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The *Helicobacter pylori* Blood Group Antigen-Binding Adhesin Facilitates Bacterial Colonization and Augments a Nonspecific Immune Response

Roland Rad,* Markus Gerhard,** Roland Lang,** Martin Schöniger,* Thomas Rösch,* Wolfgang Schepp,* Ingrid Becker,* Hermann Wagner,† and Christian Prinz‡

Presence of the *Helicobacter pylori* adherence factor blood group Ag-binding adhesin (BabA; binding to Lewis b (Le b )) is associated with ulcer disease, adenocarcinoma, and precancerous lesions. The importance of BabA for bacterial colonization and the inflammatory response is unknown. A total of 141 antral biopsies from *H. pylori*-infected patients were assessed in regard to the degree of granulocytic infiltration and lymphocytic infiltration (L1–L3). DNA genotypes of babA2 (the transcriptionally active gene of BabA), *cagA*, and *vacAsI/2* were determined by PCR. Colonization density and Le b status on gastric epithelial cells were determined by immunohistochemistry. Real-time quantitative (TaqMan) RT-PCR determined mRNA expression of IL-8, TNF-α, and the Th1 markers IFN-γ and the IL-12R β2 chain. A total of 91% of infected patients were Le b positive. The *vacAsI/2/cagA* strains harboring babA2 showed significantly higher levels of granulocytic infiltration, bacterial colonization, and IL-8 mRNA than *vacAsI/2/cagA* strains lacking babA2. IL-8 mRNA and protein production by KATO III cells in vitro increased dose dependently with addition of different numbers of type 1 strains (G27 and 2808 strains, 0.1–20 bacteria/cell). The mRNA expression of TNF-α, IFN-γ, and IL-12R β2 was higher in *H. pylori*-positive patients than in controls, but it did not differ significantly between patients infected with different strain types. These data suggest that BabA facilitates colonization of *H. pylori* and thereby increases IL-8 response, resulting in enhanced mucosal inflammation. Infection with strains harboring BabA thereby augment a nonspecific immune response, whereas the Th1 response toward *H. pylori* appears to be independent of BabA, cytotoxin-associated gene A, or vacuolating cytotoxin.

The importance of *Helicobacter pylori* for gastric pathogenesis is well documented (1–4). Bacterial virulence and adherence factors play an important role in the development of specific gastric disease. Strains expressing the cytotoxin-associated gene A (*CagA*) and secreting high amounts of the vacuolating cytotoxin (VacA) have been classified as type 1 strains (5). The *cagA*- and/or *vacAsI*-positive strains have been detected more frequently in patients with severe gastritis (6), ulcer disease (7, 8), intestinal metaplasia (IM) (9), atrophic gastritis (10), and gastric adenocarcinoma (11). In addition, recent studies demonstrated the clinical relevance of the blood group Ag-binding adhesin (BabA), encoded by the *babA2* gene. The *babA2*-positive strains were associated with ulcer disease as well as gastric carcinoma and discriminated significantly better between disease outcomes than type 1 strains (12). Moreover, *vacAsI/2/cagA* strains, which simultaneously harbored the *babA2* gene, were more frequent in patients with IM and atrophic gastritis (13).

BabA is an adherence factor expressed in a subgroup of *H. pylori* strains and binds to difucosylated Lewis b (Le b ) blood group Ags found on epithelial cells (14). The influence of BabA on bacterial colonization and on the inflammatory response is poorly understood. Mathematical as well as experimental models have hypothesized that the outcome of bacterial colonization is influenced by the ability of bacteria to adhere to epithelial cells (15, 16). Adherent bacteria are supposed to have growth advantages based on proximity to the epithelium (i.e., better availability of nutrients). Furthermore, elimination of bacteria by peristaltic movements and washout with the luminal fluid may affect adherent strains to a lesser extent. Therefore, bacteria with better adherence characteristics are supposed to colonize with higher densities.

In addition, adherent bacteria may be able to transfer their products more effectively to the host cells. Thus, a higher bacterial density and/or a more efficient delivery of bacterial products to the host may induce a stronger inflammatory response. *H. pylori* has already been shown to initiate nonspecific immune responses. Infection leads to IL-8 secretion from epithelial cells (17–19). IL-8 in turn is a potent chemoattractant factor for neutrophils and stimulates the degranulation of these cells (20), thus contributing to the *H. pylori*-induced tissue damage (21, 22).

