Synergistic Effect of *Helicobacter pylori* Virulence Factors and Interleukin-1 Polymorphisms for the Development of Severe Histological Changes in the Gastric Mucosa

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Polymorphisms of the *IL-1B* and *IL-1RN* genes (which encode interleukin [IL]–1β and IL-1 receptor antagonist, respectively) have been associated with hypochlorhydria and gastric cancer. We investigated the influence of bacterial virulence factors and host IL-1 polymorphisms on the development of histologic abnormalities in 210 *Helicobacter pylori*-infected patients with chronic gastritis. *cagA*/vacA+ *H. pylori* strains were associated with intestinal metaplasia (IM), atrophic gastritis (AG), and severe inflammation. Carriers of the proinflammatory *IL-1B* –511T/–31C and *IL-1RN* –511T alleles had an increased risk for the development of AG, IM, and severe inflammation, with odds ratios (ORs) of 1.7 (95% confidence interval [CI], 0.8–3.4) to 4.4 (95% CI, 1.5–12.9). The highest prevalence of severe gastric abnormalities was found in patients with both host and bacterial high-risk genotypes (cagA+/vacA+/IL-1B –511T/IL-1RN –511T), with ORs of 24.8 (95% CI, 5.2–117.3) for severe lymphocytic infiltration, 9.5 (95% CI, 2.8–32.1) for severe granulocytic infiltration, 6.0 (95% CI, 2.4–15.5) for IM, and 2.4 (95% CI, 0.93–6.2) for AG. Combined bacterial/host genotyping thus may provide a clinical tool to identify patients at high risk of developing cancer.

*Helicobacter pylori* has been an indigenous microbe of the human stomach since the earliest times of human evolution [1]. Infection with the bacterium regularly leads to chronic gastric inflammation. A subset of infected patients develops multifocal atrophic gastritis (AG), intestinal metaplasia (IM), dysplasia, or even distal gastric adenocarcinoma [2–4]. The course of disease is affected by bacterial virulence factors, as well as genetic predisposition and the immunological response of the host. Strains expressing the cytotoxin-associated gene A (*cagA*) and secreting high amounts of the vacuolating cytotoxin (VacA) have been found more frequently in patients with ulcer disease or gastric adenocarcinoma [5–8]. Mosaicism in the vacA signal sequence is an important determinant of cytotoxin activity. In contrast to s1, the s2 signal peptide seems to be defective and, consequently, leads to only a small release of secreted toxin [9, 10].

There are several lines of evidence showing that *H. pylori*-infected patients with duodenal ulcers seem to have a lower risk of developing gastric cancer, although bacterial virulence factors predispose to both conditions [3, 11]. This different outcome during *H. pylori* infection is thought to be directed by host factors, especially gastric acid secretion. Although high acid output observed during antral predominant gastritis predisposes to the development of duodenal ulceration, hypochlorhydria observed during corpus pangastritis is associated with increased risk of developing gastric cancer [12].
Several independent groups found a highly increased risk for the development of distal gastric cancer in patients with strong granulocytic or lymphocytic infiltration of the corpus [3, 13, 14]. Bacterial infection is first established in parts of the stomach that have a higher pH, such as the antrum. High acid production by parietal cells may protect the corpus mucosa from initial colonization. However, in hosts with low secretory capacity, *H. pylori* is capable of colonizing the corpus mucosa, which leads to further inhibition of acid secretion and more-aggressive gastritis that favors the development of gastric atrophy and carcinoma [15].

*H. pylori*-induced hypochlorhydria may be mediated, at least in part, by the proinflammatory cytokine interleukin (IL)–1β, which is up-regulated during chronic *H. pylori* infection [16–18]. IL-1β is a potent inhibitor of gastric parietal and enterochromaffin-like (ECL) cell function [19–23]. In addition to this antisecretory capacity, IL-1β plays an important role in mediating the inflammatory response to *H. pylori* [16–18]. The IL-1 gene family on chromosome 2q includes the 3 related genes *IL-1A*, *IL1-B*, and *IL-1RN*, which encode for IL-1α, IL-1β, and their endogenous receptor antagonist (IL-1RA), respectively [24]. Base exchanges at positions −31 and −511 of the *IL-1B* promoter have been associated with increased binding of nuclear factors and higher IL-1β secretion [20–24] (figure 1). The *IL-1RN* gene contains a penta-allelic 86-bp tandem repeat in intron 2, of which allele 2 (*IL-1RN*2) is also associated with enhanced IL-1β production in vivo and in vitro [24, 26].

The proinflammatory alleles of the *IL-1B* polymorphisms (−31/C and −511T) and of the *IL-1RN* gene (*IL-1RN*2) recently have been associated with hypochlorhydria and gastric cancer [25, 27–29].

