Increased Mucosal Production of Monomeric IgA1 but No IgA1 Protease Activity in *Helicobacter pylori* Gastritis

Audun E. Berstad,* Mogens Kilian,[†]
Kølbjørn N. Valnes,* and Per Brandtzaeg*

From the Laboratory for Immunohistochemistry and Immunopathology,* Institute of Pathology, University of Oslo, The National Hospital, Rikshospitalet, Oslo, Norway; and the Department of Medical Microbiology and Immunology,[†] University of Aarhus, Aarhus, Denmark

Immunoglobulin A and IgM are subjected to epithelial transport only when they are produced as polymers with incorporated J chain. Immunocytes containing various Ig isotypes and associated J chain in gastric mucosa, as well as IgA-degrading protease activity in *Helicobacter pylori* cultures, were examined. Gastric body specimens from 15 *H. pylori*-positive and 14 *H. pylori*-negative patients were studied by paired immunofluorescence for IgA, IgA1, IgA2, IgG, or IgM and concurrent cellular J chain. *H. pylori* isolates were incubated with IgA1 or secretory IgA and examined by immunoelectrophoresis for cleavage products. A substantial increase of Ig-producing cells occurred in chronic gastritis, particularly in the IgA1 isotype, but *H. pylori* was shown to possess neither IgA1-specific nor nonspecific IgA-degrading protease activity. Regardless of infection status, reduced J chain expression was observed for all immunocyte isotypes (except for IgM) in inflamed compared with normal gastric body mucosa, the median positivity for IgA1 cells being reduced to 58.7% versus 87.9% ($P = 0.0002$), and for IgA2 cells to 48.9% versus 87.8% ($P = 0.0002$). This down-regulation of the J chain suggested that a large fraction of IgA monomers is produced in gastritis. (*Am J Pathol* 1999, 155:1097-1104)

Immunological elimination of *Helicobacter pylori* from the stomach is inefficient; thus, infection with this gram-negative bacterium becomes chronic, probably persisting for life in most patients.¹ *H. pylori*-specific IgA and IgG can regularly be detected both systemically²⁻⁴ and at the gastric mucosal level^{3,4} in infected patients, whereas only some patients with chronic gastritis have IgA antibodies (and low levels of IgM antibodies) in their gastric juice.^{3,4} Importantly, despite induction of local immune responses, subsequently treated patients appear to be unprotected against reinfection.⁵ Nevertheless, a role for

secretory immunity in early *H. pylori* colonization has been suggested because sucklings are temporarily protected by specific IgA antibodies present in breast milk.⁶ Also, secretory IgA (SIgA) from colostrum can inhibit attachment of *H. pylori* to human gastric surface epithelium *in vitro*.⁷

Because *H. pylori* remains on the luminal side of the epithelial barrier,⁸ IgA and IgM antibodies produced by immunocytes (B cell blasts and plasma cells) in the lamina propria must be translocated through the epithelium before they can interact with their antigenic target. External transport of polymeric immunoglobulins (plgs) into secretions to provide SIgA and secretory IgM (SIgM) depends on production of J (joining) chain by the mucosal immunocytes. This polypeptide is necessary for appropriate assembly of dimers and larger polymers of IgA (collectively called plgA) and pentameric IgM (plgM) and their binding to epithelial transmembrane secretory component (SC) that functions as polymeric Ig receptor (plgR) by mediating active external plg transport.⁹⁻¹¹ In the normal state, plgA-producing immunocytes preferentially occur at secretory effector sites, whereas monomer producers dominate in tissues lacking glandular elements.¹² Coating of *H. pylori* with IgA in the stomach lumen,¹³ as well as up-regulated epithelial expression of IgA and SC in chronic gastritis,¹⁴ suggest that enhanced plgR-mediated transport of SIgA antibodies takes place across the gastric epithelium in infected patients.

Secretory antibodies of the IgA class are generally relatively resistant to traditional proteases, but IgA1 (including SIgA1) is selectively susceptible to IgA1 proteases. Many mucosal pathogens, including *Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumoniae*, show such specific IgA1-cleaving activity.¹⁵ Other bacterial proteases may attack human IgA nonspecifically and cause extensive molecular degradation.¹⁵ Gastric IgA responses would be severely compromised if *H. pylori* possesses such protease activity.

Supported by The Norwegian Cancer Society, the Research Council of Norway, Anders Jahre's Foundation, and the Danish Medical Research Council.

Accepted for publication July 15, 1999.

Address reprint requests to Audun E. Berstad, M.D., LIIPAT, Institute of Pathology, University of Oslo, Rikshospitalet, N-0027 Oslo, Norway. E-mail: audun.berstad@rh.uio.no.

In this study we examined the J chain-expressing capacity of mucosal immunocytes as a requisite for their pIgA and pIgM production in normal and inflamed gastric body mucosa. We used *in situ* two-color immunofluorescence staining for concomitant localization of cytoplasmic Ig isotype and J chain. Although the J chain does not associate with IgG, its expression by immunocytes of this class was also examined as a putative marker of their derivation from the mucosal *versus* the systemic immune system.^{12,16} Because the gastric B cell system is dominated by the IgA1 isotype,¹⁷ the presence in *H. pylori* cultures of IgA1-specific as well as nonspecific IgA-degrading protease activity was also examined.

