

REGULAR ARTICLE

***Helicobacter pylori* immunoproteomes in case reports of rosacea and chronic urticaria**

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Rosacea and chronic urticaria are two common skin disorders existing in idiopathic forms. A role of *Helicobacter pylori* bacterium infection in the aetiopathogenesis of rosacea or chronic urticaria has been suggested although still controversial. The aim of the present study was to establish a relationship between *H. pylori* infection and rosacea chronic urticaria by means of an immunoproteomic investigation. We analyzed immunoglobulin A (IgA)-, IgG-, and IgE-mediated immune-responses against *H. pylori* antigens and we identified some bacterial immunoresponsive proteins. A general IgA- and IgE-mediated immune response against antioxidative bacterial proteins was observed. A correlation between the bacterial occurrence and skin diseases pathogenesis is discussed.

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1 Introduction

Rosacea is one of the most common chronic skin disorders. Women are affected more frequently, but the most severe cases are often seen in men. It appears around 20 years of age with vascular abnormalities followed by erythema, flushing, papules, papulo-pustules, and erythrosis and becomes chronic around the age of 40. Rosacea is a multiphasic and multifactorial disease, in that each phase is likely to have its own etiopathogenesis. Many factors have been considered as inducing the disease or contributing to its manifestation, although none of them has been proven to be

the actual pathological cause [1]. The main factors may be grouped as follows: endocrinologic, pharmacologic, immunologic, infective, climatic, thermal, and alimentary [2–4]. Rosacea has been related to gastrointestinal disturbances, gastritis, hypochlorhydria, and abnormalities in the jejunal mucosa. The frequent association of rosacea with gastrointestinal disorders suggested a possible role of *Helicobacter pylori* in the pathogenesis of this dermatosis [7, 8].

H. pylori has been also associated with other skin disorders, such as chronic urticaria, a common dermatologic disorder with a complex pathophysiologic mechanism. The possible causes of chronic urticaria include intolerance to nonsteroidal anti-inflammatory drugs, certain foods and additives, such as preservatives and colors [9]. Moreover, many factors, including bacterial, viral, fungal, and protozoan agents, have been reported as possible initiating factors as well as autoimmune mechanisms [10]. Nevertheless, in a large number of patients the aetiology remains unknown, which makes chronic urticaria an idiopathic disease. *H. pylori* is a microaerophilic, Gram-negative spiral bacterium which

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Abbreviation: Ig, immunoglobulin

infects gastric mucosa. *H. pylori* affects approximately 50% of world population and causes chronic gastric inflammation, which in most cases remains asymptomatic. However, about 15–20% of patients develop severe gastro-intestinal pathologies including gastritis, duodenal or gastric ulcer, mucosa-associated lymphoid tissue (MALT) lymphoma or gastric carcinoma [11–15]. The different outcomes of infection are believed to be influenced by differences in bacterial genotype and by host, physiologic, immunologic, and environmental factors. The prevalence of *H. pylori* increases with the age and lower socioeconomic status, overcrowded living conditions, poor hygiene and multiple siblings [16].

More recently, clinical observations, supported by epidemiological studies, have pointed to a linkage between *H. pylori* infection and some extradigestive disorders, whose etiology is still unknown, including cardiovascular diseases (coronary heart disease, primary Raynaud phenomenon, and migraine), autoimmune disorders (Sjögrens syndrome, Schönlein Henoch purpura, and hypothyroidism) and skin diseases (rosacea, chronic urticaria, alopecia areata) [17–23]. It is unclear how a localized infection may affect areas remote from the site of primary infection. The role of *H. pylori* infection in the etiopathogenesis of rosacea and chronic urticaria is still controversial. Several studies have suggested a possible relationship between *H. pylori* and rosacea [7, 8, 24–27] and chronic urticaria [28–30], although some evidences against this association have been reported [31–35]. The aim of the present study was to investigate the relationship between *H. pylori* infection and two skin disorders (rosacea and chronic urticaria) by means of immuno-proteomic methodologies. We analyzed immunoglobulin A (IgA)-, IgG-, and IgE-mediated immune-responses against *H. pylori* antigens. With this global approach we identified some proteins that may be involved in immune response.

2 Materials and methods

2.1 Patients and sera

Human sera were obtained from three different patients, as follows. A 35-year-old woman with a rosacea came to our hospital in March 2003. The dermatitis started 2 years ago with a facial redness and flushing. In 1 year her symptoms gradually became worse with erythema accompanied by a burning sensation, telangiectasia on the surface of the nose and cheeks. In the last 6 months the patient showed erythema, papules, and pustules affecting the nose, the cheeks, and the forehead. She consulted a dermatologist that treated her with systemic antibiotics and metronidazole gel for 3 months. However, her symptoms did not respond and the disease continued to worsen. She had never been affected by chronic gastritis. The patient was examined for *H. pylori* infection by a commercially available ELISA kit and Western blotting, as well as for ova of parasites and pathogenic bacteria in faeces. We also performed serum tests for hepatitis C virus (HCV), hepatitis B