The aim of the present study was to define the role of *H. pylori* virulence factors and host IL-1 polymorphisms for the development of severe histological changes, which precede gastric cancer (i.e., severe granulocytic infiltration [G3] and severe lymphocytic infiltration [L3]), IM, and AG. As shown below, we found a synergistic effect of bacterial virulence factors and host IL-1 polymorphisms on the development of precancerous lesions, leading to dramatically increased odds ratios (ORs) of disease development among patients with high-risk bacterial and host genotypes (*cagA+/vacA*+/IL-1B−511T/IL-1RN*2 carriers). In the future, this combined bacterial/host genotyping may provide an important opportunity to identify patients who are at high risk for the development of gastric carcinoma long before malignancy occurs.

**PATIENTS, MATERIALS, AND METHODS**

**Patients and biopsy specimens.** Five antral and 5 corpus biopsy specimens were collected from each of 752 consecutive patients, after informed consent was obtained. Patients underwent endoscopy because of abdominal complaints; 210 patients (107 men and 103 women) were infected with *H. pylori*, as determined by histological staining and *vacA* polymerase chain

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**Figure 1.** Schematic illustration of polymorphisms in the *IL-1B* and *IL-1RN* genes. Allele frequencies of the single-nucleotide polymorphisms (SNPs) in the promoter region of the *IL-1B* gene at positions −511 and −31 are shown. SNP −511T (associated with higher interleukin (IL)–1β secretion [24, 26]) was in complete linkage disequilibrium with −31C. The *IL-1RN* gene has a penta-allelic 86-bp variable no. of tandem repeat region (VNTR) in intron 2, of which the allele 2 (*IL-1RA*2) was previously associated with enhanced IL-1β production [24, 26]. Relative frequencies found in this study are indicated for each allele.
reaction (PCR). Patients receiving nonsteroidal anti-inflammatory drugs or antisecretory therapy and those with ulcer disease or gastric carcinoma were excluded from the study. All 210 infected patients lived in Munich, Germany, or the immediate surroundings; 88.6% were of German nationality, 7.6% were of Turkish nationality, and the remaining were of Bosnian, Greek, Croatian, or Serbian nationalities. No patients from Africa or the Americas were included. Their mean age was 62.7 years (range, 32–92 years). Two antral and corpus sections were stained with hematoxylin-eosin for histopathological evaluation. The evaluation was performed according to the Sydney classification system in regard to the presence of IM, AG, and the degree of granulocytic infiltration (G1 [mild], G2 [moderate], or G3 [severe]) and lymphocytic infiltration (L1 [mild], L2 [moderate], or L3 [severe]). The 3 remaining antral biopsy specimens were stored in liquid nitrogen and homogenized before DNA isolation. After tissue lysis with proteinase K, DNA isolation was performed with a QIAamp tissue kit (Qiagen), according to the manufacturer’s instructions.

**PCR for H. pylori genotyping.** PCR amplification of *H. pylori* gene loci was performed for the *cagA* gene and the *vacA* mosaics *vacA*1 and *vacA*2. Two different primer sets were used to amplify the middle region and 3′ end of the *cagA* gene (data not shown). Identical results were obtained in a subset of patients (*n* = 88). Therefore, the primers located in the middle region of the *cagA* gene were used to determine the *cagA* genotype. Primer sequences for *cagA* were 5′-GTATGGGGGCAATGGTGTGTC-3′ (sense) and 5′-GATTTCTTGAGGCGTGTGATGT-3′ (antisense). Reaction mixtures were amplified for 30 cycles as follows: initial denaturation for 5 min at 94°C, 94°C for 30 s, 59°C for 30 s, 72°C for 45 s, and final extension at 72°C for 10 min. Primers for the *vacA* mosaics were *vacA*5′-ATGGAAATACAAACACACAC-3′ (forward) and *vacA*5′-CTGTTGAATGGCACCAC-3′ (reverse), as described elsewhere [9]. Amplification of either the *vacA*1 or the *vacA*2 genotype. Amplification was done using 1 µL of genomic DNA, 22 µL of Master Mix (Qiagen), and 1 µL of each primer (20 µmol). Reaction mixtures were amplified for 30 cycles as follows: initial denaturation for 5 min at 94°C, 94°C for 30 s, 56°C for 30 s, 72°C for 45 s, and final extension at 72°C for 10 min. PCR products were analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide. There were no significant differences in median age and age distribution among patient groups infected with different strain types.

