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Frequency of *vacA* Genotypes and Cytotoxin Activity in *Helicobacter pylori* Associated with Low-Grade Gastric Mucosa-Associated Lymphoid Tissue Lymphoma

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The *vacA* genotypes s1,m1 and s1,m2 were detected in 44 and 30% of *Helicobacter pylori* isolates, respectively, from patients with gastric mucosa-associated lymphoid tissue lymphoma, compared to 26 and 56% of isolates, respectively, from individuals with gastritis. The *vacA* s1 genotype was significantly associated with, but not predictive of, the presence of vacuolating cytotoxin activity.

Helicobacter pylori is linked to development of gastric mucosa-associated lymphoid tissue (MALT) lymphoma (3, 10); however, the underlying pathogenetic mechanisms are unclear. Hussell et al. demonstrated that proliferation of lymphoma cells and production of tumor-specific immunoglobulin were stimulated by *H. pylori* and that this effect is dependent on *H. pylori*-specific T cells (7). Recently, the *vacA* subtype s1 was suggested as a marker for more virulent strains (1).

We determined the frequency of *vacA* subtypes and cytotoxin activity in *H. pylori* isolates from 27 patients (12 male and 15 female patients; median age, 60 years) with low-grade gastric MALT lymphoma (stage EI_1) compared with *H. pylori* isolates from 27 age- and sex-matched symptomatic individuals with simple gastritis and no history of peptic ulcer or gastric malignancy.

H. pylori was cultured under standard conditions and identified by Gram stain morphology and biochemical testing.

Genomic DNA extraction was performed as previously described (5). Allelic regions of the *vacA* gene were PCR amplified in an automated thermal cycler (Perkin-Elmer) under previously published conditions (5) and visualized in 1% agarose gels stained with ethidium bromide.

For determination of cytotoxin activity, *H. pylori* cells were grown for 48 to 72 h in BBFKS–8% Dent liquid. Culture supernatants were centrifuged and sterilely filtered with a 0.22-μm-pore-size Millex-GV filter (Millipore, Eschborn, Germany) and tested for vacuolating cytotoxin activity with HeLa (ATCC CCL 2) and Vero (ATCC CCL 81) cells under standard conditions. After inoculation on 96-well microtiter plates with a density of 2×10^4 cells per well, serial dilutions (1:2 to 1:8) of *H. pylori* culture supernatants were inoculated onto the coated plates and incubated in a humid atmosphere with 5% CO_2 at 37°C. After 24 h, the grade of vacuolation was determined by inverse microscopy (100× to 200×). Cell lines were considered cytotoxin positive if vacuolation was observed in more than 50% of cells (positive control, *H. pylori* ATCC 49503; negative control, Tx30a).

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PCR amplification revealed a single band of the expected size for either the *vacA* s1 or s2 type and for either the *vacA* m1 or m2 type for all *H. pylori* strains investigated (Fig. 1 and 2). There was a high prevalence of *vacA* s1 (78%) in *H. pylori* strains from both study groups (Table 1). s1,m1 was numerically more common in *H. pylori* isolates from patients with MALT lymphoma than in those from patients with histologic gastritis (44 versus 26%, respectively; $P = 0.08$).

Among the 42 *H. pylori* strains containing *vacA* s1, 16 (38%) exhibited cytotoxic activity in one of the two cell lines. None of the strains containing s2 exhibited cytotoxic activity with either cell line ($P < 0.05$). Only five strains (25%) from patients with low-grade gastric MALT lymphoma containing the s1 genotype showed cytotoxin activity. Of interest, of the 16 toxin-positive (Tox^+) strains, 13 (81.3%) were Tox^+ in Vero cells but only 8 (50%) were Tox^+ in HeLa cells, suggesting that use of a single cell line may significantly underestimate the actual frequency of cytotoxin activity in *H. pylori* strains. There was no significant difference between the *vacA* s1,m1 and s1,m2 genotypes with respect to cytotoxin activity.

Although the *vacA* gene is thought to be present in all *H. pylori* strains, cytotoxin is expressed by only approximately 50% (4). The presence of cytotoxic activity has been suggested as a marker for strains with enhanced virulence acting either directly via cytotoxic action or indirectly via an increased inflammatory and immune response. *vacA* genotype s1 has been associated with enhanced activity of the vacuolating cytotoxin and with a greater degree of gastric inflammation (2).

