JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2001, p. 1339–1344 Vol. 39, No. 4
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.4.1339–1344.2001
Copyright © 2001, American Society for Microbiology. All Rights Reserved.

Assessment of *Helicobacter pylori vacA* and *cagA* Genotypes and Host Serological Response

CÉU FIGUEIREDO,[1,2] WIM QUINT,[1] NATHALIE NOUHAN,[1] HENK VAN DEN MUNCKHOF,[1] PAUL HERBRINK,[3] JOOST SCHERPENISSE,[3] WINK DE BOER,[4] PETER SCHNEEBERGER,[5] GUILLERMO PEREZ-PEREZ,[6,7] MARTIN J. BLASER,[6,7] AND LEEN-JAN VAN DOORN[1]*

Delft Diagnostic Laboratory[1] and R. de Graaf Hospital,[3] Delft, Department of Internal Medicine, Bernhoven Hospital, Oss,[4] and Department of Microbiology, Bosch Medicentrum, Den Bosch,[5] The Netherlands; IPATIMUP and Medical Faculty, University of Porto, Porto, Portugal[2]; Division of Infectious Diseases, Vanderbilt University School of Medicine, Nashville, Tennessee[6]; and Department of Medicine, New York University School of Medicine, New York, New York[7]

Received 28 September 2000/Returned for modification 22 December 2000/Accepted 26 January 2001

***Helicobacter pylori* strains can be distinguished by genotyping of virulence-associated genes, such as *vacA* and *cagA*. Because serological discrimination between strain types would reduce the need for endoscopy, 61 patients carrying *H. pylori* were studied by *vacA* and *cagA* genotyping of *H. pylori* in gastric biopsy specimens and by detection of specific serum antibodies. Serological responses to *H. pylori* were determined by Helicoblot (versions 2.0 and 2.1). Antibodies to CagA also were determined by a rapid anti-CagA assay (Pyloriset screen CagA) as well as by two noncommercially developed enzyme immunoassays, each using a recombinant CagA protein. Assessment of performance of the Helicoblot assays indicated substantial interobserver variation, with kappa values between 0.20 and 0.93. There was no relationship between the serological profiles on the Helicoblot and the genotypes from the same patients, except for strong associations between the presence of anti-CagA and the *cagA*-positive and *vacA* s1 *H. pylori* genotypes. Detection of anti-CagA by the five different assays varied considerably, with kappa values ranging from 0.21 to 0.78. Using the *cagA* genotype as the "gold standard," the sensitivity and specificity of the anti-CagA assays varied from 71.4 to 85.7% and from 54.2 to 100%, respectively. Thus, serological profiles of antibodies to *H. pylori* are heterogeneous and, with the exception of anti-CagA antibodies, show no relation to the *H. pylori vacA* and *cagA* genotypes. Detection of anti-CagA antibodies is strongly dependent on the test used.**

Helicobacter pylori persistently colonizes the mucosa of the human stomach, causes chronic gastritis, and is an important risk factor for gastroduodenal diseases, such as peptic ulcers and gastric carcinoma (11). There is increasing evidence that distinct variants of *H. pylori* exist and that these may be associated with the pathogenicity of the bacterium (41); several virulence-associated genes have been identified (1, 8).

vacA encodes a vacuolating toxin that is released by *H. pylori* and injuries epithelial cells (9, 21). *vacA* is present in all *H. pylori* strains, and includes two variable regions (1). The s region (encoding the signal peptide) is located at the 5′ end of the gene and exists as an s1 or s2 allele. Within type s1, several subtypes (s1a, s1b, and s1c) can be distinguished (39). The m region (middle) occurs as an m1 or m2 allele. The mosaic combination of s- and m-region allelic types correlates with the production of the cytotoxin and is thereby associated with virulence of the bacterial strain (1).

cagA (cytotoxin-associated gene) is considered a marker for the presence of the pathogenicity (*cag*) island of about 35 kbp (6). Carriage of *cagA*$^+$ strains increases the risk for the development of atrophic gastritis and gastric cancer (4, 19). Several studies have shown clinical relevance of specific antibodies to CagA using noncommercial assays (3, 5, 7, 20, 29, 33–35, 44), whereas others failed to confirm these findings (14, 17, 18, 23, 27, 45)

H. pylori can be diagnosed by analysis of gastric biopsy specimens by urease tests, culture of the bacterium, histopathology, or detection of bacterial DNA by PCR. Noninvasive diagnostic methods include the urea breath test and serological assays measuring antibodies to *H. pylori* in the serum (15, 22). The distinct *vacA* and *cagA* genotypes of *H. pylori* can best be identified by molecular methods, using cultured strains, or directly in gastric biopsy specimens, but this requires endoscopy. Therefore, serological typing methods analyzing specific antibodies to *H. pylori* if accurate, would be most suitable for routine clinical use. The present study assessed the relationship between the *vacA* and *cagA* genotypes of *H. pylori* and the presence of specific anti-*Helicobacter* antibodies, in particular, antibodies to CagA.

