Morphostasis of the adult gastrointestinal tract

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Citation for published version (APA):
van den Brink, G. R. (2002). Morphostasis of the adult gastrointestinal tract
CHAPTER 3

Expression and activation of nuclear factor κB in the antrum of the human stomach

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Abstract

Background
Both in vitro studies and experiments in mice suggest a key role for transcription factor nuclear factor (NF)-κB as a mediator of mucosal inflammation. Experiments in vitro show that NF-κB activation may be a critical event in the production of pro-inflammatory molecules in Helicobacter pylori associated gastritis.

Methods
This study examines the expression and activity of NF-κB in situ in antral biopsies of 69 consecutive patients with immunohistochemical techniques.

Results
In the uninflamed stomach NF-κB was highly expressed and active in a subset of epithelial cells, which were identified as predominately G cells. In accordance with this activity, G cells were shown to express high levels of the NF-κB target cytokine tumor necrosis factor (TNF)-α, a well-documented stimulator of gastrin production. In patients with Helicobacter pylori associated gastritis, NF-κB activity was markedly enhanced. Activation occurred preferentially in the epithelial cells. The number of cells showing activated NF-κB correlated with the activity of gastritis, a measure of neutrophil influx, whereas no correlation was found with the chronicity of inflammation, a measure of the presence of mononuclear inflammatory cells.

Conclusions
This correlation is direct evidence of the importance of NF-κB dependent signal transduction for neutrophil influx in Helicobacter pylori associated gastritis.
Introduction

Upon colonization by pathogenic bacteria the host cells at the site of infection respond with an induction of the innate immune response. This response is elicited after a signal from the invader triggers an alteration in host cell gene transcription. Subsequently, the host cells will produce pro-inflammatory proteins, such as cytokines, chemokines, and adhesive molecules to counteract the external threat. Moreover, at the site of colonization, both the rate of proliferation and apoptosis of the epithelial cells are often markedly affected. The molecular details of the interaction between the host epithelium and pathogenic bacterial invaders are now slowly emerging. One of the sentinel transcriptional modulators in the host response to bacterial invasion, is the NF-κB family of proteins. This transcription factor mediates both acute and chronic inflammation through the regulation of many pro-inflammatory proteins. NF-κB is present in the cell as a hetero- or homodimer, and remains inactive by binding to an inhibitory protein, IκB, within the cytoplasm. Subunits that can form these dimers are NF-κB1 and NF-κB2 (expressed as precursors p105 and p100, processed to p50 and p52, respectively), RelA (p65), RelB and c-Rel. The dimer, typically composed of a p50 and p65 subunit, is translocated to the nucleus after degradation of the inhibitory IκB in response to a wide variety of stimuli. Although it is generally assumed that NF-κB is ubiquitously expressed by all cell types, exact expression patterns remain to be studied for most human tissues.

A unique model system to study the induction of the innate immune response by a single pathogenic bacterial species exists in the human stomach. Here, colonization of the mucous layer overlaying the gastric epithelial cells by the Gram-negative bacterium Helicobacter pylori results in an acute host response, mostly followed by persistence of the bacterium and chronic gastric inflammation. The presence of Helicobacter pylori is associated with peptic ulcer disease, atrophic gastritis, gastric adenocarcinoma and gastric MALT lymphoma. NF-κB is known to play an important role in the inflammatory response in the intestine and accordingly, Helicobacter pylori activates this transcription factor. This activation may in turn cause gastritis via the induction of pro-inflammatory cytokines such as interleukin-1 and TNF-α, and chemokines like interleukin-8. We examined in this study the expression and activation of NF-κB in the antrum of the human stomach, both in the histologically uninflamed mucosa and Helicobacter pylori associated gastritis. We also investigated a possible correlation of the activity of NF-κB with the commonly used histopathological classification of the severity of gastritis, the Sidney score. Our findings show that in the antrum of the stomach activation of NF-κB correlated with the activity of gastritis, a measure of neutrophil influx, whereas this did not correlate with the chronicity of gastritis, a measure of the presence of mononuclear inflammatory cells. Furthermore, we found that expression and activation of NF-κB is associated with the epithelial cells, especially G cells.
Materials & Methods

