**Original Paper**

**Gastric mucosal cytokine and epithelial cell responses to *Helicobacter pylori* infection in Mongolian gerbils**

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**Abstract**

Experimental infection with *Helicobacter pylori* in Mongolian gerbils results in chronic gastritis and gastric cancer. To investigate epithelial cell proliferation, apoptosis, and mucosal cytokine responses in gastritis, Mongolian gerbils were infected with the *H pylori* SS1 strain. At 4 weeks post-infection, gastritis was predominantly within the antrum, but extended to the corpus in approximately 50% of gerbils by 36 weeks. Epithelial cell proliferation and apoptosis in glandular epithelial cells were increased with infection. Antral cell proliferation, but not apoptosis, correlated significantly with gastric inflammation. In female gerbils, *H pylori* significantly increased expression of transcripts for IFN-γ and IL-12p40, but not TGF-β or IL-10, in the gastric mucosa. Significantly reduced IFN-γ and IL-12p40 responses were observed in male gerbils infected with *H pylori*, but epithelial proliferative and apoptotic responses were comparable to those of females. These studies demonstrate that the female gerbil cytokine response to *H pylori* has a Th1 profile and that there are gender differences in the magnitude of the gastric cytokine responses to *H pylori*. The absence of a down-regulatory cytokine response may account for the more severe gastritis observed with *H pylori* infection in gerbils than in mice. Copyright © 2004 John Wiley & Sons, Ltd.

Keywords: Mongolian gerbil; gastritis; *Helicobacter pylori*; epithelial cell proliferation; apoptosis; gender; cytokine; Th1

**Introduction**

*H pylori* successfully colonizes the gastric mucosa of Mongolian gerbils and induces an antral predominant gastritis which progresses with time to corpus gastritis [1]. Infection with *H pylori* in gerbils results in gastric [2–4] and duodenal ulceration [5], intestinal metaplasia [1–4], and adenocarcinoma [3,6]. *H pylori* strains with a functional cag pathogenicity island (PAI) induce more severe pathology in gerbils than strains lacking a functional cag PAI [4,7,8].

Increases in epithelial cell proliferation and apoptosis are associated with gastric *Helicobacter* infection in humans [9,10] and animal models [8,11–16]. Studies in immune-deficient mice emphasize the importance of mucosal inflammation in epithelial proliferative responses to infection with gastric *Helicobacter* sp. RAG−/− mice, and mice deficient in interferon regulatory factor−/− or interferon-gamma−/− do not develop gastritis [17–19] and associated epithelial hyperproliferation [17] in response to gastric *H felis* infection. In the gerbil, comparison of proliferative and apoptotic responses to *H pylori* strains have also shown gastric epithelial responses to be increased in parallel with enhanced inflammatory responses [8].

Recent studies in the murine *H felis* model have identified gender differences in gastric epithelial proliferative and apoptotic responses [13]. Female C57BL/6 mice have enhanced proliferative and apoptotic responses to *H felis* infection compared with males [13]. Studies in the gerbil have largely used male animals [4,7,8,14,15]. However, short-term infection of female gerbils with the *H pylori* SS1 strain resulted in a rapid increase in gastric epithelial cell proliferation [16]. *H pylori* SS1 strain lacks a functional cag PAI [20]. The proliferative responses induced by the *H pylori* SS1 strain in the female gerbil contrasts with previous reports with the *H pylori* strain G1.1, which also lacks a functional cag PAI, which induces minimal proliferative responses [8,14].

Detailed study of the mucosal immune response to *H pylori* and analysis of its possible role in epithelial cell responses in gerbils have been hampered by the lack of genomic data on inflammatory mediators. The aims of the present study were three-fold: first, to use cross-species PCR to identify gerbil transcripts for key immunoregulatory genes and to examine their expression in the gastric mucosa of *H pylori*-infected gerbils; second, to examine the effects of the *H pylori* SS1 strain on gastric pathology, epithelial cell proliferation, and apoptosis in the gerbil, and to...
determine the relationship between gastric pathology, epithelial cell responses, and cytokine expression; and third, to investigate whether the gastric mucosal cytokine and epithelial responses to \textit{H pylori} infection differ between male and female gerbils.