In this study, the *vacA* s1 genotype was identified in about 75% of *H. pylori* strains from patients with low-grade gastric MALT lymphoma and in about the same proportion in strains from the control group, suggesting that the *vacA* s1 genotype is commonly present in *H. pylori* isolated from German patients. Interpretation and analysis of the role of putative *H. pylori* virulence factors have been hampered by the fact that considerable geographic variation of strains has been demonstrated, such that findings from one region may not be confirmed in another (9). Preliminary studies regarding the frequency of *vacA* genotypes in different patient populations of various geographic regions are available. Mendes et al. reported a higher prevalence of the s1 genotype in patients with peptic ulcers and in those with gastric carcinoma than in patients with simple

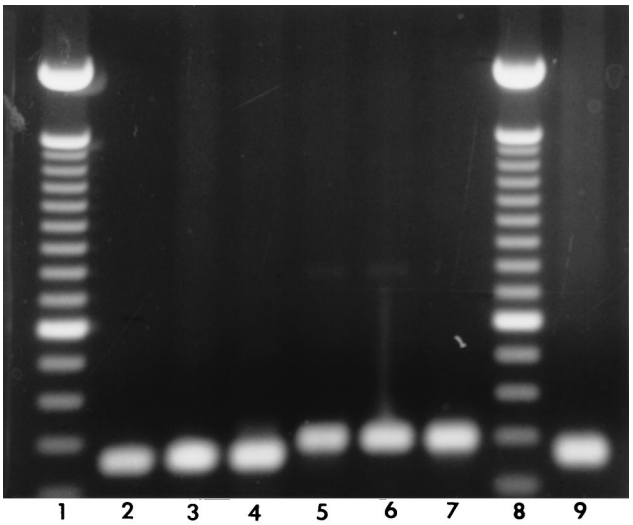

FIG. 1. One percent agarose gel electrophoresis of the 259-bp (lanes 2 to 4) and the 286-bp (lanes 5 to 7) PCR products for the *vacA* s1 and s2 genotypes, respectively. Lanes 1 and 8, 100-bp ladder; lane 9, *H. pylori* ATCC 49503 (s1).

gastritis (8). Studies performed in the United States and in the United Kingdom found no significant differences in the frequency of *vacA* s1 in strains from peptic ulcer patients compared with strains from those with simple gastritis (6, 11). These data suggest that the frequency of the *vacA* s1 genotype in isolates causing different diseases is dependent on the most prevalent genotype in a particular population or geographic region, such that the associations of the *vacA* genotype and different gastroduodenal diseases are inconsistent and spurious.

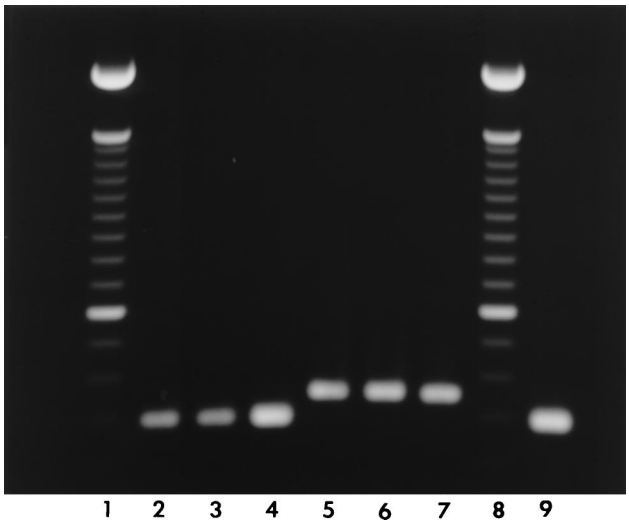

FIG. 2. One percent agarose gel electrophoresis of the 290-bp (lanes 2 to 4) and the 352-bp (lanes 5 to 7) PCR products for the *vacA* m1 and m2 genotypes, respectively. Lanes 1 and 8, 100-bp ladder; lane 9, *H. pylori* ATCC 49503 (m1).

TABLE 1. Frequency of *vacA* genotypes in *H. pylori* strains from patients with MALT lymphoma or simple gastritis

Genotype	No. of isolates (%) from patient group		
	MALT (n = 27)	Gastritis (n = 27)	Total (n = 54)
s1,m1	12 (44)	7 (26)	19 (35)
s1,m2	8 (30)	15 (56)	23 (42)
s2,m2	7 (26)	5 (18)	12 (23)
s2,m1	0 (0)	0 (0)	0 (0)

The failure of the s1 genotype to be always associated with cytotoxic activity shows that, while the s1 genotype may be necessary for the expression of vacuolating cytotoxin, its presence cannot be used as a surrogate for the presence of cytotoxin-positive *H. pylori*. Overall, cytotoxin activity was found in a minority of *H. pylori* strains obtained from patients with low-grade gastric MALT lymphoma, suggesting that cytotoxicity plays little if any role in the pathogenesis of this *H. pylori*-related disease. Importantly, we found that vacuolating cytotoxin activity was detected more frequently in Vero cells than in HeLa cells, showing that the use of a single cell line may underestimate the frequency of cytotoxic activity in *H. pylori* strains. It has been suggested that, because of the problem of geographic variation in the presence and disease associations of putative *H. pylori* virulence factors, disease-specific associations should be evaluated in isolates from different geographic regions before any claim of a possible association is made (9).

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