Key Importance of the Helicobacter pylori Adherence Factor Blood Group Antigen Binding Adhesin during Chronic Gastric Inflammation

Christian Prinz, Martin Schömiger, Roland Rad, Ingrid Becker, Erwin Keiditsch, Stephan Wagenpfeil, Meinhard Classen, Thomas Rösch, Wolfgang Schepp, and Markus Gerhard

ABSTRACT

Helicobacter pylori has been assigned as a class 1 carcinogen because of its relation to gastric adenocarcinoma. Chronic H. pylori infection may lead to severe gastritis, glandular atrophy (AT), and intestinal metaplasia (IM). Strains secreting the vacuolating toxin VacA and producing the cytotoxin-associated antigen CagA (type 1 strains), as well as the blood group antigen binding adhesin (BabA) targeting Lewis\(^b\) antigens, have been associated previously with distal gastric adenocarcinoma (M. Gerhard et al., Proc. Natl. Acad. Sci. USA, 96: 12778–12783, 1999) and may therefore also be related to lesions preceding gastric cancer. Antral and corpus biopsies were collected from 451 patients; 151 were H. pylori positive, as determined by PCR. Gastric biopsies were histologically evaluated for activity of gastritis (G0–G3, granulocyte infiltration), chronicity of gastritis (L1–L3, lymphocyte infiltration), and the presence of IM (intestinal metaplasia, G3, IM, or AT according to the Sydney classification). Simultaneously, the presence of bacterial genes encoding virulence and adherence factors (vacAs1/s2, cagA, and babA2) was determined by PCR. The presence of cagA+ and vacAs1 (alone or combined) both correlated with activity and chronicity of gastritis (P < 0.05); however, the overall prevalence of these genes was 60 or 72%, respectively, and was thus relatively frequent. The babA2 gene, encoding the adhesin BabA, was detected in 38% of infected patients and was correlated with the activity of gastritis in antrum and corpus (P < 0.005). cagA+ / vacAs1+ strains (suggesting the presence of type 1 strains) that were also babA2 positive were detected more frequently in patients with severe histological alterations (such as G3, IM, or AT) compared with subjects without these changes (P < 0.01). cagA+/ vacAs1+ strains that were babA2 negative, however, lacked a significant correlation with severe histological changes, activity, or chronicity of gastritis in antrum and corpus. Adherence of H. pylori via BabA appears to be of importance for efficient delivery of VacA and CagA and may play a special role in the pathogenesis of severe histological changes.

INTRODUCTION

Helicobacter pylori is a widespread infection in humans, being present in 30–50% of people worldwide. Infection with this pathogen is associated with the development of peptic ulcers; eradication from H. pylori prevents ulcer recurrence, indicating a causal link between the pathogen and ulcer disease (1). In addition, a strong association between H. pylori infection and gastric cancer has been reported based on epidemiological studies (2) as well as animal models, in which the infection leads to severe gastritis or intestinal metaplasia in rodents (3) and monkeys (4). Recent animal models have further revealed that H. pylori induces the formation of intestinal metaplasia and distal gastric adenocarcinoma in Mongolian gerbils (5, 6), indicating a causal link between H. pylori and distal gastric carcinoma. H. pylori-infected patients with severe gastritis are of major clinical interest because in some patients, eradication could prevent carcinogenesis. The number of H. pylori-infected patients, however, is too large to treat every infected person, because the majority of these patients will not develop any specific disease.

Further investigations are required to evaluate the bacterial mechanisms involved in H. pylori-induced gastritis and cancer development. Colonization of the gastric mucosa with H. pylori usually causes an active antral gastritis, characterized by the epithelial infiltration with polymorph mononuclear cells and leukocytes (7). Long-term infection may result in colonization of H. pylori in the gastric corpus with subsequent superficial epithelial cell injury and the presence of neutrophilic granulocytes, lymphocytes, eosinophils, and plasma cells (8–10). During chronic persistence of H. pylori, the infection may also lead to multifocal gastric atrophy and intestinal metaplasia, thereby predisposing to the development of gastric carcinoma (11, 12). Atrophic gastritis, the presence of IM, and severe corpus gastritis have been determined as possible risk factors for gastric adenocarcinoma (11–16). The reasons for the progression of chronic gastritis to gastric cancer, however, are still unknown.

