Virchows Arch (1996) 427:483–486

ORIGINAL ARTICLE

Gerhard Faller · Helmuth Steininger · Matthias Eck
Johannes Hensen · Eckhart G. Hahn
Thomas Kirchner

Antigastric autoantibodies in *Helicobacter pylori* gastritis: prevalence, in-situ binding sites and clues for clinical relevance

Received: 7 August 1995 / Accepted: 21 November 1995

Abstract Colonization of human gastric mucosa with *Helicobacter pylori* leads to chronic active gastritis and induces the occurrence of an acquired mucosa-associated lymphoid tissue (MALT) in the stomach.This remodelling of the gastric mucosa together with chronic antigen persistence may induce autoimmune reactions. The aim of this study was to investigate humoral autoimmune reactions to human gastric mucosa in *H. pylori* gastritis and their clinical relevance. Sera from patients with dyspeptic symptoms were tested for presence of IgG immunoglobulins against *H. pylori*. Gastric infection with *H. pylori* and alterations of gastric mucosa were demonstrated by histological examination of gastric biopsy specimens. All sera were tested for reactivity against human gastric mucosa by immunohistochemistry. Two different in-situ binding sites of antigastric autoantibodies were observed. Binding to canalicular structures within parietal cells was significantly correlated with antibodies to *H. pylori*, elevated basal gastrin levels and atrophy of gastric corpus glands. Our data indicate that autoimmune reactions to antigens in the human gastric mucosa occur in *H. pylori* gastritis and that they may play a role in the pathogenesis of the disease.

Key words *Helicobacter pylori* · Gastritis · Host response · Autoimmunity

Introduction

The prevalence of *Helicobacter pylori* infection in Western countries ranges between 20% and 80% in adults and it is therefore one of the most widespread of bacterial infections [11]. Colonization of *H. pylori* causes chronic active gastritis in man and an association with duodenal and gastric ulcer, stomach carcinoma and gastric malignant lymphoma has been reported [1, 14, 19, 20]. Several studies have investigated the virulence factors of *H. pylori* and the mechanisms leading to gastric epithelial lesions and dysregulation of gastrin secretion in *H. pylori* infection [3, 5, 7]. However, the pathogenic pathways of *H. pylori* gastritis and its complications are not completely understood.

One of the most interesting aspects of *H. pylori* gastritis is the acquisition of a complete mucosa-associated lymphoid tissue (MALT) following the bacterial colonization; normally only simple immunological surveillance by intraepithelial T-lymphocytes exists in gastric mucosa [8]. This remodelling of the gastric mucosa obviously requires a long term persistence of *H. pylori* leading to chronic bacterial antigen stimulation and antigen presentation of the acquired MALT, which might meet the requirements for the generation of autoimmune reactions. There is indeed some evidence of autoimmune reactions of *H. pylori* gastritis [13].

The aim of this study was to investigate the prevalence, the in-situ binding sites and the possible clinical relevance of autoantibodies to gastric mucosa in *H. pylori* gastritis.

Materials and methods

Sixty patients (age 21 to 85 years), who underwent diagnostic endoscopy because of dyspeptic symptoms, were continuously included in our study without further preconditions. Two formalin-fixed, paraffin-embedded biopsy specimens from both gastric antrum and body were histologically examined for the type and activity of gastritis according to the Sydney System in a haematoxylin-eosin stain [12, 15]. The presence and the type of gastric atrophy and intestinal metaplasia were analysed. Colonization of *H. pylori* was examined in a Warthin-Starry stain.

The study has been examined by the ethics comitee of the University of Erlangen-Nürnberg. All persons gave their informed consent prior to their inclusion in the study.