virus (HBV), syphilis, Epstein-Barr virus, and mycoplasma, antinuclear antibody (ANA), antinuclear and cytoplasmic antibody (ANCA), cryoglobulins and cold globulins, C1-esterase inhibitor. All tests were negative with the exception of *H. pylori* infection. Western blotting confirmed the infection and revealed the presence of serum antibodies to CagA. A 51-year-old Caucasian female who had a 6-month history of intensely pruritic, recurrent urticarial eruptions over her entire body consulted our department. Individual eruptions disappeared to leave normal skin within 24 h. She did not relate her symptoms to any particular food, drug, cosmetics, metals, chemical product, clothes, animals or any other factors. She did not have a personal or family history of atopy. Physical examination did not reveal any lesions at the moment. We did only observe dermatographism with erythema and slight edema. She had never been affected by chronic gastritis. Investigation revealed normal full blood count and blood film; erythrocyte sedimentation rate, complete blood biochemistry, urine analysis, immunoglobulins, total IgE, protein electrophoresis, complement C3 and C4, thyroid function, rheumatoid factor were normal. Fecal examination for ova and parasitic disease, ANA, ANCA, cryoglobulins and cold globulins, C1-esterase inhibitor, serum test for HCV, HBV, syphilis, Epstein-Barr virus, and mycoplasma were negative. The patient was examined for *H. pylori* (HP) infection. The serological diagnosis of *H. pylori* infection was determined by Western blotting, which revealed the presence of antibodies to VacA, UreB, HspB, and other antigens excepted CagA. The third serum was from a 28-year-old woman affected by a duodenal ulcer. She had a moderate inactive chronic gastritis, without atrophy. She had never been affected by any cutaneous disease. The diagnosis of *H. pylori* infection was determined by Western blotting, which revealed the presence of antibodies to VacA, UreB, HspB, and other antigens excepted CagA and by upper endoscopy. The strain (G39) isolated from gastric biopsy has been used for this study.

2.2 *Helicobacter pylori* strain and culture conditions

H. pylori strain G39 (*cagA*⁺ BD subtype, *vacA*⁺ s1/m1 subtype) was isolated from biopsy samples of a patient with duodenal ulcer. Biopsy was streaked onto the surface of Columbia agar containing 5% v/v horse blood, 10 mg/L vancomycin, 5 mg/L trimethoprim, 20 U/mL polymyxin B, and 5 mg/L cefsulodin. Plates were incubated in a microaerobic environment obtained by using an anaerobic jar with a gas-generating kit for microaerophilic atmosphere (10% CO₂, 6% O₂, 0% H₂, 84% N₂, Oxoid Camp GasPak; Oxoid, Unipath, Garbagnate Milanese, Italy). Colonies resembling *H. pylori* were identified by Gram's stain and by oxidase, catalase and urease tests.

2.3 Two-dimensional electrophoresis

H. pylori bacterial suspension was sonicated for 20 min and then it was denatured in the buffer for the first dimension of two-dimensional electrophoresis (2-D PAGE) consisting of a

solution containing 8 M urea, 4% w/v CHAPS, 40 mM Tris, 65 mM dithioerythritol (DTE), and a trace of bromophenol blue. 2-D PAGE was carried out according to procedures detailed elsewhere [36]. For analytical gels 50 µg of protein sample were applied to an Immobiline strip (IPG; Amersham Pharmacia Biotech, Uppsala, Sweden) consisting of a nonlinear gradient, pH range 3.5–10, previously rehydrated. For following Western blotting purposes and for preparative gels, 100 µg and 1 mg of protein sample were applied respectively to an Immobiline strip during its rehydration. Isoelectric focusing was carried out on Multiphor II (Amersham Pharmacia Biotech). The voltage was linearly increased from 300 to 3500 V during the first 3 h and then stabilized at 5000 V for 22 h (total 110 kV·h). The IPG strip was then equilibrated in 6 M urea, 30% w/v glycerol, 2% w/v SDS, 0.05 M Tris-HCl, pH 6.8, 2% w/v DTE, and then with 2.5% w/v iodoacetamide. Electrophoresis in the second dimension was carried out on a 9–16% polyacrylamide linear gradient gel (18 × 20 cm × 1.5 mm) at a constant current of 40 mA. Analytical gels were stained with silver nitrate as previously described [37], while preparative gels were stained with colloidal Coomassie G-250 [38].

2.4 Western blotting

Proteins were electrotransferred from 2-D PAGE gels onto nitrocellulose sheets by semidry blotting using a Novablot semidry transblot cell and a transfer buffer containing 50 mM Tris, 39 mM glycine, 1 mM SDS, and 20% v/v methanol [39]. The electrotransfer time was 75 min with a current/area of 0.7 mA/cm². The antigens were identified by incubation of the membranes with human sera from three patients infected by *H. pylori* and affected one by rosacea, one by chronic urticaria, and another one by duodenal ulcer. Membranes were blocked for 1 h in 3% w/v bovine serum albumin in PBS and washed three times for 10 min in PBS. Human sera were incubated for 2 h at room temperature at a dilution of 1:250 or 1:500. Immunodetection of specific immunoglobulins was performed in three sequential steps: (i) IgA presence was evaluated with goat anti-human IgA horseradish peroxidase-conjugated Ig (Sigma-Aldrich, Milano, Italy) at a dilution of 1:80 000 v/v; (ii) IgEs were analyzed with goat anti-human IgE horseradish peroxidase-conjugated Ig (Sigma Aldrich) at a dilution of 1:10 000 v/v; (iii) finally, IgGs were revealed with goat anti-human IgG horseradish peroxidase-conjugated Ig (Bio-Rad, Hercules, CA, USA) at a dilution of 1:3000 v/v. Enhanced chemiluminescence (ECL kit; Amersham Pharmacia Biotech) was used for the final detection. Before and after each step the membranes were extensively washed three times for 10 min in PBS.

2.5 Computer analysis

The digitalized images were obtained by a laser densitometer from Molecular Dynamics (Sunnyvale, CA, USA) scanning of the gel and then analyzed qualitatively and quanti-

tatively by the Melanie II and PDQuest softwares (Bio-Rad, Hercules, CA, USA). The spot intensities were obtained in pixel units and normalized to the total absorbance of the gel. To evaluate the experimental *pI* and *M_r* coordinates for each single spot, *H. pylori* strain protein extract was co-migrated with a protein extract of a *Saccharomyces cerevisiae* strain, whose proteomic 2-D PAGE map is well characterized [36].