Patients

Gastroduodenoscopy was performed on 69 consecutive patients referred to the endoscopy unit of the Academic Medical Center in Amsterdam for upper abdominal complaints. These patients were enrolled in a prospective study investigating the prevalence of Helicobacter pylori in patients with upper abdominal complaints in the general practitioner’s setting; the study was approved by the Ethics Committee of the Academic Medical Center. Biopsies were taken from the antrum of the stomach as part of these investigations. Specimens were fixed in Phosphate Buffered Saline (PBS)-buffered paraformaldehyde for 30 minutes (min), and embedded in paraffin with the use of standard methods. Helicobacter pylori status was determined by serology, culturing the bacteria, and by routine histology (H&E stain) by an independent pathologist. A patient was considered Helicobacter pylori positive if the bacterium was cultured or if both serology and pathology were positive. The histological grade of gastritis was scored according to the Sydney classification,\textsuperscript{17} by a pathologist blinded to the NF-κB activation score (see below).

Antibodies

Antisera to different NF-κB subunits, anti (α)-p65 rabbit polyclonal IgG antibody C-20, α-p65 mouse monoclonal IgG 1 antibody F-6, α-p50 rabbit polyclonal IgG antibody H-119, α-p52 rabbit polyclonal IgG antibody K-27, α-RelB rabbit polyclonal IgG antibody C-19, and an α-TRAF2 rabbit polyclonal IgG antibody M-19, were all purchased from Santa Cruz Biotechnology (Santa Cruz, CA). A mouse monoclonal IgG 3 antibody (Boehringer Mannheim, Mannheim, Germany), raised against the p65 nuclear localization signal, was used to detect activated NF-κB. This antibody only binds to the p65 unit after release from the inhibitory IκB subunit and thus specifically recognizes activated p65, allowing assessment of NF-κB activation in situ.\textsuperscript{14,18,32} For detection of chromogranin A and gastrin, we used α-chromogranin A mouse monoclonal antibody clone DAK-A3 and a-gastrin rabbit polyclonal antibody A 568 (DAKO, Glostrup, Denmark). A mouse monoclonal IgM antibody against TNFα, clone 4C6-H6 was purchased from Instruchemie (Hilversum, The Netherlands). For Helicobacter pylori visualization, the rabbit polyclonal antibody B0471 (DAKO) was used.

Immunohistochemistry

Paraffin sections (4mm) were dewaxed and rehydrated in graded alcohols. Endogenous peroxidase activity was quenched with 1.5% H₂O₂ in PBS for 30 min at room temperature (RT). Non-specific staining was blocked with TENG-T (10mM Tris, 5 mM EDTA, 0.15 M NaCl, 0.25% gelatin, 0.05% (v/v) Tween 20, pH 8.0) for 30 min at RT. After washing with PBS one of the following primary antibodies was applied α-p65 polyclonal (1:500), α-p65 monoclonal (1:50), α-p50 (1:50), α-p52 (1:10), α-RelB (1:20), α-TRAF2
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(1:50), α-active p65 (1:500 of 1 mg/ml stock), α-chromogranin A (1:50), α-
gastrin (1:200) in PBS containing 1% bovine serum albumin. For double
staining combinations of monoclonal and polyclonal antibodies were used.
Sections were stored overnight at 4°C. The following day, for single staining,
sections were washed in PBS, and incubated with a secondary biotinylated
Goat anti-rabbit Ig (DAKO, 1:500) or anti-mouse Ig antibody (DAKO,
1:200) for one hour at RT and washed with PBS. Hereafter, sections were
incubated with ABcomplex (DAKO) as described in the manufacturer's
instructions for one hour at RT. Peroxidase activity was detected with
diaminobenzidine (DAB, Sigma, St. Louis, MO) (5 mg DAB and 10 ml H2O2
in 10 ml 0.05 M Tris, pH 7.8), resulting in the formation of a brown reaction
product. Sections were briefly counterstained with haematoxyline, dehy-
drated in graded alcohols and mounted. For double staining experiments sec-
ondary antibodies used were combinations of alkaline phosphatase conju-
gated Goat anti-rabbit (1:100) with Goat anti-mouse biotin, or alkaline
phosphatase conjugated Goat anti-mouse (1:100) with Goat anti-rabbit
biotin. Sections were then incubated with streptavidin β-galactosidase (1:40,
Boehringer Mannheim) at RT for 30 min. The strep β-gal was detected using
1% X-gal (DAKO) in iron phosphate buffer (0.02% MgCl2-6H2O, 0.099%
potassium ferricyanide, 0.127% potassium ferrocyanide) at 37°C for 15
min, resulting in a blue color. After washing in Tris Buffered Saline, the alka-
line phosphatase was detected in purple using the Fast Red detection method
(DAKO). Double stained sections were mounted in Ultramount (DAKO),
an aqueous mounting medium. Controls consisted of omitting the primary
and secondary antibody and use of an appropriate Ig control. Single staining
in adjacent sections always preceded double staining experiments.