MATERIALS AND METHODS

Patients. Gastric (antral) biopsy specimens and serum samples were obtained from patients, undergoing upper endoscopy for routine clinical indications. Biopsy specimens were tested for *H. pylori* by a rapid urease assay (CLO test; Delta-West). A total of 61 patients were randomly selected from several hundred patients in The Netherlands to ensure that each of the *vacA* s1, *vacA* s2, *vacA* m1, *vacA* m2, *cagA*-negative, and *cagA*-positive genotypes was sufficiently represented in the test group.

Genotyping of *H. pylori*. The *vacA* and *cagA* status of *H. pylori* was determined directly in gastric biopsy specimens, as described earlier (38, 39). Briefly, total DNA was isolated from the specimens, and *vacA* s and m regions as well as part of *cagA* were simultaneously amplified by multiplex PCR. PCR products were hybridized to subtype-specific probes by reverse hybridization on a line probe

* Corresponding author. Mailing address: Delft Diagnostic Laboratory, R. de Graafweg 7, 2625 AD Delft, The Netherlands. Phone: 31-15-2604581. Fax: 31-15-2604550. E-mail: L.J.van.Doorn@ddl.nl.

TABLE 1. *H. pylori vacA* and *cagA* genotypes detected in single gastric biopsy specimens of 61 patients

Genotype			*n*	%
vacA s	*vacA* m	*cagA*		
s1a	m1	+	12	19.7
s1a	m1	−	7	11.5
s1a	m2a	+	6	9.8
s1a	m2a	−	4	6.6
s1b	m2a	+	1	1.6
s1c	m2a	+	1	1.6
s2	m2a	+	2	3.3
s2	m2a	−	21	34.4
Multiple	Multiple	+	6	9.8
Multiple	Multiple	−	1	1.6

assay (LiPA), detecting *vacA* s1a, s1b, s1c, s2, m1, m2a, and m2b and *cagA*. This assay has been extensively evaluated and showed high accuracy for detection of distinct genotypes (38–40).

Serological assays. Serum samples were analyzed by various commercially available and noncommercial assays. Immunoglobulin G antibody profiles were determined by Helicoblot versions 2.0 and 2.1 (Genelabs Diagnostics, Singapore, Singapore) and were performed according to the manufacturer's instructions. The Helicoblot assays are based on Western blot analysis of whole-cell *H. pylori* antigens. Interpretation of the serologic reactivity is restricted to antigens of various molecular masses. Version 2.0 contains antigens of 19.5, 26.5, 30, 35, 89 (VacA), and 116 (CagA) kDa. Version 2.1 contains antigens of 19.5, 30, 35, 37, 89 (VacA), and 116 (CagA) kDa. For Helicoblot 2.1, the criteria for *H. pylori* seropositivity are as follows: (i) positive result for the 116-kDa (CagA) band, where CagA has to be present with one or more bands at the following positions: 89 (VacA), 37, 35, 30 (UreA), and 19.5 kDa together, or with current infection marker (CIM); (ii) presence of any one band at 89, 37, or 35 kDa, with or without the current infection marker; (iii) presence of both the 30- and 19.5-kDa bands, with or without the CIM.

Presumably, different *H. pylori* strains have been used as the protein sources for the two versions of the Helicoblot assay. Version 2.1 also contains an additional antigen line, designated the CIM. The nature of this antigen is not explained by the manufacturer and remains unknown. Anti-CagA antibodies also were determined using a rapid assay (Pyloriset screen CagA; Orion Diagnostics, Espoo, Finland) and two specific anti-CagA enzyme immunoassays (EIAs) (DDL prototype CagA assay and Vanderbilt University anti-CagA EIA, based on recombinant CagA proteins [10, 30]).

Statistical analyses. All statistical analyses were performed using SPSS version 8.0 for Windows (SPSS, Inc., Chicago, Ill.). To assess the interobserver variation for interpretation of the Helicoblot assays, and to determine the agreement between the different anti-CagA assays, Cohen's kappa values were calculated. kappa values represent the degree of agreement between any pair of observations, and the values can be interpreted as follows: 0 to 0.2 (poor), 0.21 to 0.40 (fair), 0.41 to 0.60 (moderate), 0.61 to 0.80 (good), 0.81 to 0.99 (very good), and 1 (perfect agreement).

Relationships between serological profiles and genotypes were calculated by the chi-square test with Bonferroni correction to increase the stringency of the analysis, since multiple comparisons are being made.