2.6 Protein identification

2.6.1 MS analysis

Spots from 2-D PAGE were excised from the gel, triturated and washed with water and acetonitrile. Proteins were in gel reduced, S-alkylated, and digested with trypsin, as reported previously [40]. Protein digests were analyzed with a Voyager DE-PRO mass spectrometer (Applied Biosystems, Framingham, MA, USA) as reported previously [40]. ProteinProspector and PROWL (ProFound) software packages were used to identify spots using peptide mass fingerprint data from independent non-redundant sequence databases without any limitation in *M_r* or *pI*. Candidates with the highest scores were evaluated further by comparison of their calculated *M_r* and *pI* values with the experimental values obtained from 2-D PAGE.

2.6.2 N-Terminal microsequence analysis and database searching

Following preparative 2-D PAGE runs, the protein spots were electrotransferred onto a PVDF membrane (Bio-Rad; 0.2 µm pore size) using a semidry blotting apparatus (Novablot II; Amersham Pharmacia Biotech) [36]. Blots were stained with 0.1% w/v Coomassie Brilliant Blue R-250 in 50% v/v methanol for 1 min, and then destained in 40% v/v methanol/10% v/v acetic acid. The PVDF membrane spots were excised and transferred to a Hewlett Packard protein sequencer (Model 241; Palo Alto, CA, USA). Protein sequence tags were used as probes for searches in the Swiss-Prot and TrEMBL annotated protein sequence databases using both BLAST and Blitz softwares.

3 Results

We investigated by 2-D immunoblotting analysis the presence of IgA (Figs. 1B, 2B, 3B), IgE (Figs. 1C, 2C, 3C), and IgG (Figs. 1D, 2D, 3D) to *H. pylori* antigens in the serum of a patient with rosacea and a patient with chronic urticaria. A third serum was taken from an *H. pylori*-infected patient affected by duodenal ulcer without any skin disease. This patient was the same from whom strain G39 had been isolated and his serum was adopted as a control. All sera were tested both at a dilution of 1:250 and 1:500, giving analogous results.

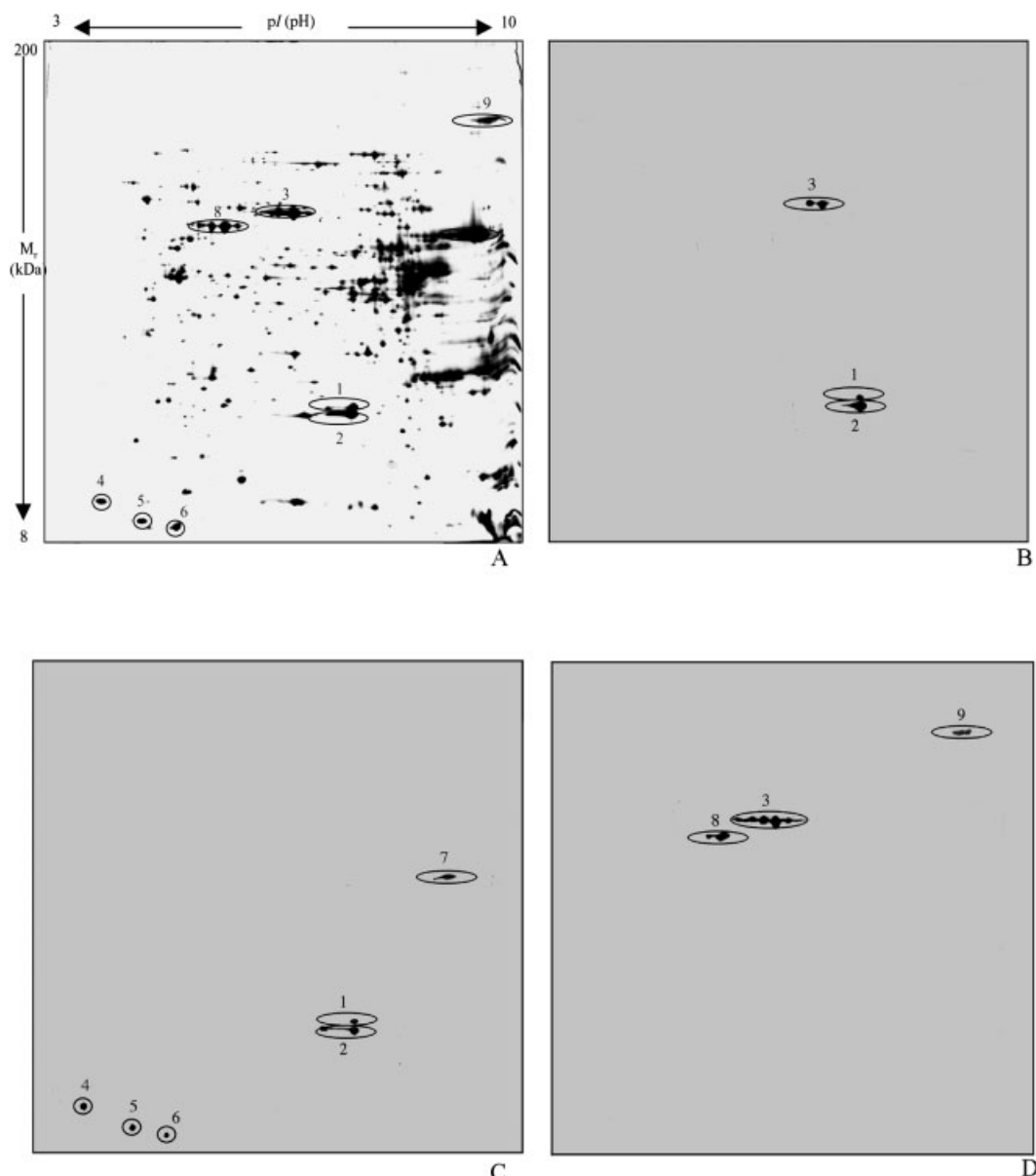


Figure 1. 2-D proteomic map of *H. pylori* strain G39 and detection of *H. pylori* antigens by immunoblot analysis with rosacea serum. Detected antigens are indicated by numbers as reported in Table 1. (A) Silver-stained 2-D PAGE map; (B) immunodetection with IgA; (C) immunodetection with IgE; (D) immunodetection with IgG.