**Materials and methods**

**Infection of Mongolian gerbils with \textit{H pylori}**

All animal experiments were conducted in accordance with the Home Office (Scientific Procedures) Act 1986 and approved by the Ethical Committee of the University. \textit{H pylori} strain SS1 (kindly provided by R Ferrero) was grown on 5\% (v/v) horse blood agar plates. Two-day cultures were harvested into sterile tryptose soya broth (Oxoid) and used immediately for inoculation into gerbils. Six- to 8-week-old female and male Mongolian gerbils (MGS/Sea, Seac Yoshimoto, Japan) were inoculated three times by oral gavage with the \textit{H pylori} SS1 strain (>10\(^8\) CFU).

**Histological and microbial analysis of infection**

Gerbils were sacrificed at 4, 12, and 36 weeks post-infection. Female infected gerbils and gender-matched controls were used at each time point. Male infected gerbils and gender-matched controls were studied at the 36-week time point only. Animals received an intra-peritoneal injection of bromodeoxyuridine (BrdU) (50 mg/kg) 1 h prior to sacrifice. At sacrifice, the number of apoptotic epithelial cells in the gastric mucosa was determined for both the superficial epithelium (top 5\%) and the glandular mucosa as previously described [13].

**Sequence analysis of Mongolian gerbil genes**

Oligonucleotide primers were designed for \textit{IL-12p40} [25] and \textit{β-actin} [26] from published sequences (see Table 1). Primers specific for \textit{IFN-γ}, \textit{IL-10}, and \textit{TGF-β} transcripts were designed by cross-species PCR after aligning the respective murine, rat, and human sequences, and identifying regions of homology (Table 1). PCR products were sequenced as previously described [21].

**RNA extraction and RT-PCR for gene expression in gastric mucosa**

RNA was extracted from gastric mucosal tissue using Catrimox™ (Qiagen, Crawley, UK) according to the manufacturer’s instructions, DNasease-treated and reverse-transcribed as previously described [27]. RT-PCR was used to amplify transcripts encoding \textit{IFN-γ}, \textit{IL-12p40}, \textit{TGF-β}, \textit{IL-10}, and \textit{β-actin} using oligonucleotide primers (Table 1). PCR amplification consisted of an initial denaturing step at 95°C for 1 min followed by 33 cycles (β-actin) or 40 cycles at 55°C (β-actin, IL-10), 60°C (TGF-β, IFN-γ,) or 63°C (IL-12p40) for annealing, followed by 1 min at 72°C for extension in all PCRs except that for IL-12p40. A specific extension step was not required for the amplification of IL-12p40 transcripts. Negative and positive controls were included in each assay. PCR was also undertaken using non-reverse-transcribed RNA as a template to confirm the absence of PCR amplions generated from small amounts of contaminating genomic DNA. PCR products were separated by 2\%(w/v) agarose gel electrophoresis. The ratio of cytokine ampolions to β-actin ampolion was determined as previously described [27].

**Table 1. Oligonucleotide primers used for reverse transcription-polymerase chain reaction (RT-PCR)**

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<tr>
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<th>Primer</th>
<th>Product size (bp)</th>
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<tbody>
<tr>
<td>β-actin</td>
<td>dGCACCACACCTTCTACAATGAG</td>
<td>164</td>
</tr>
<tr>
<td>IL-12p40</td>
<td>dTAGCACAGCCTGGATAGCAAC</td>
<td>263</td>
</tr>
<tr>
<td>IL-10</td>
<td>dGTACCTCAGACTGCGGAGAGAGG</td>
<td>196</td>
</tr>
<tr>
<td>TGF-β</td>
<td>dCTGACACCGCCAATCTCTG</td>
<td>197</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>dCTGACACCGCCAATCTCTG</td>
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<td>IFN-γ</td>
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**Statistical analysis**

Comparison of groups was undertaken using a Mann–Whitney U-test. Spearman’s correlation coefficient was used to examine the correlation between (a) gastritis scores and levels of cytokine transcripts, and (b) gastritis scores and epithelial proliferative and apoptotic indices. A p value of less than 0.05 was considered significant.

**Results**

Infection status in inoculated gerbils was assessed using the gastric biopsy urease test, microbial culture, and by immunohistology (Table 2). Age-matched uninfected female gerbils were used as controls at each time point, 4 weeks (n = 5), 12 weeks (n = 5), and 36 weeks (n = 8). At 36 weeks, age-matched uninfected male gerbils (n = 7) were used as controls. Gerbils were classified as *H pylori*-positive if they were positive by histology, culture or biopsy urease test. All female gerbils at 4, 12, and 36 weeks post-inoculation were successfully infected. However, 6/6 female and 7/10 male gerbils were negative by the biopsy urease test at 36 weeks post-infection. In contrast, 5/6 female and 9/10 male gerbils were *H pylori*-positive by gastric biopsy culture. One male gerbil was negative by histology, culture, and biopsy urease test at 36 weeks post-infection and was excluded from further analyses.

