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ORIGINAL ARTICLE

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Disparity between mucosal and serum IgA and IgG in *Helicobacter pylori* infection

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Abstract Mucosal IgA and IgG are involved in the immune defense against *Helicobacter pylori* in infected patients. In contrast to IgG, IgA is transported into the gastric lumen and is responsible for the first-line defense. Therefore antigens recognized by mucosal IgA are possible candidates for vaccination. This study compared the IgA and IgG immune response to *H. pylori* in the gastric mucosa and that in the serum of 21 patients with *H. pylori* gastritis by the immunoblotting technique. In particular, mucosal IgA immune response against the urease antigen of *H. pylori* was studied in detail, as vaccination with this antigen was not curative in men. The results show that mucosal IgA was not represented by serum IgA and IgG, and that the *H. pylori* specific mucosal IgA and IgG immune responses differ in antigen-recognition pattern. This disparity may reflect the different transport ways and functions of these two immunoglobulin isotypes. Furthermore, mucosal IgA specific for urease was found inconsistently in patients with *H. pylori* gastritis. As vaccination antigens should induce an appropriate mucosal IgA immune response against *H. pylori*, our findings may have important implications for the selection of antigens for vaccination against *H. pylori*.

Keywords *Helicobacter pylori* · Mucosal immune response · Vaccination · Urease

Introduction

Infection of the gastric mucosa by *Helicobacter pylori* triggers acquisition of the mucosa-associated lymphatic tissue (MALT), which contains plasma cells producing *H. pylori* specific antibodies. Gastric mucosal IgA, which is produced by plasma cells, is transported into the gastric lumen. It is responsible for the first-line defense against *H. pylori* and supports the cell-mediated immunity against the invader. Therefore *H. pylori* antigens recognized by mucosal IgA are possible candidates for vaccination. In contrast to mucosal IgA, mucosal IgG is not transported into the gastric lumen.

Because of the different transport way and function of these two immunoglobulin isotypes mucosal *H. pylori* specific IgA antibodies may differ as well from serum IgA as from mucosal IgG.

The humoral immune response to *H. pylori* has been investigated in many studies by testing specific IgG antibodies in the serum [3, 6, 9, 17, 16, 18, 21, 23]. Other studies have analyzed the mucosal IgA and IgG immune response in *H. pylori* infection [4, 13, 19, 20, 23]. This study for the first time compares the IgA and IgG immune response to *H. pylori* in the mucosa and the in the same patients. As in vaccination studies, urease and its subunits ureB and ureA were not curative in men [14], the mucosal IgA immune response to *H. pylori* urease was investigated in detail.

Our study demonstrated a clearcut disparity between IgA and IgG immune response in both the gastric mucosa and the serum of patients infected with *H. pylori*. Mucosal *H. pylori* specific IgA is not represented by serum IgA or mucosal IgG. Furthermore, mucosal and serum IgA immune response to ureB and ureA of *H. pylori* was inconsistent.

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Material and methods

Patients, gastric specimens, and sera

Gastric mucosa with chronic *H. pylori* gastritis of patients with *H. pylori* associated diseases (2 with gastric ulcer, 7 with gastric adenocarcinoma, 12 with gastric MALT-type lymphoma, mean age 53 years; 8 women, 13 men) was collected from gastrectomy specimens. All patients investigated were from the same ethnic

group (German) and the same geographic area (Germany). According to clinical data, no eradication therapy or chemotherapy was performed. Infection with *H. pylori* was determined by serology using the *H. pylori* western blot kit purchased from Biermann (Bad Nauheim, Germany). On the western blot we determined the 120-kDa protein (cagA gene product), 87-kDa protein (vacA gene product), 67-kDa flagellin protein, and the urease subunits. The exact position of these antigens on the nitrocellulose membrane was determined by the manufacturer. According to the manufacturer's instruction, *H. pylori* serology was positive when three of these antigens were detectable by the serum. Sera from all patients were collected at the time of surgery and stored at –20°C until analysis.

Gastric tissue culture

Specimens were taken separately from different sites of the chronically inflamed antrum and corpus mucosa which was devoid of ulcer or tumor as determined by histological examination. Immediately after surgery the mucosa was dissected from the submucosa and fragmented in small tissue pieces (about 3 mm^3). Intraepithelial and lamina propria lymphocytes were prepared by mincing these small mucosa tissue fragments in RPMI 1640 medium using a pair of scalpel blades. After vigorous vortexing the minced tissue fragments were incubated for 10 min on ice to sediment debris. To exclude that lymphoid cells were lost in tissue fragments debris was examined by histology for residual lymphoid cells. Virtually no lymphoid cells were detected in the debris. The supernatant containing the intraepithelial and lamina propria lymphocytes was centrifuged. Cells were resuspended in RPMI 1640 medium and washed three times to remove possible contamination by small volumes of serum in the tissue. Purified cells (4×10^5/ml) were cultured in RPMI 1640 medium with 10% fetal calf serum and 40 µg gentamicin at 37°C in a humidified 5% carbon dioxide, 95% air incubator. The viability of the isolated cells was tested by Trypan blue staining. After 7 days a viability of the isolated cells between 40% and 60% was found.

