Antigastric autoantibodies in *Helicobacter pylori* infection: implications of histological and clinical parameters of gastritis

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Abstract

Background—It has recently been shown that humoral antigastric autoreactivities occur in a substantial number of *Helicobacter pylori* infected patients.

Aims—To analyse the relevance of such antigastric autoantibodies for histological and serological parameters of the infection as well as for the clinical course.

Methods—Gastric biopsy samples and sera from 126 patients with upper abdominal complaints were investigated for evidence of *H pylori* infection using histology and serology. Autoantibodies against epitopes in human gastric mucosa were detected by immunohistochemical techniques. Histological and clinical findings of all patients were then correlated with the detection of antigastric autoantibodies.

Results—*H pylori* infection was significantly associated with antigastric autoantibodies reactive with the luminal membrane of the foveolar epithelium and with canalicular structures within parietal cells. The presence of the latter autoantibodies was significantly correlated with the severity of body gastritis, gastric mucosa atrophy, elevated fasting gastrin concentrations, and a decreased ratio of serum pepsinogen I:II. Furthermore the presence of anticanalicular autoantibodies was associated with a greater than twofold reduced prevalence for duodenal ulcer.

Conclusion—The data indicate that antigastric autoantibodies play a role in the pathogenesis and outcome of *H pylori* gastritis, in particular in the development of gastric mucosal atrophy.

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It is now well accepted that colonisation of the gastric mucosa with *Helicobacter pylori* leads to chronic active gastritis not only in man but also in animal models.[1–5] Moreover, the association of *H pylori* infection with further gastroduodenal diseases, such as duodenal and gastric ulcer,[6 7] gastric atrophy,[8] gastric mucosa associated lymphoid tissue (MALT) type lymphoma,[9 10] and gastric carcinoma,[11] and the significant reduced risk for ulcer relapse after *H pylori* eradication,[12 13] indicate that this bacterium plays an important role in the pathogenesis of gastroduodenal pathology.

At present it is not clear why *H pylori* infection leads to different diseases in different patients. It is possible that the diverse courses of *H pylori* infection might be due to distinct strains expressing certain bacterial virulence factors.[14 15] However, the role of host factors in *H pylori* infection which also contribute to the final outcome of infectious diseases has been far less examined.[16] In *H pylori* gastritis the most obvious host reaction is the acquisition of MALT in the gastric mucosa, which could meet the requirement for antigastric autoimmune reactions.

Our previous findings and the work of other groups have provided evidence for humoral antigastric autoimmune reactions existing in association with *H pylori* gastritis.[17–19] The aim of this study was to investigate the relevance of antigastric autoantibodies for histological and clinical parameters of *H pylori* infection.

Material and Methods

One hundred and twenty six consecutive patients (72 men, 54 women, median age 54 years, range 15–86 years), who underwent diagnostic oesophagogastroduodenal endoscopy for upper abdominal complaints, were included in our study.

From all patients four biopsy specimens, two from the gastric antrum and two from the corpus mucosa, were examined for the presence, severity, and distribution of gastritis, as well as for intestinal metaplasia and atrophy on staining with haematoxylin and eosin. Gastritis was classified according to the Sydney system[20] and to the ABC system.[21] *H pylori* colonisation was histologically detected in Warthin-Starry stains.

Serum samples were taken from all patients shortly before or soon after endoscopy and were screened for the presence of IgG antibodies reactive with *H pylori* in an enzyme linked immunosorbent assay (ELISA) as previously described.[17] Correlation of serological and histological detection of *H pylori* revealed a sensitivity of 84% and a specificity of 81% (data not shown). Forty two patients were taking proton pump inhibitors. Seven of the latter group were also taking amoxycillin for a maximum of one week. In three of these patients *H pylori* infection was only detectable by ELISA. This finding was considered when determining the performance criteria of the ELISA.