RESULTS

Genotypes of *H. pylori* strains in the gastric biopsy specimens. Gastric biopsy specimens and serum samples were obtained from 61 *H. pylori*-positive patients living in The Netherlands. DNA was isolated from the biopsy specimens, and the *H. pylori vacA* and *cagA* genotypes were determined by multiplex PCR and LiPA (Table 1). All samples could be completely genotyped. In seven (11.5%) patients, multiple *vacA* genotypes were detected, indicating the presence of multiple *H. pylori* strains in the biopsy specimens. Of the 31 s1 strains, 29 were s1a, one was s1b, and one was s1c. Twenty (64.5%) of the 31 s1 strains also were *cagA* positive, whereas only 2 (8.7%) of the 23 s2 strains contained *cagA* ($P < 0.001$).

Serological responses to *H. pylori* antigens. Serological profiles were determined by Helicoblot 2.0 and 2.1. Results were independently interpreted by three persons, the individual reactivity (positive, negative, or dubious) to each of the indicated antigens was scored, and average kappa values were calculated for each of the antigens on the blots (Table 2). For Helicoblot 2.0, the average kappa values of the 19.5-, 26.5-, 30-, 35-, 89-, and 116-kDa antigens ranged between 0.457 (19.5 kDa) and 0.692 (26.5 kDa). For Helicoblot 2.1, the average kappa values of the 19.5-, 30-, 35-, 37-, 89-, and 116-kDa antigens ranged between 0.325 (37 kDa) and 0.646 (116 kDa). The average kappa value for the CIM in Helicoblot 2.1 was 0.822. A consensus score (Table 2) also was determined for the reactivity of each serum sample to the individual antigens. If antibody reactivity was scored positive by two or more observers, it was considered a positive reaction. As such, negative and dubious scores were grouped together. The results of Helicoblot 2.0 and 2.1, using the interpretation criteria provided in the manufacturer's instructions, showed a high degree of discordance. Helicoblot 2.0 yielded positive results with 52 (85.2%) of the 61 patients tested, whereas Helicoblot 2.1 yielded positive results with 57 (93.4%) of the patients tested. The positivity rate for individual antigen bands on Helicoblot 2.0 varied from 26.2% (19.5 kDa) to 83.6% (26.5 kDa). For Helicoblot 2.1, the positivity rates varied between 54.1% (116 kDa) and 75.4% (19.5 kDa). Antibodies to the 89-kDa antigen, representing the vacuolating toxin VacA, were detected in 39.3% of the sera by using Helicoblot 2.0 and 59.0% of the sera by using Helicoblot 2.1. Antibodies to the CIM (which was applied as an additional and separate line on the Helicoblot 2.1 membrane) were found in 52 (85.2%) of the sera.

The consensus scores also were used to determine the asso-

TABLE 2. Detection of antibodies to specific antigens present on Helicoblot 2.0 and 2.1 in 61 *H. pylori*-positive patients and corresponding kappa values

Assay	Parameter	Serological reactivity to different antigen lines at indicated position (kDa)[b]								
		CIM	19.5	26.5	30	35	37	89	116	Total[c]
Helicoblot 2.0	Consensus positivity score[a] (%)		16 (26.2)	51 (83.6)	35 (57.4)	26 (42.6)		24 (39.3)	24 (39.3)	52 (85.2)
	Average kappa value		0.457	0.692	0.570	0.648		0.608	0.507	
Helicoblot 2.1	Consensus positivity score (%)	52 (85.2)	46 (75.4)		45 (73.8)	34 (55.7)	34 (55.7)	36 (59.0)	33 (54.1)	57 (93.4)
	Average kappa value	0.822	0.595		0.338	0.529	0.325	0.530	0.646	

[a] Number of patients showing a positive reaction with the indicated antigen. The consensus score was determined as stated in Materials and Methods.
[b] Different antigen lines were present on Helicoblot 2.0 and 2.1 and were interpreted by three independent observers according to the manufacturer's instructions.
[c] The overall seropositivity of each serum sample was determined according to the manufacturer's instructions.

TABLE 3. Anti-CagA reactivities in specimens from 61 *H. pylori*-positive patients as determined by five different serological assays

Assay	Result	No. of specimens with indicated reaction by the following assay								
		Helicoblot 2.1			Pyloriset		DDL		Vanderbilt	
		Negative	Positive	Dubious	Negative	Positive	Negative	Positive	Negative	Positive
Helicoblot 2.0[a]	Negative	16	6	8	28	2	20	10	30	0
	Positive	1	23	0	3	21	6	18	5	19
	Dubious	2	4	1	7	0	7	0	7	0
Helicoblot 2.1[b]	Negative				19	0	13	6	19	0
	Positive				10	23	13	20	14	19
	Dubious				9	0	7	2	9	0
Pyloriset[c]	Negative						29	9	37	1
	Positive						4	19	5	18
DDL[d]	Negative								31	2
	Positive								11	17

[a] The average kappa values (95% confidence intervals) for comparisons with the Helicoblot 2.1, Pyloriset, DDL, and Vanderbilt assays were 0.042 (0.277 to 0.607), 0.639 (0.473 to 0.806), 0.319 (0.115 to 0.522), and 0.635 (0.471 to 0.799), respectively.