Kinetic studies were performed with culture supernatant on days 1, 4, and 7.

From day 1 to day 4 the number of antigens recognized by IgA and IgG increased, as determined by immunoblotting. No qualitative or quantitative changes in the antigen pattern were detected between days 4 and 7, indicating a saturation of the kinetic on day 7. Therefore after 7 days supernatant was collected by centrifugation at 500 *g* for 10 min and stored at –20°C until use.

Immunodetection of *H. pylori* antigens

Detection of *H. pylori* specific mucosal and serum antibodies was performed by immunoblotting using a *H. pylori* western blot kit purchased from Biermann. The exact position of ureB (66 kDa) and ureA (29 kDa) on the nitrocellulose membrane was determined by the manufacturer. Supernatants from the cultured cells were used undiluted. Serum was diluted at 1:50.

Results

H. pylori infection and histology

All patients were infected with *H. pylori* as determined by serology at time of surgery. Additionally, routine biopsy specimens taken shortly before surgery showed colonization of *H. pylori* in tumor-free antrum by histopathology in these patients as determined by histopathology. In all patients a dense chronic inflammatory infiltration typical of *H. pylori* infection was found in the gastric mucosa of the surgical specimens devoid of ulcer or tumor.

Mucosal *H. pylori* specific IgA is not represented by serum IgA or IgG

Mucosal IgA in *H. pylori* gastritis of different sites of inflamed antrum and corpus recognized a broad spectrum of low and high molecular *H. pylori* antigens

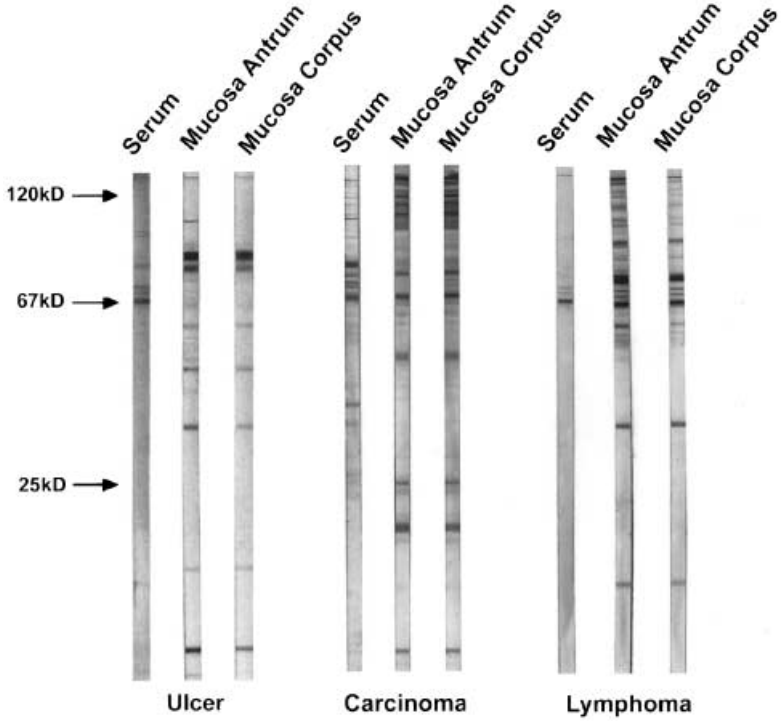

Fig. 1 Representative mucosal and serum IgA immune response to *H. pylori* in *H. pylori* gastritis of patients with gastric ulcer, carcinoma and MALT-type lymphoma. Serum IgA antibodies recognized only a subset of antigens detected by mucosal IgA, whereas the IgA immune response in antrum and corpus was identical