NF-κB activation score
To assess the activity of NF-κB in situ in the human stomach, specimens from
all patients were stained for active NF-κB. Two pictures of each section
were taken at 200x magnification (0.0325 mm²/picture) and positive cells
counted, blind to the clinical diagnosis, in each microscope field with the use
of an image analysis program (EFM Software, Rotterdam, The Netherlands).
The mean of the two fields was taken as a relative measure of NF-κB activi-
ty. To be able to compare the results between patients, it was ensured that all
sections visualized the entire axis from the superficial epithelium to the
muscularis mucosa.

Statistical analysis
Data are presented as mean ± SEM. Comparisons between groups of data
were made using a one-way analysis of variance followed by a Tukey post hoc
test. P values < 0.05 were considered statistically significant.
CHAPTER 3

Results

Patients

To investigate the expression of NF-κB in-situ in human tissue and assess the site and extent activation of this transcription factor, in response to colonization by a pathogenic bacterium, we collected biopsies of 69 consecutive patients with upper abdominal complaints (see Table 1). 26 patients were Helicobacter pylori positive, 6 after an unsuccessful attempt at eradication. Of the 43 Helicobacter pylori negative patients 8 were recently successfully eradicated. The histological grade of gastritis was scored according to the Sydney classification (Table 2).

Expression of NF-κB in the Helicobacter pylori negative stomach

It is now clear that Helicobacter pylori activates the transcription factor NF-κB and that this event plays a central role in the induction of the inflammatory reaction often associated with colonization with this bacterium. However, the expression of NF-κB in the antral mucosa, the main site of colonization, has so far not been studied. To study the expression of different NF-κB subunits in the normal gastric mucosa, we examined their expression in histologically normal gastric biopsy specimens (no activity and no chronicity of inflammation as assessed by the Sydney classification, see table 2) with the use of immunohistochemical techniques. In the antrum of the normal stomach (n = 26 patients), low but detectable expression of the p65 NF-κB subunit was found in the cytoplasm of the superficial gastric pit cells (Figure 1a). Deeper in the gastric glands however, many cells were found with a very high p65 content (Figure 1a, b and h). In some of these cells NF-κB was not only detected in large amounts in the cytoplasm but also in the nucleus, suggesting activation of NF-κB in these cells in the absence of overt signs of inflammation. The expression pattern of the p50 subunit (n = 15 patients; not shown), and RelB subunit (n = 15 patients; not shown) was similar to was clearly different, it showed a staining pattern reminiscent of the p65, p50 and RelB subunits in gastric epithelial cells, but in addition and in contrast to the other subunits a relatively high expression in clusters of inflammatory cells (Figure 1c). We concluded that NF-κB subunits are highly expressed in a specific subpopulation of cells in the uninflamed stomach.

Table 1. Patient characteristics.