Materials and Methods

Tissue Specimens

Specimens of gastric antrum and body mucosa used for immunohistochemical detection of *H. pylori* were fixed routinely in formalin (pH 7.0) overnight or directly in cold 96% ethanol for 24 hours at 4°C before being embedded in paraffin wax.¹⁸ For the study of immunocytes (Ig isotypes and J chain expression), small mucosal samples (approximately 5 mm) from the gastric body were prewashed for 48 hours at 4°C in 0.01 mol/L phosphate-buffered (pH 7.5) isotonic saline (PBS) to extract extracellular diffusible proteins before ethanol fixation and paraffin embedding.¹⁸ All mucosal specimens were collected from areas without macroscopically detectable lesions such as peptic ulcer or tumor. Most of those surgically obtained had been used in an earlier immunohistochemical study¹⁹ and were from seven subjects operated with Billroth II (BII) resection for duodenal or gastric ulcer; two operated for duodenal or gastric neoplasia; three with severe kidney failure and gastritis; and four kidney donors. In addition, biopsy specimens were retrieved endoscopically from non-ulcer patients attending an outpatient clinic for various gastric complaints. Altogether, the subjects included 13 women and 16 men with a median age of 56 years (range, 20–94 years).

Detection of *H. pylori* by Immunohistochemistry and Urease Activity

The *H. pylori* infection status of all patients was determined by immunohistochemistry on sections (5 μ m) of one to four formalin- or directly ethanol-fixed tissue specimens (median $n = 2$) from the antrum and body mucosa (only the latter type of specimen was available from one patient). The presence of *H. pylori* outside the gastric surface epithelium was demonstrated by incubation with purified IgG (33 μ g/ml) from rabbit antiserum against *H. pylori* (DAKO, Glostrup, Denmark) for 20 hours at room temperature. Formalin-fixed sections were first subjected to antigen retrieval by proteolytic digestion (10 g/l trypsin, 10 minutes at 37°C). Fluorescein isothiocyanate (FITC)-conjugated swine anti-rabbit IgG diluted 1:160 (DAKO) was applied for 3 hours as secondary reagent. Purified

rabbit IgG (33 μ g/ml, Nutritional Biochemicals Corp., Cleveland, OH) provided negative control. After being mounted, the tissue sections were examined by fluorescence microscopy (see below).

This indirect immunofluorescence *in situ* method is known to distinguish *H. pylori* from other bacteria present in the stomach and has a sensitivity of 100% and a specificity of 94% compared with cultivation results.²⁰ In an earlier study in our laboratory, the same immunofluorescence method had a sensitivity of 93% and a specificity of 85% compared with the ¹⁴C-urea breath test.^{21, 22} Omission of the primary antibody reagent abolished the staining completely. Tissue sections from routinely formalin-fixed gastric body specimens obtained by endoscopy from two patients, one positive and the other negative for *H. pylori* infection as determined by the ¹⁴C-urea breath test, were used for immunofluorescence performance control. Histological examination of H&E-stained sections revealed chronic active gastritis in the former but normal gastric mucosa in the latter patient.

Fresh gastric tissue specimens from the antrum and body were available from all patients providing endoscopic biopsy specimens. Such samples were tested for the presence of *H. pylori* urease in an urea solution at room temperature for 3 hours with phenol red pH indicator.²³

Immunohistochemistry of Cellular Ig Isotype and J Chain

Serial sections (5 μ m) of prewashed ethanol-fixed tissue specimens from the gastric body were used after dewaxing. One serial section was stained with H&E for histological examination, and the remaining five were pretreated with 6 mol/L urea (pH 3.2) for 1 hour at 4°C to unmask cytoplasmic antigenic determinants of pIg-associated J chain.²⁴ Two such denatured sections were subjected to paired immunofluorescence staining for J chain and IgA1 or IgA2. This protocol included an initial incubation step with a mixture of unlabeled murine monoclonal antibody (ascitic fluid) to human IgA1 (diluted 1:2500) or IgA2 (diluted 1:10,000) and polyclonal (rabbit) IgG (0.04 g/l) anti-human J chain conjugated with tetramethylrhodamine isothiocyanate (TRITC). Thereafter a mixture of the anti-J-chain TRITC conjugate and a rabbit anti-mouse IgG FITC conjugate (0.06 g/l) was applied. The remaining sections were subjected to paired staining for J chain and IgA, IgG, or IgM; rabbit IgG FITC conjugate specific for human IgA, IgG, or IgM mixed with the anti-J-chain TRITC conjugate was applied. The characteristics and working concentrations of the various immunoreagents as well as the reproducibility of the method have been described previously.^{17,25} All incubations took place for 20 hours at room temperature, and the sections were finally washed in PBS and deionized water, air-dried, and mounted in a buffered (pH 8) polyvinyl alcohol medium.