Our aim was to characterize the immune reactivity to this infection in these cutaneous disorders and identify specific *H. pylori* antigens that could concur to explain the pathogenesis of these dermatological diseases. The immunoblot patterns were compared with the silver-stained 2-D protein pattern of strain G39 (Figs. 1A, 2A, 3A). Western blot analysis revealed that both rosacea and chronic urticaria human sera shared an immune reactivity towards numerous *H. pylori* proteins. Most of the *H. pylori* antigens were identified. Immunoreactive spots were marked with a number on 2-D gel and on 2-D blots (Figs. 1, 2, 3) and *H. pylori* antigens

recognized by sera from patients with cutaneous disorders are listed in Table 1. From the functional point of view, four proteins are involved in the anti-oxidative stress bacterial response (AhpC, FldA, TrxA, Kata), three proteins are involved in protein synthesis and folding (L7/L12, GroEL, DnaK), two are necessary for motility (FlaA, FlaB) and, finally, two proteins play consolidated roles in pathogenesis (UreB and CagA). Moreover, catalase, flagellins A and B, urease B, and CagA are considered potent *H. pylori* virulence factors [41]. Analysis of serum from healthy donors was performed in parallel as a control; no positive staining was detected.

Table 1. *H. pylori* proteins detected by immunoblotting

Spot No.	Protein name	Gene name	Protein function	Method of identification
1	Alkyl hydroperoxide reductase	<i>AhpC</i>	Response to oxidative stress	Peptide mass fingerprint
2	Alkyl hydroperoxide reductase	<i>AhpC</i>	Response to oxidative stress	Peptide mass fingerprint
3	Urease β -subunit	<i>UreB</i>	Central intermediary metabolism	Edman degradation
4	Flavodoxin A	<i>FldA</i>	Response to oxidative stress	Edman degradation
5	Ribosomal protein L7/L12	<i>L7/L12</i>	Protein biosynthesis	Edman degradation
6	Thioredoxin	<i>TrxA</i>	Response to oxidative stress	Peptide mass fingerprint
7	Catalase	<i>Kata</i>	Response to oxidative stress	Edman degradation
8	Chaperone and heat shock protein 60	<i>GroEL</i>	Protein folding	Edman degradation
9	CagA	<i>CagA</i>	Role in pathogenesis	Edman degradation
10	Flagellin A	<i>FlaA</i>	Motility	Peptide mass fingerprint
11	Flagellin B	<i>FlaB</i>	Motility	Peptide mass fingerprint
12	Chaperone and heat shock protein 70	<i>DnaK</i>	Protein folding	Edman degradation
13	Not identified (7.9/58629)			
14	Not identified (8.4/55386)			
15	Glutamine synthetase	<i>GlnA</i>	Aminoacid biosynthesis	Edman degradation
16	Isocitrate dehydrogenase	<i>Icd</i>	TCA cycle	Edman degradation
17	Co-chaperone GroES	<i>GroES</i>	Protein folding and stabilization	Edman degradation
18	Neutrophil-activating protein A	<i>NapA</i>	Detoxification	Edman degradation
19	Not identified (5.68/17052)			
20	Not identified (5.48/17057)			
21	Hypothetical protein HPO697	<i>HPO697</i>	Unknown	Edman degradation
22	Inorganic pyrophosphatase	<i>Ppa</i>	Metabolism: pyrophosphatase activity	Edman degradation

Identified immunoresponsive proteins together with their gene name, biochemical function and identification procedure are reported. Experimental pI/M_r values are also indicated for unidentified proteins.

3.1 *H. pylori* antigens recognized by rosacea serum

The serum from the patient infected by *H. pylori* and affected by rosacea recognized some *H. pylori* antigens. Figure 1 shows both the 2-D proteomic map of *H. pylori* G39 and its relative immunoblot patterns. IgA antibodies were directed against three proteins (Fig. 1B): two isoforms of alkyl hydroperoxidase reductase (spots 1 and 2) and urease β -subunit (spot 3). The immunoblot pattern related to the IgE-mediated immune response is reported in Fig. 1C. The immunoreactive spots corresponded to two isoforms of alkyl hydroperoxide reductase (spots 1, 2), to flavodoxin A (spot 4), to ribosomal protein L7/L12 (spot 5), to thioredoxin (spot 6), and to catalase (spot 7). Figure 1D shows the bacterial antigens immune recognized by IgG: three immunoreactive spots corresponded to urease β -subunit (spot 3), chaperone and heat shock protein 60 (spot 8), and CagA (spot 9).

3.2 *H. pylori* antigens recognized by chronic urticaria serum

The serum from the patient with chronic urticaria recognized antigens of *H. pylori* marked with numbers on 2-D blots in Figs. 2B, C, D. IgA antibodies were directed against the isoforms of alkyl hydroperoxide reductase, flagellin A and flagellin B, (spots 1, 2, 10, and 11, respectively). IgE

cross-reacted with one isoform of alkyl hydroperoxidase reductase (spot 2), flavodoxin A (spot 4), ribosomal protein L7/L12 (spot 5), thioredoxin (spot 6). Figure 2D shows the antigens of *H. pylori* for IgG antibodies: spots 8 corresponded to chaperone and heat shock protein 60, spot 10 to flagellin A, spot 11 to flagellin B, and spot 12 to chaperone and heat shock protein 70.

