-744-2173 E-mail: hgoto@med.nagoya-u.ac.jp

base in the later stage of ulcer healing. Excessive deposition of collagen would interfere with gastric blood flow and may lead to ulcer recurrence. Regarding the effect of ammonia on collagen metabolism, collagen types should be considered. We showed that type lll collagen/hydroxyproline ratio in the ulcer base remained high in the late phase of ulcer healing. Type III is an immature type collagen that increases in the early stage of inflammation and forms lattice fibers, and decreases with the resolution of the inflammation. Type III collagen remains high when inflammation persists, and abnormally high proportion of type III collagen is reported in conditions associated with chronic inflammation[7-10]. Thus, the changes in the collagen types produced by chronic ammonia administration might reflect the persistence of inflammation in the submucosal connective tissue under the 'healed' or re-epithelialized ulcers. Persistent infiltration of inflammatory cells appear to confirm the persistence of inflammation. These findings suggest that re-epithelialisation can be achieved under a different condition of submucosal connective tissue. The basic fibroblast growth factor (bFGF) can accelerate healing of ulcers and is very sensitive to acid degradation[11]. Intragastric ammonia did not affect intragastric acidity, and may not reduce local levels of bFGF. Namely, chronic ammonia administration did not affect the achievement of epithelial healing of experimental gastric ulcers in rats. However, it disturbed the collagen metabolism in the ulcer base; collagen deposited excessively, and the collagen type was changed significantly. This excessive deposition and abnormally high proportion of immature type collagen may have an unfavorable effect on 'true' ulcer healing. Our observation appears to explain why *H. pylori*-induced ulcer is so difficult to treat.

H. pylori-specific IgA in gastric juice

It is widely accepted that *H. pylori* is a pathogenetic agent of chronic gastritis[12]. Chronic gastritis is characterized by mucosal infiltration of plasma cells, lymphocytes, macropharges, neutrophils, eosinophils, and mast cell. Additionally, there are systemic[13-15] and local[16-18] immune response to organisms. In the systemic immune response, IgG antibodies are of major importance against invasion by *H. pylori*. In the local immune response, IgA antibodies play an important role. It is reported that gastric juice antibodies were investigated and IgA titers to *H. pylori* were detected in a number of patients with gastritis[19]. However, there are few papers on the effects of various local immune responses upon *H. pylori* diseases. In treating the diversity of diseases of persons infected with *H. pylori,* the host's local immune responses are important as well as factors of the organism, such as cytotoxin-associated gene A (cagA). Thus, we investigated *H. pylori*-specific IgA in the gastric juice of asymptomatic persons who underwent a routine health examinations and analyzed their endoscopic and histological findings. Furthermore, cytokines in the gastric juice and cagA of *H. pylori* are measured and investigated the relationship of these with local antibody responses.

H. pylori was detected in the subjects with high IgA titer; therefore, IgA is considered to be insufficient eliminating the organism. Interestingly, the group with high IgA and low IgA titer differed in histological findings. The degree of neutrophil infiltration and density of colonization of *H. pylori* were lower in the group with high titer in gastric juice. This suggest that IgA still functions protectively, although it may be insufficient for eliminating organisms. Furthermore, the degree of metaplasia was high IgA titer in gastric juice. These results suggest that the local immune system appears to function efficiently in gastric mucosa with intestinal metaplasia. Immunohistological findings suggested that intestinal metaplasia showing an enhanced local immunity could be a positive and purposeful host reaction or adaptation against persistent inflammatory process in the gastric mucosa[20]. Endoscopic findings varied according to local IgA

response: peptic ulcers, especially duodenal ulcers, found more in the $IgG^{pos}IgA^{lo}$ group. These findings suggest that difference in local IgA response might decide the state of disease after infection; in other words, that there are two states of diseases according to local IgA response. We suggest the following; in states where local humoral response is not activated, polymorphonuclear leukocytes can infiltrate severely into gastric mucosa, which becomes prone to peptic ulcer occurrence. However, in states where local immune response is activated, organisms can be excluded and few polymorphonuclear leukocytes can infiltrate into the gastric mucosa, so gastritis with metaplasia gradually. It is important to understand why some infected persons develop clinical manifestations, for example, peptic ulcer, whereas others don't. One reason to explain this diversity is the difference among strains of *H. pylori*. It is reported that cytotoxigenisity of *H. pylori* could be involved in the development of peptic ulcers. Recently, attention has focused on cagA, and it is suggested that bacteria harbouring this protein are associated with disease[21]. We suggested that local antibody response to *H. pylori* showed no significant correlation with the existence of cag A and the diversity of diseases showed no correlation either, and that in addition to the possibility of varying pathogenicity according to strains, local immune response may also be one of the factors that decide the state of disease.