Microscopy and Cell Counting

The H&E-stained tissue sections were graded blind for gastritis by one observer according to the updated Sydney system.²⁶ Inflammation was evaluated by the presence and density of mononuclear cells in the lamina propria and scored on a 4-point scale: 0, absent; 1, mild; 2, moderate; and 3, marked. In our laboratory, two independent observers reported discrepant results with regard to gastric body inflammation in only 8% of the cases when the schematic description of the system was used.²¹

Parallel immunostained sections were examined by the same investigator throughout the study in a Leitz DMR-DXE microscope equipped with a Ploem-type vertical illuminator system (Leica, Wetzlar, Germany) containing interference filter blocks for selective observation of green (FITC) or red (TRITC) emission. The filter blocks could easily be switched, thus facilitating repeated observations of single cells after paired staining. Counting of immunocytes that showed a discernible nucleus within a positive cytoplasm was performed with an $\times 40$ oil immersion objective and an $\times 10$ ocular lens.

The intensity of J chain staining was graded from negative or negligible (0) to moderate (+) and bright (++), with reference to the overall impression of the J chain-positive cells as contrasted against the background fluorescence in each section.²⁴ Only immunocytes with distinct diffuse cytoplasmic red staining were considered as J chain-positive. The enumeration of cells was carried out in a systematic manner throughout each section with an optical grid ($250\ \mu\text{m} \times 250\ \mu\text{m}$) in a $250\text{-}\mu\text{m}$ -high luminal zone and in a basal zone that represented the remaining mucosa, and cell density was expressed per mm^2 mucosal section area. In each zone, more than 100 IgA cells were evaluated for cytoplasmic J chain in every patient on the basis of one to five (median two) tissue specimens from the same location of the body of the stomach. However, a similar number of IgA1, IgA2, IgG, and IgM immunocytes was not always present in the parallel sections, especially in normal mucosa. Six weeks after completion of the study, 30 sections were randomly selected for blind re-evaluation; the coefficient of variation was 20% for immunocyte density and 18% for J chain expression.

For each immunocyte isotype, J chain expression was defined as the percentage of cells positive (+ or ++) for J chain. For photographic documentation of J chain expression, single and double exposures were recorded digitally with a Nikon E-800 fluorescence microscope (Nikon Corporation, Tokyo, Japan) equipped with a Hamamatsu C-5810 3-CCD cooled video camera (Hamamatsu Photonics KK, Hamamatsu-City, Schizuoka-ken, Japan) connected to a personal computer using PhotoShop (Adobe Systems Inc.) and PhotoStation (Interfoto A.S., Høvik, Norway).

Bacterial Strains and Growth Conditions

H. pylori NCTC 11637 (cytotoxin-associated gene A⁺, cagA⁺) was obtained from the National Collection of Type

Cultures (London, England). Eight *H. pylori* isolates (6 cagA⁺, 2 cagA⁻) were cultivated from endoscopic biopsy specimens of eight patients attending an outpatient clinic. Strains were maintained at -70°C in 10% glycerol-heart infusion broth (Difco Laboratories, Detroit, MI) before use. The presence of *H. pylori* was confirmed by growth characteristics, colony morphology, urease, oxidase, and catalase production. Polymerase chain reaction (PCR) for cagA was performed as described.²⁷ Strains were grown on blood plates (tryptose agar base, Oxoid, Basingstoke, UK containing 5% human blood) for 72 hours under microaerobic conditions (Anoxomat, Mart Microbiology BV, Lichtenvoorde, The Netherlands) before being harvested. The clinically isolated strains had been subcultivated less than 10 times on blood agar before the examination of protease activity.

Examination of IgA Protease Activity

A small loopful of *H. pylori* growth on the agar medium was suspended in $40\ \mu\text{l}$ of a solution of purified human myeloma IgA1 or colostral SIgA ($1.5\ \mu\text{g}/\text{ml}$) in $0.05\ \text{mol}/\text{L}$ Tris (pH 7.4) with 0.85% NaCl and incubated overnight at 37°C . One strain of *H. influenzae*, known to produce IgA1 protease, was used as a positive control.²⁸ Solutions of IgA without added bacteria provided negative controls. The reaction mixtures were examined for cleavage by immunoelectrophoresis²⁸ and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by Western blotting. The bands were visualized with affinity-purified rabbit anti-human α -chain (DAKO) followed by alkaline phosphatase-conjugated swine anti-rabbit Ig (DAKO).

Peptide sequences of the serine type IgA1 proteases of *H. influenzae*²⁹ and *N. meningitidis*³⁰ and the metallo-type IgA1 protease of *S. pneumoniae*³¹ IgA1 proteases were retrieved from the SWISSPROT and PIR databases. Search queries were made against predicted coding regions of the entire genome of *H. pylori* strain 26695³² by peptide sequences to the search form of The Institute for Genomic Research (<http://www.tigr.org/>).