[b] The average kappa values (95% confidence intervals) for comparisons with the Pyloriset, DDL, and Vanderbilt assays were 0.483 (0.329 to 0.636), 0.213 (0.027 to 0.399), and 0.389 (0.240 to 0.537), respectively.

[c] The average kappa values (95% confidence intervals) for comparisons with the DDL and Vanderbilt assays were 0.565 (0.359 to 0.771) and 0.783 (0.620 to 0.946), respectively.

[d] The average kappa value (95% confidence interval) for comparison with the Vanderbilt assay was 0.560 (0.359 to 0.762).

ciation between the detection of antibodies in the serum and the presence of specific *vacA* and *cagA* genotypes in the stomach. For Helicoblot 2.0, no significant associations were found between *vacA* s, *vacA* m, and *cagA* genotypes, and antibodies against the antigens of 19.5, 26.5, 30, 35, and 89 kDa (all P values > 0.05). In contrast, there was a strong association between the presence of *vacA* s1 and *cagA*-positive strains, and the presence of anti-CagA (116 kDa) antibodies ($P < 0.001$). For Helicoblot 2.1, no significant associations were found between the *vacA* s, *vacA* m, and *cagA* genotypes, and antibodies against the antigens of 19.5, 30, 35, 37, and 89 kDa. As for Helicoblot 2.0, there was a strong association between the presence of *vacA* s1 and *cagA*-positive strains, and the presence of anti-CagA antibodies ($P < 0.001$).

Anti-CagA antibodies. Antibodies to CagA also were measured by the Pyloriset screen rapid assay and two independent anti-CagA EIAs based on recombinant CagA proteins (Table 3). The detection rate of anti-CagA differed considerably between the five different assays, and kappa values, measuring interassay agreement, ranged from 0.213 to 0.783. The agreement between the two versions of the Helicoblot with respect to anti-CagA (116 kDa) was only 0.557.

Finally, the association between the *H. pylori cagA* genotype detected in the gastric biopsy specimens and the anti-CagA reactivity in the serum was assessed (Table 4). Despite the clear association between the *cagA* genotype and detection of anti-CagA, a considerable number of discrepant results were observed. The kappa values for comparisons between the *cagA* genotype and the antibody detection were 0.437 and 0.332 for Helicoblot 2.0 and 2.1, respectively. The DDL prototype CagA EIA showed the highest agreement with the *cagA* genotype ($\kappa = 0.736$), but this was not significantly higher than that for the Pyloriset screen test (0.632) and the Vanderbilt EIA (0.696). Using the *cagA* genotype as the "gold standard," the sensitivity of the assays ranged from 71.4% (Helicoblot 2.0 and Pyloriset) to 85.7% (DDL EIA). The specificity of the assays ranged from 54.2% (Helicoblot 2.1) to 100% (Vanderbilt EIA). The positive predictive values varied between 66.6% (Helicoblot 2.1) and 100% (Vanderbilt EIA), and the negative predictive values were between 68.4% (Helicoblot 2.1) and 87.9% (DDL EIA) (Table 4).

DISCUSSION

Serological methods are valuable tools for detecting the presence of *H. pylori* but do not allow direct characterization of the *H. pylori* strain. The present study compared the serum antibody responses to *H. pylori* antigens with the *H. pylori vacA* and *cagA* genotypes in antral biopsy specimens. That the *vacA* and *cagA* genotypes could be determined for all 61 patients by the extensively validated PCR LiPA method facilitated this analysis (37–39, 42). Specimens from approximately 10% of the patients contained multiple genotypes, which is in agreement with earlier findings (37).

Serum antibodies to several *H. pylori* antigens, identified by their molecular weight, were determined by two different versions of the Western blot-based Helicoblot assay, which generate particular serological profiles. The reactivity toward each antigen was assessed visually, and signal intensities may vary considerably. Consequently, interobserver variation of both the 2.0 and 2.1 versions was substantial. Of all the individual antigens, an additional antigen line, designated the CIM, which is of unknown clinical relevance, performed best ($\kappa = 0.822$), but antibodies to the CIM were found in only 85.2% of the *H. pylori*-positive patients. That the CIM was present on Helicoblot 2.1 as a separate line at a clearly defined position probably limited erroneous interpretations. Taken together, our results demonstrate that the reproducibility of the visual scoring of the Helicoblot results is limited, in part due to the low intensity of some signals and due to variation between different strip batches, preventing accurate alignment with the provided template. Scanning of blots and computer-assisted

TABLE 4. Association between *cagA* genotype and detection of anti-CagA antibodies by various assays