The clinical outcome of H. pylori-induced gastritis may be related to differences in virulence among specific strains. Several bacterial virulence factors, especially the vacuolating cytotoxin (VacA) and the cytotoxin-associated antigen (CagA), have been identified and used to describe more virulent “type 1” bacteria when both factors are produced (17). VacA is an oligomeric toxin that becomes activated at low pH and leads to gastric epithelial erosions after active bacterial secretion (18, 19). The vacA gene encoding the vacuolating toxin is present in all H. pylori strains. The signal region of vacA, located at the 5′ end, occurs as an s1 or s2 allele; vacAs1 strains produce moderate to large amounts of toxin, whereas s2 strains produce very little amounts of toxin (19). Therefore, detection of vacAs1 in gastric biopsies via PCR can be used as a marker for the secretion of the vacuolating toxin. CagA is a M90,000 immunodominant antigen of H. pylori, the serological or genetic detection of which indicates the presence of the cag pathogenicity island (cag-PAI) encoding a type IV bacterial secretion machinery (20–22). Very recently, independent groups provided evidence that, after attachment of H. pylori to AGS cells, CagA is secreted into the cells, becomes phosphorylated, and is thus involved in bacteria-induced signal transduction (23–25). Thus, the presence of VacA and CagA is of special importance during the process of epithelial damage and chronic gastric inflammation, but the virulence may depend on a close cell-to-cell interaction between these factors and the host cells.

Bacterial adherence factors mediating such close attachment to the epithelium may therefore contribute to pathogenicity, but the importance of H. pylori adherence factors for induction of severe gastritis, glandular AT, and IM is poorly understood. The recently discovered bacterial adherence factor “BabA” (blood group antigen binding adhesin) targeting Lewis\(^b\) blood group antigens (26, 27) could possibly
be relevant for bacterial pathogenicity by enabling direct bacterial contact to epithelial cells and subsequent delivery of virulence factors such as VacA or CagA. We have shown previously an association between the presence of the \(babA2\) gene (encoding BabA) in clinical isolates and the presence of ulcer disease and gastric adenocarcinoma (28). Therefore, the presence of the \(babA2\) gene in gastric biopsies, in addition to \(vacAs1\) and \(cagA\), is of clinical interest to identify \(H. pylori\)-infected patients who are at increased risk for developing gastric malignancies years before a malignant transformation is irreversible.

To further evaluate this hypothesis, we correlated the presence of \(babA2\) in biopsies from \(H. pylori\)-infected patients to the activity and chronicity of gastritis. These results were compared with the data obtained with \(cagA+\)/\(vacAs1+\) strains (suggesting the presence of type 1 \(H. pylori\)) because these bacterial subtypes have already been correlated with the increased activity of antral gastritis (29, 30). Because of the high prevalence of type 1 strains (60–90%) in Western populations, prophylactic eradication strategies to prevent cancer cannot be based on VacA or CagA presence alone, and further criteria are necessary to define a smaller subgroup of pathogenic strains.

In the present study, we correlated gene presence of \(babA2\), \(vacAs1\), and \(cagA\) genotypes with the activity and chronicity of gastritis and the presence of AT and IM in a large number of patients. As shown below, our data indicate that the presence of the bacterial adhesin, encoded by \(babA2\), specifically when present in combination with \(cagA\) and \(vacA\) (“triple-positive” strains), may play an important role in the induction of severe histological changes. Patients infected with triple-positive strains are at increased risk for developing such changes, and this subgroup of patients may profit from eradication therapy to prevent gastric cancer.

**MATERIALS AND METHODS**

**Patients.** A total of five antrum and five corpus biopsies were collected from each of the 451 consecutive patients (242 males and 209 females) who underwent routine upper gastrointestinal endoscopy because of abdominal complaints. The mean age was 64.2 years, ranging from 23 to 99 years; 88% had German nationality, and 12% were from other European countries. Patients taking nonsteroidal anti-inflammatory drugs or antisecretory medications as well as patients with peptic ulcer disease or gastric adenocarcinoma were excluded from the study. Patient’s informed consent was obtained before the examination for additional histological and molecular analysis, because of ethical guidelines of the Technical University of Munich. After each endoscopic examination, endoscopes were cleaned over 45 min in an Olympus endoscope cleaning system, endoscopes were cleaned over 45 min in an Olympus endoscope cleaning system, and strain characteristics had not been used in previous studies.