Antigastric autoantibodies reactive with human gastric mucosa were detected using an immunohistochemical method described previously.[17] Briefly, heterologous formalin fixed gastric biopsy samples from antrum and corpus without pathological alterations were incubated overnight with the patient's serum diluted 1/100. After washing, 1/10 diluted alkaline phosphatase conjugated rabbit antihuman IgG (Dako, Hamburg, Germany) was added. Colour was developed with fast red. In a negative control with omission of serum, immunohistochemical reactivity could not be detected in the antrum or corpus.

All serum samples were also tested by immunofluoresence for classic antiparietal cell antibodies using native rat tissues (HISS Diagnostics, Freiburg, Germany).

Fasting gastrin concentrations were measured by a gastrin radioimmunoassay kit (GASK-PR, CIS Biointernational, France). Normal values ranged between 28 and 115 µU/ml. The concentrations of pepsinogen I and II were determined using a radioimmunoasssay kit (Sorin Biomedica, Saluggia, Italy).

Statistical correlations of qualitative parameters were tested with the χ^2 method. Statistical analysis of gastrin concentrations and of the pepsinogen I:II ratio was performed by means of the Mann-Whitney U test. A value of $p \leq 0.05$ was considered significant. With regard to gastrin and pepsinogen concentrations, patients treated with proton pump inhibitors were excluded from the analysis, since it is known that these drugs lead to higher fasting gastrin and pepsinogen concentrations.[22]

The study protocol was examined by the ethics committee of the University of Erlangen-Nürnberg. All patients gave their informed consent prior to inclusion in the study.

Results

PATIENTS AND HISTOLOGY

Thirty two patients had normal gastric mucosal histology. Four patients had autoimmune gastritis (type A gastritis), three of which had pernicious anaemia. Five patients had gastric mucosal alterations diagnosed as chemically induced gastritis (type C gastritis). Eighty four patients had type B chronic gastritis, 79 of whom (94%) were histologically or serologically positive for *H pylori*. The age profile of *H pylori* infected patients did not differ from that of uninfected patients (mean age 54.5 years, range 25–85 years; and 53.7 years, range 15–86 years, respectively). One patient had coeliac sprue and showed moderate chronic gastritis in the antrum and lymphocytic gastritis in the antrum and body.
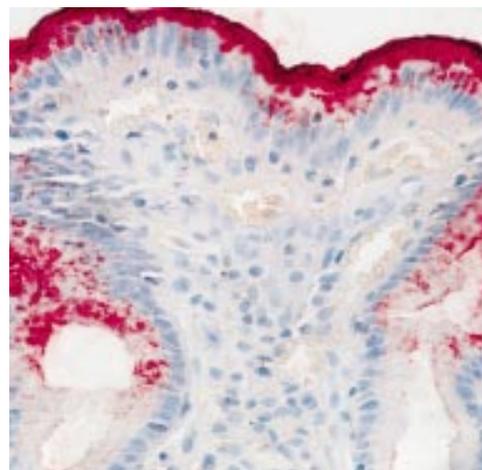

Figure 1:   Antiluminal antigastric reactivity against the apical membrane of foveolar epithelium cells in the human gastric antrum and body (original magnification ×40).

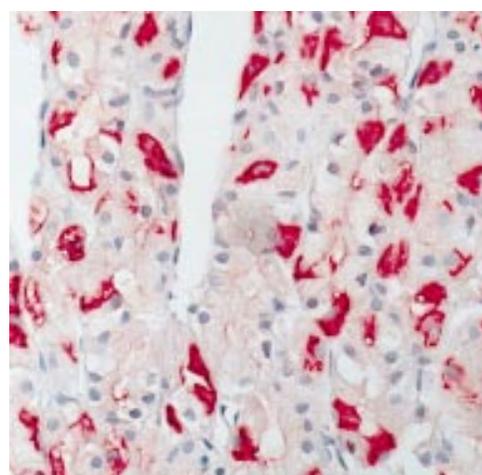*Figure 2:   Anticanalicular antigastric autoreactivity against canaliculi within parietal cells of the human gastric body (original magnification ×40).*

Twenty eight patients had intestinal metaplasia. Atrophic mucosal alterations—that is, complete or incomplete reduction of gastric glands, were detectable in 18 patients.