Finally, the lymphocytic infiltrate in the gastric mucosa suggests that *H. pylori* also elicits adaptive immune responses. Indeed, *H. pylori*-specific T cells have been isolated from infected patients (23). Gastric T cells from infected mice (24), rhesus macaques (25), and humans (26) produce predominantly IFN-γ and TNF-α, but not IL-4, which is typical for a Th1-polarized T cell response.

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R.R., M.G., and R.L. contributed equally to this work.

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Abbreviations used in this paper: *CagA*, cytotoxin-associated gene A; VacA, vacuolating cytotoxin; BabA, blood group Ag-binding adhesin; Le b , Lewis b ; AT, gastric atrophy; IM, intestinal metaplasia.
Because production of IFN-γ by Th1 T cells is pivotal in the control of intracellular pathogens, the Th cell response toward specific *H. pylori* Ags may generally amplify local inflammatory responses and promote tissue destruction. Although the Th helper cell response to *H. pylori* is generally considered to be of the Th1 phenotype leading to a cell-mediated immune response (23, 27), the importance of bacterial virulence and adherence factors for the induction and intensity of a specific Th1 response remains controversial.

The present study investigates the influence of BabA on bacterial colonization and on the induction of specific and nonspecific inflammatory responses. Using quantitative real-time TaqMan PCR, we determined mRNA copy numbers of characteristic cytokines and receptors describing the profile of granulocytic and lymphocytic responses. Our study supports a crucial role of BabA for the pathogenesis of chronic gastric inflammation and describes possible mechanisms mediating this effect.

**Materials and Methods**

**Patients and biopsies**

Five antral biopsies were collected from each of the 451 consecutive patients (242 male and 209 female) after they received informed consent. A total of 141 patients were *H. pylori* positive. Patients underwent routine gastroscope examination because of abdominal complaints. The mean age was 64.2 years, ranging from 23 to 92 years; 88% had German nationality and 12% were from other European countries. Patients taking nonsteroidal anti-inflammatory drugs or receiving antiserotherapy were excluded from the study. Two antral sections were stained with H&E for histopathological evaluation. The inflammatory response toward *H. pylori* in the mucosa was characterized by histopathological evaluation (updated Sydney classification system) in regard to the degree of granulocytic infiltration (G1*, mild; G2*, moderate; G3*, severe) and lymphocytic infiltration (L1°, G1°, moderate; G3°, severe) and the etiopathogenesis of the gastritis. Gastritis is differentiated into autoimmune, *H. pylori*-associated, and chemically induced reactive gastritis, as well as other infrequent forms. The group of *H. pylori*-associated gastritis is characterized in regard to the activity and chronicity of inflammation and differentiates several degrees of granulocytic and lymphocytic infiltration (none, mild, moderate, and severe), respectively. Moreover, the presence of gastric atrophy (AT) and IM is assessed.

The three remaining antral biopsy specimens were stored in liquid nitrogen and homogenized before DNA or RNA isolation. After tissue lyssation with proteinase K, DNA isolation was performed with a QiAamp tissue kit (Qiagen, Hilden, Germany), according to the manufacturer’s instructions. RNA was extracted by the phenol/chloroform method.

**Immunhistochemical analysis of Leb and determination of colonization densities**

Formalin-fixed paraffin-embedded tissue sections were deparaffinized with xylene and ethanol and then incubated with a 1/10000 dilution of mouse anti-human Leb mAb (MAB2102; Chemicon International, Temecula, CA) and xylene and ethanol and then incubated with a 1/1000 dilution of mouse monoclonal anti-human Leb mAb (MAB2102; Chemicon International, Temecula, CA). Immunohistochemical analysis of Leb and determination of colonization densities was assessed.

**PCR for *H. pylori* genotyping**

PCR amplification of *H. pylori* gene loci was performed for the caga gene as published previously (12); vacA primers were used as described before. (30). PCR primers for amplification of babA2 were as follows: sense, 5′-AATCCAAAAAGGAGAAAAACATGAAA-3′; antisense, 5′-TGT TAGTGATTTCGGTGTAGGACA-3′. Amplification was conducted using 1 μl of genomic DNA, 22 μl of Master Mix (Qiagen), and 1 μl of each primer (20 μM). MgCl2 concentrations were adjusted for each primer pair. Reaction mixtures were amplified for 30 cycles as follows: initial denaturation at 94 °C for 45 s, 55–62 °C for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 10 min. PCR products were analyzed on 1–2% agarose gels stained with ethidium bromide.