Statistical Analysis

Comparisons between or within patient groups were based on median values and the nonparametric Mann-Whitney or Wilcoxon's matched pairs rank sum tests. Two-tailed *P* values smaller than 0.05 were considered statistically significant.

Results

Infection Status and Degree of Pathology

Of the 29 included patients, 15 were deemed to be infected with *H. pylori* as determined by immunofluorescence *in situ* staining. There was agreement between the immunofluorescence and rapid urease tests in 12 of the 13 patients for whom endoscopic biopsies were available; the urease test was negative in one patient with a

small number of immunohistochemically detectable *H. pylori* (therefore regarded infected).

All *H. pylori*-positive subjects had body gastritis, either grade 1 ($n = 5$) or grade 2 ($n = 10$). In the *H. pylori*-negative subjects, the body mucosa was normal ($n = 8$) or showed grade 1 ($n = 5$) or 2 ($n = 1$) gastritis.

Immunocyte Distribution

Immunocytes of all isotypes were clearly visualized by their cytoplasmic fluorescence in prewashed body mucosa and were particularly numerous in the luminal zone between the gastric pits. Only scattered immunocytes occurred basally between the oxyntic glands in histologically normal mucosa, but their number increased in gastritis both at this level and in the luminal zone (Figure 1). Compared with normal mucosa, the median density of IgA- and IgM-producing cells in the luminal zone with grade 2 gastritis was 4.6 and 5.5 times increased, respectively. This increase was dominated by IgA1 cells, the density of which was higher than that of IgA2 cells in every subject. In normal mucosa, the median density of IgA1 and IgA2 cells was 79.3 cells/mm² and 45.6 cells/mm², respectively ($P = 0.01$); in grade 2 gastritis, these figures were 308.6 cells/mm² for IgA1 and 160.0 cells/mm² for IgA2 ($P = 0.0008$). Thus, the relative expansion of immunocytes was 3.9 and 3.5 times for IgA1 and IgA2, respectively, and appeared to be related to the grade of inflammation rather than infection status. The largest relative increase was observed for IgG immunocytes ($\times 16$), the density in grade 2 gastritis being 157.0 cells/mm² compared with 9.9 cells/mm² in normal mucosa.

Compared with normal mucosa, the median density of IgA- and IgM-producing cells of the basal zone in grade 2 inflammation was 12.7 and 6.7 times increased, respectively. The IgA elevation was dominated by IgA1 immunocytes also in this zone (Figure 1).

J-Chain Expression by Mucosal Immunocytes

Concurrent expression of cytoplasmic J chain and Ig isotype was clearly discernible by paired immunofluorescence (Figure 2). Faint double staining was occasionally seen in the extracellular matrix due to incomplete extraction of pIgA and/or pIgM by the prefixation washing process, but this did generally not disturb evaluation of the much brighter cellular staining. Immunocytes of all isotypes (except for IgM) showed reduced J chain expression in gastritis (Figure 3). Thus, the median J chain positivity (+ or ++) for all IgA cells was 50.7% in the luminal zone with grade 1 or 2 gastritis versus 92.3% in histologically normal mucosa ($P < 0.0001$). The corresponding figures were 58.7% versus 87.8% ($P = 0.0002$) for IgA1 cells, 48.9% versus 87.8% ($P = 0.0002$) for IgA2 cells, and 36.6% versus 87.4% ($P < 0.0001$) for IgG cells. No significant difference was demonstrated for IgM cells (95.0% versus 100%, $P = 0.1$). From grade 1 to grade 2 gastritis, only a small additional decrease occurred for all immunocyte subsets (except for IgM that remained high). Notably, reduced J chain expression was observed in the

Figure 1. Box and whiskers diagrams depicting numbers of mucosal Ig-producing cells/mm² of gastric body tissue section in relation to grade of inflammation as indicated (n = number of subjects). Results from a 250- μ m high mucosal luminal (A) and the remaining basal (B) zone are given as median and observed range (boxes indicate 25 to 75 percentiles).

six uninfected subjects with gastritis. Thus, their median J chain expression (+ or ++) of all IgA cells in the luminal zone was 66.1% versus 92.3% in those with normal mucosa ($P = 0.003$). The corresponding figures were 68.7% versus 87.9% ($P = 0.001$) for IgA1 cells, 62.1% versus 87.8% ($P = 0.01$) for IgA2 cells, and 45.0% versus 87.4% ($P = 0.001$) for IgG cells. Again, no significant difference was demonstrated for IgM cells (93% versus 100%, $P = 0.2$).

