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Immunoproteomics of *Helicobacter pylori* infection in patients with atrophic body gastritis, a predisposing condition for gastric cancer

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ABSTRACT

Atrophic body gastritis is considered an outcome of *H. pylori* infection at high risk for gastric cancer. Immunoproteomics has been used to detect *H. pylori* antigens, which may act as potential markers for neoplastic disease and may be used in specific serological tests. We used immunoproteome technology to identify *H. pylori* antigens, recognized by sera from patients with atrophic body gastritis.

Here, we performed 2DE protein maps of *H. pylori* strain 10K, probed against single sera from 3 groups of *H. pylori*-positive patients (atrophic body gastritis; intestinal-type gastric cancer; peptic ulcer) and negative controls. Immunoreactive spots were identified by MALDI-TOF–MS.

A total of 155 immunoreactive spots were detected corresponding to 14.1% of total spots detected in our reference map of *H. pylori* strain 10K. Sera from atrophic body gastritis ($40.5 \pm 2\%$) and gastric cancer patients ($25.9 \pm 1.8\%$) showed a significantly higher and stronger mean immunoreactivity versus *H. pylori* antigens compared to peptic ulcer patients ($11.2 \pm 1.3\%$). The average intensity of immunoreactivity of sera from atrophic body gastritis and gastric cancer patients was significantly stronger compared to peptic ulcer patients. Sera from atrophic body gastritis and gastric cancer patients differentially recognized 17 *H. pylori* spots.

Immunoproteome technology may discriminate between different *H. pylori*-related disease phenotypes showing a serological immunorecognition pattern common to patients with gastric cancer and atrophic body gastritis, its precursor condition. This tool may be promising for developing specific serological tests to identify patients with gastritis at high risk for gastric cancer, to be evaluated in prospective investigations.

Introduction

Helicobacter pylori (*H. pylori*), a Gram-negative flagellate bacterium that infects the stomach of more than half of the global population, is regarded as the leading cause of chronic gastritis, peptic ulcer (PU), and gastric cancer (GC). WHO classifies the bacterium as a class I carcinogen and a close association between infection and GC has been reported (Marshall and Windsor, 2005; Graham, 2000). The incidence of *H. pylori* infection has been decreasing in industrialized countries due to improved sanitation, smaller family sizes, and decreased overcrowding (Parsonnet, 1995). Anyway, the prevalence of GC remains very high in various regions of the world such as the Far East, the Middle East, and Eastern Europe (Parkin et al., 2005).

Although *H. pylori* infection gives rise to substantial systemic and mucosal immune responses, these responses are usually not protective, as the infection once established often remains for life. Thus, humoral immune responses to *H. pylori* infection represent more a marker of infection than an indication of protection (Kabir, 2007).

In most individuals, *H. pylori* infection is asymptomatic, approximately 10–15% of infected individuals have been shown to develop PU disease, and a few percent may develop GC. These different outcomes of infection may be due to differences in virulence factors of the infecting strains, differences in immunogenic and physiologic factors of the host, and to different genetic susceptibility of the infected individuals (Svennerholm and Lundgren, 2007).

Corpus-predominant gastritis, in particular when associated with gastric body atrophy, is considered a clinical outcome of *H. pylori* infection at high risk for developing GC (Correa, 1992; Haber,

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Table 1
Clinical and histological features of patients whose sera were investigated in this study.

	Patient group			
	Atrophic body gastritis group (ABG)	Gastric cancer group (GC)	Peptic ulcer group (PU)	Normal group (N)
No. of patients	9	10	10	10
No. of females	6	5	8	6
Mean age (years)	53.8	57.1	46.7	43.4
H. pylori status[a]	All positive	All positive	All positive	All negative
Gastric histology:				
Corporal mucosa	AG + IM	AG + IM	Normal	Normal
Antral mucosa	CSG	CSG	CSG	Normal

AG, atrophic gastritis; IM, intestinal metaplasia; CSG, chronic superficial gastritis.
[a] *H. pylori* status was examined by histology (Giemsa stain) and ELISA serology (IgG) in patients with atrophic body gastritis and by histology and biopsy-based urease testing in the other 3 groups.

2002), and the pivotal role of hypochlorhydria as a consequence of severe inflammation and/or atrophy of the gastric body mucosa has been highlighted (Haber, 2002). This type of gastritis has been indicated as 'gastritis of the carcinoma phenotype' and may be viewed as the opposite of the antrum-predominant gastritis, indicated as 'gastritis of the duodenal ulcer phenotype', which is characteristic in patients with antral *H. pylori* gastritis and duodenal ulcer, who almost never develop GC (Uemura et al., 2001; Hansson et al., 1996).