ENDOSCOPY

At endoscopy, 24 patients were shown to have duodenal ulcer, 16 of whom were positive for *H pylori* infection detected by serology or histology. Fifteen patients had gastric ulcer, 11 of whom were *H pylori* positive. Of the 42 patients treated with proton pump inhibitors, 28 had duodenal and/or gastric ulcers. The age profile of *H pylori* infected patients with duodenal and/or gastric ulceration was not significantly different from that of non-ulcer patients (mean
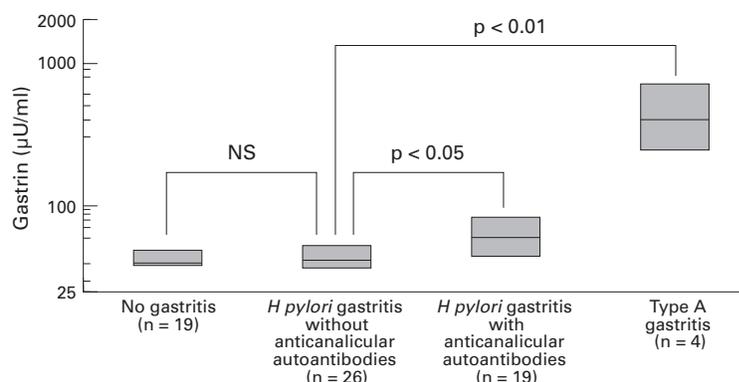
TABLE 1   *Type of gastritis, H pylori infection, and antigastric autoantibodies*

	No. of cases	Positive H pylori histology	Positive H pylori serology	Anticanalicular autoantibodies	Antiluminal autoantibodies	Classic APC autoantibodies
Normal mucosa	32	1	3	2	4	0
Autoimmune (type A) gastritis	4	0	0	4	1	3
Type B gastritis	84	63	66	27	26	6
Chemically induced gastritis	5	0	0	0	0	0
Lymphocytic gastritis	1	0	0	1	0	0

Figure 3:   Fasting serum gastrin concentrations in healthy controls, patients with H pylori gastritis with and without anticanalicular autoantibodies, and patients with type A gastritis.
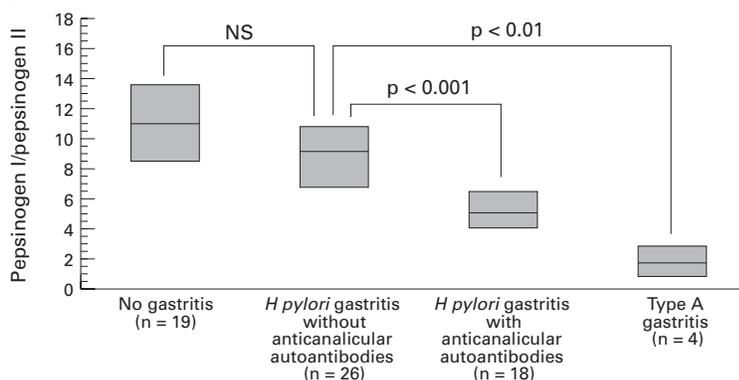

Figure 4:   Fasting pepsinogen I:II ratio in healthy controls, patients with H pylori gastritis with and without anticanalicular autoantibodies, and patients with type A gastritis.

age 55.9 years, range 15–86 years; and 52.7 years, range 21–78 years, respectively).

ANTIGASTRIC AUTOANTIBODIES
Antigastric autoantibodies could be detected by immunohistochemistry in 51/126 patients when tested on human gastric tissue. Seventeen patients showed only autoantibodies reactive with the luminal membrane of foveolar epithelial cells in the antrum and the body (fig 1), and 20 patients showed only a distinctive immunoreactivity with canalicular structures within the parietal cells in the body (fig 2). Fourteen patients showed both types of autoreactivity.

Using immunofluoresence on rat tissue, antiparietal cell antibodies could be found in nine patients, three with classic autoimmune gastritis and six with *H pylori* gastritis. All of these nine patients were also positive for anticanalicular autoantibodies using human gastric tissue (table 1).