3.3 *H. pylori* antigens recognized by duodenal ulcer serum

As far as it regards the serum G39, used as a control, IgA antibodies were directed against three proteins (Fig. 3B): ribosomal protein L7/L12 (spot 5), thioredoxin (spot 6), and isocitrate dehydrogenase (spot 16). Figure 3C shows the antigens of *H. pylori* for IgE antibodies: two isoforms of alkyl hydroperoxidase reductase (spot 1, 2), ribosomal protein L7/L12 (spot 5), glutamine synthetase (spot 15), isocitrate dehydrogenase (spot 16), co-chaperone GroES (spot 17), neutrophil-activating protein A (spot 18). IgE antibodies were directed against four unidentified proteins (spots 13, 14, 19, and 20). Finally, IgG cross-reacted with to two isoforms of alkyl hydroperoxide reductase (spots 1, 2), flavodoxin A (spot 4), ribosomal protein L7/L12 (spot 5), thioredoxin (spot 6), hypothetical protein HP0697 (spot 21), inorganic pyrophosphatase (spot 22), and one unidentified protein (spot 19).

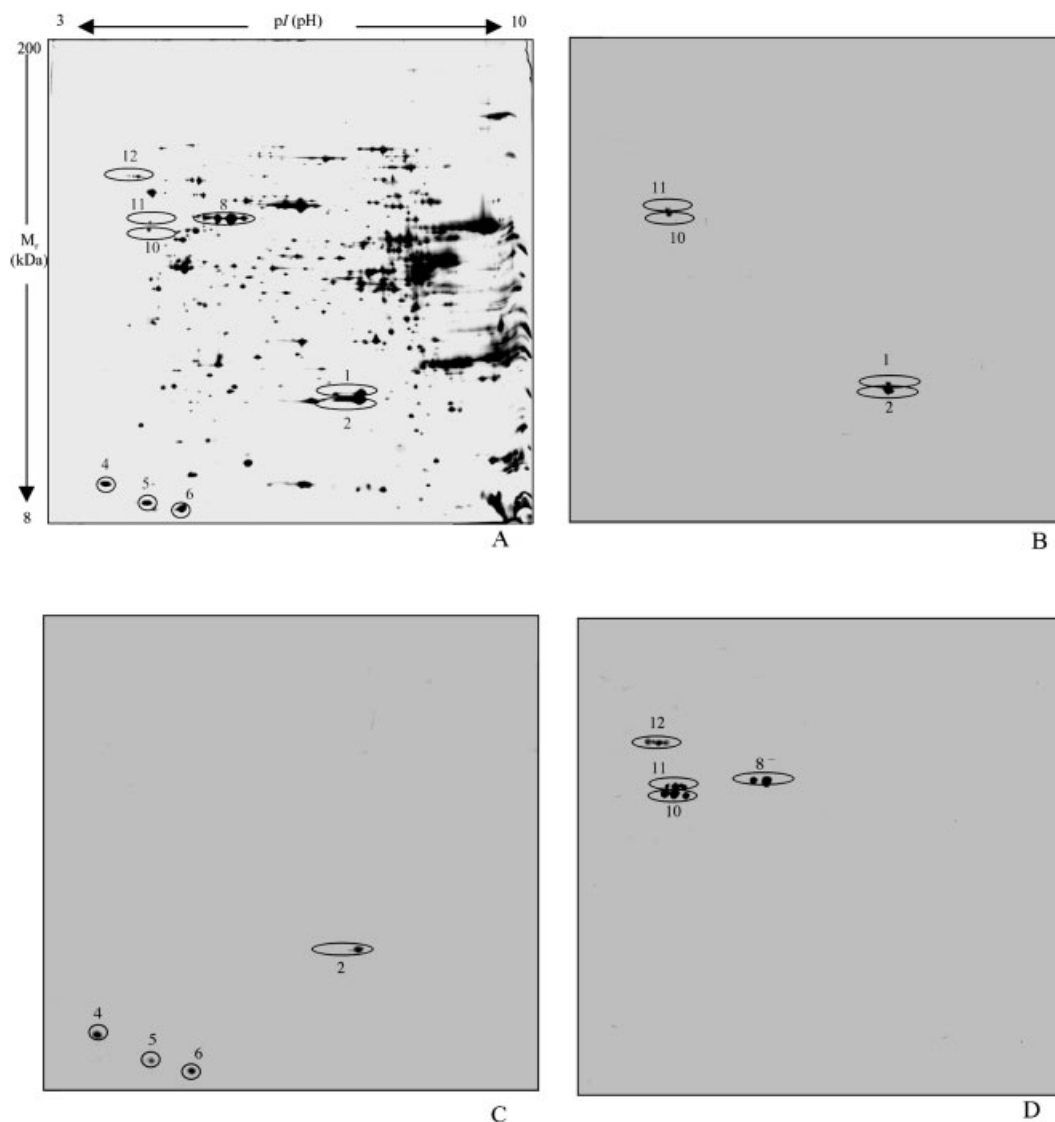


Figure 2. 2-D proteomic map of *H. pylori* strain G39 and detection of *H. pylori* antigens by immunoblot analysis with chronic urticaria serum. Detected antigens are indicated by numbers as reported in Table 1. (A) Silver-stained 2-D PAGE map; (B) immunodetection with IgA; (C) immunodetection with IgE; (D) immunodetection with IgG.

4 Discussion

A linkage between idiopathic forms of rosacea or chronic urticaria and *H. pylori* infection has been long debated. In fact, no definitive indications at the molecular level have been provided so far. Although *H. pylori* infection is the most diffused chronic bacterial infection in man, and host response is considered a key factor in the comprehension of pathogenesis and natural history of the bacterium, the relationships between systemic, humoral, and local immune responses and their pathogenetic roles are still poorly defined. In general, *H. pylori* is known to induce serum, salivary, gastric, and rectal specific isotype antibody responses [42]. Nevertheless, the study of such responses has been

focused on their potential diagnostic importance rather than pathogenic role. For example, it is quite recent the observation that the gastric mucosal IgA-mediated immune response can represent one of the main mechanisms in the pathogenesis of *H. pylori*-associated diseases [43, 44].