<table>
<thead>
<tr>
<th></th>
<th>H. pylori negative</th>
<th>H. pylori positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex M/F ratio</td>
<td>17/26 (1.5)</td>
<td>10/16 (1.6)</td>
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<tr>
<td>Mean age</td>
<td>50.9 +/- 13.2</td>
<td>50.3 +/- 14.8</td>
</tr>
<tr>
<td>No. of patients</td>
<td>43</td>
<td>26</td>
</tr>
<tr>
<td>Treated patients</td>
<td>6</td>
<td>8</td>
</tr>
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</table>
NF-κB expression in the human stomach

Table 2. Severity of inflammation according to the Sidney classification, number of patients per group

<table>
<thead>
<tr>
<th>Chronicity</th>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>26 Hp-</td>
<td>0 Hp+</td>
<td>10 Hp-</td>
<td>2 Hp+</td>
<td>4 Hp-</td>
</tr>
<tr>
<td></td>
<td>0 Hp-</td>
<td>0 Hp+</td>
<td>1 Hp-</td>
<td>2 Hp+</td>
<td>1 Hp-</td>
</tr>
<tr>
<td></td>
<td>0 Hp-</td>
<td>0 Hp+</td>
<td>0 Hp-</td>
<td>1 Hp+</td>
<td>0 Hp-</td>
</tr>
<tr>
<td>Severe</td>
<td>0 Hp-</td>
<td>0 Hp+</td>
<td>0 Hp-</td>
<td>0 Hp+</td>
<td>0 Hp-</td>
</tr>
<tr>
<td>Total</td>
<td>26 Hp-</td>
<td>0 Hp+</td>
<td>11 Hp-</td>
<td>5 Hp+</td>
<td>5 Hp-</td>
</tr>
</tbody>
</table>

High NF-κB expression in the endocrine cell region

The immunohistochemical determination of NF-κB protein expression showed that high levels of NF-κB subunits are expressed in a subset of epithelial cells deeper in the gastric glands. The morphology and localization of the NF-κB-expressing cells suggested that NF-κB expression in the stomach corresponded to the endocrine cell population. To investigate this, sections were double-stained with a monoclonal α-chromogranin A antibody, a marker for enteroendocrine cells, and a polyclonal α-NF-κB p65 antibody. We observed that the NF-κB expressing region in stomach indeed corresponded to the chromogranin A-positive cells and those cells immediately adjacent to the chromogranin A positive cells (n = 69 patients; Figure 1d). An almost complete correlation was observed in double-staining experiments in which sections were stained with a polyclonal antibody against gastrin and an α-NF-κB p65 monoclonal antibody (n = 10 patients; Figure 1e,f). Apparently, many of the highly NF-κB expressing cells are G cells. These results were further confirmed by single staining experiments of adjacent sections (not shown). We concluded that expression of NF-κB in the uninflamed stomach is associated with the G cells.

Activity of NF-κB in the uninflamed stomach

The nuclear localization of NF-κB found staining with an antibody that recognizes total p65 protein suggested that this transcription factor may be active in the uninflamed mucosa. The possible activation of NF-κB was further investigated using an antibody directed against the nuclear localization sequence of the p65 subunit.14,18,32 Since this epitope is only exposed after degradation of the inhibitory protein I-κB this antibody recognizes activated p65. In adjacent sections, this antibody (Figure 1h) identified the same cells that displayed nuclear localization of the protein when stained with the polyclonal α-total p65 antibody (Figure 1g,h). For further proof of acti-
vation we also examined if these cells showed expression of TRAF2, a transcriptional target of NF-κB. Staining for TRAF2 revealed high expression of this protein in the same cells that contain activated NF-κB (n = 12 patients; Figure 1h,i). Furthermore, double staining for activated p65 and gastrin in the uninflamed, Helicobacter pylori negative stomach (n = 26 patients) revealed that activation of NF-κB occurs preferentially in the endocrine cell region of the stomach, mostly in the G cells (Figure 3b,e and f). We concluded that the uninflamed stomach shows constitutive activation of NF-κB, in the same cells that maintain high NF-κB expression, and that these cells are mainly endocrine G cells.

