

Original article

High prevalence of IgG and IgA antibodies to 19-kDa *Helicobacter pylori*-associated lipoprotein in chronic urticaria

Background: Chronic urticaria has been described in patients with *Helicobacter pylori* infection. We studied the titer of IgG and IgA type antibodies against *H. pylori* in patients with and without urticaria of unknown etiology. We also investigated the prevalence of antibodies against *H. pylori*-associated lipoprotein 20 (lpp20) in patients with and without chronic urticaria.

Methods: The concentration of anti-*H. pylori* antibodies (IgG and IgA) was determined by the RIDA test. The level of anti-lpp20 antibodies was determined by Western blot using various *H. pylori* antigens (from 19 to 120 kDa).

Results: Patients with chronic urticaria and *H. pylori* infection (subgroup 1, $n = 33$) had high IgG and IgA titers whereas all patients with chronic urticaria and without *H. pylori* infection (subgroup 2, $n = 23$) were seronegative ($P = 0.0128$ for IgG and $P = 0.003$ for IgA). Titers in subgroup 1 did not differ significantly from a control group ($n = 33$) with severe *H. pylori*-associated gastritis without urticaria. The prevalence of the anti-lpp20 antibodies was significantly higher in subgroup 1 compared to the control group (93.9 vs 21.2%, $P < 0.0001$ for IgG, and 46.1 vs 6.3%, $P < 0.0029$ for IgA).

Conclusions: We suggest that IgG and IgA antibodies to *H. pylori*-associated lpp20 may play role in the pathogenesis of chronic urticaria.

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Chronic urticaria may result from several causes, but the etiology for most cases remains unknown and therapy is largely directed at symptomatic care. Immunoglobulin E (IgE)-mediated allergy, pseudoallergic reactions against food additives and gastrointestinal disorders and chronic 'focal' infections represent common etiologic factors in chronic urticaria (1). Autoantibodies directed against either IgE or the α -chain of the high-affinity IgE receptor (Fc ϵ RI α) can be detected in 30–50% of patients, suggesting an autoimmune origin (2). Skin test with autologous serum is a reasonably sensitive and specific marker of histamine-releasing activity on basophils and is widely used for the diagnosis of chronic autoimmune urticaria (3, 4).

Most recently, numerous publications have appeared advocating *Helicobacter pylori* as causative in chronic urticaria (5–9). Despite conflicting results of eradication therapy, *H. pylori* may have an indirect role in urticaria (10). There seems to be a positive correlation between a positive autologous test and *H. pylori* suggesting its role in autoimmune pathogenesis (11).

The outer membrane is a continuous structure on the surface of Gram-negative bacteria, and has particular significance as a potential target for immunity. Immunoblot analysis indicates that several immunoreactive proteins are present in broth culture supernatant of

H. pylori. Thus, an 87-kDa band in fractions 25–29 represents vacuolating cytotoxin (VacA), 66- and 31-kDa bands in fractions 29–34 represent two urease subunits, and a 56-kDa band in fractions 29–34 represents HspB. Furthermore, multiple immunoreactive bands ranging in size from about 20 to 100 kDa are identified in fractions 35–45 (12, 13). Finally, immunoblotting identified a specific serum IgG response to an 18-kDa outer membrane protein in immunized animals (14).

Previously we found that the prevalence of anti-*H. pylori*-associated lipoprotein20 (lpp20) antibody, analyzed by Western blot, was considerably higher in *H. pylori*-positive patients with chronic urticaria than in *H. pylori*-positive patients without it (15). In order to assess the occurrence of *H. pylori* infection in chronic urticaria we extended our preliminary observation of the high prevalence of anti-lpp20 antibodies in *H. pylori*-positive patients with chronic urticaria.

Patients and methods

Fifty-six patients (34 women and 22 men, 14–75 years of age; mean $\pm$ SD, 40.8 $\pm$ 16.4 years) with chronic urticaria were investigated. The duration of the disease varied between 2 and 240 months (median: 6 months, IQ range: 3.5–18.0).

The patients underwent an extensive investigation to establish the cause of the urticaria including thorough history taking, complete blood count with differential panel, erythrocyte sedimentation rate, liver and kidney function tests, hepatitis serology, complement activation, search for bacterial foci, including chest and sinus X-ray, cultures of bile and stool, TSH serologic tests for autoimmune diseases (antinuclear factor, anti-dsDNA titer, ANCA, rheumatoid factor, anti-SSA, anti-SSB, anti-ENA), cryoglobulin, circulating immunocomplex C3-, C4-, C1-inhibitor. Skin testing and investigation of specific IgE were performed with the most common food and aeroallergens; additional tests for physical and contact urticaria were also done.