In the present work, we adopted a proteomic approach which allowed identifying new *H. pylori* antigens recognized by specific immunoglobulin isotypes in the sera of patients affected by idiopathic rosacea and chronic urticaria. We found that the immunoreactivity against some antigens was shared by both patients, while the reaction with other antigens was specific for one pathology only. Since sera were probed against the same *H. pylori* strain, these differences may be imputable to the different skin pathology.

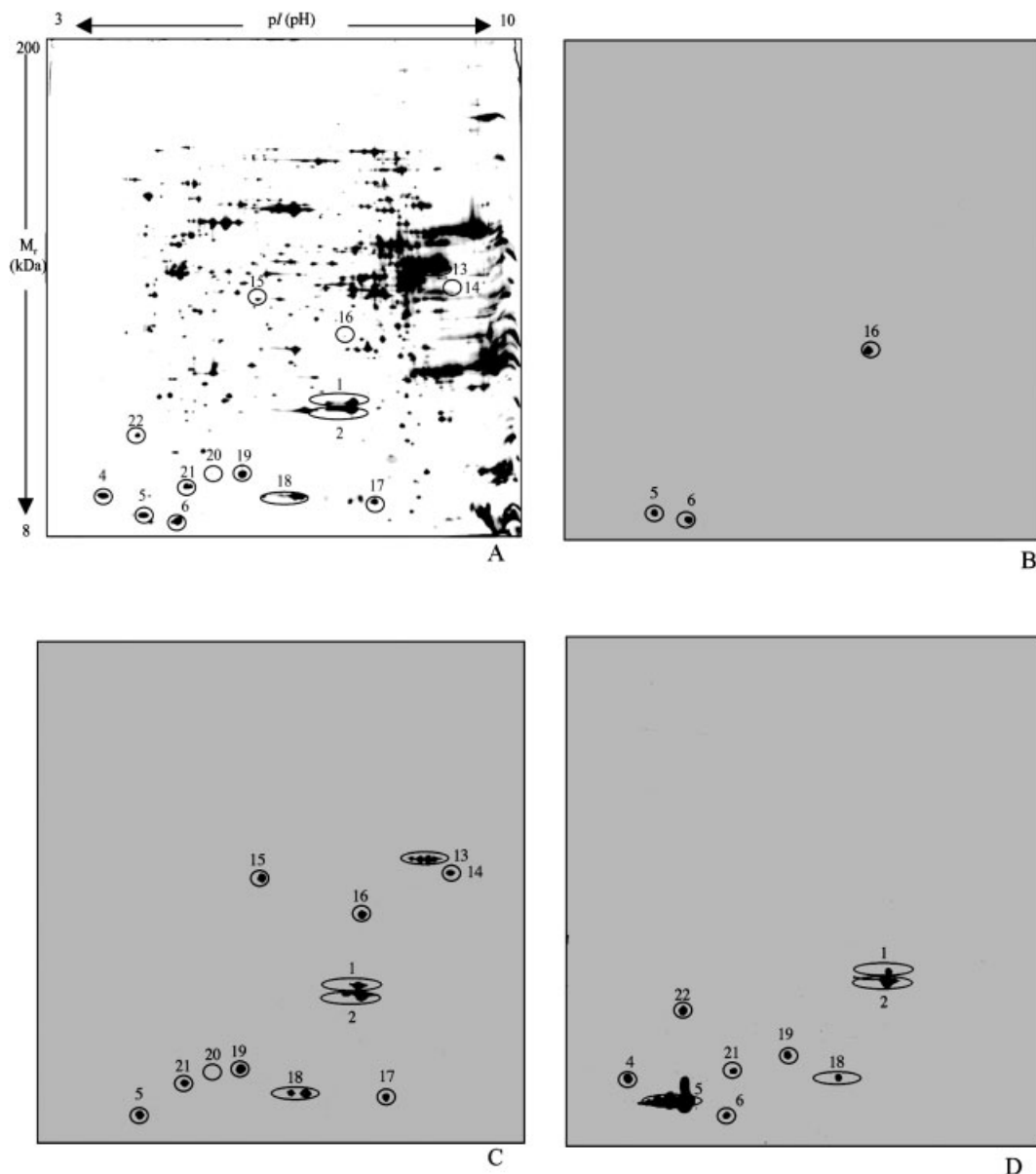


Figure 3. 2-D proteomic map of *H. pylori* strain G39 and detection of *H. pylori* antigens by immunoblot analysis with duodenal ulcer serum. Detected antigens are indicated by numbers as reported in Table 1. (A) Silver-stained 2-D PAGE map; (B) immunodetection with IgA; (C) immunodetection with IgE; (D) immunodetection with IgG.

4.1 Shared antigens

Both rosacea and chronic urticaria sera contained IgG against GroEL (Hsp60). GroEL is not known as an immunodominant antigen in *H. pylori*-associated gastric diseases, and, consequently, it could be a potential antigen for extra-digestive *H. pylori*-associated diseases, particularly skin disorders. Interestingly, GroEL has been recently correlated to an extra-gastric *H. pylori*-related disease, atherosclerosis, through a molecular mimicry mechanism [45] and, previously, in the induction of *H. pylori*-related autoimmune

disorders [46]. It has been hypothesized that Hsp proteins play specific roles during infection. Particularly, GroEL has been involved in urease stability, an enzyme protecting gastric lumen from low pH [47].