TABLE 2   *Correlation between antigastric autoantibodies and severity of H pylori gastritis and gastric atrophy*

	Antiluminal autoantibodies		Anticanalicular autoantibodies	
	Negative	Positive	Negative	Positive
Severity of body gastritis				
–/+	33	8	34	7
++/+++	23	15*	18	20***
Atrophy				
No	49	17	48	18
Yes	7	6 (NS)	4	9**

–/+, no or mild chronic body gastritis; ++/+++, moderate or severe chronic body gastritis.
NS, not significant; * p ≤ 0.05; ** p < 0.01; *** p < 0.001.

H PYLORI INFECTION AND ANTIGASTRIC AUTOANTIBODIES
The presence of anticanalicular as well as antiluminal autoantibodies was significantly correlated with detection of *H pylori* by both ELISA and histology: of the 34 patients positive for anticanalicular autoantibodies, 27 were positive for *H pylori* by ELISA and 23 showed *H pylori* infection at histology (p<0.001 and p<0.05, respectively). Of the 31 patients positive for antiluminal autoantibodies, 22 were positive for *H pylori* by serology and 21 were positive by histology (p<0.05 for both). Of the 79 patients positive for *H pylori* by either serology or histology, 16 had anticanalicular autoantibodies, 12 had antiluminal autoantibodies, and 11 showed both types of autoreactivity. The age profile of *H pylori* infected patients with antigastric autoantibodies was not significantly different from that of those without these autoantibodies (mean age 54.9 years, range 25–83 years; and 53.2 years, range 15–86 years, respectively).

HISTOLOGICAL ALTERATIONS AND ANTIGASTRIC AUTOANTIBODIES
When the group of patients positive for *H pylori* by histology or serology was analysed, a significant association of either anticanalicular or antiluminal antigastric autoantibodies with the presence of moderate to severe corpus gastritis could be found (p<0.001 and p≤0.05, respectively). Furthermore, a strong association between anticanalicular autoantibodies and atrophic changes in the gastric mucosa of the antrum or body was detectable (p<0.01). No significant correlation between the severity of antral gastritis and the presence of either type of antigastric autoantibodies could be found (table 2).

GASTRIN CONCENTRATIONS AND ANTIGASTRIC AUTOANTIBODIES
Fasting serum gastrin concentrations in patients with histologically or serologically proved *H pylori* infection with anticanalicular autoantibodies were significantly higher than both those in *H pylori* infected patients without these autoantibodies and in healthy controls (mean values 56.2, 43.1, and 39.6 µU/ml, respectively; p<0.05). No significant association between antiluminal autoantibodies and raised gastrin concentrations could be found. The four patients with type A gastritis had the highest gastrin concentrations (mean value 387 µU/ml) (fig 3).

PEPSINOGEN I:II RATIO AND ANTIGASTRIC AUTOANTIBODIES
In the group of *H pylori* positive patients with anticanalicular autoantibodies the pepsinogen I:II ratio was significantly decreased compared with that in *H pylori* positive patients without these autoantibodies or in the healthy controls (mean values 5.10, 9.01, and 10.61, respectively; p<0.001). Patients with classic autoimmune gastritis had the lowest pepsinogen I:II ratio (fig 4). Detection of antiluminal autoantibodies was not significantly associated with an altered pepsinogen I:II ratio.

ANTIGASTRIC AUTOANTIBODIES AND PREVALENCE OF DUODENAL ULCER

At endoscopy 16/79 *H pylori* positive patients were shown to have duodenal ulcer. In three, anticanalicular autoantibodies were present; 13 showed no anticanalicular autoreactivity. The prevalence of duodenal ulcers in the group of patients without anticanalicular autoantibodies was more than twice as high as that in the group with anticanalicular autoantibodies (25% *v* 11%). However, because the number of patients with duodenal ulcer was low, a significant correlation could not be shown. There was no association between the presence of antigastric autoantibodies of either type and gastric ulceration.