Our patients had no other underlying causes of urticaria such as food allergy or intolerance, contact allergy, bacterial focus or parasitic infection, physical urticaria, urticarial vasculitis, hypocomplementemic urticaria or polysystemic autoimmune diseases. They had no dyspeptic symptoms in their history and over the observation period.

The control group comprised a selected group of 33 *H. pylori* positive, severely dyspeptic but nonurticarial patients (12 men and 21 women, 24–75 years of age; mean, 51.1 ± 13.3 years) with high-grade histologically proven *H. pylori*-associated gastritis. All patients underwent gastroscopy, histology of the gastric mucosa and urease testing. The degree of gastritis was estimated by Updated Sydney System.

Anti-*H. pylori*-specific IgG and IgA were determined by the RIDA® test (R-Biopharm GmbH, Darmstadt, Germany). Titers 10 IU/l or higher were considered positive, according to the values given by the manufacturer. Immunoblotting was performed by RIDA® test (R-Biopharm GmbH, Darmstadt, Germany) with the following molecular weights: 120 kDa (CagA), 87 kDa (VacA), 67 kDa (OMP 67), 62 kDa (urease B), 58 kDa (heat shock protein), 54 kDa (flagellin), 47, 33 and 29 kDa (urease H), 28 kDa (hcpA – *H. pylori* cysteine-rich protein A), 25 kDa (urease D) and 19 kDa (lpps20). In the test are anti-human-IgG and -IgA conjugates (IgG antibodies from rabbit conjugated with peroxidase).

Fisher's exact test was applied to compare the anti-*H. pylori* antibody profiles and score of gastritis of the two study groups. Analysis of variance between the values of *H. pylori*-specific IgG and IgA was performed using with the Mann–Whitney test. $P \leq 0.05$ was considered statistically significant.

Results

Helicobacter pylori-specific IgG and IgA titers in patients with and without chronic urticaria

The 56 patients with urticaria were divided into two subgroups based on the findings of gastroscopy and the urease test. Subgroup 1 comprised 33 urease-positive patients with mild gastritis on histology (score 1.3), and 23 urease-negative, nongastritic patients formed subgroup 2 (Table 1). They are categorized as 'idiopathic' urticarial patients.

Whereas *H. pylori*-specific IgG was elevated (≥ 10 IU/l) in each patient in subgroup 1, all patients remained seronegative in subgroup 2. In each patient of the control group, endoscopy and the histology showed severe gastritis (score 3.8) and the *H. pylori*-specific IgG proved to be positive. The score of gastritis was significantly higher in the control group than in subgroup 1 (Table 1).

The *H. pylori*-specific IgA in subgroup 1 was positive in 13 out of the 33 patients. In subgroup 2, the anti-*H. pylori* IgA levels were not elevated. In the control group, high anti-*H. pylori* IgA levels were detected in 28 out of 33 patients (84.8%) (Table 1). Titers of anti-*H. pylori* IgG and IgA antibodies were significantly higher in the control group than in subgroup 1 ($P = 0.0128$, and $P < 0.0001$) (Table 1).

Patients with and without chronic urticaria show different IgG and IgA profiles against *Helicobacter pylori* antigens

Western blot analysis was performed in 33 *H. pylori* positive patients with urticaria (subgroup 1) and in the control group. In subgroup 2, the low specific IgG or IgA (0–10 IU/l) did not establish measurable values in Western blot analysis, accordingly these patients were not

Table 1. The titers of *Helicobacter pylori*-specific IgG and IgA in patients with and without chronic urticaria

	Subgroup 1 (chronic urticaria with <i>H. pylori</i> infection)	Subgroup 2 (chronic urticaria without <i>H. pylori</i> infection)	Control group (<i>H. pylori</i> -associated gastritis without urticaria)
Number of patients	33	23	33
Histologic score P^*	1.3	0	3.8
<i>H. pylori</i> -specific IgG			
Number of patients ≥ 10 IU/l	33 (100%)	0	33 (100%)
Median P^{**} , P_+	48.0 IU/l	3.6 IU/l	76.2 IU/l
IQ range	23.8–116.6 IU/l	0–5.9 IU/l	41.3–112.6 IU/l
<i>H. pylori</i> -specific IgA			
Number of patients ≥ 10 IU/l	13 (39.4%)	0	28 (84.8%)
Median P_+ , $P_+^\dagger$	11.9 IU/l	2.4 IU/l	16.5 IU/l
IQ range	3.8–22.7 IU/l	0–4.0 IU/l	8.0–22.5 IU/l

* $P = 0.001$ between subgroup 1 and control group.