Namely, persons infected with *H. pylori* were divided into two histologically and endoscopically different states of disease according to *H. pylori*-specific IgA titer in gastric juice.

Autoantibodies against Lewis antigens

H. pylori is a Gram-negative bacterium, and has lipopolysaccharide (LPS) as an endotoxin. *H. pylori* infection may induce antibodies cross-reacting with human gastric mucosa and cross-reactivity may lead to atrophic involution[22]. LPs from some *H. pylori* strains was reported to display molecular mimicry of human cell surface Lewis x (Le^x) or Lewis y (Le^y) determinations[23,24]. The expression of these determinations on the bacterial surface may vary according to strains and their culturing conditions. Lewis blood group related antigens were expressed in the gastric epithelium[25]. It was suspected that anti-Le^x and -Le^y autoantibodies induced by *H. pylori* LPS would react to epithelial cells of the stomach and cause gastritis[23]. On the other hand, there are papers which the lack of correlation between Lewis antigen expression by *H. pylori* and gastric epithelial cells in infected patients[26]. We investigated serum anti-Le^x and -Le^y autoantibodies in peptic ulcer patients up to 16 months after successful eradication of *H. pylori*, and discussed the significance of these autoantibodies in ulcer development.

If the anti-Le antibodies would be involved in the pathogenesis of *H. pylori*-induced peptic ulcer, reduction of the antibody titer might be expected in patients with no recurrence of peptic ulcer after eradication. According to this hypothesis, we started a long-term follow-up study of the change of anti-Le antibody titers in sera of patients. The sera were taken at various intervals after successful eradication. The longest interval after eradication was 481 days. Eleven out of 48 patients examined had no recurrence of peptic ulcer or re-infection of *H. pylori* in the examined interval. We also identified Le^x and Le^y antigen determinations in the LPS of some *H. pylori* strains and detected autoantibodies in patients sera, which were predominantly IgG, against these antigens in the sera of patients[27]. These autoantibodies were absorbed, at least in part, with Le^x antigen prepared from human milk.

Although the examined cases were limited, 5 out of 11 cases had no change in titers of anti-Le^x and -Le^y autoantibodies in their sera during the experimental interval with no recurrence of peptic ulcer. These autoantibodies alone, therefore, seemed to have no critical role in the development of *H. pylori*-induced peptic ulcer. However, since the eradication of *H. pylori*

was successful in these patients and no re-infection was observed, the possible additive effect of these autoantibodies on the induction of peptic ulcer caused by *H. pylori* can not be excluded. Although more than 85% of *H. pylori* isolates express Le antigens, the titer of *H. pylori*-induced anti-Le^x and -Le^y antibodies in a patient's sera may not necessarily be related with the epitope of *H. pylori* that infected the patient[23,28]. *H. pylori* infection induces strong antibody responses in the human gastric mucosa[29,30]. Most of the *H. pylori*-specific IgA antibody-screening cells isolated from gastric mucosa are reactive with *H. pylori* membrane proteins, flagellin and urease, whereas they react only poorly with LPSs[29]. This is consistent with the observation that serum anti-Le^x and -Le^y antibodies are predominantly IgG. The involvement of local immune response against Le antigens in the development of peptic ulcer therefore might be unlikely.

Tumor necrosis factor (TNF) and soluble TNF receptors

Recent studies have indicated an increase in gastric epithelial cell apoptosis in mucosal biopsy specimens from patients with *H. pylori* associated gastritis[30-33], and it is possible that excessive apoptosis caused by *H. pylori* infection may favour carcinogogenesis. A variety of stimuli can induce apoptosis, such as TNF[34-36]. Several studies have suggested that TNF is produced by *H. pylori* infection[37-39] and is closely related to epithelial injury[37]. TNF exerts pleiotropic effects by linking two high affinity TNF receptors (TNF-Rs) of 55 and 75kDa on a variety cells[40]. Soluble forms of human 55 kDa TNF-R (sTNF-RI) and the 75 kDa (sTNF-RII) appear to be related from the cell surface by proteolytic cleavage of the extracellular domains of these membrane associated receptors[41,42]. Soluble receptors inhibit TNF activity by binding to TNF thus preventing its binding to membrane associated TNF-Rs[43,44]. We investigated gastric TNF expression and sTNF-R release in patient with *H. pylori* infection, and the protective effect of sTNF-Rs against the cytotoxic and apoptptic activity of TNF.