Both rosacea and chronic urticaria sera also contained IgA and IgE recognizing AhpC, a peroxidase subunit involved in the defence response against oxidative stress. No anti-AhpC IgG-mediated immune response was found. This implies that antibacterial immunity in this case exclusively involves the local (IgA) and antiallergic (IgE) responses, thus suggesting a pathology specificity. The alkyl hydroperoxide

Table 2. Antigenic pattern obtained with serum from patient with rosacea (R), chronic urticaria (CU), and duodenal ulcer (DU) detected by Western blotting with three different classes of Ig's

Spot No.	Gene name	IgA			IgE			IgG		
		R	CU	DU	R	CU	DU	R	CU	DU
1	<i>AhpC</i>	+	+	–	+	–	+	–	–	+
2	<i>AhpC</i>	+	+	–	+	+	+	–	–	+
3	<i>UreB</i>	+	–	–	–	–	+	+	–	–
4	<i>FldA</i>	–	–	–	+	+	+	–	–	+
5	<i>L7/L12</i>	–	–	+	+	+	+	–	–	+
6	<i>TrxA</i>	–	–	+	+	+	+	–	–	+
7	<i>Kata</i>	–	–	–	+	–	–	–	–	–
8	<i>GroEL</i>	–	–	–	–	–	+	+	+	–
9	<i>CagA</i>	–	–	–	–	–	–	+	–	–
10	<i>FlaA</i>	–	+	–	–	–	–	–	+	–
11	<i>FlaB</i>	–	+	–	–	–	–	–	+	–
12	<i>DnaK</i>	–	–	–	–	–	–	–	+	–
13	–	–	–	–	–	–	+	–	–	–
14	–	–	–	–	–	–	+	–	–	–
15	<i>GlnA</i>	–	–	–	–	–	+	–	–	–
16	<i>Icd</i>	–	–	+	–	–	+	–	–	–
17	<i>GroES</i>	–	–	–	–	–	+	–	–	–
18	<i>NapA</i>	–	–	–	–	–	+	–	–	–
19	–	–	–	–	–	–	+	–	–	+
20	–	–	–	–	–	–	+	–	–	–
21	<i>HPO697</i>	–	–	–	–	–	–	–	–	+
22	<i>Ppa</i>	–	–	–	–	–	–	–	–	+

reductase enzyme system is endowed with an antioxidant activity [48]. AhpC acts in synergy with a 57 kDa flavoprotein which has been identified as the thioredoxin reductase, to form a complex named AhpR essential for a complete antioxidative defence. AhpC was also shown to belong to the thioredoxin-dependent peroxidase family called peroxiredoxin [48]. Both sera also contained IgE against FldA and TrxA, two other proteins involved in the bacterial antioxidative response to fight the oxidative action of macrophages and neutrophils. The thioredoxin reductase/thioredoxin system is another fundamental antioxidative *H. pylori* defence. Its functional mechanism is analogous to the above-mentioned AhpC/AhpF system, to which is partially overlapped. FldA is the electron acceptor of the essential pyruvate-oxireductase enzyme complex and proposed to be involved in the pathogenesis of *H. pylori*-related gastric MALToma [49]. Summarizing, the shared immune response for rosacea and chronic urticaria seems to be mainly IgA- and IgE-mediated and directed against antioxidative and folding proteins.

4.2 Chronic urticaria-specific antigens

The patient affected by chronic urticaria presented both serum IgA and IgG specific for FlaA and FlaB, two essential component of bacterial flagellum conferring upon *H. pylori*

the motility necessary to colonize the gastric mucosa and to attain robust infection [50]. Both flagellar proteins are immunodominant antigens in the immune response against *H. pylori*. Some researchers have recently reported an immune response in IgE of patients with chronic urticaria against *H. pylori* “flagellar structures”, without specifying any molecular component [51]. The fact that in the serum sample from the patient with rosacea no reactivity against flagella was found, could possibly indicate a sort of pathology specificity.

IgGs against the DnaK (Hsp70) protein were also found. DnaK is a bacterial chaperone and heat shock protein, which, together with GroEL, can be found on *H. pylori* cell surface, both proteins playing a role in bacterial adhesion to glycolipids of gastroepithelial cells [52]. Moreover, it seems that transcription of GroEL and DnaK is negatively regulated by the same repressor [53]. Summarizing, the specificity of the immune response in chronic urticaria seems to involve IgA and IgG against bacterial motility and protein folding.

Finally, it is worth noting the absence of IgG against the CagA protein. The lack of an immune reactivity against CagA, which is an immunodominant antigen, suggests that the *H. pylori* strain that infected the patient with chronic urticaria was *cag*- or *cagA*-negative, thus providing an important indirect indication on the strain virulence.

4.3 Rosacea-specific antigens

The most remarkable data in the rosacea-specific serum immune response is indeed the IgE-mediated reactivity against Kata protein, the bacterial catalase. It is an antioxidant enzyme required for a complete protection against superoxide radicals. Together with urease, catalase is an *H. pylori* virulence factor and a strong immunogen. The present one is the first report on IgE reactivity against catalase, which could be a rosacea-specific anti-allergic response antigen. It has been reported that one of the causes of rosacea is a reduced catalase activity in patients [54], which implies an insufficient anti-oxidant response. It could be possible that *H. pylori* infection could induce an antibacterial catalase immune response which could be later revealed against host catalase, thus making human antioxidative defence inadequate. In this case, *H. pylori* bacterial infection could be at the basis of the so-called “antioxidant defect hypothesis”, recently reported as a rosacea cause [54]. Thus, catalase could be considered as a new molecule implied in a novel case of molecular mimicry related to rosacea pathology. A comparative sequence analysis between human and *H. pylori* catalase indicates a significant primary structure identity (52%, 255 residues out of 482). This identity is distributed in hydrophilic portions of the sequence and involves regions long enough to justify the presence of shared epitopes, even in their continuous forms. The patient affected by rosacea also had anti-cagA IgG in her serum, thus indicating that *H. pylori* infecting strain was in that case *cagA*-positive. This

would support a recent hypothesis that rosacea could be associated with infections due to *cagA*-positive strains [55], as a strain-specific feature of the pathology.