** $P < 0.0001$ between subgroup 1 and subgroup 2.

+ $P = 0.0128$ between subgroup 1 and the control group, compared by the Mann–Whitney test.

† $P = 0.0035$ between subgroup 1 and subgroup 2.

†† $P < 0.0001$ between subgroup 1 and the control group, compared by the Mann–Whitney test.

Table 2. Western blot analysis of the IgG profile against *Helicobacter pylori* in chronic urticaria and control group

<i>H. pylori</i> antigens	Subgroup 1 (chronic urticaria with <i>H. pylori</i> infection) (<i>n</i> = 33)	Control group (<i>H. pylori</i> -associated gastritis without urticaria) (<i>n</i> = 33)
120 kDa	28† (84.8%)	33† (100%)
87 kDa	24 (72.7%)	28 (84.8%)
67 kDa	26 (78.8%)	27 (81.8%)
62 kDa	12 (36.4%)	21 (63.6%)
58 kDa	33 (100%)	33 (100%)
54 kDa	27 (81.8%)	22 (66.7%)
47 kDa	15 (45.5%)	19 (57.6%)
44 kDa	19 (57.6%)	18 (54.5%)
33 kDa	20 (60.6%)	12 (36.4%)
29 kDa	23 (69.7%)	20 (60.6%)
28 kDa	31 (93.9%)	32 (96.9%)
25 kDa	26 (78.8%)	22 (66.7%)
19 kDa	31 (93.9%)*	7 (21.2%)

* $P < 0.0001$ compared to the control group, Fisher's test.

† Number of positive patients.

included in the Western blot study. We observed a surprisingly higher prevalence of anti-lpp20 IgG antibodies (93.9%) in subgroup 1 than in the control group (21.2%, $P < 0.0001$) (Table 2). Immunoglobulin G levels to other *H. pylori* antigens showed no significant difference between these two groups (Fig. 1).

Immunoblotting for IgA antibodies against *H. pylori* antigen determinants was performed in 13 *H. pylori*-positive patients with chronic urticaria (in all patients in subgroup 1 with elevated *H. pylori*-specific IgA) and in 16 control patients. There was a significant difference in the prevalence of anti-lpp20 IgA antibodies between the urticarial and the nonurticarial patients (46.1 vs 6.3%, $P = 0.0029$) (Table 3). There were no significant differences between the two groups regarding the prevalence of other anti-*H. pylori* IgA antibodies.

Discussion

There is evidence that chronic urticaria can be of autoimmune origin in 30–50% of the cases (2, 16, 17). Immunoglobulin G autoantibodies directed against FcεRIα occur in 23–39% of patients with chronic urticaria. The anti-FcεRIα autoantibodies in chronic urticaria are related predominantly to the complement fixing subtypes IgG1 and IgG3 (18). Among other factors, complement activation is of pathogenic relevance in chronic urticaria (19). In this context, there is growing evidence that the phenomenon of parasite–host mimicry may initiate or maintain autoimmunity (20). A great number of cross-reacting (auto)antibodies may contribute to the pathogenesis of the stomach and duodenal alterations caused by *H. pylori* (21). Various findings have previously been published suggesting an association between *H. pylori* infection and some extradigestive

