Epithelium related deposition of activated complement in *Helicobacter pylori* associated gastritis

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Abstract

Background and Aims—It is unknown whether *Helicobacter pylori* infection activates complement in vivo. Mucosal deposition of various activation products of the complement system may contribute to the pathogenesis of chronic gastritis and was therefore studied by immunohistochemistry.

Patients and Methods—Ethanol fixed antrum or body gastric tissue sections from 24 patients infected with *H pylori* (determined by bacterial immunohistochemistry) and 22 uninfected patients were examined by immunofluorescence with monoclonal antibodies to activation neopeptides in C3b and in the terminal complex (TCC). As a control group, biopsy samples from the gastric stump of 23 Billroth II operated patients were studied.

Results—Patchy, bright staining for TCC occurred below the surface epithelium and around the glands in *H pylori* positive and negative gastritis as well as in stump gastritis but seldom in normal mucosa. Activated C3 was present at the apical face of the surface epithelium, significantly more often in the antrum and body from patients with than without *H pylori* infection ($p=0.05$ and $p=0.03$ respectively), and particularly in samples with granulocyte infiltration ($p=0.04$). Many bacteria were coated with activated C3 towards the pit openings but seldom within the foveolae.

Conclusions—Local complement activation was shown to take place in simple chronic gastritis, associated as well as unassociated with *H pylori* infection, and also in stump gastritis. The fact that activated C3 was seldom seen on *H pylori* within the foveolae, suggested that the bacterium evades complement attack in this location.

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Helicobacter pylori colonises the gastric epithelium and leads to a chronic inflammatory reaction.¹ It is intriguing that this organism persists despite the induction of both humoral and cellular immunity. *H pylori* specific IgG and IgA antibodies occur in peripheral blood

as well as in the gastric mucosa of infected subjects.^{2,3} The antibodies are probably important in restricting the infection to the mucosal surface but are not sufficient to eliminate the bacteria.⁴ Furthermore, it is unknown to what extent humoral or cell mediated immunopathology is involved in chronic gastritis.

The systemic antibody response to *H pylori* includes all four subclasses of IgG although IgG1 and IgG2 antibodies predominate.⁵ Antral and body IgG and IgA antibodies measured in homogenates of mucosal biopsy specimens, are significantly increased in *H pylori* positive patients – the IgG response being particularly noticeable in those with active chronic pangastritis.⁶ Externally translocated antibodies of the IgA, IgG, and IgM classes bind in vivo to *H pylori* and the frequency of coated bacteria is higher when neutrophils are present in the mucosa than in inactive gastritis.⁷ Furthermore, *H pylori* infection can apparently induce gastric autoantibodies⁸ that to some extent are directed against the gastrin producing cells^{9,10}; antibodies to interleukin 8 (IL 8) have also been reported.¹¹ In addition, *H pylori* seems to share antigenic determinants with foveolar epithelial cells and pyloric glands.¹² The induction of various types of gastric autoantibodies could be one way in which the bacterium causes full thickness lesions in the mucosa.

H pylori specific antibodies of the IgG class are not regularly detected in gastric juice^{2,6} because this class is not actively transported to the gastric lumen.¹³ However, small amounts of IgG can passively diffuse between epithelial cells,¹³ particularly in inflammation where *H pylori* infection can lead to a weakening of the tight junction complexes between surface mucous cells.¹⁴ Because IgG does not combine with secretory component (SC), this antibody class is rapidly degraded in gastric juice.¹⁵

The numbers of mucosal IgA, IgM, and IgG producing plasma cells and blasts increase significantly with increasing degree of gastritis; the IgG class shows the largest relative increase, particularly in the basal part of the mucosa.¹⁶ *H pylori* antigens that cross the stomach epithelium may form subepithelial immune complexes with locally produced antibodies and thereby cause complement activation and inflammation (type III hypersensitivity) as recently discussed by Ernst *et al.*¹⁷ Based on the detection of neopeptides in the terminal complement complex (TCC) and C3b, activated complement has been observed