**Calibration of the quantitative TaqMan PCR system**

Recently, a new technique for the detection of PCR-amplified nucleic acids using the 5′--3′ nuclease activity of Taq polymerase has been reported (31–33). To determine absolute cytokine mRNA copy numbers, standard curves were generated for each cytokine using plasmid dilution series containing the unknown target sequences. Over a wide dynamic range, the threshold cycle value was a linear function of the starting cDNA input with coefficients of correlation of 0.95–1. For each sample, copy numbers of GAPDH, TNF-α, IFN-γ, IL-12R β2, and IL-8 were determined. Cytokine and cytokine receptor copy numbers were presented in copies per 10,000 GAPDH copies.

**TaqMan primers and fluorogenic probes**

TaqMan primers (MWG Biotec, Ebersberg, Germany) and probes (PerkinElmer, Weiterstadt, Germany) were designed using the primer design software Primer Express (PE Applied Biosystems, Foster City, CA). All probes were synthesized by PerkinElmer and labeled with the reporter dye 6-carboxyfluorescein at the 5′ end and the quencher dye 6-carboxytetramethylrhodamin at the 3′ end. Primer and probe sequences for TNF-α, IFN-γ, IL-12R β2, and IL-8 were determined. Cytokine and cytokine receptor copy numbers were presented in copies per 10,000 GAPDH copies.

**TaqMan PCR procedure**

Five microliters of RNA was transcribed into cDNA in a total volume of 50 μl using 50 U of MultiScribe reverse transcriptase (PerkinElmer) according to the manufacturer’s instructions. PCR was performed in a volume of 30 μl on the ABI PRISM 7700 sequence detection system (PerkinElmer). For each run, a master mix was prepared on ice containing 15 μl of Universal Master Mix (PE Applied Biosystems), primers (0.5 μmol/l for GAPDH, TNF-α, and IL-12R β as well as 1 μmol/l for IFN-γ and IL-8), fluorogenic probes (0.16 μmol/l for GAPDH, TNF-α, and IL-12R β as well as 0.32 μmol/l for IFN-γ and IL-8), and H2O. To each well of a 96-well plate, 25 μl of master mix and 5 μl of cDNA samples were added. All PCRs were performed in duplicate. Thermal cycling was initiated with an incubation step at 50 °C for 2 min, followed by a first denaturation step at 95 °C for 10 min, and continued with 40 cycles of 95 °C for 15 s, 58 °C for 20 s, and 72 °C for 30 s.

**Statistical analysis**

Statistical analysis was performed using the χ2 test, Mann-Whitney rank sum test, or a Spearman rank test, depending on the data set of concern. Values of p < 0.05 were considered significant. The tests applied are indicated in the figures.
Table I. Sequences of primers and probes used for quantitative TaqMan-PCR

<table>
<thead>
<tr>
<th>Target</th>
<th>Sequences of Primers and Probes</th>
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<tr>
<td>GAPDH</td>
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<tr>
<td>FP</td>
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<tr>
<td>RP</td>
<td>TTAGGTGCTGAGCTGAGTT</td>
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<tr>
<td>P</td>
<td>CACACAAGCAAGGTTGCTGAGTT</td>
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<tr>
<td>IL-1ββ2</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>TTAGCTGAGCATGAGTT</td>
</tr>
<tr>
<td>P</td>
<td>TATGTTTATAATCACGGGCTGATTAAGGC</td>
</tr>
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<td>IFN-γ</td>
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<tr>
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</tr>
<tr>
<td>P</td>
<td>AGAGCTCCTGCTGAGGACCAAGAGAAC</td>
</tr>
</tbody>
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*FP, Forward primer; RP, reverse primer; P, fluorogenic internal probe.