G. Faller (✉) · H. Steininger · T. Kirchner
Institute of Pathology, University of Erlangen-Nürnberg, Krankenhausstrasse 8–10, D-91054 Erlangen, Germany

M. Eck
Institute of Pathology, University of Würzburg, Würzburg, Germany

J. Hensen · E.G. Hahn
Department of Internal Medicine I, University of Erlangen-Nürnberg, Erlangen, Germany

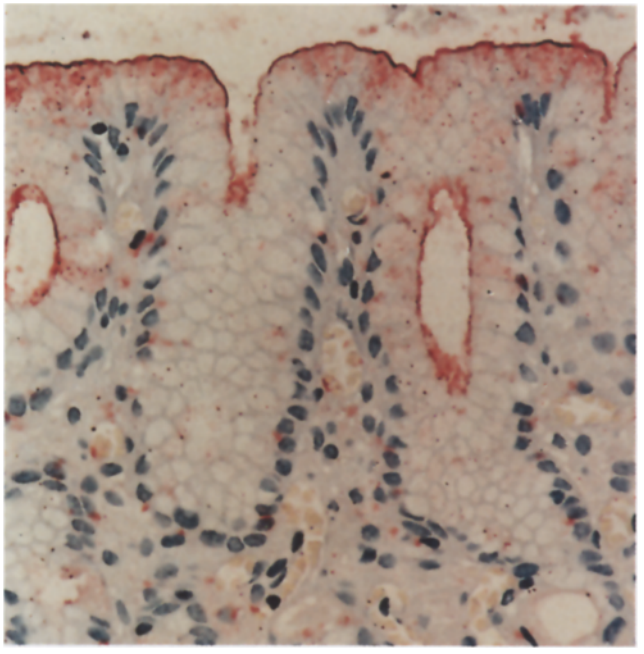

Fig. 1 Detection of antiluminal autoantibodies reactive with the cytoplasm and the luminal membrane of foveolar epithelial cells in human gastric body mucosa. Immunostaining, ×200

Sera of all patients were taken shortly before or soon after endoscopy and were screened for presence of IgG-antibodies reacting to *H. pylori* by an enzyme linked immunosorbent assay (ELISA) procedure as previously described [4] with certain modifications. Briefly, sonicated *H. pylori* was used as antigen. This strain was isolated from a patient with *H. pylori* gastritis and was identified by routine microbiological assays. Flat-bottom microtitre plates were coated overnight with 100 µl antigen solution at room temperature (2 µg sonicate/ml carbonate buffer, pH 9.6). After washing and blocking of non-specific binding sites, plates were incubated for 2 h with 100 µl serum diluted 1:500 in phosphate-buffered saline (pH 7.4) containing 0,05% Tween (PBST). After being washed plates were then incubated with 100 µl diluted peroxidase-conjugated rabbit to human IgG (Dako, Hamburg, Germany) for 1 h. After final washing plates were incubated with substrate and hydrogen peroxide and were read by optical density (OD) measurements at 492 nm. The cut-off value was calculated by adding 2 SD to the mean OD of 4 healthy subjects. All assays were carried out in duplicate.

Gastric biopsy samples were formalin-fixed and paraffin-embedded according to routine methods. For immunohistochemical studies sections were treated with xylol (30%) and alcohol (100% and 70%) to remove paraffin. Sections were then washed in TRIS-buffer and incubated overnight with human sera diluted 1:100 in RPMI 1640 medium (Biochrom, Berlin, Germany). After washing, alkaline phosphatase-conjugated rabbit to human IgG (Dako, Hamburg, Germany) diluted 1:10 in RPMI was added. After final washings colour was developed with fast red, sections were counterstained with haematoxylin, mounted and examined by light microscopy. Controls were performed omitting the sera and no immunohistochemical reaction in the corpus or in the antrum was found.

Fasting gastrin levels were measured by a gastrin radioimmunoassay kit (GASK-PR, CIS bio international, Gif-sur-Yvette, France). Normal values ranged between 28 and 115 µgU/ml.

Statistical correlation between positive *H. pylori* serology and detection of antigastric antibodies were tested with the χ^2-method.