Assay and result	No. with genotype[a]		Kappa value	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)[c]	NPV (%) (95% CI)[d]
	cagA negative	*cagA* positive					
Helicoblot 2.0			0.557 (0.339–0.776)	71.4 (52.9–84.7)	84.6 (66.5–93.8)	83.3 (64.1–93.3)	73.3 (55.6–85.8)
Negative	22	8					
Positive	4	20					
Dubious	7	0					
Helicoblot 2.1			0.332 (0.079–0.586)	78.6 (60.4–89.8)	54.2 (35.1–72.1)	66.6 (49.6–80.2)	68.4 (46.0–84.6)
Negative	13	6					
Positive	11	22					
Dubious	9	0					
Pyloriset			0.632 (0.438–0.826)	71.4 (52.9–84.7)	90.9 (76.4–96.8)	87.0 (67.9–95.5)	79.0 (63.8–88.9)
Negative	30	8					
Positive	3	20					
DDL			0.736 (0.565–0.906)	85.7 (68.5–99.3)	87.9 (72.7–95.2)	85.7 (68.5–94.3)	87.9 (72.7–95.2)
Negative	29	4					
Positive	4	24					
Vanderbilt			0.696 (0.521–0.870)	67.9 (49.3–82.1)	100 (89.6–100)	100 (83.2–100)	78.6 (64.1–88.3)
Negative	33	9					
Positive	0	19					

[a] Including patients whose specimens contained multiple *vacA* genotypes.
[b] CI, confidence interval.
[c] PPV, positive predictive value.
[d] NPV, negative predictive value.

interpretation may improve the performance of these assays. Although Helicoblot 2.0 and 2.1 contain different antigens, reactivities against the shared 19.5-, 30-, 35-, 89-, and 116-kDa proteins differed considerably. This may be due to differences in *H. pylori* strains used in both assays, in batches of strips, and in observers. Therefore, to adequately compare serological results from various patients or use for follow-up studies, strips from the same batch should be used and need to be interpreted by the same observer.

The only significant associations were found between the presence of anti-CagA on the Helicoblot and the *vacA* s1 and *cagA*-positive genotypes. Since strains lacking *cagA* do not produce the CagA antigen, the lack of anti-CagA antibodies in hosts carrying *cagA*-negative strains was expected. The association we found between colonization with *vacA* s1 strains and presence of anti-CagA antibodies confirms earlier findings, since most *cagA*-positive strains also have the *vacA* s1 genotype (41).

The 89-kDa antigen represents the mature vacuolating toxin VacA (9). Although antibodies to VacA might have been expected in all *H. pylori*-positive patients, in our patient group only 39.3 to 59.0% of the sera contained antibodies to the 89-kDa antigen, confirming earlier studies (12, 32), but with no significant association with the *vacA* genotype of *H. pylori*. This result may indicate that most antibodies against VacA recognize conformational epitopes (25), whereas the Helicoblots only present denatured, linear epitopes, in contrast to studies using purified VacA protein (31).

The great majority of *cagA*-positive strains produce the CagA protein (26). Therefore, if *cagA*-positive strains are present, the immune system usually will have been exposed to the CagA antigen, especially since CagA is injected into gastric epithelial cells by a type IV secretion apparatus encoded by the *cag* pathogenicity island (28). Absence of anti-CagA antibodies in such patients may be due to sequence variation in *cagA*, resulting in different epitopes (24, 43). Several *cagA* variants have been described that have particular geographical distribution (36, 40). The anti-CagA assays we studied are based on different CagA proteins, which may contain different B-cell epitopes. The Vanderbilt and DDL EIAs were both based on recombinant CagA protein, derived from Western *H. pylori* strains. No information is available on the source of CagA used for the Helicoblot 2.0, 2.1, and Pyloriset assays. However, specimens from patients from different parts of the world appear able to recognize a single recombinant CagA protein (16, 30).

The presence of antibodies may reflect recent past carriage, and therefore, antibodies may be found when the bacteria are no longer present in the stomach. However, none of the patients in the present study had been treated, and all were *H. pylori* positive at the time of investigation, as determined by PCR. Similarly, it is possible that *cagA*-positive strains were present in the stomach but were not detected due to sampling error, since each antral gastric biopsy specimen only represents a very small sample of the entire gastric mucosa.

The five assays comprised three different test formats, i.e., Western blot (Helicoblot), rapid immunoassay using a lateral flow system (Pyloriset screen CagA), and microtiter EIA (DDL and Vanderbilt EIAs), and were not in full agreement with each other (Table 3). Although different assay formats may influence the test performance of anti-CagA measurements (2, 13), using the *cagA* genotype as the standard, both Helicoblot assays show limited sensitivity and specificity. In contrast, the DDL assay was most sensitive, while the Vanderbilt assay was most specific (Table 4).

In conclusion, measurement of anti-CagA antibodies by different test formats revealed considerable differences in detection rates. Both in-house EIAs and the Pyloriset screen CagA showed good agreement, but the Helicoblots were not highly reproducible or accurate. Therefore, in future studies in which anti-CagA measurements are used, investigators should include evaluation of the serological assays used in the population studied.

ACKNOWLEDGMENTS

We thank Hans Pottel for statistical advice.