Expression of TNFα in the uninflamed mucosa

As active NF-κB was found predominantly in the G cells, we were interested to see if these cells would express the cytokine TNFα, since the pro-
**Figure 1.** Immunohistochemical staining of the antrum of the uninflamed human stomach. (A-C): DAB stain (brown precipitate), nuclear counterstain with haematoxyline, arrows show high NF-κB expression. (A) Total p65 NF-κB. p65 NF-κB is differentially expressed in the antrum of the stomach. (B) Higher magnification of the boxed area in A, showing cells with high p65 content. (C) Adjacent section, p52 NF-κB subunit, in addition to the epithelial cells, some clusters of inflammatory cells (large asterisks) show intense staining. (D-F) Double stainings for p65 and enteroendocrine cells as described in methods section, no nuclear counterstain. (D) Total p65 NF-κB (red) chromogranin A (blue), arrows show examples of double staining cells. (E) p65 NF-κB (red) and gastrin (blue). (F) Blow-up of boxed area in E almost all high p65 expressing cells were G cells (arrows denote examples of double staining cells). (G-I) Immunohistochemical staining with DAB, no haematoxyline in G. Three adjacent sections, of non-inflamed mucosa, showing constitutive activity of p65 NF-κB. (G) active p65 NF-κB, note the nuclear staining (arrows). (H) Adjacent section, total p65 NF-κB, stained the same cells (arrows). (I) Adjacent section, TRAF2, a transcriptional target of NF-κB, many of the cells with high NF-κB expression also showed high TRAF2 expression. (Original magnifications: a, 330x; b,c, 660x; d, 650x e, 200x, f, 1000x; g, 660x; h, 1220x; a-c, 1600x).

Production of this cytokine is stimulated by NF-κB. Therefore, we stained for TNFα and gastrin in adjacent sections (n=20 patients; Figure 2 a,b) and double staining experiments (n=10 patients, Figure 2 c,d). As shown in figure 2 these stainings showed that the G cells are the main site of production of TNFα in the histologically uninflamed stomach. Thus in accordance with the observed activity of NF-κB, G cells produce the proinflammatory cytokine TNFα.

**NF-κB activation in the Helicobacter pylori infected stomach**

The activation of NF-κB in the gastric mucosa in response to colonization by a pathogenic bacterium was studied in patients with *Helicobacter pylori* infection. To this end, a double stain of active NF-κB and gastrin was performed for all 69 patients studied. The number of cells with activity of NF-κB in the *Helicobacter pylori*-colonized stomach was markedly enhanced, as compared to uninflamed tissue (Figure 3a,b). Whereas p65 NF-κB was found in all cell types (Figure 3c), the active NF-κB was mainly detected in the epithelial cells. Remarkably, whereas some isolated inflammatory cells scattered throughout the mucosa often also stained with the anti-active p65 antibody, the large infiltrates of neutrophils did not (Figure 3d). Double staining of active NF-κB and *Helicobacter pylori* showed that many of the NF-κB-positive cells were not in direct contact with the bacteria (Figure 3b). Active NF-κB was primarily detected in the cells deeper in the gastric glands, many of them G cells (Figure 3e-h). Thus, we found markedly enhanced activity of NF-κB in *Helicobacter pylori* infected patients, predominantly in the epithelial cells deeper in the gastric glands.
Active NF-κB and the histopathological severity of gastritis