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locally associated with an increased mucosal IgG response in patients with inflammatory bowel disease,¹⁸ and related to increased concentrations of serum IgG and IgM antibodies to gluten in coeliac disease.¹⁹ In view of the large amounts of IgG produced locally in *H pylori* associated gastritis,¹⁶ it would be of interest to look for complement activation also in this mucosal lesion. Furthermore, in vitro studies have suggested that *H pylori* can activate the classic pathway of complement even in the absence of antibodies.²⁰

In this study we applied monoclonal antibodies (mAbs) against neoepitopes of C3b (early activation) and TCC (late activation) to sections of directly ethanol fixed mucosal tissue samples with or without gastritis. Mechanisms of complement activation were also studied. Such in situ studies have to our knowledge not been performed earlier in gastritis.

Methods

Specimens and tissue preparation

Specimens of gastric antral and body mucosa obtained from nine women and 12 men with a mean age of 63 years (range, 26–81), were fixed directly in cold 96% ethanol for 24 hours at 4°C and then embedded in paraffin wax; or they were washed for 48 hours at 4°C in 0.01 M phosphate buffer (pH 7.5) containing 0.15 M NaCl (phosphate buffered saline, PBS) to extract diffusible proteins before ethanol fixation.²¹ Most of these samples had been used in an earlier immunohistochemical study¹⁶; they were collected surgically from 21 people from the following groups: patients operated with Billroth II (BII) resection for duodenal or gastric ulcer (n=10); patients operated for duodenal neoplasia (n=2); severe kidney failure and gastritis (n=5); and kidney donors (n=4). Two to four additional formalin fixed, paraffin wax embedded mucosal samples from the antrum and body were used for immunohistochemical detection of *H pylori* (see later).

Additional material consisted of endoscopic biopsy specimens, one antrum and one body sample, obtained from each of 25 patients attending an outpatient gastroenterology clinic for various abdominal complaints. These subjects included 13 women and 12 men with a mean age of 47 years (range, 20–79). One specimen from the antrum and one from the corpus were used for the immunohistochemical detection of *H pylori*. A separate antral specimen from each patient was used in the rapid urease test for detection of *H pylori* (see later). Informed consent was obtained from all these subjects, and the project was approved by the Clinical Research Ethics Committee of Health Region II. Neither patients with malignant diseases nor those taking immunosuppressive drugs or having received antimicrobial treatment during the past two months were included. A blood sample was available from 20 of the patients for determination of serum *H pylori* IgG antibodies.

Endoscopic biopsy specimens (n=37) were also obtained from the gastric stump of 23 patients with a mean age of 64 years (range, 51–75) who had been subjected to BII resection for duodenal ulcer 27–32 years earlier (mean, 29.5). The specimens were taken from the lesser and the greater curvature no more than 2 cm beyond the stoma. This group served solely as controls and was not included in the statistical analysis.

All endoscopic biopsy samples were collected from areas without macroscopical affection except for gastritis; they were fixed directly in cold 96% ethanol and embedded in paraffin wax.²¹

Determination of H pylori infection

The *H pylori* infection status of patients in the study group was determined by immunohistochemistry on sections (5 µm) of formalin or ethanol fixed tissue from both the antrum and body mucosa. A rabbit antiserum (1/10) to *H pylori* (DAKO, Glostrup, Denmark) was applied for 20 hours at room temperature. Antigenic retrieval by proteolytic digestion (10 g/l trypsin, 10 min, 37°C) was first performed for formalin fixed sections. The antiserum distinguished *H pylori* from other curved bacteria present in the stomach and had a sensitivity of 100% and a specificity of 94% compared with cultivation results.²² Fluorescein isothiocyanate (FITC) conjugated swine antirabbit IgG (1/160; DAKO) was applied for three hours as secondary reagent. Omission of the primary antiserum provided a negative control. After mounting, the tissue sections were examined by fluorescence microscopy (see later).