Discussion

This paper presents a systematic analysis of the relevance of antigastric autoantibodies in *H pylori* gastritis. We and others have previously shown that autoantibodies against gastric epitopes occur in about 50% of *H pylori* infected patients with upper gastrointestinal complaints. We have described two different in situ binding sites for these autoantibodies, one at the luminal membrane of the foveolar epithelium, the other at canalicular structures within parietal cells; the latter showed the highest correlation with *H pylori* infection.[17 18] This high prevalence of antigastric autoreactivity was confirmed in this study. Furthermore, according to our data, this type of autoantibody is correlated with histological and clinical manifestations of *H pylori* infection. Thus, the occurrence of anticanalicular autoantibodies correlates with the severity of body gastritis, and atrophic changes of the gastric mucosa, as well as with elevated basal serum gastrin concentrations and a decreased pepsinogen I:II ratio. Interestingly, the presence of anticanalicular autoantibodies is correlated with a greater than twofold reduced prevalence of duodenal ulcer. Although it is evident that the parameters mentioned are not independent of each other, they represent different facets of gastric mucosal atrophy. This indicates that autoantibodies to canaliculi are either responsible for the development of atrophy or are at least a marker for other pathogenic factors leading to atrophic changes in the mucosa.

Recently, Negrini *et al* also reported two different in situ binding sites for antigastric autoantibodies in *H pylori* gastritis.[23] These autoreactivities were not simultaneously present in all patients positive for antigastric autoantibodies, indicating two different types of autoantibodies. In their study the presence of these autoantibodies seemed to be relevant for certain histological alterations such as the density of periglandular lymphocytic infiltrations and glandular atrophy, which is comparable with our results. However, in our study a more detailed evaluation for both types of autoreactivity—against mucous cells and against canaliculi of parietal cells—was performed. Furthermore, we also analysed certain clinical parameters, such as fasting gastrin concentrations and the pepsinogen I:II ratio,

which support the histological diagnosis of gastric mucosal atrophy.

The mechanisms leading to the formation of antigastric autoantibodies in chronic *H pylori* infection and to atrophic gastritis are still under investigation. Negrini *et al* and Appelmelk *et al* suggest molecular mimicry between epitopes on *H pylori* and Lewis blood group antigens expressed on gastric epithelial cells.[18 19 23]

It is possible however, that apart from this hypothesis other autoimmune mechanisms are responsible for the generation of antigastric autoantibodies. Since almost no immunological surveillance exists in the normal gastric mucosa, one can describe this region as an immunologically privileged site,[24] which loses its virginal status by colonisation with *H pylori*, leading to expression of major histocompatibility complex class II antigens on gastric epithelial cells[25–27] and aggregation of a dense lymphoplasmacellular infiltrate within the gastric mucosa.[28] Thus, in immunological terms, *H pylori* gastritis represents chronic antigen presentation and immunostimulation in acquired MALT. It is possible that this unique condition leads to adequate immunological exposure of self epitopes to reactive immunocompetent cells. Cellular and/or humoral autoreactivity against autoantigens located on cells in the body mucosa could result in an increased rate of apoptosis and lead to atrophic alterations.[29] Furthermore, autoantibodies against epitopes on canalicular structures within parietal cells, where the major autoantigen in classic autoimmune gastritis is located,[30] could also inhibit parietal cell function in patients with *H pylori* gastritis.[31] This could lead to the unchanged or even decreased basal acid secretion despite raised gastrin concentrations in *H pylori* infected subjects without duodenal ulcer[32–36]—a possible explanation for the reduced rate of duodenal ulcers in the group with anticanalicular autoantibodies found in our study. However, these hypotheses need to be investigated further.

Our data provide evidence that autoimmune reactions play a role in the final outcome of *H pylori* gastritis. Therefore one might discuss whether a strict aetiological distinction between autoimmune gastritis and *H pylori* associated gastritis is still possible. One might even speculate that the *H pylori* associated multifocal atrophic gastritis proposed by Correa[37] or the so called AB gastritis proposed by Glass and Pitchumoni[38] corresponds to a special type of *H pylori* gastritis which develops anticanalicular autoreactivity and gastric atrophy. Further investigations into antigastric autoimmune reactivity in *H pylori* infection might lead to an understanding of the long term outcome of gastritis.

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