PCR amplification of \(H. pylori\) gene loci was performed for \(ureB\), \(cagA\), the \(vacA\) mosaic \(vacAs1/2\), and the \(babA2\) genotype. Primer sequences for \(babA2\) were the same as published previously (28). Other primers were: \(ureB\), sense 5'-5TACCCCAAAATCCCTACAG-3' and antisense 5'-ACGGCCCATCCGGTCTAGAT-3'; \(cagA\), sense 5'-GTATGGGGGACTGTTGG-3' and antisense 5'-GATTCTTGGGCTGTTG-3'; \(vacAs1\), \(vacAs1a\), \(slh\), and \(vacAs2\) primers were synthesized as published previously (19). Amplification was carried out in a total volume of 25 µl using the Taq PCR Master Mix (Qiagen), 1 µl of template DNA (20 ng), and 0.5 µl of each primer (20 µm). MgCl₂ concentrations were adjusted for each primer pair. Cycling was performed in a Primus PCR Cycler (MWG Biotech, Ebersberg, Germany) as follows: initial denaturation for 5 min at 94°C, 94°C for 30 s, 55-62°C for 30 s, and 72°C for 30 s (25–30 cycles); and final extension at 72°C for 5 min. PCR products were analyzed on 1–2% agarose gels stained with ethidium bromide.

**Statistical Analysis.** The \(\chi^2\) test (Figs. 1–5) and logistic regression analysis (Table 1) were used to compare the differences among the various isolates. \(P\) and the applied tests are indicated in the text and in the figure legends. \(P < 0.05\) is considered to be significant.

**RESULTS**

**Histological Evaluation of Gastric Biopsies.** Two biopsies (of antrum and corpus, total of four) were fixed in formalin and were finally evaluated by one pathologist (I.B.) at the Institute for Pathology (Technical University of Munich). Activity of gastritis (G0–G3), chronicity of gastritis (L1–L3), glandular AT, and IM were classified according to pathological guidelines and visual scales, i.e., the Sydney classification (31). The presence of \(H. pylori\) was determined in the sections using a modified Giemsa staining protocol. Biopsies and strain characteristics had not been used in previous studies.

**Preparation of DNA and PCR.** For DNA analysis, three antral and corpus biopsies were taken from every patient and were snap frozen in liquid nitrogen immediately after endoscopy. Biopsies were homogenized in 2.0-ml safe lock tubes (Eppendorf, Germany) and stored in liquid nitrogen until DNA preparation. For DNA isolation, tissue was lysed with proteinase K. DNA was isolated using the QIAamp Tissue kit (Qiagen, Munich, Germany) according to the manufacturer’s instructions.

**MATERIALS AND METHODS**

**Patients.** A total of five antrum and five corpus biopsies were collected from each of the 451 consecutive patients (242 males and 209 females) who underwent routine upper gastrointestinal endoscopy because of abdominal complaints. The mean age was 64.2 years, ranging from 23 to 99 years; 88% had German nationality, and 12% were from other European countries. Patients taking nonsteroidal anti-inflammatory drugs or antisecretory medications as well as patients with peptic ulcer disease or gastric adenocarcinoma were excluded from the study. Patient’s informed consent was obtained before the examination for additional histological and molecular analysis, because of ethical guidelines of the Technical University of Munich. After each endoscopic examination, endoscopes were cleaned over 45 min in an Olympus washing machine at 60°C. Autoclaved biopsy forceps were used for each patient separately.

**Histological Evaluation of Gastric Biopsies.** Two biopsies (of antrum and corpus, total of four) were fixed in formalin and were finally evaluated by one pathologist (I.B.) at the Institute for Pathology (Technical University of Munich). Activity of gastritis (G0–G3), chronicity of gastritis (L1–L3), glandular AT, and IM were classified according to pathological guidelines and visual scales, i.e., the Sydney classification (31). The presence of \(H. pylori\) was determined in the sections using a modified Giemsa staining protocol. Biopsies and strain characteristics had not been used in previous studies.