Since NF-κB seems to play a pivotal role in the induction of inflammation in the gastric mucosa, we compared a measure of the activity of this transcription factor with the severity of gastritis as scored according to the Sydney criteria. To get a quantitative measure of NF-κB activity in the stomach biopsies, the active NF-κB positive cells were counted, blind to the clinical diagnosis. As depicted in Figure 4a, Helicobacter pylori infection results in a marked increase in active NF-κB positive cells (uninflamed: 870 cells/mm² vs. inflamed: 2430 cells/mm²; p << 0.01). Separate analysis of the patients that received treatment (Figure 4b) revealed that in patients successful eradication of the bacterium restored the mean active NF-κB score to normal whereas in those patients where treatment was unsuccessful this score remained high. To investigate the relation of the number of active NF-κB positive cells to the severity of gastritis, we examined a possible correlation between the active NF-κB score and the histopathological score based on the Sydney classification system. The number of active NF-κB positive cells correlated well with the activity of gastritis, a measure of neutrophil influx (Figure 5a). Interestingly however, no such correlation was found between the activity of NF-κB and the chronicity of inflammation, a score of the number of mononuclear inflammatory cells (Figure 5b). As shown in figure 5b, Helicobacter pylori-negative patients with chronic gastritis (n = 17 patients, with moderate to severe chronicity of gastritis, see table 2) did not show
Figure 3. Immunohistochemical double staining performed as described in the methods. (A) The mucosa of the uninflamed antrum of the stomach, gastrin (red), active p65 NF-κB (blue). (B) Helicobacter pylori (red), active p65 NF-κB (blue). The bacteria resided mostly in the superficial mucous layer (denoted by #), and although some of the epithelial cells that contained active p65 NF-κB were in direct contact with the Helicobacter pylori (asterix), most of them were not (arrows). (C and D) Adjacent sections, Helicobacter pylori infected patient. (C) Total p65 NF-κB (blue), chromogranin A (red), all cells expressed p65 NF-κB. (d) Active p65 NF-κB (blue), gastrin (red), p65 NF-κB was predominantly activated in the epithelial cells (arrows), whereas no active p65 NF-κB was detected in the neutrophil infiltrate (asterix). (E-F) Helicobacter pylori infected patient, active p65 NF-κB (blue), gastrin (red). p65 NF-κB activation in the epithelial cells was highest deeper in the epithelium. (F) Blow-up of boxed area in E, in many patients NF-κB was preferentially activated in the G cells. (H and I) Double stain of gastrin and active p65 NF-κB, clearly showing nuclear staining with the α-active p65 NF-κB antibody (blue) and cytoplasmic staining with the α-gastrin antibody (red) in the G cells. (Original magnification A, 660x; B-D, 500x; E, 500x; F, 1000x; G and H 1000x).
enhanced activation of NF-κB compared to patients without chronic gastritis (n=26). The significantly higher NF-κB activity in patients with severely chronic gastritis is most likely explained by the fact that 9 out of 10 of these patients have a marked to severe activity of disease (see table 2). We concluded that activation of NF-κB correlates the activity of gastritis and thus with neutrophil influx in the gastric mucosa in response to colonization with Helicobacter pylori.

**Discussion**

The present study investigated the expression and activation of NF-κB in the uninflamed and inflamed stomach. NF-κB was differentially expressed in the antrum of the human stomach. Although most cell types expressed some NF-κB, the G cells displayed particularly high levels of this transcription factor. We found activity of p65 NF-κB in the G cells in the histologically uninflamed gastric mucosa. Activation was judged by four different criteria: nuclear localization of the protein, staining with an antibody against active
NF-κB and high expression of the NF-κB target genes, TRAF2 and TNFα. The activity was markedly enhanced activation in Helicobacter pylori infected patients in these cells.

NF-κB is a key regulator of the innate immune response and its high expression and activity in the G cells, which are not known to be involved in the immune response, is a surprising finding. However, G cells stimulate release of acid into the stomach via the production of gastrin,¹⁹,²⁰ and therefore these cells may help combat bacterial infection of the stomach by increasing their gastrin secretion and subsequent bactericidal acidification of the stomach. Indeed, the production of gastric acid provides an essential non-immunological first line of defense against colonization by enteropathogenic bacteria.²¹ Exogeneous bacteria are usually rapidly destroyed at a pH up to 4.0, and impaired gastric acid production caused by use of antacids, stomach resections and especially chronic auto-immune gastritis with parietal cell
destruction and pernicious anemia is associated with colonization of the stomach and small intestine with fecal type bacteria and for example increased risk of disease during cholera epidemics. The notion that G cells are capable of reacting to bacterial infection is further supported by the hypergastrinemia observed in *Helicobacter pylori* infected patients. Indeed, three reports describe enhanced production of gastrin after exposure of isolated antral G cells to live *Helicobacter pylori* or *Helicobacter pylori* extracts. Thus the expression and activity of NF-κB in the G cells may be a bona fide reflection of a role of these cells in innate immunity.