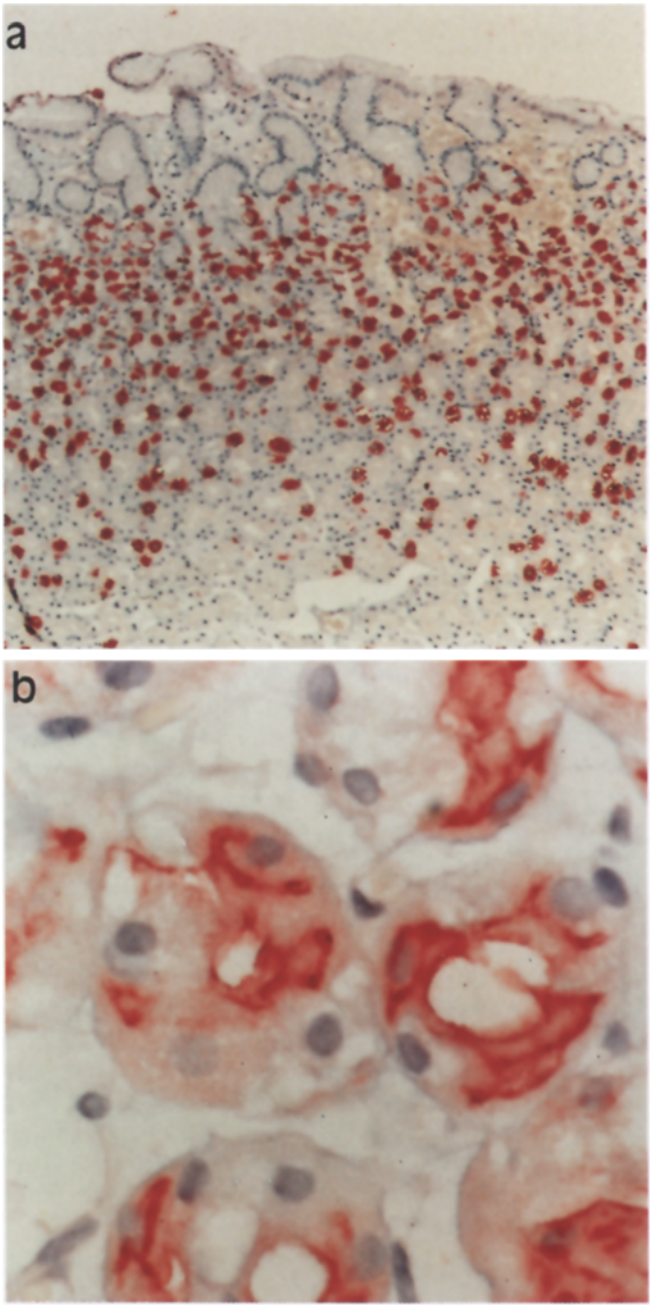

Fig. 2a, b Detection of anticanalicular autoantibodies reactive with canalicular structures within parietal cells of human gastric body mucosa. Immunostaining, a: ×50; b: ×1000

Results

Histological examination revealed no gastritis in 13 patients, type B gastritis in 40 patients, autoimmune gastritis in 4 patients, chemically or drug induced gastritis in 2 patients and gastric atrophy in antrum and body accompanied by malignant lymphomatous polyposis (mantle cell lymphoma) of the gastrointestinal tract in 1 patient. Thirty-five out of 60 patients showed colonization of the gastric mucosa with *H. pylori*.

Table 1 Correlation of positive *H. pylori* serology and detection of antigastric antibodies

	H. pylori-ELISA positive (n=32)	*H. pylori*-ELISA negative (n=28)	Significance
Antiluminal autoantibodies	10	4	Not significant
Anticanalicular antibodies	14	3	P<0,01

According to our cut-off values antibodies to *H. pylori* were demonstrated in 32 patients. Using the detection of *H. pylori* in the Warthin-Starry stain as standard, the *H. pylori*-ELISA gave a sensitivity of 77%, a specificity of 80% and a positive predictive value of 84%.

In 25 out of the 60 sera, antigastric autoantibodies were demonstrated; 18 were also positive for antibodies against *H. pylori* in the ELISA. The prevalence of antigastric auto-antibodies in microscopically confirmed *H. pylori* colonization and in sera with positive *H. pylori*-ELISA reached 54% and 56% respectively. Two different binding sites of antigastric autoantibodies were observed (Figs. 1 and 2, Table 1). One binding site was cytoplasmic and at the luminal membrane of the foveolar and glandular epithelial cells in antral and body mucosa. This luminal staining pattern was found in 14 cases, 10 out of which were also positive for antibodies against *H. pylori* (Fig. 1).

A second binding site was detected at canalicular structures within parietal cells of the body (Fig. 2A, B). This canalicular staining pattern was observed in 17 cases, 14 out of which were positive in the *H. pylori*-ELISA. Two of the three patients with parietal cell antibodies and negative *H. pylori* serology suffered from autoimmune gastritis, the third from coeliac disease. Correlation between anticanalicular and positive *H. pylori*-ELISA reached statistic significance (P<0,01).