**Preparation of DNA and PCR.** For DNA analysis, three antral and corpus biopsies were taken from every patient and were snap frozen in liquid nitrogen immediately after endoscopy. Biopsies were homogenized in 2.0-ml safe lock tubes (Eppendorf, Germany) and stored in liquid nitrogen until DNA preparation. For DNA isolation, tissue was lysed with proteinase K. DNA was isolated using the QIAamp Tissue kit (Qiagen, Munich, Germany) according to the manufacturer’s instructions.

**PCR amplification of \(H. pylori\) gene loci was performed for \(ureB\), \(cagA\), the \(vacA\) mosaic \(vacAs1/2\), and the \(babA2\) genotype. Primer sequences for \(babA2\) were the same as published previously (28). Other primers were: \(ureB\), sense 5'-5TACCCCAAAATCCCTACAG-3' and antisense 5'-ACGGCCCATCCGGTCTAGAT-3'; \(cagA\), sense 5'-GTATGGGGGACTGTTGG-3' and antisense 5'-GATTCTTGGGCTGTTG-3'; \(vacAs1\), \(vacAs1a\), \(slh\), and \(vacAs2\) primers were synthesized as published previously (19). Amplification was carried out in a total volume of 25 µl using the Taq PCR Master Mix (Qiagen), 1 µl of template DNA (20 ng), and 0.5 µl of each primer (20 µm). MgCl₂ concentrations were adjusted for each primer pair. Cycling was performed in a Primus PCR Cycler (MWG Biotech, Ebersberg, Germany) as follows: initial denaturation for 5 min at 94°C, 94°C for 30 s, 55-62°C for 30 s, and 72°C for 30 s (25–30 cycles); and final extension at 72°C for 5 min. PCR products were analyzed on 1–2% agarose gels stained with ethidium bromide.

**Statistical Analysis.** The \(\chi^2\) test (Figs. 1–5) and logistic regression analysis (Table 1) were used to compare the differences among the various isolates. \(P\) and the applied tests are indicated in the text and in the figure legends. \(P < 0.05\) is considered to be significant.

**RESULTS**

**Histological Evaluation of Antral and Corpus Biopsies.** Antral and corpus biopsies were obtained from 451 patients undergoing routine endoscopy. In total, 151 patients had \(H. pylori\) infection as determined by PCR. One hundred forty-five patients had chronic antral and/or corpus gastritis. The distribution of each histological diagnosis in regard to the activity of gastritis and IM is shown in Fig. 1. Forty-two patients had IM and/or glandular atrophy in the antrum, but only 8 patients had IM and/or AT in the corpus. Patients with IM also had chronic antral or corpus gastritis in all cases. The chronicity of the gastritis was assessed by lymphocyte infiltration and was determined as degrees L1–L3. L2 was the predominant degree of...
lymphocytic infiltration in the antrum, whereas most patients had a lower degree of lymphocyte infiltration in the corpus (L1, 101 patients).

**Correlation of the babA2, vacAs1, and cagA Genotypes with the Degree of Granulocytic or Lymphocytic Infiltration in Antrum or Corpus.** Initially, the correlation between the babA2, vacAs1, and cagA genotypes with the activity and chronicity of gastritis was determined for each genotype (Figs. 2–4). babA2 genotype was detected in only 38% of *H. pylori*-infected patients in the antrum and corpus. As shown in Fig. 2, a statistically significant correlation between the activity of gastritis and the babA2 genotype was detected in antral and in corpus biopsies (Fig. 2, A and B; *P* = 0.01 and *P* = 0.05, respectively). Regarding the chronicity of gastritis, the relative percentage of babA2-positive biopsies increased linearly in the antrum (Fig. 2) but was almost identical in the corpus. Therefore, a significant correlation between babA2 strains and lymphocyte infiltration was obtained only in the antrum (Fig. 2A, *P* = 0.0018) but not in the corpus (Fig. 2B, *P* = 0.84).