PCR analysis of DNA extracted from the cultured bacteria using primers specific for *H pylori* 16S rRNA or cagA confirmed successful *H pylori* infection. The glmM sequence of SS1 *H pylori* strains cultured from the gastric mucosa at 4 and 36 weeks post-infection was identical to the glmM sequence of the pre-infection *H pylori* SS1 strain (data not shown).

**Gastric pathology in Mongolian gerbils infected with *H pylori***

Uninfected male and female control gerbils had histologically normal gastric mucosa. Antral inflammation was evident in all female infected gerbils at 4 weeks post-infection. Both active inflammation and atrophy increased at later time points in the antrum (Table 3). By 36 weeks post-infection, four of six female gerbils had corpus gastritis. In most infected stomachs, there was a dense lymphoid response occupying both mucosa and submucosa. Frequent glandular herniations into the submucosa (Figure 1A) were also observed by 36 weeks post-infection with *H pylori*. At 36 weeks post-infection, all male gerbils had antral gastritis, with mild or moderate chronic inflammation. In contrast, three of six female gerbils had severe inflammation at 36 weeks, compared with none of ten male animals. At 36 weeks post-infection with *H pylori*, 33.3% of male gerbils developed corpus gastritis in comparison with 66.6% of female gerbils.

**Sequence analysis of gerbil cytokine transcripts**

RT-PCR products produced from transcripts coding for *IFN-γ* (230 bp), *IL-10* (196 bp), and *TGF-β* (197 bp) were analysed by agarose gel electrophoresis. DNA bands were isolated and sequenced. Novel gerbil DNA sequences that were flanked by the oligonucleotide primers used for their amplification were determined (Figure 2). A high degree of homology was observed when these sequences were aligned with respective rodent and human sequences (Figure 2). For example, the 180 bp nucleotide sequence coding for Mongolian gerbil *IFN-γ* was 71%, 77%, 71%, and 64% identical to rat, hamster, murine, and human orthologues, respectively (Figure 2C). Similarly, high degrees of homology were observed for *IL-10* and *TGF-β* (Figures 2A and 2B, respectively). The 263 bp nucleotide sequence of *IL-12p40* and the 164 bp sequence of β-actin demonstrated 100% homology with previously published data [25,26].

**Effects of *H pylori* infection on gastric cytokine gene expression in Mongolian gerbils**

Infection with the *H pylori* SS1 strain resulted in significant increases in *IFN-γ* transcripts (Figure 3) at 12

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**Table 2. Determination of infection status of Mongolian gerbils inoculated with the *H pylori* SS1 strain at 4, 12 and 36 weeks post-infection**

<table>
<thead>
<tr>
<th>Weeks post-infection</th>
<th>4 female</th>
<th>12 female</th>
<th>36 female</th>
<th>36 male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric biopsy urease test</td>
<td>7/8</td>
<td>3/4</td>
<td>0/6</td>
<td>3/10</td>
</tr>
<tr>
<td>Culture from gastric biopsy</td>
<td>8/8</td>
<td>4/4</td>
<td>5/6</td>
<td>9/10</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>8/8</td>
<td>4/4</td>
<td>6/6</td>
<td>9/10</td>
</tr>
<tr>
<td><em>H pylori</em>-positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Animals were classified as successfully infected if they were Helicobacter-positive by histology, culture or urease test. Control animals for each group were negative for each test at all time points.
Figure 1. Pathology and immunohistological analysis of gastric mucosa of Mongolian gerbils infected with the SS1 strain of *H. pylori*. (A) Haematoxylin and eosin-stained section of gastric antral mucosa of a female Mongolian gerbil 36 weeks post-infection with the SS1 strain of *H. pylori*, illustrating large epithelial invaginations into the submucosa (arrows). Bar = 200 μm. (B, C) Immunohistological detection of proliferating epithelial cells in the gastric mucosa of female Mongolian gerbils using an anti-bromodeoxyuridine (BrdU) monoclonal antibody. Gerbils were injected 1 h prior to sacrifice with BrdU. (B) Corpus mucosa 12 weeks post-infection with the *H. pylori* SS1 strain. (C) Antral mucosa 36 weeks post-infection with *H. pylori* SS1. Bar = 200 μm. (D) TUNEL assay performed on gastric mucosa of an uninfected gerbil showing apoptotic cells (arrows) in the surface epithelium. Bar = 50 μm. (E) TUNEL assay performed on gastric mucosa of a 36-week *H. pylori* SS1-infected female Mongolian gerbil showing an apoptotic epithelial cell (arrow) in the glandular epithelium. Bar = 50 μm.