This work was supported in part by grant R01 DK53707 from the National Institutes of Health and by the Medical Research Service of the Department of Veterans Affairs.

REFERENCES

1. **Atherton, J. C., P. Cao, R. M. J. Peek, M. K. Tummuru, M. J. Blaser, and T. L. Cover.** 1995. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific *vac*A types with cytotoxin production and peptic ulceration. J. Biol. Chem. **270:**17771–17777.
2. **Basso, D., A. Stefani, L. Brigato, F. Navaglia, E. Greco, C. F. Zambon, M. G. Piva, A. Toma, F. Di Mario, and M. Plebani.** 1999. Serum antibodies anti-*H. pylori* and anti-CagA: a comparison between four different assays. J. Clin. Lab Anal. **13:**194–198.
3. **Beales, I. L., J. E. Crabtree, D. Scunes, A. Covacci, and J. Calam.** 1996. Antibodies to CagA protein are associated with gastric atrophy in *Helicobacter pylori* infection. Eur. J. Gastroenterol. Hepatol. **8:**645–649.
4. **Blaser, M. J., G. I. Perez-Perez, H. Kleanthous, T. L. Cover, R. M. Peek, P. H. Chyou, G. N. Stemmermann, and A. Nomura.** 1995. Infection with *Helicobacter pylori* strains possessing *cagA* is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res. **55:**2111–2115.
5. **Bonvicini, F., L. Baldini, S. Pretolani, N. Figura, G. Epifanio, A. Armuzzi, F. Miglio, and G. Gasbarrini.** 1997. Anti-CagA antibodies are associated with atrophic gastritis in a population at high gastric cancer risk: a morphometric study by computerized image analysis. Ital. J. Gastroenterol. Hepatol. **29:** 409–414.
6. **Censini, S., C. Lange, Z. Xiang, J. E. Crabtree, P. Ghiara, M. Borodovsky, R. Rappuoli, and A. Covacci.** 1996. *cag*, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. Proc. Natl. Acad. Sci. USA **93:**14648–14653.
7. **Chow, W. H., M. J. Blaser, W. J. Blot, M. D. Gammon, T. L. Vaughan, H. A. Risch, G. I. Perez-Perez, J. B. Schoenberg, J. L. Stanford, H. Rotterdam, A. B. West, and J. F. Fraumeni.** 1998. An inverse relation between *cagA*+ strains of *Helicobacter pylori* infection and risk of esophageal and gastric cardia adenocarcinoma. Cancer Res. **58:**588–590.
8. **Covacci, A., S. Censini, M. Bugnoli, R. Petracca, D. Burroni, G. Macchia, A. Massone, E. Papini, Z. Xiang, and N. Figura.** 1993. Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. Proc. Natl. Acad. Sci. USA **90:** 5791–5795.
9. **Cover, T. L.** 1996. The vacuolating cytotoxin of *Helicobacter pylori*. Mol. Microbiol. **20:**241–246.
10. **Cover, T. L., Y. Glupczynski, A. P. Lage, A. Burette, M. K. Tummuru, G. I. Perez-Perez, and M. J. Blaser.** 1995. Serologic detection of infection with *cagA*$^+$ *Helicobacter pylori* strains. J. Clin. Microbiol. **33:**1496–1500.
11. **Dunn, B. E., H. Cohen, and M. J. Blaser.** 1997. *Helicobacter pylori*. Clin. Microbiol. Rev. **10:**720–741.
12. **Elitsur, Y., C. Neace, M. C. Werthammer, and W. E. Triest.** 1999. Prevalence of CagA, VacA antibodies in symptomatic and asymptomatic children with *Helicobacter pylori* infection. Helicobacter **4:**100–105.
13. **Fusconi, M., D. Vaira, M. Menegatti, S. Farinelli, N. Figura, J. Holton, C. Ricci, R. Corinaldesi, and M. Miglioli.** 1999. Anti-CagA reactivity in *Helicobacter pylori*-negative subjects: a comparison of three different methods. Dig. Dis. Sci. **44:**1691–1695.
14. **Graham, D. Y., R. M. Genta, D. P. Graham, and J. E. Crabtree.** 1996. Serum CagA antibodies in asymptomatic subjects and patients with peptic ulcer: lack of correlation of IgG antibody in patients with peptic ulcer or asymptomatic *Helicobacter pylori* gastritis. J. Clin. Pathol. **49:**829–832.
15. **Herbrink, P., and L. J. van Doorn.** 2000. Serological methods for diagnosis of *Helicobacter pylori* infection and monitoring of eradication therapy. Eur. J. Clin. Microbiol. Infect. Dis. **19:**164–173.
16. **Hook-Nikanne, J., G. I. Perez-Perez, and M. J. Blaser.** 1997. Antigenic characterization of *Helicobacter pylori* strains from different parts of the world. Clin. Diagn. Lab. Immunol. **4:**592–597.
17. **Kato, S., T. Sugiyama, M. Kudo, K. Ohnuma, K. Ozawa, K. Iinuma, M. Asaka, and M. J. Blaser.** 2000. CagA antibodies in Japanese children with nodular gastritis or peptic ulcer disease. J. Clin. Microbiol. **38:**68–70.
18. **Kikuchi, S., J. E. Crabtree, D. Forman, and M. Kurosawa.** 1999. Association between infections with CagA-positive or -negative strains of *Helicobacter pylori* and risk for gastric cancer in young adults. Research Group on Prevention of Gastric Carcinoma Among Young Adults. Am. J. Gastroenterol. **94:**3455–3459.
19. **Kuipers, E. J., G. I. Perez-Perez, S. G. Meuwissen, and M. J. Blaser.** 1995. *Helicobacter pylori* and atrophic gastritis: importance of the *cagA* status. J. Natl. Cancer Inst. **87:**1777–1780.