Results

Patient population and *H. pylori* strain characteristics

A total of 451 patients underwent endoscopy of the gastrointestinal tract at the Technical University of Munich (Munich, Germany) and were examined; 141 were infected with *H. pylori*, as determined by histological staining and *H. pylori* PCR. Simultaneously, strain characteristics of *H. pylori* were determined by PCR. As shown in Fig. 1A, the vacA strains characterized in 74%, cagA in 65%, and babA2 in 38% of all *H. pylori*-positive antral biopsies. The babA2 strains were associated with cagA and vacAs1 gene presence (Fig. 1B) because nearly all babA2-positive (babA2+) strains (50 of 53) were simultaneously vacAs1/cagA positive (vacAs1+/cagA+). To determine the importance of BabA for pathogenesis, vacAs1+/cagA+ biopsies (suggesting the presence of type 1 strains) were divided into two subgroups dependent on the babA2 status (Fig. 1B). Of a total of 88 vacAs1+/cagA+ biopsies, 38 were babA2 negative (babA2−), whereas 50 were babA2+.

**Leb** immunohistochemistry of the gastric mucosa

Leb expression was assessed in 131 *H. pylori*-positive gastric sections by immunohistochemical staining. A total of 90.7% of the sections were found to be Leb-positive. Leb staining of the gastric mucosa was negative in only 12 patients (9.3%). Three of these patients were infected with vacAs1+/cagA+/babA2− strains, four of them with vacAs1+/cagA+/babA2−/babA2+ strains and five of them with vacAs1+/cagA−/babA2− strains, but none of them developed severe gastritis (G3 or L3), IM, or AT. Leb status was also determined in 141 *H. pylori*-negative biopsies. Again, Leb was abundantly detected in 127 patients (90.1%). Thus, among *H. pylori*-positive and -negative biopsies, the Leb status seems to be almost identical.

Importance of babA2 presence for granulocytic infiltration and the development of IM and AT

To investigate the role of babA2 for the pathogenesis of gastritis, we correlated the presence of different strain types to the varying levels of granulocytic infiltration. Patients infected with *H. pylori* strains lacking babA2, vacAs1, and cagA genes predominantly had mild or moderate degrees of gastritis, as shown in Fig. 2A. Patients infected with vacAs1+/cagA+/babA2− strains had higher degrees of gastritis, but this difference did not reach significant levels compared with those infected with cagA+/vacAs1+/babA2− strains. The highest degrees of granulocytic infiltrations were reached in patients infected with vacAs1+/cagA− strains that additionally harbored babA2, with significant differences from the other two groups (Fig. 2A).

Comparing the distribution of bacterial subtypes with the presence of IM or AT, we found that these histological alterations were more frequent in patients infected with vacAs1+/cagA−/babA2−

**FIGURE 1.** A, Prevalence of vacAs1, cagA, and babA2 in a population of 141 *H. pylori*-positive patients. B, Schematic illustration characterizing the presence or absence of the babA2 gene in vacAs1+/cagA+ gastric biopsies. The vacAs1+/cagA+ biopsies are enclosed in the filled circle. The hatched circle represents the whole group of babA2+ biopsies. Nearly all babA2+ strains simultaneously harbored the other two genes (50 of 53). To determine the functional importance of babA2, vacAs1+/cagA+ strains were divided into two subgroups depending on the presence or absence of babA2.
Strains (Fig. 2, B and C). Infection with these strains was associated with an ~2-fold increase in the diagnosis of IM or AT compared with infection with other strain types.

Furthermore, the presence of AT/IM was determined in patients with different degrees of antral gastritis. AT and/or IM were detected in 33% (11 of 33) of \( H. pylori \)-positive patients with lower degrees of granulocytic infiltration (G0°/G1°), in 40% (32 of 80) of patients with G2° gastritis, and in 46% (13 of 28) with G3°.

Similarly, the rising degrees of lymphocytic infiltration correlated with the presence of AT and/or IM: L1°, 26% (8 of 31 patients); L2°, 39% (33 of 84); and L3°, 58% (15 of 26).

Importance of \( \text{babA2} \) presence for IL-8 secretion

Next, the relationship between IL-8 mRNA levels and \( \text{babA2} \) presence was determined (Fig. 3). IL-8 mRNA amounts were measured by quantitative TaqMan-PCR in the gastric mucosa. Median IL-8 mRNA amounts were significantly higher in \( H. pylori \)-positive biopsies compared with noninfected patients (data not shown). Furthermore, IL-8 mRNA amounts were found to differ in patient groups infected with different strain types. The lowest level of IL-8 mRNA was detected in patients infected with \( \text{cagA}^-/\text{vacA}^-/\text{babA}^- \) strains (Fig. 3). The \( \text{vacA}^+/\text{cagA}^-/\text{babA}^- \) strains induced higher levels of IL-8. The highest IL-8 mRNA amounts were measured in patients infected with \( \text{vacA}^+/\text{cagA}^+/\text{babA}^+ \) strains. IL-8 mRNA levels also correlated significantly with increasing degrees of granulocytic infiltration (\( p < 0.05; r = 0.33; \) Spearman rank test; data not shown), rising from mild (G0°–G1°) to severe (G3°) gastritis.