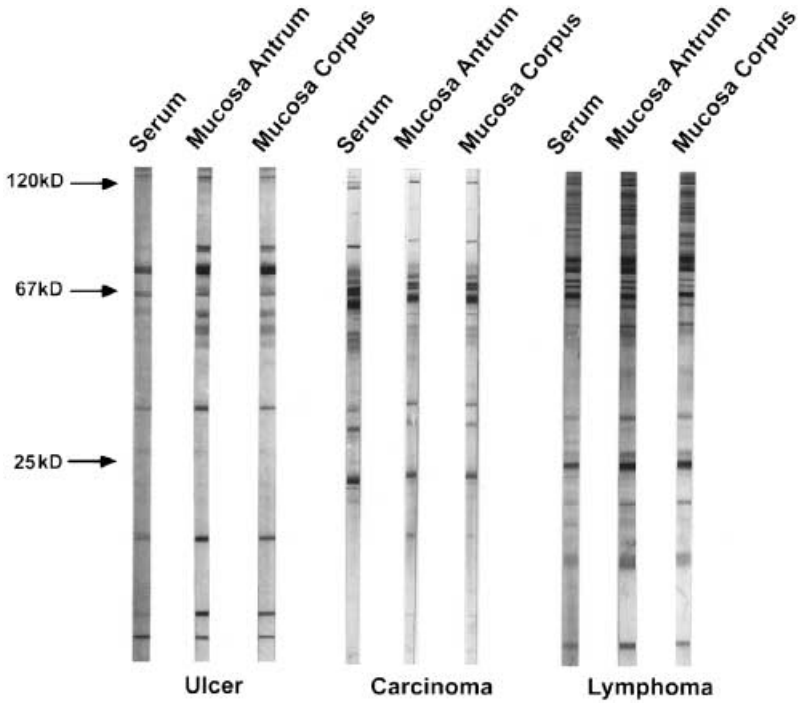

Fig. 2 Representative mucosal and serum IgG immune response to *H. pylori* in the *H. pylori* gastritis of patients with gastric ulcer, carcinoma and MALT-type lymphoma. The IgG immune response in antrum, corpus and serum was identical

(Fig. 1). No significant differences in the mucosal IgA antigen pattern were found between antrum and corpus mucosa. However, serum IgA antibodies of all patients recognized only a subset of antigens detected by mucosal IgA (Fig. 1). There was no correlation between the pattern of antigens recognized by IgA or IgG and the underlying disease (lymphoma, carcinoma, or ulcer). Mucosal IgG recognized a broad spectrum of low and high molecular *H. pylori* antigens. In contrast to IgA, mucosal IgG did not differ from serum IgG (Fig. 2). Comparing humoral immune response in the mucosa and the serum clearcut differences between *H. pylori* specific IgG and IgA between these two compartments were found in all patients (Fig. 3). Its unlikely that differences between mucosal and serum immunoglobulin response to *H. pylori* are due to different immunoglobulin contents in the mucosal preparation, and that the serum as the immunoblot pattern between mucosal IgG and serum IgG are identical (Fig. 2). In conclusion, our data show that mucosal IgA was not represented by serum IgA and IgG, and that the *H. pylori* specific mucosal IgA and IgG immune responses differ in antigen recognition pattern.

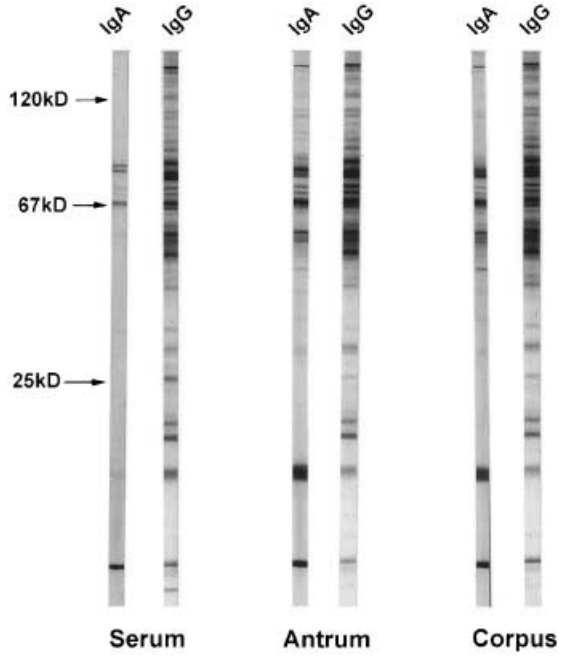

Fig. 3 In example, differences between the IgA and IgG immune response to *H. pylori* in the mucosa (antrum, corpus) and in the corresponding serum are displayed. In the gastric mucosa and the serum the IgA and IgG antibodies recognized different *H. pylori* antigens

Mucosal and serum IgA immune response to ureB and ureA of *H. pylori*

As *H. pylori* urease or its subunits ureA and ureB were used as vaccination antigens, the mucosal IgA immune response to the subunits ureB and ureA was examined. Mucosal IgA antibodies directed to ureB were found in 10 of 21 patients (48%) with *H. pylori* gastritis. UreA specific mucosal IgA antibodies were detected in 14 of 21 of these patients (67%). Serum IgA showed reactivity to ureB in none of the patients and to ureA in only five (Fig. 4, Table 1). Our results demonstrate that mucosal IgA specific for urease was found only in some of the patients with *H. pylori* gastritis.