20. **Lamarque, D., T. Gilbert, F. Roudot-Thoraval, L. Deforges, M. T. Chaumette, and J. C. Delchier.** 1999. Seroprevalence of eight *Helicobacter pylori* antigens among 182 patients with peptic ulcer, MALT gastric lymphoma or non-ulcer dyspepsia. Higher rate of seroreactivity against CagA and 35-kDa antigens in patients with peptic ulcer originating from Europe and Africa. Eur. J. Gastroenterol. Hepatol. **11:**721–726.
21. **Leunk, R. D.** 1995. Production of a cytotoxin by *Helicobacter pylori*. Rev. Infect. Dis. **13:**S686.
22. **Logan, R. P.** 1999. Urea breath tests in the management of *Helicobacter pylori*. Gut **43**(Suppl.)**:**47–50.
23. **Maeda, S., F. Kanai, K. Ogura, H. Yoshida, T. Ikenoue, M. Takahashi, T. Kawabe, Y. Shiratori, and M. Omata.** 1997. High seropositivity of anti-*cagA* antibody in *Helicobacter pylori*-infected patients irrelevant to peptic ulcers and normal mucosa in Japan. Dig. Dis. Sci. **42:**1841–1847.
24. **Maeda, S., H. Yoshida, K. Ogura, Y. Yamaji, T. Ikenoue, T. Mitsushima, H. Tagawa, R. Kawaguchi, K. Mori, K. Mafune, T. Kawabe, Y. Shiratori, and M. Omata.** 2000. Assessment of gastric carcinoma risk associated with *Helicobacter pylori* may vary depending on the antigen used: CagA specific enzyme-linked immunoadsorbent assay (ELISA) versus commercially available *H. pylori* ELISAs. Cancer **88:**1530–1535.
25. **Manetti, R., P. Massari, D. Burroni, M. de Bernard, A. Marchini, R. Olivieri, E. Papini, C. Montecucco, R. Rappuoli, and J. L. Telford.** 1995. *Helicobacter pylori* cytotoxin: importance of native conformation for induction of neutralizing antibodies. Infect. Immun. **63:**4476–4480.
26. **Miehlke, S., M. F. Go, J. G. Kim, D. Y. Graham, and N. Figura.** 1998. Serologic detection of *Helicobacter pylori* infection with *cagA*-positive strains in duodenal ulcer, gastric cancer, and asymptomatic gastritis. J. Gastroenterol. **33**(Suppl. 10)**:**18–21.
27. **Mitchell, H. M., S. L. Hazell, T. D. Bohane, P. Hu, M. Chen, and Y. Y. Li.** 1999. The prevalence of antibody to CagA in children is not a marker for specific disease. J. Pediatr. Gastroenterol. Nutr. **28:**71–75.
28. **Odenbreit, S., J. Puls, B. Sedlmaier, E. Gerland, W. Fischer, and R. Haas.** 2000. Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion. Science **287:**1497–1500.
29. **Parsonnet, J., G. D. Friedman, N. Orentreich, and H. Vogelman.** 1997. Risk for gastric cancer in people with CagA positive or CagA negative *Helicobacter pylori* infection. Gut **40:**297–301.
30. **Perez-Perez, G. I., N. Bhat, J. Gaensbauer, A. Fraser, D. N. Taylor, E. J. Kuipers, L. Zhang, W. C. You, and M. J. Blaser.** 1997. Country-specific constancy by age in *cagA*+ proportion of *Helicobacter pylori* infections. Int. J. Cancer **72:**453–456.
31. **Perez-Perez, G. I., R. M. J. Peek, J. C. Atherton, M. J. Blaser, and T. L. Cover.** 1999. Detection of anti-VacA antibody responses in serum and gastric juice samples using type s1/ml and s2/m2 *Helicobacter pylori* VacA antigens. Clin. Diagn. Lab. Immunol. **6:**489–493.
32. **Rocha, G. A., A. M. Oliveira, D. M. Queiroz, A. S. Carvalho, and A. M. Nogueira.** 2000. Immunoblot analysis of humoral immune response to *Helicobacter pylori* in children with and without duodenal ulcer. J. Clin. Microbiol. **38:**1777–1781.
33. **Rudi, J., C. Kolb, M. Maiwald, I. Zuna, A. von Herbay, P. R. Galle, and W. Stremmel.** 1997. Serum antibodies against *Helicobacter pylori* proteins VacA and CagA are associated with increased risk for gastric adenocarcinoma. Dig. Dis. Sci. **42:**1652–1659.
34. **Shimoyama, T., S. Fukuda, M. Tanaka, T. Mikami, A. Munakata, and J. E. Crabtree.** 1998. CagA seropositivity associated with development of gastric cancer in a Japanese population. J. Clin. Pathol. **51:**225–228.
35. **Sozzi, M., M. Valentini, N. Figura, P. P. De, R. M. Tedeschi, A. Gloghini, D. Serraino, M. Poletti, and A. Carbone.** 1998. Atrophic gastritis and intestinal metaplasia in *Helicobacter pylori* infection: the role of *CagA* status. Am. J. Gastroenterol. **93:**375–379.
36. **van der Ende, A., Z. J. Pan, A. Bart, R. W. van der Hulst, M. Feller, S. D. Xiao, G. N. J. Tytgat, and J. Dankert.** 1998. *cagA*-positive *Helicobacter pylori* populations in China and The Netherlands are distinct. Infect. Immun. **66:** 1822–1826.
37. **van Doorn, L. J., C. Figueiredo, F. Mégraud, A. S. Pena, P. Midolo, D. M. Queiroz, F. Carneiro, B. Vandenborght, M. G. F. Pégado, R. Sanna, W. A. de Boer, P. Schneeberger, P. Correa, E. K. Ng, J. C. Atherton, M. J. Blaser, and W. G. V. Quint.** 1999. Geographic distribution of *vacA* allelic types of *Helicobacter pylori*. Gastroenterology **116:**823–830.
38. **van Doorn, L. J., C. Figueiredo, R. Rossau, G. Jannes, M. van Asbroeck, J. C. Sousa, F. Carneiro, and W. Quint.** 1998. Typing of the *Helicobacter pylori vacA* gene and detection of the *cagA* gene by PCR and reverse hybridization. J. Clin. Microbiol. **36:**1271–1276.
39. **van Doorn, L.-J., C. Figueiredo, R. Sanna, A. S. Pena, P. Midolo, E. K. Ng,**