Immunoproteomics has been used to detect *H. pylori* antigens (Mini et al., 2005, 2006a), to be used as potential markers of different *H. pylori*-related diseases (Mini et al., 2006b; Bernardini et al., 2007) to improve serological tests for detecting and monitoring *H. pylori* infection.

An important clinical question is whether a correlation exists between the presence of antibodies directed against specific *H. pylori* antigens and particular *H. pylori*-related clinical outcomes. Few previous studies on *H. pylori* immunoproteomics have addressed this item investigating disease-associated immunoreactive *H. pylori* antigens recognized by sera from patients with PU, gastritis, and GC (Lin et al., 2006, 2007; Krah et al., 2004; Haas et al., 2002; Kimmel et al., 2000). Albeit showing a highly variable humoral immune response, some reports have observed immunoreactive *H. pylori* antigens occurring specifically in patients with GC suggesting that this approach might be useful to identify specific disease-related serologic indicators (Lin et al., 2006; Krah et al., 2004; Haas et al., 2002). In theory, GC-related immunoreactive *H. pylori* antigens may act as potential markers for neoplastic disease, although, in order to be clinically useful, they should yet be detectable in patients with gastritis at high risk for GC such as atrophic body gastritis (ABG), its precursor condition. None of the previous studies on *H. pylori* immunoproteomics has taken into consideration patients with gastritis at high risk for GC, and, to our knowledge, the *H. pylori* immunoproteomic pattern in patients with ABG has never been investigated. In the present study, we used immunoproteomics to identify *H. pylori* antigens recognized by sera from ABG patients considered at high risk for GC.

Materials and methods

Bacterial strains and culture conditions

H. pylori strain 10K [*cagA*₋ A(I) subtype, *vacA*₋ s1/m1 subtype], isolated from biopsy samples of a patient with intestinal-type gastric carcinoma, was cultured as reported (Mini et al., 2006a,b).

Immunoproteomics of H. pylori

2DE (two-dimensional polyacrylamide gel electrophoresis)

Sonicated *H. pylori* bacterial suspensions were solved in Reswelling Buffer (Bernardini et al., 2004). A total of 100 μg of protein samples were adsorbed onto a ReadyStrip (Immobilized pH Gradient, 11 cm, pH 3–10 NL, Bio-Rad) and submitted to 2DE as described elsewhere (Mini et al., 2006a). SDS-PAGE was carried out on a Criterion XT precast gels (Bio-Rad) with a 4–20% polyacrylamide linear gradient gel at 200 V for 1 h. Gels were silver-stained (Mini et al., 2006a), and digitalized images were obtained by ImageScanner (GE-Healthcare) and then analyzed qualitatively and quantitatively by the ImageMaster software (GE-Healthcare).

Human sera

Human sera were obtained from 3 groups of *H. pylori*-positive patients affected by different *H. pylori*-related diseases (Table 1):

- ABG group ($n = 9$, female $n = 6$, mean age: 53.8 years): *H. pylori*-positive ABG.
- GC group ($n = 10$, female $n = 5$, mean age: 57.1 years): *H. pylori*-positive intestinal-type GC.
- PU group ($n = 10$, female $n = 8$, mean age: 46.7 years): *H. pylori*-positive PU and non-atrophic antral gastritis as well as from subjects with a healthy stomach as negative controls who underwent gastroscopy plus antral and body biopsies due to dyspepsia (N group, $n = 10$, female $n = 6$, mean age: 43.4 years).

Sera were obtained at the time of clinical diagnosis and stored at −20 °C until usage. Exclusion critera were previous attempts to eradicate *H. pylori*, use of antibiotics, proton pump inhibitors or bismuth compounds within the last 2 weeks prior to endoscopy, and previous gastric surgery. All patients/subjects were white and recruited from the same geographical area (central Italy). All patients/subjects underwent gastroscopy for dyspepsia or anaemia during which a standardized biopsy sampling (3 from the antrum and 3 from the body mucosa) for conventional histopathological examination was performed (Marignani et al., 1999). Clinical information such as age, gender, and histological data was recorded.

ABG was defined on the basis of the presence of hypergastrinaemia and histological confirmation of body atrophy (Annibale et al., 2000). Degree of gastritis was assessed according to updated Sydney System (Price, 1991). Presence of *H. pylori* infection was defined on the basis of a positive Giemsa stain. In addition, as a second standard, for patients with ABG, a positive serological titre of *H. pylori* IgG antibodies determined by ELISA (GAP test IgG, Bio-Rad, Milan, Italy), and for patients with GC and PU, a positive urease biopsy test was used. Intestinal-type gastric adenocarcinoma was diagnosed according to Padova classification (Rugge et al., 2000). PU was defined as a lesion with loss of mucosal integrity and continuity of ≥0.5 cm with apparent depth of ≥1 mm in the antrum or the duodenum (Cappell and Friedel, 2008).

The study was approved by local ethical committees, and subjects provided signed informed consent.