(*p < 0.05*) and 36 weeks (*p < 0.01*) post-infection in female gerbils compared with controls (Figure 4A.). *IFN*-γ transcript levels at 12 (*p < 0.05*) and 36 weeks (*p < 0.01*) post-infection were significantly greater than at 4 weeks post-infection. Gastric mucosal *IL-12p40* transcript levels were also significantly (*p < 0.05*) increased at 36 weeks post-infection with *H. pylori* in female gerbils compared with controls (Figures 3 and 4B). *IL-12p40* transcript levels at 36 weeks post-infection were significantly greater (*p < 0.05*) than at 4 weeks post-infection (Figure 4B). *IL-10* transcripts were detected in the gastric mucosa of both uninfected and *H. pylori*-infected female gerbils, but levels did not vary significantly with infection status. An age-related increase in *TGF-β* transcript levels was observed in both uninfected (*p < 0.05*) and *H. pylori*-infected female gerbils. There was no significant difference between control and infected gerbils (Figure 4C).

A comparison of the gastric mucosal transcripts coding for *IFN*-γ and *IL-12p40* between male and female 36-week infected gerbils and age- and gender-matched controls is shown in Figure 5. In males, there was
Figure 2. A comparison of orthologous IL-10, TGF-β, and IFN-γ transcript sequences of gerbil (Ge), rat (Ra), hamster (Ha), mouse (Mo), and human (Hu). Gerbil transcripts were amplified by PCR using the oligonucleotide primers described in Table 1. Nucleotides that are identical to those in the Mongolian gerbil are indicated by an asterisk (*); — indicates a shift of sequence to maintain maximum sequence homology. Numbers in the right-hand column relate to the nucleotide position assigned in each of the respective database entries. The sequences described are flanked by the oligonucleotide primers described in Table 1. (A) Alignment of 120 bp of IL-10 sequences for the Mongolian gerbil, rat (X60675), mouse (M37897), and human (M57627; Alu sequence is inserted between positions 1173 and 1489). The degrees of identity observed between gerbil and rat, mouse or human IL-10 sequences were 88%, 89%, and 88%, respectively. (B) Alignment of 149 bp of TGF-β sequences of Mongolian gerbil, rat (X52498), hamster (AF191297), mouse (M13177), and human (BT007245). The degrees of identity observed between gerbil and rat, hamster, mouse or human TGF-β sequences were 91%, 89%, 91%, and 87%, respectively. (C) Alignment of 180 bp of IFN-γ nucleotide sequences of Mongolian gerbil, rat (AF010466), hamster (AF034482), mouse (M28621), and human (X01992) transcripts. The degrees of identity observed between Mongolian gerbil and rat, hamster, mouse, or human IFN-γ sequences were 71%, 77%, 71%, and 64%, respectively.
no significant increase in IFN-γ transcript levels after *H pylori* infection compared with the levels observed in control animals. In addition, in infected males, IFN-γ transcript levels were significantly lower (*p < 0.05*) than in infected females (Figure 5). In males, *IL-12p40* transcripts were significantly increased at 36 weeks post-infection with *H pylori* infection (*p < 0.01*). However, *IL-12p40* transcript levels in both control (*p < 0.05*) and infected (*p < 0.05*) males were significantly lower than in female control and infected gerbils, respectively. Similar to the observations with females, there was no significant increase in gastric mucosal *IL-10* transcripts in infected males.

In *H pylori*-infected male and female gerbils, there was a significant (*p < 0.001, r = 0.648*) correlation between IFN-γ and *IL-12p40* transcript levels in the gastric mucosa. IFN-γ, but not *IL-12p40*, transcript levels correlated significantly with chronic (*p < 0.001, r = 0.673*) and active (*p < 0.05, r = 0.463*) inflammation scores in the antrum.