To confirm the bacterial immunostaining in the patient group providing fresh endoscopic biopsy specimens from the antrum and body, one antral sample was tested for the presence of *H pylori* urease in a urea solution at room temperature for three hours with phenol red pH indicator.²³ A colour change from yellow to pink was regarded as positive. In the same group of patients, serum IgG antibodies to *H pylori* were tested for by an enzyme immunoassay method (Pyloriset EIA-G, Orion Diagnostica, Espoo, Finland; sensitivity 92%, specificity 84%).²⁴ The serum samples from 20 patients attending the outpatient clinic were examined in duplicate at a dilution of 1/200. If the antibody level was higher than that of reference serum no 2, the result was considered positive for *H pylori* antibodies. The same test was used to screen the infection status of the control group of stump gastritis patients.

Immunohistochemical detection of activated complement

The three step two colour biotin/avidin enhanced immunofluorescence staining procedure used was a slight modification of a previously described method.¹⁸ Serial paraffin wax sections (5 µm) from directly ethanol fixed specimens were dewaxed and incubated for 20 hours with mAb to TCC (Clone aE11, murine

IgG2a; 1 mg/l)²⁵ or to a C3b activation neopeptide in the C3c part of C3b/iC3b (Clone bH6, murine IgG2a; 7.5 mg/l),²⁶ in combination with rabbit antiserum to cytokeratin (1/100)²⁷ or to S protein/vitronectin (1/5000).²⁸ Selected specimens from five patients found to have apical complement deposition of C3b were, in addition, examined with rabbit antiserum to C1q (1/500; DAKO) and C4c (1/500; DAKO) in combination with mAb to C3b. The mAbs and antisera were appropriately diluted in PBS containing bovine serum albumin (12.5 g/l) on the basis of performance testing. Biotinylated horse anti-mouse IgG (2 mg/l; Vector Laboratories, Burlingame, CA) was incubated for three hours, followed by FITC-conjugated swine antirabbit IgG (1/160; DAKO) and Streptavidin Texas red conjugate (1/200; Gibco BRL, Gaithersburg, MD) for 30 minutes.

Nine tissue samples positive for C3b apically on the epithelium, were restained with the three step immunofluorescence method but with antiserum to *H. pylori* (1/10) included instead of antiserum to cytokeratin. By this modification the bacteria appeared green (FITC) and the complement neopeptides red (Texas red). Dewaxed sections of 12 prewashed tissue samples were examined for TCC and C3b. These washed tissue samples had been obtained concomitantly with and from the same areas as those subjected to direct alcohol fixation in which complement deposits were observed.

Staining specificity was ensured by a dissimilar (or negative) decoration pattern obtained with different antibodies applied to serial sections. Omission of the primary antibody also provided a negative control, as did application of an irrelevant mAb (mouse IgG2a, 5 mg/l; Becton Dickinson, CA).

To visualise neutrophilic granulocytes, mAb to human neutrophil elastase (Clone NP57, murine IgG1, 12.6 µg/l; DAKO) was applied in alkaline phosphatase antialkaline phosphatase (APAAP) staining²⁹ to parallel sections. This method also facilitated the grading of intestinal metaplasia, as the epithelial brush border stained bright red because of endogenous alkaline phosphatase.

Fluorescence microscopy

The sections were examined blind by the same investigator using a Leitz DMR-DXE microscope camera equipped with a Ploem-type vertical illuminator system (Leitz, Wetzlar, Germany). A dual filter for the simultaneous observation of red (Texas red) and green (FITC) emission made both colours appear together as yellow. Pictures were recorded on Ektachrome 800/1600 ISO daylight film pushed to 800 ISO. Subepithelial TCC and apical C3b were semiquantitatively scored from no (–) to intense (3) as done earlier.¹⁹ Grade 1 indicated distinct but patchy staining in a few areas, and grade 3 continuous intense staining. Deposits of TCC were scored separately in the foveolar and glandular part of the mucosa. TCC staining of elastic fibrils, muscular tissue, and vessels³⁰ was not recorded.