FIGURE 2. Granulocytic infiltration and prevalence of IM as well as atrophy in patients infected with different strain types. A, Degrees of granulocytic infiltration in gastric biopsies infected with different strain types as indicated on the x-axis (G1°, mild; G2°, moderate; G3°, severe granulocytic infiltration). A Mann-Whitney \( U \) test was applied to compare statistical differences. B and C, Prevalence of IM (B) or AT (C) in patients infected with different strain types. The \( \chi^2 \) test was applied to compare groups statistically.

FIGURE 3. IL-8 mRNA amounts in gastric biopsies infected with different bacterial strain types. A Mann-Whitney \( U \) test was applied to compare statistical differences.
Role of babA2 gene presence for colonization density

A total of 95 antral sections were evaluated for colonization density by immunohistochemical staining. Colonization densities were significantly lower in patients infected with strains lacking babA2 than in patients infected with babA2+ strains \((p < 0.005)\). The cagA+/vacAs1+ strains lacking babA2− showed higher colonization densities than cagA−/vacAs1− strains (Fig. 4), but this difference did not reach a statistically significant level. The highest colonization density was observed in patients infected with vacAs1+/cagA+/babA2+ strains, and the difference to the vacAs1−/cagA+ group was highly significant \((p < 0.005)\).

Correlation of IL-8 secretion from epithelial cells with bacterial densities in vitro

To evaluate the effect of the bacterial load on IL-8 secretion, we cocultured KATO III cells with different amounts of the type I H. pylori strain G27. Similar results were observed with the laboratory strain H. pylori 2808. Both laboratory strains are cagA+/vacAs1+/babA2+. Clinical isolates of type I strains with differential expression of babA2 were not available. IL-8 mRNA levels were determined by TaqMan PCR (Fig. 5A) after 2 h of stimulation, and IL-8 protein levels were measured in the supernatant by ELISA after 24 h of incubation (Fig. 5B). IL-8 mRNA and protein concentrations increased continuously with increasing bacterial densities up to 20 bacteria/cell. At concentrations above 20 H. pylori per cell, IL-8 secretion decreased slightly.

Lymphocytic and Th1 response toward infection with different H. pylori strains

Gastric biopsies were also evaluated in regard to lymphocytic infiltration, and the results were correlated to the presence of different strains types. As shown in Fig. 6A, the highest degrees of lymphocytic infiltration were observed in patients infected with vacAs1+/cagA+/babA2+ strains, which was significantly different from the two other groups, both lacking babA2. Furthermore, several markers of a Th1 response were determined. A typical receptor found on Th cells is the IL-12R, composed of two β-type cytokine receptor subunits (34). The IL-12R β2 chain has been shown to be selectively expressed on Th1 cells, whereas the β1 chain is present on both Th1 and Th2 cells (35, 36). Expression of IL12R β2 is up-regulated in diseases characterized by a Th1-skewed immune status (37, 38). Cytokines that further characterize a Th1-driven immune response are IFN-γ and TNF-α. Besides the classical Th1 marker IFN-γ, we included TNF-α in our analysis because it is produced by Th1 cells (but also by other cell types, like macrophages) and, like IFN-γ, may play an important role in tissue damage.

To investigate the influence of H. pylori and its virulence factors on the Th1 response, mRNA amounts of IFN-γ, TNF-α, and the
IL-12R β2 chain were determined in antral biopsies using quantitative TaqMan PCR. The mean cytokine mRNA amounts were 2- to 3-fold higher in the *H. pylori*-positive biopsies than in the non-infected samples, confirming that *H. pylori* induces a Th1 response (data not shown). As shown in Fig. 6, B–D, all Th1 markers correlated with the increasing degrees of lymphocytic infiltration. However, we found no differences in the expression of TNF-α, IFN-γ, or IL-12R β2 among patients infected with different strain types (Fig. 7). The cytokine amounts were similar in vacA*s1/cagA+ and -negative biopsies; the additional presence of babA2 among vacA*s1/cagA+ strains did not affect the expression of Th1 markers either.