TNF level correlates significantly with the inflammatory severity but not with active severity, suggesting that TNF may be released from inflammatory cells, mainly mononuclear cells. On the other hand, the sTNF-R levels did not correlate with the inflammatory and active severity but correlated significantly with TNF level. This suggest that sTNF-Rs may be related by cells within the gastric mucosa, such as epithelial cells, rather than by inflammatory cells. We showed that TNF induced apoptosis was regulated by both increased sTNF-R release from gastric epithelial cells and decreased membrane surface TNF-R expression on the cells. Indeed, the host defence mechanism is thought to involve shedding of the extracellular domain of the receptor, which not only reduces the number of binding sites at the cell surface, but also increases binding protein for circulating TNF and thus prevents attachment to cell surface receptors. Our investigations were also unable to clarify the differences in the function of the two sTNF-Rs. Neutralisation of either sTNF-RI or sTNF-RII did not reduce cell viability very much but neutralisation of both significantly reduce cell viability. These results suggest that each sTNF-R may have the ability to block the effect of TNF. It has been reported that apoptosis in gastric epithelial cell triggers glandular atrophy, reducing secretion of acid and pepsin, and thus is connected with carcinogenesis[32]. It is also said that increased apoptosis may be the stimulus for a compensatory hyperproliferative and potentially preneoplastic response in chronic *H. pylori* infection[30]. We surmise that gastric epithelial cells release sTNF-Rs as a protection against these dangers, thereby controlling the action of TNF. However, it is conjectured that, when the balance between TNF and sTNF-Rs is upset, the action of TNF becomes predominant and causes cell damage, beginning with apoptosis.

Namely, TNF is produced after infection by *H. pylori*, and this is accompanied by sTNF-R release from gastric epithelial cells. Moreover, release of sTNF-Rs from gastric epithelial cells and apoptosis induced by anti-TNF-R monoclonal antibodies combined with TNF suggest that the soluble receptors regulate the action of TNF. In addition, TNF-R expression was confirmed to be downregulated. However, elucidation of the mechanism involved requires further research.

TNF_{α} gene polymorphism

Gastric mucosal folds are associated with a variety of pathologic conditions such as inflammation, neoplasia, and anatomic variants[45,46]. These folds are generally considered to be enlarged when the width is 5mm or more[47,48]. Several studies have suggested that *H. pylori*-associated gastritis may be one of the causes of hyperrugosity[49-54]. After eradication of *H. pylori*, gastric body fold width is reduced with an associated decrease in inflammatory infiltrates in the body mucosa[49,50,53]. *H. pylori*-associated gastritis is characterized by infiltration of neutrophils and mononuclear cells in the gastric mucosa[55]. TNF_{α} is a proinflammatory cytokine produced mainly by activated macrophages, causing tissue damage by recruitment and activation of the host's leukocytes. Many studies have reported that TNF_{α} is produced in *H. pylori*-infected gastric mucosa and involved in gastric inflammation as well as apoptosis[56-58].

Polymorphisms are associated with various kinds of diseases[59-62]. These polymorphisms, however, are very uncommon in Japanese[63-65]. Recently, polymorphisms in the 5'-flanking region of *TNFA* gene at positions -857, -863 and -1031, were identified[63] and found in a substantial proportion of the Japanese population. Moreover, -857T, -863A and -1031C alleles were shown to be associated with higher transcriptional activity of TNF_{α}[66-68]. These alleles may be associated with Crohn's disease and other diseases. We determined the association of polymorphism of the *TNFA* gene at position -857. We also determined the association of its polymorphism with diseases such as gastric carcinoma and peptic ulcers. Our findings demonstrate that -857 T/T genotype in the *TNFA* gene was significantly associated with susceptibility to *H. pylori*-infected rugal hyperplastic gastritis. Gastric and duodenal ulcers were not associated with *TNFA -857* single nucleotide polymorphism (SNP) compared with controls, nor was the positivity of anti-*H. pylori* IgG antibody. TNFα has been reported to be involved in the pathogenesis of *H. pylori*-infected chronic gastritis and expression levels of its mRNA and protein are closely correlated with the severity of the mononuclear and polymorphonuclear cell infiltration both in the gastric body and antrum[69,70]. It induces endothelial cell activation, apoptosis, and up-regulation of adhesion molecules followed by attraction of leukocytes through interleukin 8 (IL-8) and monocyte chemotactic protein-1 (MCP-1)[71-73]. Chronic gastritis with rugal hyperplasia, a special form of gastritis, is defined by the Sydney system when the thickness of non-flattening folds is approximately 5mm or wider[48]. Our data detected no association of *TNFA -857* SNP and total non-cardia gastric carcinomas in Japan, suggesting the difference in ethnicities or *TNFA* SNPs. In addition, our data may suggest an association of the high producer allele, -857T, with gastric carcinomas, which arise from background mucosa that is severe in activity and inflammation rather than atrophy. Further histological examination of the gastric mucosa surrounding carcinomas is needed.

Namely, not only *H. pylori* infection but also *TNFA -857* SNP were associated with susceptibility to rugal hyperplastic gastritis. *TNFA -857* SNP may be associated with gastric carcinomas without severe atrophy.

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