**Genotyping of IL-1 polymorphisms.** IL-1B polymorphisms were distinguished by 2 methods, 5′ nucleic acid PCR assay (allelic discrimination TaqMan PCR; Perkin Elmer) and restriction fragment—length polymorphism analysis (RFLP). DNA was obtained from gastric tissue samples, as described above. Results were identical to blood samples obtained from 20 patients used as a control. TaqMan assay was performed as described elsewhere [25]. Primer (MWG Biotech) and probe (PE Applied Biosystems) sequences were provided by Dr. E. M. El Omar (Aberdeen University, Aberdeen, UK). Probes for the T or C allele were 5′-labeled with either FAM or VIC fluorescence dyes, and 3′-labeled with TAMRA. PCR and end-point analyses were performed in a volume of 25 µL on an ABI PRISM 7700 Sequence Detection System (Perkin-Elmer). For RFLP analysis of the −511 polymorphism, the region containing the polymorphic site was amplified using the primers 5′-TGGCATGTGATC-TGGTTCACT-3′ (sense) and 5′-GTATAGGAAT-CTTCCTCACTT-3′ (antisense). PCR conditions were as follows: 95°C for 5 min, then 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, and, finally, 72°C for 5 min. The products were digested with 10 U of *Ava*I at 37°C for 3 h. Fragments were analyzed by electrophoresis on a 3% agarose gel stained with ethidium bromide. This yielded products of 190 and 114 bp (allele 1) and 304 bp (allele 2). The IL-1RN exon 2 polymorphism was analyzed by PCR using the primers 5′-CTCACCAACTCTTATCATGTTGTC-3′ (sense) and 5′-TCCTGTCAGGAGTTAGAAGGTAA-3′ (antisense). Conditions used were as follows: 95°C for 5 min, then 35 cycles of 95°C for 30 s, 50°C for 30 s, 72°C for 30 s, and, finally, 72°C for 5 min. The PCR products were analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide. Allele 1 (4 repeats) was 410 bp, allele 2 (2 repeats) was 240 bp, allele 3 (5 repeats) was 300 bp, allele 4 (3 repeats) was 325 bp, and allele 5 (6 repeats) was 395 bp in length.

**RESULTS**

**Patient population, distribution of IL-1 polymorphisms, and *H. pylori* strain characteristics.** Strain characteristics of *H. pylori* were investigated in 210 *H. pylori*-infected patients by PCR. The *vacA*1 genotype was found in 78% (164/210) of patients, and *cagA* was found in 71% (149/210) of patients. The presence of the *cagA* gene was associated with the *vacA*1 genotype, because almost all *cagA*+ strains were simultaneously *vacA*1+ (146/149). Allele frequencies of the IL-1 polymorphisms are shown in figure 1. IL-1B −511C was in complete linkage disequilibrium (100%) with IL-1B −31T. Among western European populations, frequencies of IL-1B −511/−31 have been described to be 59% for the −31T/−511C allele and 41% for the −31C/−511T allele [24, 25]. In the present study, the −31T/−511C allele was more frequent (70%); errors were excluded by applying 2 methods for polymorphism genotyping (allelic discriminating TaqMan PCR and RFLP, with >99% concordance).

When we analyzed the distribution of bacterial virulence factors and host polymorphisms, we found no significant as-

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sociations between certain IL-1 polymorphisms and the presence of vacAs1 or cagA. The frequency of infection with vacAs1+ or cagA+ strains among patients with different IL-1 genotypes was as follows: IL-1B−511CC, 83% (85/102) for vacAs1+ and 71% (72/102) for cagA+; IL-1B−511T carrier, 73% (79/108) for vacAs1+ and 75% (77/102) for cagA+; IL-1RN*1/2, 80% (98/122) for vacAs1+ and 70% (85/122) for cagA+; and IL-1RN*2 carrier, 76% (58/76) for vacAs1+ and 75% (57/76) for cagA+. These results indicate that colonization with specific H. pylori strain types is not influenced by IL-1 polymorphisms.

Because the development of severe histological changes is influenced by age and sex, these parameters were investigated in different patient groups. There were no significant differences in age and sex among carriers of different IL-1 polymorphisms and patients infected with different strain types. The mean age and sex distribution of patients harboring different IL-1 genotypes was as follows: IL-1B−511CC, 63.4 (50 women and 52 men); IL-1B−511T carrier, 61.9 (53 women and 55 men); IL-1RN*1/2, 63.8 (59 women and 63 men); and IL-1RN*2 carrier, 61.5 (38 women and 38 men). The mean age and sex distribution of patients infected with different H. pylori strain types was as follows: vacAs1+, 61.1 (80 women and 86 men); vacAs1−, 64.2 (23 women and 23 men); cagA+, 62.1 (74 women and 75 men); and cagA−, 63.4 (29 women and 32 men).