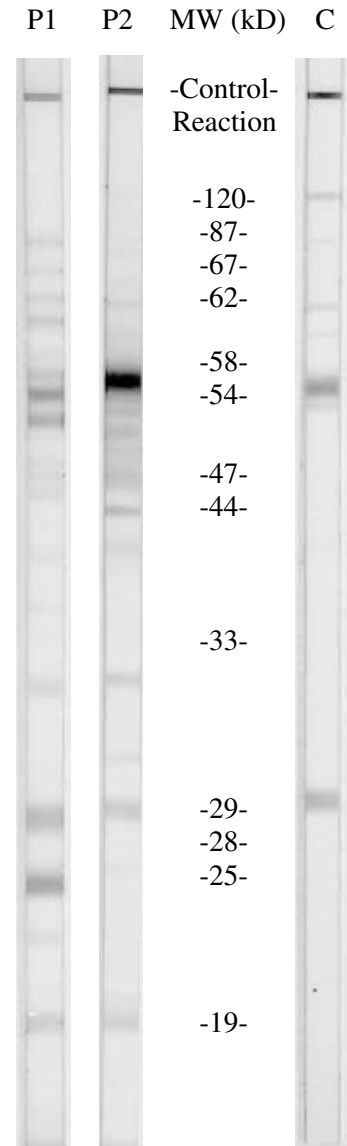


Figure 1. IgG Western blot result. P1, P2 are chronic urticaria patients infected with *Helicobacter pylori* and C is a patient from control group.

ailments such as Sjögren's syndrome (22), scleroderma (23), Henoch-Schönlein purpura (24) and thyroiditis (25). It is of special interest that in autoimmune thyroiditis there seems to be a preferential prevalence of chronic urticaria (26–28).

In this study, we pointed out a characteristic difference in humoral immunoreactivity to *H. pylori*-associated lpps20 between *H. pylori*-infected patients with chronic urticaria, and *H. pylori*-positive patients without chronic urticaria. As to the other *H. pylori*-associated antigen moieties, we found practically identical antibody patterns both in patients with and without urticaria.

The 19-kDa band represents lipoprotein 20, a conserved *H. pylori*-associated lipoprotein that contains

Table 3. Western blot analysis of IgA profile against *Helicobacter pylori* in chronic urticaria and in the control group

<i>H. pylori</i> antigens	Subgroup 1 (chronic urticaria with <i>H. pylori</i> infection) (n = 13)	Control group (<i>H. pylori</i> -associated gastritis without urticaria) (n = 16)
120 kDa	9† (69.2%)	12† (75%)
87 kDa	8 (61.5%)	10 (62.5%)
67 kDa	5 (38.5%)	8 (50.0%)
62 kDa	12 (92.5%)	15 (93.8%)
58 kDa	11 (84.6%)	15 (93.8%)
54 kDa	6 (46.2%)	6 (37.5%)
47 kDa	3 (23.1%)	4 (25.0%)
44 kDa	4 (30.7%)	4 (25.0%)
33 kDa	1 (7.7%)	2 (12.5%)
29 kDa	5 (38.5%)	5 (31.3%)
28 kDa	9 (69.2%)	7 (53.8%)
25 kDa	2 (15.4%)	3 (18.7%)
19 kDa	6 (46.1%)*	1 (6.3%)

* $P < 0.0029$ compared to the control group, Fisher's test.

† Number of positive patients.

a classical lipoprotein signal sequence (29). Like many other Gram-negative bacteria, in some special conditions *H. pylori* may shed part of its highly antigenic outer membrane as vesicles, resulting in portions of the membrane blebbing off the surface of growing cells (30). They could then be released into the extracellular space and enter the gastric mucosa. This phenomenon may have important functional consequences, including a potential role in inciting a gastric mucosal response (31, 32). Bacterial lipoproteins are not only targets of immune response, but also act as immunostimulatory molecules (33). They may play a role in the pathogenesis of several

autoimmune diseases, e.g. rheumatoid arthritis, systemic lupus erythematosus (34). Furthermore, the 19-kDa lpp20 may act as a protective antigen, but this protection depends on the magnitude and subclass of the response; for example, an IgG1 subclass monoclonal antibody raised against *H. pylori* lpp20 can reduce or even prevent *H. pylori* colonization (35). This protective antibody response may have a functional role in prevention or mitigation of gastritis associated with *H. pylori* infection.

Our urticaria patients had no dyspeptic symptoms in their history and over the observation period and only a mild gastritis was seen on endoscopy. This phenomenon may lie in the protective effect of anti-lpp20 IgG antibody. On the other hand, people harboring *H. pylori* with zero or low levels of anti-lpp20 may develop a severe gastritis with overt dyspeptic symptoms as it has been observed in our selected dyspeptic control group.

Our findings suggest that IgG and partly IgA antibodies to *H. pylori* associated lpp20 – in addition to their putative gastroprotective effect – could act as a source of autoimmunity, and may play a role in the pathogenesis of a subtype of chronic urticaria supposedly via cross-reactivity between the bacterial lpp20 and some skin antigen components. Further investigation is needed to establish cross-reactivity between lpp20 and skin antigens.

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