In accordance, we show that these cells are the main site of production of the NF-κB regulated cytokine TNFα in the uninflamed stomach. It is interesting to note that addition of exogenous TNFα to isolated G cells stimulates the production of Gastrin in vitro. Our finding that G cells produce TNFα and respond to *Helicobacter pylori* with enhanced NF-κB activation may suggest that colonization by this bacterium increases expression of TNFα in the G cells through activation of NF-κB and causes increased gastrin production in an autocrine manner. Thus, the high levels of NF-κB and TNFα expression in the G cells may couple the innate immune response to the production of bactericidal gastric acid. Additionally, NF-κB may play a specific role in the cell fate and/or differentiation of these cells. Since NF-κB plays an important role in the protection of cells from apoptosis via stimulation of the production of anti-apoptotic proteins, such as TRAF2, the activity of this transcription factor is likely to play a role herein. Indeed, whereas the superficial gastric epithelial cells undergo enhanced apoptosis in *Helicobacter pylori* infected patients, G cells may be protected from this process by their high expression of NF-κB and increased G cell mass has been reported in these patients. *Helicobacter pylori* infection greatly increased the number of cells containing active NF-κB, activation occurred predominantly in the epithelial cells and little activation was detected in neutrophils. This is in agreement with findings by Rogler and colleagues in patients with inflammatory bowel disease, who showed that NF-κB was mainly activated in epithelial cells and macrophages. These authors also demonstrated a correlation between the activity of NF-κB and an endoscopic score of inflammation. In our study, the increase in NF-κB activity correlated well with the activity of gastritis, a measure of neutrophil influx, as scored according to the Sydney classification system. Together these studies support an important role for epithelial NF-κB activation in the production of neutrophil chemoattractants like IL-8. No correlation was found with the chronicity of inflammation, a measure of the number of mononuclear inflammatory cells in the mucosa, as *Helicobacter pylori* negative patients with chronic inflammation did not have a higher NF-κB activity score than those without. This lack of correlation is further supported by our finding that the NF-κB activity score was restored to normal values in recently eradicated patients whereas it is well known that the chronic component of gastritis may last for up to a year after successful treatment.
NF-κB expression in the human stomach

Double staining for *Helicobacter pylori* and active p65 NF-κB, showed that most of the epithelial cells exhibiting activation of NF-κB were not in direct contact with the bacteria. The activation occurred preferentially deeper in the gastric glands, in the endocrine cell region, where no *Helicobacter pylori* are found since the bacterium resides in the superficial mucous layer. Therefore, NF-κB activation in the stomach does not seem to require direct contact with bacteria, and may thus be depended on a secreted factor. Accordingly, *Helicobacter pylori* virulence and induction of epithelial IL-8 expression is strongly associated with a group of 31 bacterial genes, the pathogenicity island,\(^{33,34}\) which encodes a type IV toxin secretion system.\(^{35}\)

In conclusion, our findings show that in the uninflamed antrum of the stomach high NF-κB expression and activity are associated with the G cells that produce TNFα, a well-documented stimulator of gastrin release. This suggests an autocrine mechanism of gastrin production by TNFα that may be enhanced upon colonization by pathogenic bacteria. Furthermore our data show a strong correlation between epithelial NF-κB activation and neutrophil influx in *Helicobacter pylori* associated gastritis, a clear illustration of the importance of NF-κB activation in chemokine production.

**Acknowledgements**

The authors wish to thank Dr. W.P. van den Brink for expert statistical advice.
References


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