IL-1B and IL-1RN polymorphisms are associated with the presence of severe histological changes in the gastric mucosa. Table 1 shows the frequency of L3, G3, IM, and AG in patients with different IL-1B−511CC alleles (PPC+ patients). The lowest prevalence of L3, G3, IM, or AG was found in patients harboring the IL-1B−511CC−31T allele. Among carriers of the IL-1B−511T−31C allele, the frequency of these abnormalities was 1.2–2.1-fold higher. However, these differences did not reach statistical significance, except for the frequency of L3 in the antrum (table 1). The highest frequencies of L3, G3, IM, or AG were found in patients with the IL-1B−511TT−31CC genotype. Because of the small number of patients with this genotype (n = 19), the differences compared with other groups were not significant. The influence of the different IL-1RN polymorphism alleles on the presence of L3, G3, IM, or AG is shown in table 2. The lowest prevalence of all abnormalities was observed in patients with the IL-1RN1/1 genotype, whereas IL-1RN*2 homozygotes had the highest prevalence of L3, G3, IM, or AG. Among IL-1RN*1/2 heterozygotes, severe abnormalities occurred at an intermediate frequency, compared with other groups. Similar to the associations with the IL-1B single-nucleotide polymorphisms, the differences between groups were not significant in most cases.

Next, we were interested in the influence of combined IL-1 polymorphisms on the development of lesions preceding gastric cancer. Therefore, patients simultaneously harboring the proinflammatory IL-1RN*2 and IL-1B−511T−31C alleles (proinflammatory polymorphism combination–positive [PPC+]) were compared with patients lacking the IL-1B−511T−31C or/and IL-1RN*2 alleles (PPC−). Figure 2 shows the frequency of severe gastric histopathologic abnormalities in carriers of the 2 genotype combinations. The prevalence of L3, G3, IM, or AG was higher in PPC+ patients than in PPC− patients. All P values but one (for AG in the antrum) were significant. ORs for PPC−

Table 1. Prevalence of severe histological changes in patients with different alleles of the IL-1B−511 locus.

<table>
<thead>
<tr>
<th>Disease, location</th>
<th>No. (%) of subjects</th>
<th>511CC/31TT</th>
<th>511CT/31T</th>
<th>511CC/31C carriers</th>
<th>511CC vs. 511CT</th>
<th>511CC vs. 511TT carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>L3 Antrum</td>
<td>14.7 (15)</td>
<td>9.0 (8)</td>
<td>15.8 (3)</td>
<td>10.2 (11)</td>
<td>.03</td>
<td>.31</td>
</tr>
<tr>
<td>Corpus</td>
<td>4.9 (5)</td>
<td>9.0 (8)</td>
<td>15.8 (3)</td>
<td>10.2 (11)</td>
<td>.39</td>
<td>.11</td>
</tr>
<tr>
<td>G3 Antrum</td>
<td>15.7 (16)</td>
<td>9.0 (8)</td>
<td>15.8 (3)</td>
<td>10.2 (11)</td>
<td>.35</td>
<td>.11</td>
</tr>
<tr>
<td>Corpus</td>
<td>4.9 (5)</td>
<td>10.1 (9)</td>
<td>10.5 (2)</td>
<td>10.2 (11)</td>
<td>.27</td>
<td>.30</td>
</tr>
<tr>
<td>IM Antrum</td>
<td>28.4 (29)</td>
<td>31.5 (28)</td>
<td>42.1 (8)</td>
<td>33.3 (36)</td>
<td>.75</td>
<td>.28</td>
</tr>
<tr>
<td>Corpus</td>
<td>5.9 (6)</td>
<td>6.7 (6)</td>
<td>15.8 (3)</td>
<td>8.3 (9)</td>
<td>1.0</td>
<td>.15</td>
</tr>
<tr>
<td>AG Antrum</td>
<td>21.6 (22)</td>
<td>24.7 (22)</td>
<td>31.6 (6)</td>
<td>25.9 (28)</td>
<td>.61</td>
<td>.38</td>
</tr>
<tr>
<td>Corpus</td>
<td>5.9 (6)</td>
<td>6.7 (6)</td>
<td>15.8 (3)</td>
<td>8.3 (9)</td>
<td>1.0</td>
<td>.15</td>
</tr>
</tbody>
</table>

NOTE. AG, atrophic gastritis; G3, severe granulocytic infiltration; IM, intestinal metaplasia; L3, severe lymphocytic infiltration. * Calculated by Fisher’s exact test.
versus PPC+ patients were 1.7–4.4 (figure 2). These data suggest that a synergistic interaction exists between polymorphisms in the IL-1RN and IL-1B genes, leading to the highest prevalence of gastric histopathologic abnormalities in the PPC+ group. Most of the patients with L3, G3, IM, or AG in the corpus showed these histological changes simultaneously in the antrum. These results indicate that proinflammatory IL-1 polymorphisms enhance both the severity of corpus gastritis and the severity of antral gastritis. However, the influence on the development of corpus gastritis was more pronounced. The prevalence of the PPC+ genotype was higher in patients with severe histological changes in the corpus than in patients with severe changes in the antrum: L3, 39% (16/41) in the antrum and 50% (8/16) in the corpus; G3, 39% (16/41) in the antrum and 43.8% (7/16) in the corpus; IM, 33.9% (22/65) in the antrum and 46.6% (7/15) in the corpus. Because most of the patients with severe corpus gastritis simultaneously had severe antral gastritis, corpus gastritis serves as an indicator for pangastritis in this study.