Similar gastritis-associated reduction of J chain positivity was noted in the basal mucosal zone. In subjects with grade 1 or 2 gastritis, the median J chain expression

Figure 2. Two-color immunofluorescence staining for IgA or IgG (FITC, green) in section of prewashed ethanol-fixed body mucosa from patient with *H. pylori* gastritis (A-C) and uninfected subject (D). Selective separation of red emission (TRITC) shows all J chain-expressing cells (A), whereas green emission shows all IgA-producing cells (B). One IgA-producing cell that does not express J chain appears purely green (arrow), whereas cells with both IgA and J chain appear variably yellow (arrowhead) in double exposure of the same field (C). One IgG-producing cell (FITC, green) that is negative for J chain (TRITC, red) appears purely green (arrow), whereas one cell with both IgG and J chain appears yellow (arrowhead) in double exposure of the same field (D) in section of prewashed ethanol-fixed body mucosa from an uninfected subject. Original magnification, $\times 400$.

of IgA cells was 56.8% versus 91.0% in normal mucosa ($P < 0.0001$); the corresponding figures were 68.3% versus 92.2% ($P = 0.0002$) for IgA1 cells, 64.3% versus 89.4% ($P = 0.002$) for IgA2 cells, and 43.6% versus 86.0% ($P < 0.0001$) for IgG cells. The six uninfected subjects with gastritis were all affected by J chain reduction.

IgA Protease Activity

Cleavage of IgA1 was clearly demonstrated by a positive control bacterium (Figure 4), whereas none of the nine *H. pylori* strains induced detectable specific or nonspecific degradation of IgA1 or SIgA. Homology search with published peptide sequences of IgA1 proteases identified 17.5% identity in 309 peptides of *H. influenzae* IgA1 protease and HP0887 (vacuolating cytotoxin), and 34% identity in a 32-peptide overlap of *N. meningitidis* and HP0922 (toxin-like outer membrane protein). Otherwise, no or only low percentage of identity or similarity with *H. pylori* open reading frames or entire genome was detected.

Discussion

This study demonstrated for the first time that mucosal immunocytes show markedly reduced J chain expression in chronic gastritis, regardless of the presence or absence of *H. pylori* infection. Incorporation of J chain into pIgA and pIgM provides a binding site necessary for noncovalent interaction of these polymers with the pIgR to provide SIgA and SIgM.¹¹ Thus, only J chain-positive IgA and IgM immunocytes can contribute to secretory immunity.^{9,16} Reduced J chain expression in gastritis suggested that a large proportion of the IgA immunocytes are chiefly producers of monomers in contrast to the normal situation. A high level of J chain expression is a characteristic previously recognized for immunocytes (regardless of isotype) present in various other normal secretory effector tissues, including the intestine.^{12,16}

Our observation probably reflected an influx from the systemic immune system of relatively mature B cell memory clones with down-regulated J chain.^{12,16} This accords with the recent report that *H. pylori*-specific IgA in gastric juice is mainly of the monomeric form, whereas

A

B

Figure 3. Box and whiskers diagrams depicting cytoplasmic J chain expression in gastric body mucosa related to Ig isotype of immunocytes and grade of inflammation as indicated (n = number of subjects). Results from a 250- μ m high mucosal luminal (A) and the remaining basal (B) zone are given as median and observed range (boxes indicate 25 to 75 percentiles).

total IgA is predominantly bound to SC, thus being SIgA.³³ Most likely, inflammatory up-regulation of intercellular adhesion molecule 1 (ICAM-1 or CD54),³⁴ and perhaps other endothelial receptors, results in less restricted extravasation of immune cells. The J chain is not incorporated into IgG and accumulates for degradation

Figure 4. IgA1 protease activity of *H. pylori* strains examined by immunoelectrophoresis with polyclonal antibody against the Fc portion of IgA. Well a shows negative control, ie, intact IgA1 preparation without bacteria; well b shows positive control, ie, IgA1 preparation after incubation with *H. influenzae* strain producing IgA1-specific protease (note cleaved fragment, arrowhead). Remaining wells (c–e) show IgA1 incubated with representative isolates of *H. pylori* (note no evidence of IgA cleavage products). Anode is at the top.

in IgG immunocytes.⁹ Therefore, depletion of cellular J chain in gastritis by increased output appears unlikely because J chain expression was reduced also in IgG immunocytes. Surprisingly, however, J chain expression was unaltered in IgM-producing cells. Unfortunately, not much is known about the regulation of J chain, but its high level in IgM immunocytes even in gastritis suggested that they represent relatively early memory clones.¹⁶

Our laboratory has previously reported that reduced J chain expression is a common feature of mucosal inflammatory diseases and chronic lesions in various exocrine tissues,¹² including the inflamed colon.³⁵ Here we found that the median J chain positivity was 92% for IgA immunocytes in normal gastric body mucosa, but only 50% in gastritis. However, the reduced J chain expression was more than compensated for by a concomitant 4-fold (luminal) to 12-fold (basal) numerical increase of the total IgA immunocyte density. Increased IgA production in chronic gastritis,¹⁹ and enhanced epithelial transport of pIgA,¹⁴ have been demonstrated in earlier immunohistochemical studies from this laboratory. Altogether, therefore, the overall generation of SIgA appears to be elevated in gastritis, a response that involves many more IgA1 than IgA2 immunocytes. This accords with the predominant IgA1 production in normal gastric mucosa as shown both here and earlier.¹⁷ The same is true for the proximal small intestine.¹⁷ Furthermore, *H. pylori* IgA antibodies have been detected mainly within this subclass, both in serum and in homogenized endoscopic gastric biopsy specimens.³⁶ Notably, contamination by serum antibodies could have affected the mucosal results of the latter study, but such diffusible IgA was efficiently removed by extensive prewashing of our tissue specimens.