J. C. Atherton, M. J. Blaser, and W. Quint. 1998. Expanding allelic diversity of *Helicobacter pylori vacA*. J. Clin. Microbiol. **36:**2597–2603.

40. **van Doorn, L.-J., C. Figueiredo, R. Sanna, M. J. Blaser, and W. G. V. Quint.** 1999. Distinct variants of *Helicobacter pylori cagA* are associated with *vacA* subtypes. J. Clin. Microbiol. **37:**2306–2311.
41. **van Doorn, L.-J., C. Figueiredo, R. Sanna, A. Plaisier, P. Schneeberger, W. A. de Boer, and W. Quint.** 1998. Clinical relevance of the *cagA, vacA*, and *iceA* status of *Helicobacter pylori*. Gastroenterology **115:**58–66.
42. **van Doorn, L.-J., Y. Henskens, N. Nouhan, A. Verschuuren, R. Vreede, P. Herbrink, G. Ponjee, K. van Krimpen, R. Blankenburg, J. Scherpenisse, and W. Quint.** 2000. The efficacy of laboratory diagnosis of *Helicobacter pylori* infections in gastric biopsy specimens is related to bacterial density and *vacA, cagA*, and *iceA* genotypes. J. Clin. Microbiol. **38:**13–17.
43. **Vaucher, C., B. Janvier, J. B. Nousbaum, B. Grignon, L. Pezennec, M. Robaszkiewicz, H. Gouerou, B. Picard, and J. L. Fauchere.** 2000. Antibody response of patients with *Helicobacter pylori*-related gastric adenocarcinoma: significance of anti-CagA antibodies. Clin. Diagn. Lab. Immunol. **7:**463–467.
44. **Vicari, J. J., R. M. Peek, G. W. Falk, J. R. Goldblum, K. A. Easley, J. Schnell, G. I. Perez-Perez, S. A. Halter, T. W. Rice, M. J. Blaser, and J. E. Richter.** 1998. The seroprevalence of *cagA*-positive *Helicobacter pylori* strains in the spectrum of gastroesophageal reflux disease. Gastroenterology **115:**50–57.
45. **Yamaoka, Y., T. Kodama, K. Kashima, and D. Y. Graham.** 1999. Antibody against *Helicobacter pylori* CagA and VacA and the risk for gastric cancer. J. Clin. Pathol. **52:**215–218.