Bacterial virulence factors are associated with a high prevalence of severe histological changes in the mucosa. Next, we investigated the influence of bacterial virulence factors on the development of severe mucosal alterations. Therefore, the presence of vacAs1 and cagA was correlated with the presence of L3, G3, IM, or AG. Table 3 shows that all abnormalities were associated with the vacAs1 and cagA genotypes of H. pylori.

None of the patients infected with cagA− or vacAs1− strains developed severe gastric inflammation, IM, or AG in the corpus. Because the event rate of severe corpus abnormalities in patients with cagA− or vacAs1− strains was 0, ORs could not be calculated for the corpus. The prevalence of L3 and G3 in the antrum also was very low among patients infected with cagA− or vacAs1− strains. ORs comparing the prevalence of L3, G3, IM, or AG in the antrum of vacAs1+ versus vacAs1− strain carriers were 2.1–16.5 (table 3).

Interaction between IL-1 polymorphisms and bacterial strain types. To further investigate possible interactions between host IL-1 polymorphisms and H. pylori virulence factors, we assessed the frequency of severe gastric histopathologic abnormalities in patients with combined host or bacterial genotypes. Figure 3 summarizes the results of these evaluations.

First, the prevalence of L3, G3, IM, and AG was investigated in patients infected with cagA+ and/or vacAs1− strains (figure 3, white bars). The prevalence of all pathologic abnormalities was low (antrum) or absent (corpus) in this group, independent of the polymorphism status. This result suggests that infection with cagA+/vacAs1− strains represents a major risk factor for the development of mucosal alterations. In patients infected with cagA− and/or vacAs1− strains, IL-1 polymorphisms had little or no influence on the development of L3, G3, IM, or AG.

The gray bars in figure 3 represent patients infected with cagA+/vacAs1+ strains but who lack the proinflammatory polymorphisms IL-1B −511T/−31C and IL-1RN*2 (cagA+/vacAs1+/PPC−). These patients had a markedly higher prevalence of L3, G3, IM, or AG in both the antrum and the corpus, underlining the key importance of bacterial virulence factors for pathogenesis. ORs comparing these patients with cagA− and/or vacAs1− strain carriers (figure 3, white bars) were as follows in the antrum: L3, 10.8 (95% confidence interval [CI], 2.3–44.1); G3, 4.0 (95% CI,

### Table 2. Prevalence of severe histological changes in patients with different alleles of the variable-number-of-tandem-repeats region in the IL-1RN gene.

<table>
<thead>
<tr>
<th>Disease, location</th>
<th>IL1RN-1/1 (n = 122)</th>
<th>IL1RN-1/2 (n = 55)</th>
<th>IL1RN-2/2 (n = 17)</th>
<th>IL1RN*2 carriers (n = 76)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L3 Antrum</td>
<td>18.9 (23)</td>
<td>27.3 (15)</td>
<td>29.4 (5)</td>
<td>12.5 (2)</td>
</tr>
<tr>
<td>Corpus</td>
<td>4.9 (6)</td>
<td>12.7 (7)</td>
<td>17.7 (3)</td>
<td>0</td>
</tr>
<tr>
<td>G3 Antrum</td>
<td>18.0 (22)</td>
<td>23.6 (13)</td>
<td>29.4 (5)</td>
<td>6.3 (1)</td>
</tr>
<tr>
<td>Corpus</td>
<td>5.7 (7)</td>
<td>10.9 (6)</td>
<td>5.9 (1)</td>
<td>12.5 (2)</td>
</tr>
<tr>
<td>IM Antrum</td>
<td>25.4 (31)</td>
<td>38.2 (21)</td>
<td>47.1 (8)</td>
<td>31.3 (5)</td>
</tr>
<tr>
<td>Corpus</td>
<td>4.1 (5)</td>
<td>12.7 (7)</td>
<td>17.6 (3)</td>
<td>0</td>
</tr>
<tr>
<td>AG Antrum</td>
<td>20.5 (25)</td>
<td>23.6 (13)</td>
<td>35.5 (6)</td>
<td>37.5 (6)</td>
</tr>
<tr>
<td>Corpus</td>
<td>4.1 (5)</td>
<td>10.9 (6)</td>
<td>23.5 (4)</td>
<td>0</td>
</tr>
</tbody>
</table>

**NOTE.** AG, atrophic gastritis; G3, severe granulocytic infiltration; IM, intestinal metaplasia; L3, severe lymphocytic infiltration.