### Discussion

Recent studies provide biochemical as well as clinical evidence that the *H. pylori* adherence factor BabA contributes to the specific tropism and pathogenicity of *H. pylori* in the human gastric epithelium (12–14). The functionally active gene encoding BabA has been cloned and termed babA2 (14). The corresponding receptors for BabA are Leb (α-1, 3/4-difucosylated) Ags present on epithelial cells (14, 39, 40). However, little is known about the presence of these epitopes in the human gastric epithelium, especially during *H. pylori* infection. In the human blood system, Leb is detected in 70–80% of patients, but this distribution does not reflect the expression on epithelial cells (41). Therefore, we initially assessed the presence of Leb Ags in a total of 131 *H. pylori*-positive and 141 *H. pylori*-negative gastric biopsies. The percentage of Leb-negative patients was <10% in both groups, which is remarkably smaller than the prevalence of nonsecretors (20–30%) in Western populations. Thus, Leb epitopes, functioning as target molecules for BabA-expressing strains, are present in the gastric epithelium of the vast majority of our population. Therefore, Leb expression is

![Image of Figure 6](http://www.jimmunol.org/)
not a limiting parameter in *H. pylori* infection, enabling almost all BabA+ strains to attach to the gastric epithelium.

The focus of the current study was to define the importance of BabA for the development of gastric inflammation and to describe possible mechanisms involved in this process. As summarized and illustrated in Fig. 8, our data suggest a sequence of events by which BabA-mediated adherence leads to increased mucosal damage of the gastric mucosa. We initially observed that patients infected with strains harboring the babA2 gene had higher degrees of granulocytic infiltration in gastric biopsies than patients infected with vacAs1/cagA+ strains lacking babA2. This finding in humans is in agreement with the investigations of Guruge et al. (42), who studied the role of the BabA/Leb interaction in transgenic mice expressing the Leb epitope on their gastric epithelial cells. Attachment of babA2-positive *H. pylori* in such mice resulted in the development of more severe degrees of gastritis than in wild-type mice lacking Leb.

An important chemotactic factor for granulocytes is IL-8, which is mainly secreted by epithelial cells in response to *H. pylori* infection. Several studies have shown that induction of IL-8 secretion is dependent on the presence of different genes of the cagPAI (43, 44). In the current study, the highest levels of IL-8 were detected in patients infected with babA2+ strains. BabA presence, therefore, seems to enhance the capability of cagA+ *H. pylori* strains to induce IL-8 in the gastric mucosa. One possible mechanism underlying this effect appears to be the influence of BabA on colonization density. BabA seems to ameliorate the colonization properties of *H. pylori*, in that the highest colonization densities were observed in patients infected with babA2+ strains. Bacteria with better adherence characteristics are supposed to be more resistant to clearance by gastric motility or washout with the luminal fluids (15, 16). Higher colonization densities then induce a higher IL-8 response and subsequently an enhanced granulocytic infiltration in the gastric mucosa. This idea is supported by our current

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**FIGURE 8.** Schematic illustration of the suggested sequence of events leading to the higher pathogenicity of babA2+ *H. pylori* strains.

**FIGURE 7.** mRNA amounts of IL-12Rβ2 (A), IFN-γ (B), and TNF-α (C) in patients infected with different *H. pylori* strain types. Statistical differences between the different groups were calculated by Mann-Whitney U test but did not reach significant levels.
finding in vitro in which the amount of IL-8 secretion from gastric cell lines in response to type 1 strains was dependent on the bacterial load, increasing up to 20 bacteria per cell. Furthermore, these findings are in agreement with a previous in vivo study (45). In that report, IL-8 secretion and colonization densities were found to be higher in ulcer patients infected with cagA strains compared with non-ulcer cagA patients; however, increase of antral IL-8 production in the ulcer group was related to an increased number of bacteria and not to an increase in the in vitro cytokine production per cagA isolate.

In seeming contradiction of our finding is one recent report, which investigated the influence of the H. pylori outer membrane protein OipA on IL-8 secretion (46). In that study, babA2 knockout strains were analyzed in regard to the ability to induce IL-8 secretion, but the differences between babA2 knockouts and wild-type strains were not significant. However, Lea receptor presence of the cell lines used was not assessed in that study, which may explain the diverging data. Moreover, static in vitro assays, in which high numbers of bacteria are allowed to sink down and passively get into contact with epithelial cells, do not reflect the complex situation in vivo, which is characterized by clearance mechanisms like peristaltic movements and bacterial washout.