Six sera with positive *H. pylori*-ELISA showed antiluminal and anti-canalicular autoantibodies simultaneously.

Like Negrini et al. [13] and in contrast to other workers [17, 18] we did not observe any reaction to gastrin cells in the antrum.

The presence of anticanalicular autoantibodies was significantly associated with atrophic changes of gastric body glands: 7 out of the 17 patients (41%) with anticanalicular autoantibodies showed initial or advanced atrophic changes in the body, 4 of them suffered from *H. pylori* gastritis and three from autoimmune gastritis. In contrast, only 3 out of the 43 patients (7%) without these antibodies showed equivalent alterations, one patient with autoimmune gastritis, the second with *H. pylori* gastritis and finally the patient with malignant lymphomatous polyposis.

Detection of anticanalicular autoantibodies was positively correlated with hypergastrinaemia: 10 patients showed hypergastrinaemia, which could be explained by autoimmune gastritis in 3 patients and by pretreatment with omeprazole in further 3 patients. All of the 4 remaining cases with hypergastrinaemia had anticanalicular serum autoantibodies associated with *H. pylori* gastritis, and positive *H. pylori*-serology. Gastrin levels in these patients ranged between 121 and 295 µU/ml. One of these four was also positive for antiluminal antibodies. However, presence of antiluminal autoantibodies was neither associated with atrophic changes nor with hypergastrinaemia. There was no case of hypergastrinaemia in the remaining patients without these autoantibodies, autoimmune gastritis or omeprazole pretreatment.

Discussion

Colonization of the human gastric mucosa with *H. pylori* induces chronic active gastritis, which is characterized by abundant neutrophil and lymphoid cells in the mucosa. The accumulation of lymphocytes and plasma cells is of particular interest since – in contrast to the gut – no mucosa associated lymphoid tissue (MALT) is found in normal gastric mucosa. Despite the extensive inflammatory response, *H. pylori* can persist for years resulting in a chronic bacterial antigen presentation from the acquired MALT, without substantial bacterial invasion or spread. Together with the epithelial expression of class II MHC [16] these changes may fulfill the requirements for autoimmune reactions at the site of the disease (the gastric mucosa). A previous study found antigastric autoantibodies with reactivity to foveolar and glandular cells of the antrum in 84% of patients with positive *H. pylori* serology. However, only three sera were tested on the mucosa of the body of the stomach, one of which reacted with the cytoplasm of parietal cells [13].

In addition to the high prevalence of antigastric autoantibodies, all autoantibodies showed an antiluminal, an anticanalicular, or both types of reactivity in our study. The occurrence of anticanalicular autoantibodies was positively correlated with the presence of antibodies of *H. pylori*. The canalicular binding site may correspond to the tubulovesicular membranes of parietal cells, where the proton pump, the main antigen of anti-parietal cell antibodies of classical autoimmune gastritis, is located [2]. Most interestingly the presence of anticanalicular autoantibodies can be related to hypergastrinaemia and to atrophic changes in the body mucosa not only in autoimmune, but also in *H. pylori* gastritis. In this context our results support the recently reported observation that a certain group of patients with *H. pylori* gastritis shows atrophic mucosal alterations in the gastric body, when reassessed after a period of 10 years [9].

Our data provide evidence for the clinical relevance of some of the antigastric autoantibodies in *H. pylori* gastritis. It is possible that autoimmune mechanisms induced by *H. pylori* colonization temporarily reduce parietal cell function and contribute the hitherto unexplained reduction in acid secretion and upregulation of gastrin secretion in *H. pylori* gastritis [3, 5–7]. In contrast to this, some studies have found no impairement of parietal cell function in studies with duodenal ulcer patients [3, 13].

The presence and specificity of antigastric autoantibodies differs in patients with *H. pylori* infection. The characterization of the autoimmune response may define groups of *H. pylori* infected patients with different risk for the development of the clinical complications associated with *H. pylori* gastritis [10].

Acknowledgements We thank Drs. H. Ernst, A. Greiner, H. Marquardt and C. Zechmeister for kind support and C. Winkelmann for excellent technical assistance. This work was supported by the Johannes and Frieda Marohn Foundation, Erlangen, Germany.