* Calculated by Fisher’s exact test.
Figure 2. Correlation of interleukin (IL)–1 polymorphism with histological alterations. Gray bars, Patients harboring simultaneously the proinflammatory IL-1RN*2 allele as well as the proinflammatory IL-1B −511T/−31C alleles (proinflammatory polymorphism combination–positive [PPC+]); white bars, patients lacking IL-1B −511T/−31C or/and IL-1RN*2 alleles (PPC−). Fisher’s exact test was used to calculate P values. Odds ratios and corresponding 95% confidence intervals were calculated by use of StatXact software (version 4.0.1; Cytel). AG, atrophic gastritis; G3, severe granulocytic infiltration; IM, intestinal metaplasia; L3, severe lymphocytic infiltration.

1.3–12.1); IM, 2.5 (95% CI, 1.2–5.5); and AG, 1.7 (95% CI, 0.8–3.6). Because the event rate of severe corpus abnormalities in patients with cagA+ or vacA s1+ strains was 1 (figure 3B, white bars), ORs could not be calculated for the corpus.

Finally, patients who were infected with cagA+/vacAs1+ strains and also harbored the proinflammatory IL-1 polymorphism combination (cagA+/vacAs1+/PPC+ patients; figure 3, black bars) had the highest prevalence of all histological changes. ORs comparing cagA+/vacAs1+/PPC+ patients with cagA+ and/or vacAs1− strain carriers were 2.4–24.8 (figure 3, white bars). These data show that, in the presence of cagA+/vacAs1+ strains, IL-1 polymorphisms further enhance the risk for the development of severe gastric pathologic abnormalities, which suggests that bacterial virulence factors and host IL-1 polymorphisms act synergistically during gastric carcinogenesis.

DISCUSSION

H. pylori infection is the primary cause for the development of gastric cancer [2–4]. Because only a small percentage of infected patients develop malignancy, it is of major interest to identify bacterial and host genetic markers associated with cancer development. Recent studies have reported a close correlation between IL-1 polymorphisms and hypochlorhydria, gastritis, atrophy, and carcinoma during chronic H. pylori infection [25, 27–29]. Our current results are in agreement with these studies but indicate that, long before manifestation of malignancy, IL-1 polymorphisms are associated with the presence of severe histological alterations that precede gastric cancer.

Because severe gastritis, IM, and AG were rare or absent in patients infected with cagA+ or vacAs1− strains, bacterial virulence factors seem to be a primary trigger leading to those alterations. For example, 0 of 64 patients infected with cagA− or vacAs1− strains developed severe gastric inflammation in the corpus, and only 3%–6% of patients infected with these strains had severe abnormalities in the antrum. Therefore, the correlation of IL-1 polymorphisms with lesions preceding gastric cancer seems to be dependent on the presence of more virulent cagA+/vacAs1+ H. pylori strains. Although epidemiological studies correlating CagA presence with gastric cancer have been controversial [6, 30–35], there are several lines of evidence suggesting a major role of the cag pathogenicity island (cag PAI) for the induction of inflammatory reactions. In vitro experiments have shown that cag PAI genes up-regulate a superset of host genes, including immune response genes [36], leading to a heightened inflammatory response [37]. Furthermore, studies in animal models revealed that cagA− strains are more virulent than cagA+ strains. In contrast to infection with cagA+ strains of H. pylori Virulence Factors and IL-1 Polymorphisms • JID 2003:188 (15 July) • 277
**Table 3. Prevalence of severe histological changes in patients infected with different *Helicobacter pylori* strain types.**