Besides the effect on bacterial colonization we have described, it could be possible that BabA-mediated adherence also favors a direct interaction between bacteria and epithelial cells. In one previous study, IL-8 secretion from epithelial cells could not be detected when adhesion was prevented by separating bacteria from the cells through filter membranes (47). Furthermore, attachment of type 1 H. pylori to epithelial cells leads to a couple of events, such as translocation of CagA into the host cell, induction of signal transduction pathways, cytoskeletal rearrangements, and cellular growth changes (48). Although BabA is not the only adherence factor of H. pylori (49), it appears reasonable that a tight attachment via BabA leads to a more efficient induction of these pathways.

To investigate the direct effect of BabA on IL-8 secretion without considering the effect of BabA on colonization density, it appears reasonable to compare type1/babA2- and type1/babA2- strains. However, BabA is not the only virulence factor associated with increased IL-8 production. For example, OipA presence has been associated with increased IL-8 levels. Therefore, an objective in vitro comparison should be based on large numbers of type1/babA2- and type1/babA2- strains to avoid a sampling error. At present, large numbers of clinical isolates of type 1 strains with differential BabA expression are not available in our facility. Alternatively, type 1 BabA-knockout strains could be compared with type 1 wild-type strains in regard to the induction of cytokine levels in Lea-positive cell lines. Such knockout experiments are currently under investigation in our laboratory.

Finally, the activation of an adaptive immune response by more virulent H. pylori strains could represent another mechanism responsible for the induction of gastric inflammation and tissue destruction. For example, increased MHC class II expression on gastric epithelial cells has been observed during H. pylori infection, mediating apoptosis in the gastric mucosa (50). Previous studies have revealed that H. pylori infection is associated with a Th cell response of the Th1 phenotype (23, 27, 51, 52). In the current study, we also observed an increased Th1 response during infection with H. pylori. However, mRNA amounts of the Th1 markers IFN-γ, TNF-α, and IL-12R β2 did not differ between patient groups infected with different strain types. Therefore, although the degree of lymphocytic infiltration was found to depend on the presence of cagA/vacA1 and was further strongly enhanced in patients infected with babA2+ strains, the extent of the Th1 response was independent of cagA, VacA, or BabA.

One previous report determined the presence of CagA-specific T cells in patients with gastritis and ulcer disease (53). T cells from H. pylori-infected patients proliferated and expressed predominately a Th1 cytokine profile in response to exogenous addition of the CagA protein in vitro, suggesting the expansion of clonal, CagA-specific Th1 cells and the recognition of this protein by the immune system, whereas the majority of T cell lines specific for other H. pylori Ags were of the Th0 type (53). However, we and other investigators found that the extent of the H. pylori-induced Th1 response in vivo appears to be independent of vacA1, cagA, or babA (54, 55). A possible explanation for this phenomenon may be that H. pylori strains expressing these genes are capable of attenuating the specific immune response. Indeed, a most recent study revealed H. pylori-induced apoptosis in T cells that was dependent on the presence of the cagPAI (56). The resulting down-regulation of T cell responses by strains bearing cagPAI may mask the existence of a specific Th1 response toward CagA, VacA, or BabA.

Our studies provide a new insight into how bacterial adherence factors contribute to the pathogenicity of H. pylori in the stomach. Previous studies emphasized the importance of this adherence factor for the induction of gastric inflammation, ulcer disease, and also gastric adenocarcinoma. Our current data support the view that bacterial colonization densities are of great importance for the degree of mucosal inflammation and damage, which is in accordance with previous observations (45, 57). BabA appears to be a key factor favoring higher colonization densities. Thus, this adherence factor achieves its importance for the induction of a non-specific immune response through a rather indirect mechanism. Because babA2 presence was previously correlated with both ulcer disease and adenocarcinoma, it appears that genetic susceptibilities may influence the further development of disease once the bacterial colonization is established. Individual hyperacidity or achlorhydria in response to the infection may determine the progression of gastritis to ulcer disease or gastric adenocarcinoma, respectively. In this point, IL-1β polymorphisms, which previously have been associated with gastric achlorhydria and adenocarcinoma (58), may be of importance in determining the further course of events.

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References