4.4 Mechanisms of oxidative stress and antioxidative defence

Skin is at greater risk than other tissues since it is exposed to oxidative stress both from endogenous and exogenous sources [56]. Reactive oxygen species (ROS) are toxic chemicals playing an important role in many inflammatory skin diseases and organisms, including aerobic and microaerophilic microorganisms, developed defence antioxidative protection systems. In many skin disorders, a significant decrease of antioxidative enzymatic systems has been observed. Particularly, in rosacea, an “antioxidant system defect hypothesis” was postulated to explain the pathogenesis of the disease [54]. The clinically positive effect of the therapy with the antioxidant metronidazole was proven. Nevertheless, this treatment, besides its action to decrease ROS generated by neutrophils in the inflammation site, could more powerfully act directly on *H. pylori* whose toxins are well-known ROS inducers. Antibacterial activity of metronidazole is exerted via one-electron reduction of the nitro group leading to the production of radical species that cause DNA damage. The drug is effective only with organisms having a low intracellular redox status. *H. pylori* has a pyruvate/flavodoxin oxidoreductase complex and is metronidazole-sensitive. The mechanisms leading to a metronidazole resistance are still unclear. Recently, it has been reported that AhpC is required for metronidazole sensitivity and that the absence of such a protein is directly related with the development of metronidazole resistance [57]. Interestingly, the immune response shared by sera of both skin pathologies analyzed was mainly directed against two isoforms of AhpC. The unsuccessful metronidazole treatment in the patient affected by rosacea may be imputable to a metronidazole resistance due to the immune response raised against the essential AhpC. Analogously, the immune response raised against FldA could have contributed to the induction of drug resistance, being such a protein a metronidazole electron donor.

In an overall vision, it is clear that an alteration of the redox status occurs, both in host tissue and bacterium, in both skin pathologies. On the other hand, only two other tissues are exposed as skin to oxidative stress: lung and gastric epithelium. Being *H. pylori* a bacterium able to survive in the stomach, it is not surprising that it can provide defence mechanisms which subsequently induce biochemical mechanisms causing a direct or indirect tissue damage at the gastric mucosa or skin levels.

The observed immune response against *H. pylori* antioxidant proteins could: (i) cause a cross-reaction against homologue human proteins (molecular mimicry); (ii) let neutrophils and macrophages act with their own produced ROS against the bacterium causing a skin tissue damage, which is then perpetuated since the immune response is known to be

an ineffective antibacterial factor [58]. In other words, at the skin level a phenomenon could occur analogously to that at the gastric mucosa level, where the host inflammatory response, which is unable to eradicate the bacterium [59], contributes to further injure the gastric mucosa [60, 61].

The systemic inflammatory status secondary to chronic infection or a cross-molecular mimicry existing between bacterial and host antigens have been already suggested for other *H. pylori* extra-gastric manifestations. *H. pylori* colonization, although spatially confined to the stomach, results in the continuous release of various soluble cytotoxic substances, either of bacterial origin or produced by the host, that may be transported in the blood stream and promote an inflammatory response in organs distant from the stomach [62, 63].

4.5 Roles of immunoglobulin isotypes

In the present work, the most remarkable finding is the presence of serum IgA and IgE to *H. pylori* antigens that had not aroused an antibody response in IgG. First, because studies investigating an IgE response to *H. pylori* infection are very rare. Secondly, because such antibody isotypes were found to be reactive against unusual bacterial antigens, which are not recognized as immunodominant in *H. pylori*-associated gastro-intestinal pathologies. The role of serum IgA is still quite obscure, but an anti-inflammatory activity has been proposed for IgA [64]. In our case, IgA could play a role of secondary antibacterial defence, once the bacterium has penetrated the mucosal barrier.

We have produced novel data on an IgE-mediated immune response against *H. pylori* AhpC, FldA, TrxA, L7/L12 (both sera), and Kata (rosacea serum). These data indicate a powerful allergic immune response towards the bacterial antioxidative system. Thus, IgE could seemingly play a fundamental role in both skin pathologies. *H. pylori* was found to induce an IgE-mediated response in patients affected by chronic gastritis [65]. Moreover, mastocytes involved in IgE-mediated reactions have been proposed as inducers of chronic inflammation due to *H. pylori* infection [65]. Particularly, for chronic urticaria it has been suggested that IgE binding *H. pylori* antigen could activate mastocytes and that the subsequent release of histamine could provoke the symptoms of the disease [66] although it is not known which is the mastocytes activator in such a context. Our results suggest that IgE raised by the bacterium could be such mastocytes activators.

Moreover, it has been proven that a relationship exists between *H. pylori* infection and anti-IgE autoimmune reaction in chronic urticaria [67]. It has been hypothesized that *H. pylori* could stimulate the production of anti-*H. pylori* IgE able to cross-react with cells of the gastric wall and facilitate the absorbance of antigens or the unmasking of pre-existing antigens. Should this be the case, the production of IgE responsible of the symptoms of chronic urticaria could persist even after *H. pylori* eradication.

One-third of patients affected by chronic urticaria have circulating anti-F_{ce}RI autoantibodies [68, 65]. Since it has been proven that IgE induce an over-regulation of F_{ce}RI [69], we hypothesize that *H. pylori*-induced IgE production in chronic urticaria could be the cause of both the above-mentioned autoimmune phenomena. Finally, IgE could be correlated with allergy mechanisms triggered by or worsened by the injury of the gastric mucosal barrier following *H. pylori* infection, which could cause the penetration of macromolecules into the epithelium with subsequent allergic and molecular mimicry phenomena [65].

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