The same investigator performed the immunofluorescence evaluation throughout the study. Six weeks after the completion of the study, 10 sections were randomly selected for blind re-evaluation.

Grading of gastritis

Parallel tissue sections from each specimen were stained with haematoxylin and eosin and graded for gastritis by one observer according to the Sydney system.³¹ The histological variables were scored on a four point scale: 0, absent; 1, mild; 2, moderate; and 3, severe. Inflammation was examined for the presence and density of mononuclear cells in the lamina propria; activity for the presence and density of neutrophils in the lamina propria as well as in the epithelium; atrophy for the loss of gastric glands; and intestinal metaplasia for the presence of intestinal glandular tissue. Randomly selected sections were graded by two observers with excellent agreement. Grading of gastritis was performed without knowledge of the patients' clinical or infection status.

Statistical analysis

Samples with inflammation were combined, and complement activation was recorded as present or absent before statistical analysis was performed with Fisher's exact test, including the Bonferroni correction for multiple comparisons. Reproducibility of immunofluorescence scores was determined by the weighted κ test.³² The Spearman's rank correlation test was used to evaluate any association between the degree of subepithelial TCC deposition and serum IgG antibody titres to *H. pylori*. A value of $p < 0.05$ was considered significant.

Results

Infection status and degree of pathology in gastritis

Directly ethanol fixed tissue samples from antral (n=43) and body (n=43) mucosa of 46 patients were studied. Of these patients, 24 were considered to be infected with *H. pylori* (52%) as determined by immunofluorescence in situ. The Table lists the results of immunostaining, the rapid urease test, and the serum IgG antibody determination.

All gastric biopsy specimens (n=46) from *H. pylori* positive subjects were inflamed: 24 antral samples showed inflammation grade 1 (n=9), grade 2 (n=13) or grade 3 (n=2); and 22 body samples showed grade 1 (n=14) or grade 2 (n=8). Four antral samples from *H. pylori* negative subjects and 17 from *H. pylori* positive subjects showed epithelial neutrophil infiltration.

Directly ethanol fixed specimens (n=40) from *H. pylori* negative subjects were less inflamed: 19 antral samples were either considered normal (n=12), of grade 1 (n=6), or of grade 2 (n=1); 21 body samples were either considered normal (n=13) or of grade 1 (n=8). One body sample from a *H. pylori*

H pylori status of patients based on in situ immunofluorescence*

Sample category	No of observations	Immunofluorescence		Rapid urease test		H pylori antibodies	
		Positive	Negative	Positive	Negative	Positive	Negative
Archival material	21	12	9				
Outpatient biopsy specimens	5	2	3	2	3		
	20	10	10	8	12	7	13
Total	46	24	22				

*From patients providing archival material, immunofluorescence alone was performed. For five outpatients, immunofluorescence was complemented by the rapid urease test, and there was complete agreement. In 20 outpatients, serology was also performed and this turned out to be the least sensitive test.

negative subject and 11 from *H pylori* positive subjects showed epithelial neutrophil infiltration.

Biopsy samples were available from both antrum and body mucosa in 40 subjects: 10 had predominantly antral gastritis; 18 had pangastritis; and one had predominantly body gastritis. Eleven patients showed no inflammation in either the antral or the body samples.

Deposition of activated complement in gastritis

Bright staining for the TCC neopeptide was found below the epithelium related to the basement zone in 23 of 43 antral (53%) and in 20 of 43 body (47%) samples. These deposits occurred in both *H pylori* positive and negative gastritis. TCC was located in a patchy manner below the surface epithelium but was seen only seldom related to the foveolar pits or necks (Fig 1A). TCC also occurred deep in the mucosa along and below the basement membrane zone and scattered between the glands (Fig 1B) in 26 of 43 antral samples (60%) and in 21 of 40 body samples (53%). Glands were not adequately represented in three body samples. Bright staining of large blood vessels, and of the mucus above the surface epithelium as well as faint staining in muscular tissue were seen in some specimens but not recorded systematically.