References

1. Berstad K, Berstad A (1993) *Helicobacter pylori* infection in peptic ulcer disease. Scand J Gastroenterol 28:561–567
2. Callaghan JM, Khan MA, Alderuccio F, Van Driel IR, Gleeson PA, Toh B-H (1993) a and b subunits of the gastric H+/K+-ATPase are concordantly targeted by parietal cell autoantibodies with autoimmune gastritis. Autoimmunity 16:289–295
3. Dunn BE (1993) Pathogenic mechanisms of *Helicobacter pylori*. Gastroenterol Clin North Am 22:43–57
4. Faller G, Vollmers HP, Weiglein I, Marx A, Zink C, Pfaff M, Müller-Hermelink HK (1990) HAB-1, a new heteromyeloma for continuous production of human monoclonal antibodies. Br J Cancer 62:595–598
5. Graham DY, Go MF, Lew GM, Genta RM, Rehfield JF (1993) *Helicobacter pylori* infection and exaggerated gastrin release. Effects of inflammation and progastrin processing. Scand J Gastroenterol 28:690–694
6. Haruma K, Kawaguchi H, Kohmoto K, Okamoto S, Yoshihara M, Sumii K, Kajiyama G (1995) *Helicobacter pylori* infection, serum gastrin, and gastric acid secretion in teen-age subjects with duodenal ulcer, gastritis, or normal mucosa. Scand J Gastroenterol 30:322–326
7. Haruma K, Kawaguchi H, Yoshihara M, Okamoto S, Sumii K, Kishimoto S, Kajiyama G (1994) Relationship between *Helicobacter pylori* infection and gastric acid secretion in young healthy subjects. J Clin Gastroenterol 19:20–22
8. Kirchner T, Melber A, Fischbach W, Heilmann KL, Müller-Hermelink HK (1990) Immunohistological patterns of the local immune response in *Helicobacter pylori* gastritis. In: Malfertheiner P, Ditschuneit H (eds) *Helicobacter pylori*, gastritis and peptic ulcer. Springer, Berlin Heidelberg New York, pp 213–222
9. Kuipers EJ, UA, Pena AS, Roosendaal R, Pals G, Nelis GF, Festen HPM, Meuwissen SGM (1995) Long-term sequelae of *Helicobacter pylori* gastritis. Lancet 342:1525–1528
10. Lee A (1993) *H. pylori*-initiated ulcerogenesis: look to the host. Lancet 341:280–281
11. Marshall BJ (1994) Epidemiology of *H. pylori* in Western countries. In: Hunt RH, Tytgat GNJ (eds) *Helicobacter pylori* – basic mechanisms to clinical cure. Kluwer Academic Publishers, Dordrecht, pp 75–84
12. Misiewicz JJ (1991) The Sydney System: a new classification of gastritis. Introduction. J Gastroenterol Hepatol 6:207–208
13. Negrini R, Lisato L, Zanella I, Cavazzini L, Gullini S, Villanacci V, Poiesi C, Albertini A, Ghielmi S (1991) *Helicobacter pylori* infection induces antibodies cross-reacting with human gastric mucosa. Gastroenterology 101:437–445
14. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich H, Sibley R (1991) *Helicobacter pylori* infection and the risk of gastric carcinoma. N Engl J Med 325: 1127–1131
15. Price AB (1992) The Sydney System: histological division. J Gastroenterol Hepatol 6:209–222
16. Scheynius A, Engstrand L (1991) Gastric epithelial cells in *Helicobacter pylori* associated gastritis express HLA-DR but not ICAM-1. Scand J Immunol 33:237–241
17. Uibo R, Krohn JE (1984) Demonstration of gastrin cell autoantibodies in antral gastritis with avidin-biotin complex antibody technique. Clin Exp Immunol 58:341–347
18. Vandelli C, Botazzo GF, Doniach D, Franceschi F (1979) Autoantibodies to gastrin-producing cells in antral (type B) chronic gastritis. N Engl J Med 300:1406–1410
19. Warren JR, Marshall BJ (1983) Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1:1273–1275
20. Wotherspoon AC, Ortitz-Hidalgo C, Falzon MR, Isaacson PG (1991) *Helicobacter pylori*-associated gastritis and primary B-cell gastric lymphoma. Lancet 338:1175–1176