IgA1 is highly susceptible to a specific group of bacterial proteases that may enable the bacteria to evade secretory immunity.¹⁵ Because SIgA1 predominates in breast milk and saliva, representing 85% of salivary IgA in infancy,³⁷ IgA1-specific proteases might facilitate early colonization of *H. pylori*. Its vacuolizing cytotoxin precursor shows structural organization resembling the IgA protease type of exoprotein produced by pathogenic *Neisseriae* and *Haemophilus* spp.³⁸ Moreover, *H. pylori*

produces a metalloprotease that may be involved in degradation of host proteins.³⁹ However, in our *in vitro* test system, *H. pylori* did not show IgA1-specific or nonspecific protease activity that could degrade IgA1 in its monomeric (serum) or secretory form. Although a search of the *H. pylori* genome confirmed some structural similarity between IgA1 proteases and vacuolating cytotoxin, our *in vitro* findings were supported by no or (only) low identity of *H. pylori* genes with sequenced IgA1 proteases.

The secretory immune system appears unable to eradicate *H. pylori*, perhaps because little or no SIgA antibodies are elicited against this bacterium.³³ Local IgA responses may nevertheless be of importance in restricting the severity of inflammation.⁴⁰ Constituents of *H. pylori* such as urease, can penetrate into the lamina propria mucosa⁴¹; if *H. pylori* toxins and/or antigens are retained there, chronic gastritis might be caused by IgG and IgM antibodies. However, this proinflammatory development could be dampened by corresponding IgA antibodies that do not activate complement.⁴² It is interesting that patients lacking IgA (selective IgA deficiency or hypogammaglobulinemia) relatively often develop gastric atrophy and show a markedly increased risk for gastric malignancies.^{43,44} In addition, SIgA may play a protective role against *H. pylori* colonization^{6,7} in early childhood or after therapeutic eradication, a possibility supported by results obtained by active or passive (IgA) local vaccines in experimental animals.^{45,46} However, the role of antibodies has been questioned by recent vaccination results obtained in B cell knockout mice.⁴⁷

In conclusion, reduced J chain expression in gastritis suggested a shift from local production of pIgA to monomeric IgA. The negative consequence of this alteration for the generation of SIgA in the stomach appeared to be more than compensated for by a marked increase of the total IgA cell population in chronic gastritis. In view of the mucosal dominance of IgA1-producing cells, it was interesting to note that *H. pylori* did not possess IgA-degrading protease activity.

Acknowledgments

The excellent technical assistance of the LIIPAT laboratory staff is gratefully acknowledged. We thank Finn-Eirik Johansen for help with the *H. pylori* genome search. The cost of the color plate was kindly borne by AstraZeneca.