Seventy-two % of *H. pylori*-positive patients were vacAs1 positive, and 28% had the vacAs2 genotype. As shown in Fig. 3, A (antrum) and B (corpus), the association between vacAs1 and activity of gastritis reached significance levels in the antrum (*P* = 0.038) and corpus (*P* = 0.019). Regarding chronicity of gastritis, the percentage of vacAs1 increased linearly from L1 through L3 gastritis in the antrum (*P* = 0.001) but not in the corpus (*P* = 0.12). In 2 patients, there was evidence of mixed infections, as reflected by positive results for both vacAs1 and vacAs2 in the same sample. babA2 and CagA were also present in these biopsies, and the histological investigations showed mild granulocytic (G1–G2) and a low degree of lymphocytic infiltration (L1–L2) but no G3, AT, or IM. Moreover, a further subclassification of *sl* strains was performed. *vacAs1a* strains are the predominant type (>90%) in Northern America; *slb* strains are most frequent in South America, in which the rate of gastric cancer is high, and *sle* strains have been detected in Asian populations but are rare in Western countries (32–34). Using identical primers as originally proposed by Atherton *et al.* (19), we found that 84 of 104 of our German *vacAs1* strains were *vacAs1a* strains (78.8%). Similar to the distribution of *vacAs1*, high-risk groups with G3, AT, or IM had >70% *sl* strains. The previously observed clinical correlation of *sl* strains with gastric cancer may therefore reflect the geographic distribution of different *vacA* alleles and may not be of relevance in our German population.

The *cagA* gene was detected in a total of 61% of *H. pylori*-infected patients. Fig. 4 illustrates that *cagA* was the only gene associated with the activity of gastritis in antrum and corpus (antrum, *P* = 0.001; corpus, *P* = 0.01) and, moreover, also with the degree of lymphocytic infiltration in the antrum (*P* = 0.001) and in the corpus (*P* = 0.04). *cagA+/vacAs1* strains were detected in a total of 60% of *H. pylori*-infected patients and showed a statistically significant correlation with the activity (*P* = 0.04 in the antrum; *P* = 0.03 in the corpus) and with the chronicity of gastritis (antrum, *P* = 0.01; corpus, *P* = 0.02; data not shown).

Subsequently, the simultaneous distribution of the *cagA*, *vacAs1*, and babA2 genes was determined. Eighty-seven patients were infected with *cagA+/vacAs1* strains, and 50 of them had the babA2 gene simultaneously. Thirty-seven patients were infected with strains expressing *cagA+/vacAs1* but lacking the babA2 gene. In contrast, of 52 *vacAs1/babA2* strains, only 2 strains did not express *cagA*. Of 51 *cagA/babA2* strains, only one strain did not harbor the *vacAs1* genotype. Thus, only the subclassification of *cagA+/vacAs1* strains

![Fig. 2. Presence of babA2-positive stains (relative %, in each group) in biopsies from *H. pylori*-infected patients suffering from various degrees of granulocyte infiltration (activity of gastritis) or lymphocyte infiltration (chronicity) in the antrum (A) and corpus (B).](image-url)
by the presence or absence of babA2 yielded subgroups with numbers large enough to allow statistical evaluation. These subgroups were then correlated with the different histological scores.

**Correlation of cagA+/vacAs1+ Strains Harboring or Lacking the babA2 Gene with the Activity and Chronicity of Gastritis.** Fig. 5 illustrates the distribution of cagA+/vacAs1+ strains subdivided by the absence or presence of the babA2 gene. As shown in Fig. 5, cagA+/vacAs1+ strains that were babA2 negative showed no association with the activity of gastritis in antrum or corpus, respectively. In contrast, cagA+/vacAs1+ strains that were babA2 positive (triple-positive strains, detected in a total of 34% of all H. pylori-positive biopsies) showed a significant correlation with the activity of antral and corpus gastritis (P = 0.002) and corpus gastritis (P = 0.019) gastritis. Thus, the presence of babA2 was the important parameter determining the association of cagA+/vacAs1+ strains with the degree of granulocyte infiltration in the gastric mucosa. Triple-positive strains also showed a close correlation with lymphocyte infiltration in the antrum (P = 0.01) but not in the corpus (P = 0.54). cagA+/vacAs1+ strains that lacked babA2 did not show a significant correlation with the chronology of gastritis in antrum or corpus (data not shown).