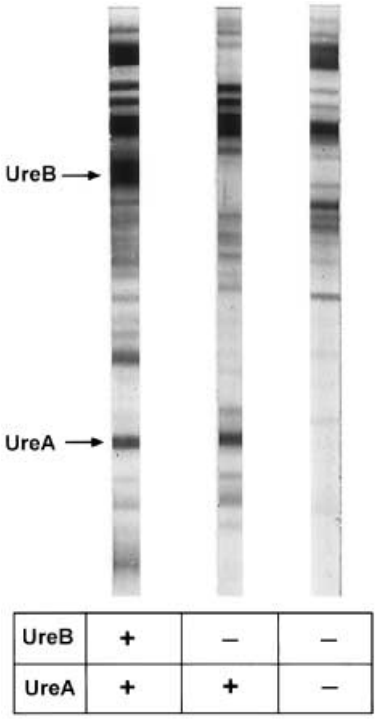

Fig. 4 Mucosal IgA immune response to urease B and urease A illustrated in patients infected by *H. pylori*

Table 1 IgA immune response to urease B and urease A in patients infected by *H. pylori* (n=21)

	Positive	
	n	%
UreB specific IgA		
Mucosa	10	48
Serum	0	0
UreA specific IgA		
Mucosa	15	67
Serum	5	24

Discussion

In *H. pylori* gastritis the mucosal IgA immune response to *H. pylori* was not represented by serum IgA and IgG, which is usually tested by investigating the immune response to *H. pylori*. Therefore testing only serum antibodies provides only limited insight to the humoral mucosal immune response to *H. pylori*.

Mucosal IgA is produced by mucosal plasma cells in a di- or polymeric form linked by the J-chain. Via the J-chain it can be transported directly into the gastric lumen, where it is able to block bacterial adhesion to gastric epithelial cells [15] and to neutralize bacterial toxins. Therefore mucosal IgA plays the major role in the first line defense to *H. pylori*, which inhabits the mucus layer overlying gastric epithelium. To exclude that IgA immunoglobulins produced in the mucosa are not completely secreted into the gastric lumen we investigated IgA in the mucous in two patients and compared it with mucosal IgA. Mucosal IgA and IgA from mucous showed the same antigen pattern (data not shown), indicating that IgA found in the mucosa is secreted into the gastric lumen.

H. pylori specific IgG antibodies are also produced by mucosal plasma cells in *H. pylori* gastritis. IgG triggers the complement cascade and enhances phagocytosis by Fc binding to polymorphonuclear leukocytes and mononuclear cells. This mechanism may amplify the nonspecific immune response in the lamina propria [20]. In contrast to IgA, mucosal IgG cannot be actively secreted into the gastric lumen and is consequently not involved in the first-line defense to *H. pylori*. These functional differences may be reflected by the different IgA and IgG *H. pylori* antigen recognition pattern found in this study.

As IgA antibodies provide the first-line defense in the stomach, antigens used for vaccination should be able to induce an appropriate mucosal IgA immune response against *H. pylori*. *H. pylori* urease or the subunits ureB and ureA were recently used as vaccination antigens. In the mouse model immunization with urease or its subunits protected against *H. pylori* infection or was curative [2, 5, 8, 10, 11, 12]. Vaccination with urease in men has not been curative so far [14].

To our surprise, the mucosal IgA response to ureB or ureA was found in only about one-half of our series. The absence of mucosal urease specific IgA antibodies in many patients may be one reason that vaccination with this antigen was not curative in humans infected by *H. pylori*. Otherwise, in our study patients with longstanding chronic *H. pylori* infection were investigated whose immune response obviously had failed to eradicate the bacterium. Therefore we cannot exclude that the humoral immune response in patients chronically infected by *H. pylori* differed from that which is needed for successful eradication of the bacterium.

Recently it was speculated that the synthesis of mucosal IgA results predominantly from plasma cells of the intestinal mucosa and is transported via serum into the gastric mucosa [1, 7]. As in our study no ureB-specific IgA antibodies were found in the serum, an ureB-specific IgA production in another site of the gastrointestinal tract and a transport via serum to the gastric mucosa seems to be unlikely. In all patients with a serum immune response to ureA, ureA-specific IgA was also produced by local plasma cells in the gastric mucosa. Therefore it is unlikely that IgA directed to *H. pylori* ureA is transported to the gastric mucosa from other sites of the gastrointestinal tract.

In conclusion, our results show that mucosal IgA was not represented by serum IgA and IgG, and that the *H. pylori* specific mucosal IgA and IgG immune response differ in antigen recognition pattern. This disparity may reflect the different functions of these two immunoglobulin isotypes. Moreover, our findings may have important implications for the selection of antigens for vaccination against *H. pylori*. As vaccination antigens should induce a mucosal IgA immune response against

H. pylori, and serum IgA and IgG does not represent mucosal IgA, mucosal IgA antibodies may be more appropriate for detecting vaccination antigens for *H. pylori*.

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