Statistical analysis showed that subepithelial deposition of TCC in the antrum and body (Fig 2) was significantly more often present in specimens with than in those without

Figure 1: Two colour immunofluorescence staining (double exposure) of complement deposits in sections of directly ethanol fixed antral mucosa from patients with *H pylori* gastritis. (A) TCC (Texas red) is located beneath the surface epithelium that is visualised by staining for cytokeratin (FITC, green). (B) Same staining combination shows TCC around glandular elements. (C) Two colour staining for TCC (Texas red) and S protein (FITC) shows colocalisation (yellow) around glandular elements. (D) Two colour staining for C3b (Texas red) and cytokeratin (FITC) shows luminal deposits. (E) Two colour staining for C3b (Texas red) and *H pylori* (FITC) shows coated bacteria (yellow) on the surface epithelium, whereas bacteria in the gastric pits are uncoated (pure green). Original magnifications: A, $\times 1000$; B, $\times 400$; C, $\times 250$; D, $\times 400$; E, $\times 1000$.

inflammation ($p=0.001$ and $p=0.004$ respectively). The same was true ($p=0.02$) for glandular TCC deposition in the antrum (Fig 3). Specimens with mild to severe loss of glandular structures were found in 15 of 43 antral specimens and in six of 40 body specimens. Of the 15 antrum and six body specimens with atrophy, TCC staining around and in relation to remaining glands was found in 10 and four specimens respectively.

In patients who provided both antral and body specimens ($n=40$), the frequency of TCC deposition was similar in the antrum and body. In these patients, subepithelial deposition of TCC was found in 21 of 40 antral samples (53%) and in 20 of 40 body samples (50%).

Figure 2: Scatter diagram depicting subepithelial deposition of TCC in gastric antral (A) and body (B) mucosa in relation to grade of inflammation. Both specimens from *H. pylori* positive (△) and *H. pylori* negative (●) patients are included. TCC in the antrum and body was present significantly more often in specimens with than in those without inflammation.

Only faint staining for TCC was present in prewashed tissue specimens; the intensity was clearly decreased compared with that seen in directly alcohol fixed tissue taken from adjacent tissue sites. This suggested that TCC was present in a fluid phase rather than being membrane bound. Staining for S protein in directly alcohol fixed tissue samples showed colocalisation with TCC (Fig 1C).

Epithelium related deposits of C3b were not seen in prewashed tissue except in two specimens from the same patient where the staining was clearly reduced compared with similar staining after direct ethanol fixation. In such specimens, C3b appeared to be related to the apical face of the surface epithelium (Fig 1D) in 16 of 43 antral samples (37%) and in 10 of 43 body samples (23%), a location remarkably different from that of TCC. In the patients who provided both antral and body samples ($n=40$), C3b was more frequently found in the antrum (38%) than in the body (25%). The C3b staining varied from an almost continuous band to scattered patches and often extended between the adjacent epithelial cells as well as into the mucus layer. It was neither found in the neck nor in the glandular epithelial areas and was likewise absent from the lamina propria. When biopsy samples from patients with or without detectable infection were compared, apical C3b was found more often in antral and body samples from the *H. pylori* positive group ($p=0.05$ and 0.03 respectively). Moreover, C3b was significantly more often present in antral samples with granulocytes present in the lamina propria and epithelium than in those not showing such a sign of inflammatory activity ($p=0.04$, Fig 4).

Antiserum to the classic activation components C1q and C4c produced faint co-staining with C3b in directly ethanol fixed specimens, but considerable background staining rendered these findings inconclusive. Such costaining could not be performed reliably on prewashed tissue specimens because of the considerable reduction in staining intensity for C3b.

H. pylori produced a strong staining signal with the polyclonal antibody reagent applied to alcohol fixed samples. Towards the openings of the gastric pits, bacteria were found to be positive for C3b as shown by paired immunofluorescence staining, while they were negative deeper in the pits (Fig 1E). Readily identifiable positive bacteria were seen in four of 16 patients with C3b staining related to the antral surface epithelium.