<table>
<thead>
<tr>
<th>Disease, location</th>
<th>vacAs1+</th>
<th>vacAs1−</th>
<th>cagA+</th>
<th>cagA+/vacAs1−</th>
<th>OR (95% CI)</th>
<th>P*</th>
<th>OR (95% CI)</th>
<th>P*</th>
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<tbody>
<tr>
<td><strong>L3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antrum</td>
<td>2.2 (1)</td>
<td>26.8 (44)</td>
<td>3.3 (2)</td>
<td>28.9 (43)</td>
<td>3.1 (2)</td>
<td>29.5 (43)</td>
<td>16.5 (2.2–123)</td>
<td>.0001</td>
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<tr>
<td>Corpus</td>
<td>0</td>
<td>9.8 (16)</td>
<td>0</td>
<td>10.7 (16)</td>
<td>0</td>
<td>11.0 (16)</td>
<td>NA</td>
<td>.03</td>
</tr>
<tr>
<td><strong>G3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antrum</td>
<td>6.5 (3)</td>
<td>23.2 (38)</td>
<td>4.9 (3)</td>
<td>25.5 (38)</td>
<td>6.3 (4)</td>
<td>25.3 (37)</td>
<td>4.3 (1.3–14.7)</td>
<td>.01</td>
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<td>9.8 (16)</td>
<td>0</td>
<td>10.7 (16)</td>
<td>0</td>
<td>11.0 (16)</td>
<td>NA</td>
<td>.03</td>
</tr>
<tr>
<td><strong>IM</strong></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Antrum</td>
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<td>34.1 (56)</td>
<td>14.8 (9)</td>
<td>37.6 (56)</td>
<td>15.6 (10)</td>
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<td>2.1 (0.96–4.7)</td>
<td>.07</td>
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<td>9.1 (15)</td>
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<td>10.1 (15)</td>
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<td>10.3 (15)</td>
<td>NA</td>
<td>.05</td>
</tr>
<tr>
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<td>13.1 (8)</td>
<td>28.2 (42)</td>
<td>17.2 (11)</td>
<td>27.4 (39)</td>
<td>2.4 (0.97–6.2)</td>
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<td>10.1 (15)</td>
<td>0</td>
<td>10.3 (15)</td>
<td>NA</td>
<td>.05</td>
</tr>
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</table>

**NOTE.** AG, atrophic gastritis; CI, confidence interval; G3, severe granulocytic infiltration; IM, intestinal metaplasia; L3, severe lymphocytic infiltration; NA, not available; OR, odds ratio.

* StatXact software (version 4.0.1; Cytel) was used to determine ORs and corresponding 95% CIs. Because the event rate of severe corpus pathologies was 0, ORs could not be calculated for the corpus.

b Calculated by Fisher’s exact test.

**pylori**, inoculation of Mongolian gerbils with cagA− strains does not induce severe gastric inflammation or atrophy and does not lead to cancer development, which suggests that there is an essential role of the cag PAI in pathogenesis [38, 39].

Bacterial adherence factors further enhance the virulence of cagA+/vacAs1− *H. pylori*. Recently, the blood group antigen–binding adhesin, BabA, has been associated with higher bacterial pathogenicity [40–44]. BabA ameliorates the colonization properties of the bacterium, leading to higher colonization densities, an augmented nonspecific immune response, and, subsequently, more-aggressive gastritis [42]. Therefore, multiple bacterial virulence genes seem to affect the outcome of infection and the simultaneous presence of different virulence factors in one strain enhances its pathogenicity.

However, the currently known bacterial virulence and adherence factors are associated not only with gastric carcinoma but also with duodenal ulceration [7]; therefore, host factors seem to influence the diverging outcome. In the present study, we found an association between proinflammatory IL-1 polymorphisms and the presence of L3, G3, IM, and AG. Carriers of the proinflammatory IL-1β−31T/T−31C and IL-1RN*2* alleles had an increased risk for the development of L3, G3, IM, or AG, especially when both proinflammatory polymorphisms were present simultaneously, with ORs of 1.7 (95% CI, 0.8–3.4) to 4.4 (95% CI, 1.5–12.9).

In recent years, it has been recognized that patients with low acid output have an increased risk for the development of distal gastric cancer [12, 45, 46]. IL-1β, which is up-regulated during chronic *H. pylori* infection, is a powerful inhibitor of gastric acid secretion [19]. In previous articles, we and others have shown that this antisecretory effect is mediated through inhibition of gastric parietal and ECL cell function. IL-1β decreases acid secretion from parietal cells via protein kinase C–dependent mechanisms through binding to IL-1 receptors [21, 22, 47]. In addition, IL-1β impairs histamine secretion from ECL cells in the gastric corpus via NO-dependent mechanisms and by inducing ECL cell apoptosis [20, 23]. This phenomenon indicates that IL-1β has a crucial role in the development of gastric hypochlorhydria, an idea which is further supported by a recent study reporting that administration of recombinant IL-1 receptor antagonist to *H. pylori*-infected gerbils increases gastric acid secretion [48]. Because excessive acid secretion has a growth inhibitory effect on *H. pylori* [49], colonization of the acid-secreting corpus region may, therefore, be promoted by IL-1β–induced hypochlorhydria, especially in patients with proinflammatory IL-1 polymorphisms.