References

- Blaser MJ, Parsonnet J: Parasitism by the "slow" bacterium *Helicobacter pylori* leads to altered gastric homeostasis and neoplasia. *J Clin Invest* 1994, 94:4–8
- Perez-Perez GI, Dworkin BM, Chodos JE, Blaser MJ: *Campylobacter pylori* antibodies in humans. *Ann Intern Med* 1988, 109:11–17
- Rathbone BJ, Wyatt JI, Worsley BW, Shires SE, Trejdosiewicz LK, Heatley RV, Losowsky MS: Systemic and local antibody responses to gastric *Campylobacter pyloridis* in non-ulcer dyspepsia. *Gut* 1986, 27:642–647
- Veenendaal RA, Schroijen JM, Götz JM, Peña AS, Roosendaal R, Veselic M, Lamers CBHW: Salivary, systemic, gastric juice, and gastric mucosal IgA and IgG *Helicobacter pylori* antibodies in patients with active chronic *Helicobacter pylori* associated antral and pangastritis. Dissertation. Leiden, University of Leiden, 1994, pp 61–77
- Borody T, Andrews P, Mancuso N, Jankiewicz E, Brandl S: *Helicobacter pylori* reinfection 4 years post-eradication (letter). *Lancet* 1992, 339:1295
- Thomas JE, Austin S, Dale A, McClean P, Harding M, Coward WA, Weaver LT: Protection by human milk IgA against *Helicobacter pylori* infection in infancy. *Lancet* 1993, 342:121
- Falk P, Roth KA, Borén T, Westblom TU, Gordon JI, Normark S: An *in vitro* adherence assay reveals that *Helicobacter pylori* exhibits cell lineage-specific tropism in the human gastric epithelium. *Proc Natl Acad Sci USA* 1993, 90:2035–2039
- Hessey SJ, Spencer J, Wyatt JI, Sobala G, Rathbone BJ, Axon ATR, Dixon MF: Bacterial adhesion and disease activity in *Helicobacter* associated chronic gastritis. *Gut* 1990, 31:134–138
- Brandtzaeg P: Molecular and cellular aspects of the secretory immunoglobulin system. *APMIS* 1995, 103:1–19
- Mestecky J, Zikan J, Butler WT: Immunoglobulin M and secretory immunoglobulin A: presence of a common polypeptide chain different from light chains. *Science* 1971, 171:1163–1165
- Brandtzaeg P, Prydz H: Direct evidence for an integrated function of J chain and secretory component in epithelial transport of immunoglobulins. *Nature* 1984, 311:71–73
- Brandtzaeg P, Korsrud FR: Significance of different J chain profiles in human tissues: generation of IgA and IgM with binding site for secretory component is related to the J chain expressing capacity of the total local immunocyte population, including IgG and IgD producing cells, and depends on the clinical state of the tissue. *Clin Exp Immunol* 1984, 58:709–718
- Wyatt JI, Rathbone BJ, Heatley RV: Local immune response to gastric *Campylobacter* in non-ulcer dyspepsia. *J Clin Pathol* 1986, 39:863–870
- Valnes K, Brandtzaeg P, Elgjo K, Stave R: Specific and nonspecific humoral defense factors in the epithelium of normal and inflamed gastric mucosa: immunohistochemical localization of immunoglobulins, secretory component, lysozyme, and lactoferrin. *Gastroenterology* 1984, 86:402–412
- Kilian M, Reinholdt J, Lomholt H, Poulsen K, Frandsen EV: Biological significance of IgA1 proteases in bacterial colonization and pathogenesis: critical evaluation of experimental evidence. *APMIS* 1996, 104:321–338
- Brandtzaeg P, Baekkevold ES, Farstad IN, Jahnsen FL, Johansen F-E, Nilsen EM, Yamanaka T: Regional specialization in the mucosal immune system: what happens in the microcompartments? *Immunol Today* 1999, 20:141–151
- Kett K, Brandtzaeg P, Radl J, Haajman JJ: Different subclass distribution of IgA-producing cells in human lymphoid organs and various secretory tissues. *J Immunol* 1986, 136:3631–3635
- Brandtzaeg P: Mucosal and glandular distribution of immunoglobulin components. *Immunohistochemistry with a cold ethanol-fixation technique*. *Immunology* 1974, 26:1101–1114
- Valnes K, Brandtzaeg P, Elgjo K, Stave R: Quantitative distribution of immunoglobulin-producing cells in gastric mucosa: relation to chronic gastritis and glandular atrophy. *Gut* 1986, 27:505–514
- Andersen LP, Holck S, Povlsen CO: *Campylobacter pylori* detected by indirect immunohistochemical technique. *APMIS* 1988, 96:559–564
- Berstad AE, Hatlebakk JG, Maartmann-Moe H, Berstad A, Brandtzaeg P: *Helicobacter pylori* gastritis and epithelial cell proliferation in patients with reflux oesophagitis after treatment with lansoprazole. *Gut* 1997, 41:740–747
- Berstad K, Wilhelmsen I, Berstad A: Biometric evaluation of gastric urease activity in man. *Scand J Gastroenterol* 1992, 27:977–983
- Ruiz B, Janney A, Diavolitsis S, Correa P: One-minute test for *Campylobacter pylori* (letter). *Am J Gastroenterol* 1989, 84:202
- Brandtzaeg P: Immunohistochemical characterization of intracellular J-chain and binding site for secretory component (SC) in human immunoglobulin (Ig)-producing cells. *Mol Immunol* 1983, 20:941–966
- Brandtzaeg P: Prolonged incubation time in immunohistochemistry: effects on fluorescence staining of immunoglobulins and epithelial components in ethanol- and formaldehyde-fixed paraffin-embedded tissues. *J Histochem Cytochem* 1981, 29:1302–1315
- Dixon MF, Genta RM, Yardley JH, Correa P, Batts KP, Dahms BB, Filipe MI, Haggitt RC, Haot J, Hui PK, Lechago J, Lewin K, Offerhaus JA, Price AB, Riddell RH, Sipponen P, Solcia E, Watanabe H: Clas-