**Detection of H. pylori Subtypes in the Presence or Absence of Severe Histological Changes (G3, AT, and IM).** Severe histological changes, such as severe granulocyte infiltration (G3), glandular AT, or IM can be regarded as lesions possibly preceding gastric cancer. Therefore, we specifically investigated the association between several bacterial genotypes and the presence or absence of G3, AT, and IM in the antrum and corpus (Table 1). As shown in Table 1, the presence of triple-positive strains showed a significant correlation with severe granulocyte infiltration (G3) in the antrum (P = 0.001), and P was lower compared with vacAs1, cagA, or cagA+/vacAs1+ strains. Moreover, the additional presence of babA2 in cagA+/vacAs1+ strains correlated with G3 in the corpus (P = 0.004). Similar to the observations stated above, cagA+/vacAs1+ strains lacking babA2 also showed no correlation with G3 status in antrum or corpus (P = 0.63–1.0), as calculated by exact logistic regression.

babA2 and triple-positive strains were the only subtypes associated with the presence of IM and glandular AT in the antrum (Table 1) and yielded the best significance levels (P = 0.001–0.0045). vacAs1+ or cagA+ strains were inconsistently associated with AT or IM in the antrum and showed no significant correlation in the corpus. Most prominently, babA2-negative cagA+/vacAs1+ strains lacked an association with these histological alterations in antrum and corpus and were found at a similar frequency. A close association of triple-positive strains and AT/IM was obtained in the corpus, where these strains were twice as frequent compared with the absence of AT or IM. However, because of the small number of IM- and AT-positive biopsies (n = 8) in the corpus, P did not reach significance.

**DISCUSSION**

Since the discovery of H. pylori in 1983, this microorganism has been implicated in gastric carcinogenesis based on various epidemiological and animal studies (5, 6, 35, 36). Controversy remains why only a minority of H. pylori-infected persons will develop gastric
adenocarcinoma. Because bacterial adherence and virulence factors have been identified among different strains, these strain characteristics may explain the progression of gastritis to precancerous lesions or even gastric adenocarcinoma in a subgroup of patients. In the current study, we investigated the presence of different H. pylori adherence and virulence factors in human gastric mucosa and correlated these results with the severe histological changes in the mucosa that may precede gastric cancer.

VacA- and CagA-positive strains ("type 1 strains") have already been implicated in the development of more severe gastritis (7, 30, 37, 38). Exposure of epithelial cells to VacA leads to a swelling of endosomal compartments (39), alterations in cellular trafficking (40), and inhibition of antigen processing within the prelysosomal compartment (41). The presence of vacAs1 has been reported previously to correlate with the activity and chronicity of antral gastritis (34), which is confirmed by our current results. We also found a strong correlation between vacAs1 and severe granulocyte infiltration in antrum and corpus as well as a correlation between vacAs1 and lymphocytic infiltration in the gastric antrum.

Our data also confirm a close correlation of cagA-positive strains not only with severe granulocyte but also with lymphocyte infiltration in antrum and corpus, which is in accordance with previous reports (7, 30). This correlation suggests that H. pylori leads to a direct damage of the gastric mucosa by secretion of CagA into epithelial cells, inducing a granulocytic response (23–25), as well as an indirect damage via activation of the immune response (20, 37, 38). Several studies have determined that cagA+ H. pylori strains contain variations in the 3’ repeat regions, which have been associated with differing risks for gastric cancer, implying that not all cagA+ strains are identical (42). Therefore, we used primer pairs located in the middle region of the cagA gene to reduce the impact of genetic diversity. Although cagA had the lowest Ps of all markers with which we investigated the correlation with gastritis, the overall prevalence of cagA+ or cagA+/vacAs1+ strains was 61–100%, depending on the different degrees of gastritis. Treatment of patients infected with these strains may be problematic in regard to treatment costs and efficacy, because only a minority will develop gastric disease. Thus, it is of special importance to define a subgroup of cagA+/vacAs1+ strains that show a close association with severe histological changes. We therefore investigated the presence of the adherence factor BabA alone or with the simultaneous presence of VacA and CagA. We hypothesized that these virulence factors may obtain a higher pathogenic potential if the bacteria are tightly attached to the epithelium.

Previous studies have already suggested a key role of the M, 78,000 adhesin BabA and of the corresponding Lewis β antigens for adherence of H. pylori to gastric surface mucosal cells in vitro and in vivo (26, 43, 44). Two corresponding genes encoding the BabA protein have been cloned and termed babA1 and babA2, but only the babA2 gene is functionally active (27). Our previous studies have shown that the presence of babA2, especially when present in combination with vacAs1 and cagA, is associated with ulcer disease and adenocarcinoma (28), and this association led to the proposal of triple-positive strains.