There was good agreement between the first and second observations of C3b and TCC depositions ($\kappa_s=0.71$). Of 30 repeat observations, 22 were identical, four provided a negative difference of one grade, and four a positive difference of one grade.

Observations in gastric stumps

Biopsy specimens from 23 BII operated patients were studied. Two samples were available from each of 14 patients, and one

Grade of activity	C3b deposition (Y-axis)	Symbol
0	0	Solid circle
0	0	Solid circle
0	0	Solid circle
0	0	Solid circle
0	0	Solid circle
0	0	Solid circle
0	0	Solid circle
0	0	Solid circle
0	0	Solid circle
0	0	Solid circle
0	1	Open triangle
0	1	Open triangle
0	2	Open triangle
1	-0.1	Solid circle
1	-0.1	Solid circle
1	0	Open triangle
1	0	Open triangle
1	0	Open triangle
1	1	Open triangle
1	1	Open triangle
1	2	Open triangle
1	2	Open triangle
1	3	Open triangle
1	3	Open triangle
2	0	Open triangle
2	1	Open triangle
2	1	Open triangle
2	2	Open triangle
2	2	Open triangle
3	3	Solid circle

epithelially in 19 of 23 patients (83%) and in the glandular compartment of 14 of 22 patients (64%) in whom glands were sufficiently represented. C3b was found apically on the surface epithelium in only two of 23 patients (9%). Eighteen of 23 patients (78%) had a serum IgG antibody concentration considered to be positive for *H. pylori* infection. The values were unrelated to TCC deposition ($r=-0.004$).

This is apparently the first study of complement activation in human gastric mucosa. Immunohistochemistry with mAbs to neopeptides of C3b (early activation) and TCC (late activation) performed on ethanol fixed antral and body specimens showed that such activation occurs locally in gastritis regardless of whether *H. pylori* is detectable or undetectable in the patient's stomach. No difference was noted between the antrum and body in the frequency of TCC deposition in the patients who provided both antral and body samples. This was unexpected because the grade of inflammation usually declines in patients with active chronic gastritis the closer to the cardia the biopsy specimen is taken.³³ One reason might be the inclusion of a rather high number of patients with pangastritis. Conversely, C3b was more frequently found in the antrum (38%) than in the body (25%), and this activation product was significantly related to *H. pylori* infection. It should be noted that in situ immunofluorescence was used to document the infection state, and this method distinguishes *H. pylori* from other curved bacteria present in the stomach.²² However, because of uneven bacterial distribution, some patients with gastritis might have falsely been classified as *H. pylori* negative. Nevertheless, the immunofluorescence result was supported by the rapid urease test and by serology in a subgroup of patients.

Except in one case, C3b was found in the body mucosa only when it was also present in

the antrum. This early activation product was distributed in a patchy manner apically on the surface epithelium but not in the foveolae. Although this deposition resembled that seen apically on the colonic surface epithelium of patients with active ulcerative colitis, the latter was more extensive and generally associated with TCC.¹⁸

Because C3b was localised to the epithelial surface in chronic gastritis, a dynamic interaction may exist between the bacterium and the host – perhaps induced by *H pylori* antigens. Complement activation via the classic pathway that involves C3b, is initiated by soluble antigen-antibody complexes or by the binding of antibody to target antigens such as bacteria. Antibodies of IgA, IgG, and IgM isotypes have been shown to coat *H pylori* in vivo, the two latter classes (which are complement activating) usually in the presence of epithelial neutrophil infiltration.⁷

In this study, we were unable to draw any conclusion as to whether the classic or alternative activation pathway was involved in the deposition of C3b. *H pylori* is not overtly invasive, but proinflammatory bacterial factors may reach the lamina propria, particularly if the epithelial barrier is damaged. It has also been proposed that bacterial antigens may form immune complexes with IgG and activate complement.^{15–17} Lipopolysaccharides from Gram negative bacteria are known to be initiators of the alternative pathway of complement activation and C3 has a labile internal thioester bond that is activated as C3b is formed, allowing the C3b fragment to bind to free hydroxyl or amino groups on a cell membrane.³⁶ It is therefore likely that both complement activation pathways are involved in *H pylori* associated gastritis.