- sification and grading of gastritis—the updated Sydney system. *Am J Surg Pathol* 1996, 20:1161–1181
27. Peek RM, Jr., Miller GG, Tham KT, Perez-Perez GI, Zhao X, Atherton JC, Blaser MJ: Heightened inflammatory response and cytokine expression *in vivo* to *cagA*+ *Helicobacter pylori* strains. *Lab Invest* 1995, 73:760–770
28. Mestecky J, Kilian M: Immunoglobulin A (IgA). *Methods Enzymol* 1985, 116:37–75
29. Poulsen K, Reinholdt J, Kilian M: A comparative genetic study of serologically distinct *Haemophilus influenzae* type 1 immunoglobulin A1 proteases. *J Bacteriol* 1992, 174:2913–2921
30. Lomholt H, Poulsen K, Kilian M: Comparative characterization of the *iga* gene encoding IgA1 protease in *Neisseria meningitidis*, *Neisseria gonorrhoeae* and *Haemophilus influenzae*. *Mol Microbiol* 1995, 15:495–506
31. Poulsen K, Reinholdt J, Kilian M: Characterization of the *Streptococcus pneumoniae* immunoglobulin A1 protease gene (*iga*) and its translation product. *Infect Immun* 1996, 64:3957–3966
32. Tomb J-F, White O, Kerlavage AR, Clayton RA, Sutton GG, Fleischmann RD, Ketchum KA, Klenk HP, Gill S, Dougherty BA, Nelson K, Quackenbush J, Zhou LX, Kirkness EF, Peterson S, Loftus B, Richardson D, Dodson R, Khalak HG, Glodek A, McKenney K, Fitzgerald LM, Lee N, Adams MD, Hickey EK, Berg DE, Gocayne JD, Utterback TR, Peterson JD, Kelley JM, Cotton MD, Weidman JM, Fujii C, Bowman C, Watthey L, Wallin E, Hayes WS, Borodovsky M, Karp PD, Smith HO, Fraser CM, Venter JC: The complete genome sequence of the gastric pathogen *Helicobacter pylori*. *Nature* 1997, 388:539–547
33. Birkholz S, Schneider T, Knipp U, Stallmach A, Zeitz M: Decreased *Helicobacter pylori*-specific gastric secretory IgA antibodies in infected patients. *Digestion* 1998, 59:638–645
34. Hatz RA, Rieder G, Stolte M, Bayerdörffer E, Meimarakis G, Schildberg F-W, Enders G: Pattern of adhesion molecule expression on vascular endothelium in *Helicobacter pylori*-associated antral gastritis. *Gastroenterology* 1997, 112:1908–1919
35. Kett K, Brandtzaeg P, Fausa O: J-chain expression is more prominent in immunoglobulin A2 than in immunoglobulin A1 colonic immunocytes and is decreased in both subclasses associated with inflammatory bowel disease. *Gastroenterology* 1988, 94:1419–1425
36. Van der Est MMC, Veenendaal RA, Peña AS, Kuiper I, Lamers CBHW: Local immunoglobulin A subclass alteration in the gastric mucosa of *Helicobacter pylori*-infected patients. *Helicobacter pylori* and Gastrointestinal Pathology. Edited by JM Pajares, AS Peña, and P Malfertheimer. Berlin, Springer-Verlag, 1993, pp 170–176
37. Fitzsimmons SP, Evans MK, Pearce CL, Sheridan MJ, Wientzen R, Cole MF: Immunoglobulin A subclasses in infants' saliva and in saliva and milk from their mothers. *J Pediatr* 1994, 124:566–573
38. Schmitt W, Haas R: Genetic analysis of the *Helicobacter pylori* vacuolating cytotoxin: structural similarities with the IgA protease type of exported protein. *Mol Microbiol* 1994, 12:307–319
39. Windle HJ, Kelleher D: Identification and characterization of a metalloprotease activity from *Helicobacter pylori*. *Infect Immun* 1997, 65:3132–3137
40. Watanabe T, Goto H, Arisawa T, Hase S, Niwa Y, Hayakawa T, Asai J: Relationship between local immune response to *Helicobacter pylori* and the diversity of disease: investigation of *H. pylori*-specific IgA in gastric juice. *J Gastroenterol Hepatol* 1997, 12:660–665
41. Mai UE, Perez-Perez GI, Allen JB, Wahl SM, Blaser MJ, Smith PD: Surface proteins from *Helicobacter pylori* exhibit chemotactic activity for human leukocytes and are present in gastric mucosa. *J Exp Med* 1992, 175:517–525
42. Russell MW, Reinholdt J, Kilian M: Anti-inflammatory activity of human IgA antibodies and their Fab alpha fragments: inhibition of IgG-mediated complement activation. *Eur J Immunol* 1989, 19:2243–2249
43. Schaffer FM, Monteiro RC, Volanakis JE, Cooper MD: IgA deficiency. *Immunodeficiency Rev* 1991, 3:15–44
44. Kinlen LJ, Webster AD, Bird AG, Haile R, Peto J, Soothill JF, Thompson RA: Prospective study of cancer in patients with hypogammaglobulinaemia. *Lancet* 1985, 1:263–266
45. Suerbaum S, Josenhans C: Virulence factors of *Helicobacter pylori*: implications for vaccine development. *Mol Med Today* 1999, 5:32–39
46. Czinn SJ, Cai A, Nedrud JG: Protection of germ-free mice from infection by *Helicobacter felis* after active oral or passive IgA immunization. *Vaccine* 1993, 11:637–642
47. Ermak TH, Giannasca PJ, Nichols R, Myers GA, Nedrud J, Weltzin R, Lee CK, Kleanthous H, Monath TP: Immunization of mice with urease vaccine affords protection against *Helicobacter pylori* infection in the absence of antibodies and is mediated by MHC class II-restricted responses. *J Exp Med* 1998, 188:2277–2288