It has been shown that patients with gastric cancer often have corpus predominant or pangastritis, whereas duodenal ulcers are associated with antral predominant gastritis [15]. Of interest, our present results indicate that proinflammatory IL-1 polymorphisms enhance the severity of both corpus gastritis and antrum gastritis. The influence on the development of corpus gastritis, however, was more pronounced. Furthermore, because most of the patients with severe corpus gastritis simultaneously had severe antral gastritis, corpus gastritis serves as an indicator for pangastritis in our study. Nevertheless, because proinflammatory IL-1 polymorphisms also predispose to higher antral gastritis scores, one could assume
Figure 3. Prevalence of atrophic gastritis (AG), severe granulocytic infiltration (G3), intestinal metaplasia (IM), and severe lymphocytic infiltration (L3) in patients infected with different *Helicobacter pylori* strain types and harboring or lacking proinflammatory polymorphism combinations (PPCs) in the antrum (A) and corpus (B). White bars, Patients infected with cagA⁺/vacAs1⁺ strains (not regarding the polymorphism genotype); gray bars, patients infected with cagA⁺/vacAs1⁺ *H. pylori* strains, but not harboring IL-1B −511T/−31C and IL-1RN*2* simultaneously (PPC⁻); black bars, patients infected with cagA⁺/vacAs1⁺ *H. pylori* strains and possessing simultaneously IL-1B −511T/−31C and IL-1RN*2* alleles (PPC⁺). Fisher’s exact test was used to calculate P-values. Odds ratios (ORs) and corresponding 95% confidence intervals were calculated by use of StatXact software (version 4.0.1; Cytel). Because the event rate of severe corpus pathologies in patients with cagA⁻ or vacAs1⁻ strains was 0, ORs could not be calculated for the corpus.
that these polymorphisms may also be associated with duodenal ulceration. However, a key feature of duodenal ulceration is high acid secretion [15]. Because IL-1β is a potent and very effective suppressor of gastric acid secretion leading to hypochlorhydria [20–22, 48], the presence of the proinflammatory IL-1 polymorphisms is an unlikely predictor of duodenal ulcer. Indeed, a most recent study found a reduced risk for the development of duodenal ulcers in patients with the above-mentioned proinflammatory polymorphisms [50].

Because we found that bacterial virulence factors have a crucial role in the development of precancerous lesions, it was important to exclude that the associations of IL-1 polymorphisms with gastric histopathologic abnormalities were not due to a random coincidence of these polymorphisms with more-virulent H. pylori strains. Analyzing the distribution of bacterial virulence factors and host IL-1 genotypes, there were no significant associations between certain IL-1 polymorphisms and the presence of vacAs1 or cagA. This finding excludes the possibility of false-positive associations of IL-1 polymorphisms with gastric histopathologic abnormalities and indicates that colonization with specific H. pylori strain types is not influenced by IL-1 genotypes.

The highest prevalence of severe gastric histopathologic abnormalities (L3, G3, IM, and AG) was found in patients with both host and bacterial high-risk genotypes (cagA+/vacAs1+/IL-1B − 511T/H11002/IRN*2 carriers), with ORs of 2.4 (95% CI, 0.93–6.2) to 24.8 (95% CI, 5.2–117.3), compared with patients harboring cagA− or vacAs1− strains. These dramatically increased values suggest that there is a synergistic interaction between bacterial virulence factors and host IL-1 polymorphisms, leading to a very high potential for the development of lesions preceding gastric cancer. Such synergism may be of special importance during the development of hypochlorhydria. High IL-1β secretion in patients with proinflammatory IL-1 polymorphisms leads to reduced acid secretion and a subsequent bacterial colonization of the corpus. If cagA+/vacAs1+ strains are present, inhibition of gastric acid secretion may be more pronounced, because cagA+ strains, but not cagA− strains, have been shown to induce apoptosis of parietal cells [51]. Furthermore, cagA+ strains induce higher levels of IL-1β expression than do cagA− strains in the chronically infected gastric mucosa [18], leading to more-aggressive gastritis. These results are in agreement with a very recently published work [29], which found that the prevalence of gastric carcinoma was highest in patients infected with vacAs1+/cagA+ strains and simultaneously harboring the IL-1B − 511T and IL-1RN*2 alleles. Similar to our observations, the authors concluded that combined bacterial and host genotyping may provide an important tool in identifying individuals who are at a high risk for developing cancer.

To summarize, our study supports a sequential model of gastric carcinogenesis, in which H. pylori virulence factors and host IL-1 polymorphisms both play a crucial role. Bacterial virulence factors are important determinants of histopathological changes, and, if virulent strains are present, IL-1 polymorphisms are crucial for potentiating their pathogenic effect and for directing disease toward cancer development. The current data underline that bacterial and host immune factors act in a synergistic manner during gastric carcinogenesis, providing a better understanding of this complex multifactorial disease. Furthermore, the findings may be of clinical relevance, because genetic combined bacterial and host genotyping may allow for the identification of patients at high risk of gastric cancer before malignant transformation occurs. Those patients would benefit from preventive eradication therapy. Considering the high prevalence of H. pylori infection, antibiotic resistance, and costs of therapy, this approach may provide a reasonable basis for therapeutic decisions at the stage of chronic gastritis.

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