In the current study, babA2 was significantly associated with granulocyte infiltration in antral and corpus mucosa and increased almost linearly from G0 to G3 stages. These observations suggest that the adhesion of H. pylori to Lewis antigens is important in the pathogenesis of severe gastritis. babA2 was also significantly correlated with lymphocytic infiltration in the antrum but was not associated with the chronicity of gastritis in the corpus (L1–L3, 36–43%). This observation may be attributable to the fact that the adhesin per se is not associated with a specific immune, i.e., lymphocyte, response and may not serve as a bacterial epitope or antigen for these immune cells. The association with the chronicity in the antrum may therefore only reflect the course of disease and the chronic infection in the antrum.

Therefore, we focused on the presence of babA2 together with vacAs1 and cagA. As shown in Fig. 4, triple-positive strains were significantly associated with the activity of gastritis, whereas babA2-negative cagA+/vacAs1+ strains lacked a correlation. Thus, babA2 was an important parameter determining the correlation of especially virulent H. pylori strains with the activity of gastritis. This finding suggests that adherence via BabA is of special importance in the process of bacterial attachment and delivery of products. Clearly, the presence of BabA is of high clinical relevance, especially when it is expressed together with VacA and CagA. This observation was obvious when comparing presence or absence of severe histological changes.

Three histological conditions have been investigated previously in relation to the development of gastric adenocarcinoma: severe granulocyte infiltration in the corpus, glandular AT, and IM. Severe granulocyte infiltration in the corpus (G3) and especially an increased score of corpus versus antral gastritis have been reported by several authors as an independent risk factor for the development of gastric cancer (13–15, 45, 46). As shown in Table 1, characterization of cagA+/vacAs1+ strains by the additional presence of babA2 (triple-positive strains) showed a significant correlation with the presence of G3 in antrum and corpus. In contrast, cagA+/vacAs1+ strains that were babA2 negative were almost equally distributed. Similarly, the presence of triple-positive strains correlated with the presence of glandular AT and IM in the antrum, whereas cagA+/vacAs1+ strains
lacking babA2 were equally distributed. In the corpus, no significance level was reached, which appears to be because of the low number of IM- and AT-positive patients (n = 8).

These findings imply that a close attachment of the bacteria to epithelial cells via BabA may be of special importance for effective delivery of bacterial products in cagA+/vacA+/ babA2+ strains, which may directly or indirectly lead to increased mucosal injury. The development of severe gastritis, glandular AT, and finally IM may be initiated and facilitated by a close contact of babA2-positive bacteria to the gastric epithelium. After attachment via BabA, exposure to VacA toxins and/or CagA antigens may further enhance epithelial damage, achlorhydria, glandular AT, and IM.

AT and IM, especially the sulfomycin-secreting type III of IM, have been determined to be risk factors for the development of gastric cancer (12, 16, 47–51) and may lead to the generation of N-nitroso compounds, thereby predisposing to gastric carcinogenesis. Even metaplastic cells may represent a possible target for attachment of bacteria via BabA and Lewisβ interaction, because these cells have been shown previously to exhibit an increased expression of Lewis antigens (52). Therefore, the correlation of babA2-positive strains with glandular AT and IM may reflect continuous bacterial adherence, even during severe changes of the gastric architecture.

Our current data support the view that the presence of babA2, particularly in combination with vacA/S1 and cagA, is of special importance for the pathogenesis of specific gastric disease. Simultaneous expression of BabA with other virulence factors may lead to severe histological alterations and thereby predispose to gastric carcinogenesis. We suggest that classification of H. pylori by molecular genotyping should be routinely performed to identify the bacterial strain type. Further follow-up, prospective studies will determine whether patients infected with triple-positive strains will benefit from eradication in early stages of gastritis.

REFERENCES


Key Importance of the *Helicobacter pylori* Adherence Factor Blood Group Antigen Binding Adhesin during Chronic Gastric Inflammation

Christian Prinz, Martin Schöniger, Roland Rad, et al.


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/5/1903

Cited articles  This article cites 47 articles, 20 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/5/1903.full.html#ref-list-1

Citing articles  This article has been cited by 26 HighWire-hosted articles. Access the articles at:
/content/61/5/1903.full.html#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.