It was interesting to notice that *H pylori* was costained for C3b near the openings of gastric pits and on the luminal surface. The fact that several bacteria were negative in the same section excluded unwanted antibody cross reactivity between the C3b neoepitope and *H pylori*. Bacteria deeper in the pits remained unstained, perhaps reflecting evasion of complement attack; this would agree with the absence of IgG and IgM antibody coating in this location.⁷ C3b coating of the bacteria was not present in all *H pylori* infected patients. We cannot explain why C3b was seen to coat *H pylori* in some patients, but not in others. The ethanol fixation had apparently removed bacteria and the mucus layer from the surface, which made evaluation of C3b coating even more difficult.

Binding of C3b to *H pylori* may facilitate opsonisation by cells bearing complement receptors, thereby enhancing phagocytosis by neutrophils, monocytes, and macrophages.³⁷ In vitro studies have shown that complement is an efficient opsonin for *H pylori*, either activation pathway being sufficient for opsonisation on its own.³⁸ If in vivo activation of complement takes place on the surface of *H pylori*, rapid hydrolysis would limit binding of C3b to nearby host cells.³⁶ Mammalian cells have high levels of sialic acid, which

contributes to rapid inactivation of bound C3b molecules.³⁶ Microorganisms generally lack mammalian complement regulatory proteins and can activate complement in the absence of antibody via the alternative pathway. This activation leads to accumulation of a large number of C3b molecules on their surface, but many bacteria have developed strategies to evade destruction.³⁹ However, surprisingly little is known about the local protective measures possessed by *H pylori* and by the gastric epithelial cells.

The actual role of chemotactic substances (C3a, C4a, and C5a) released by complement activation in gastritis associated with *H pylori* colonisation remain undetermined, but we noticed that the C3b deposits were significantly related to the presence of neutrophils in the mucosa. However, these products are unlikely to be the only factors attracting neutrophils to gastric lesions; for example, increased epithelial expression of the chemokine interleukin 8 has been seen in gastritis compared with normal mucosa.⁴⁰ Complement could also be important in neutrophil activation in *H pylori* associated gastritis. In an earlier study,²⁰ *H pylori* did not induce a significant increase in neutrophil chemiluminescence in the absence of opsonins, but this was considerably increased by the addition of fresh agammaglobulinaemic serum as a source of complement. The complement activating abilities of different *H pylori* strains (type I, type II) should be tested in future studies, as the ability to activate complement might be an important virulence marker.

TCC was present in mucosal samples from most (83%) BII operated patients, whereas C3b was found in only few (9%). Preferential deposition of TCC beneath the epithelium and in the glandular areas probably reflected chronic immunopathology; these patients were relatively old and had longstanding gastric lesions. Serum IgG antibodies to *H pylori* were often found at a level indicating infection in this group (78%), apparently without being related to the TCC deposition. This supported our finding in the gastritis group that deposition of TCC along the basement membrane is not necessarily related to *H pylori* infection. The fact that *H pylori* is seldom found near the stoma in gastric stumps⁴¹ suggests that other factors (for example, bile reflux) maintain the inflammation in stump gastritis.

In conclusion, complement activation takes place in chronic gastritis, both associated and unassociated with *H pylori* infection, as well as in stump gastritis after BII resection. However, the pattern of complement deposition differs, which suggests separate immunopathological mechanisms. The role of activated complement for neutrophil chemotaxis and epithelial destruction in gastritis remains undetermined; but it is of considerable interest that activated C3 was seen to coat *H pylori* and to be deposited at the apical face of the surface epithelium preferentially in infected patients.

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