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

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Received 5 December 1997/Returned for modification 17 February 1998/Accepted 24 April 1998

\textit{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 \textit{H. pylori} and that this effect is dependent on \textit{H. pylori}-specific T cells (7). Recently, the \textit{vacA} subtype s1 was suggested as a marker for more virulent strains (1).

We determined the frequency of \textit{vacA} subtypes and cytotoxin activity in \textit{H. pylori} isolates from 27 patients (12 male and 15 female patients; median age, 60 years) with low-grade gastric MALT lymphoma (stage EI1) compared with 26 and 56% of isolates, respectively, from individuals with gastritis. The \textit{vacA} s1 genotype was significantly associated with, but not predictive of, the presence of vacuolating cytotoxin activity. PCR amplification revealed a single band of the expected size for either the \textit{vacA} s1 or s2 type and for either the \textit{vacA} m1 or m2 type for all \textit{H. pylori} strains investigated (Fig. 1 and 2).

The frequency of \textit{vacA} genotypes s1,m1 and s1,m2 were detected in 44 and 30% of \textit{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 \textit{vacA} s1 genotype was significantly associated with, but not predictive of, the presence of vacuolating cytotoxin activity.

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Among the 42 \textit{H. pylori} strains containing \textit{vacA} s1, 16 (38%) exhibited cytotoxic activity in one of the two cell lines. None of the strains containing \textit{vacA} 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 \textit{s1} genotype showed cytotoxic activity. Of interest, of the 16 toxin-positive (\textit{Tox}+) strains, 13 (81.3%) were \textit{Tox}+ in Vero cells but only 8 (50%) were \textit{Tox}+ in \textit{HeLa} cells, suggesting that use of a single cell line may significantly underestimate the actual frequency of cytotoxic activity in \textit{H. pylori} strains. There was no significant difference between the \textit{vacA} s1,m1 and s1,m2 genotypes with respect to cytotoxin activity.

Although the \textit{vacA} gene is thought to be present in all \textit{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. \textit{vacA} genotype \textit{s1} has been associated with enhanced activity of the vaculating cytotoxin and with a greater degree of gastric inflammation (2).

In this study, the \textit{vacA} \textit{s1} genotype was identified in about 75% of \textit{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 \textit{vacA} \textit{s1} genotype is commonly present in \textit{H. pylori} isolated from German patients. Interpretation and analysis of the role of putative \textit{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 \textit{vacA} genotypes in different patient populations of various geographic regions are available. Mendes et al. reported a higher prevalence of the \textit{s1} genotype in patients with peptic ulcers and in those with gastric carcinoma than in patients with simple
respectively. Lanes 1 and 8, 100-bp ladder; lane 9, _H. pylori_ and the 352-bp (lanes 5 to 7) PCR products for the _vacA_ and the 286-bp (lanes 5 to 7) PCR products for the _H. pylori_ respectively. Lanes 1 and 8, 100-bp ladder; lane 9, _H. pylori_ ATCC 49503 (s1).

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).

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).

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, cytotoxic 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 cytotoxic 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).

TABLE 1. Frequency of _vacA_ genotypes in _H. pylori_ strains from patients with MALT lymphoma or simple gastritis

<table>
<thead>
<tr>
<th>Genotype</th>
<th>MALT (n = 27)</th>
<th>Gastritis (n = 27)</th>
<th>Total (n = 54)</th>
</tr>
</thead>
<tbody>
<tr>
<td>s1, m1</td>
<td>12 (44)</td>
<td>7 (26)</td>
<td>19 (35)</td>
</tr>
<tr>
<td>s1, m2</td>
<td>8 (30)</td>
<td>15 (56)</td>
<td>23 (42)</td>
</tr>
<tr>
<td>s2, m2</td>
<td>7 (26)</td>
<td>5 (18)</td>
<td>12 (23)</td>
</tr>
<tr>
<td>s2, m1</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

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.

REFERENCES