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**Figure 3.** Detection of *IL-12p40*, IFN-γ, and β-actin transcripts in RNA extracted from the gastric mucosa of female Mongolian gerbils 36 weeks post-infection with the *H pylori* SS1 strain. Transcripts of (A) *IL-12p40*, (B) IFN-γ, and (A, B) β-actin in RNA extracted from gastric mucosa of female Mongolian gerbils 36 weeks post-infection with the SS1 strain of *H pylori* and age- and sex-matched uninfected control gerbils were analysed by RT-PCR and agarose gel electrophoresis. Lane L: 100 base pair ladder. Lanes 1–6 (A) and 1–7 (B): uninfected controls. Lanes 7–12 (A) and 8–13 (B): *H pylori*-infected. −ve = PCR negative control.

**Figure 4.** IFN-γ, *IL-12p40*, and TGF-β transcript levels in the gastric mucosa of female Mongolian gerbils infected with the *H pylori* SS1 strain for 4, 12, and 36 weeks and age- and gender-matched controls. Levels of IFN-γ (A), *IL-12p40* (B), and TGF-β (C) transcripts relative to β-actin at 4, 12, and 36 weeks are indicated on the y-axis. Data are expressed as mean ± SEM. Statistical analysis: Mann–Whitney U-test, *p < 0.05; **p < 0.01.*
Gerbil cytokine and epithelial responses to *H. pylori*

Figure 5. Comparison of IFN-γ and IL-12p40 transcript levels in the gastric mucosa of male and female Mongolian gerbils 36 weeks post-infection with the SS1 strain of *H. pylori*. Levels of IFN-γ and IL-12p40 transcripts relative to β-actin in infected male and female gerbils and age- and gender-matched controls are indicated on the y-axis. Data are expressed as mean ± SEM. Statistical analysis: Mann–Whitney U-test, *p < 0.05, **p < 0.01.

Figure 6. Gastric epithelial cell proliferation and apoptosis in female Mongolian gerbils infected with the SS1 strain of *H. pylori* for 4, 12, and 36 weeks and age- and gender-matched controls. The epithelial cell proliferation labelling index (LI %) in the antrum (A) and corpus (B) was determined immunohistologically using a monoclonal antibody to BrdU. The epithelial cell apoptosis index (AI %) in the glandular epithelium in the antrum (C) and corpus (D) was determined by the TUNEL assay. Data are expressed as mean ± SEM. Statistical analysis: Mann–Whitney U-test, *p < 0.05; **p < 0.005; †p = 0.08. *band †c, p < 0.05 from a.

Epithelial cell proliferation in Mongolian gerbils infected with *H. pylori*

Epithelial cell proliferation was assessed following BrdU incorporation (Figures 1B and 1C). At 4 weeks (*p < 0.05), 12 weeks (*p < 0.05), and 36 weeks (*p = 0.08) post-infection, an increase in epithelial cell proliferation was evident in the antrum of *H. pylori*-infected female gerbils compared with uninfected gender-matched controls (Figure 6A). At 36 weeks post-infection, males similarly had a significant increase in antral epithelial cell proliferation (*p < 0.05) compared with uninfected gender-matched controls (Table 4). In *H. pylori*-infected female and male gerbils, there was a significant correlation between antral epithelial proliferation indices and chronic (*p < 0.05, r = 0.479) and active (*p < 0.05, r = 0.487) inflammation scores. There was no significant correlation between IFN-γ transcripts and proliferation indices in the antrum (*p = 0.09, r = 0.392).

In the corpus, a trend towards increased gastric epithelial cell proliferation (Figure 1B) was observed...
Table 4. Epithelial cell proliferation labelling index (LI %) and glandular apoptosis index (AI %) in male and female Mongolian gerbils 36 weeks post-infection with the H pylori SS1 strain

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th></th>
<th>Female</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Infected</td>
<td>Control</td>
<td>Infected</td>
</tr>
<tr>
<td>Proliferation LI %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antrum</td>
<td>4.07 ± 0.88</td>
<td>16.11 ± 3.12*</td>
<td>7.09 ± 1.77</td>
<td>16.39 ± 4.30</td>
</tr>
<tr>
<td>Corpus</td>
<td>0.97 ± 0.13</td>
<td>5.31 ± 2.79</td>
<td>0.95 ± 0.13</td>
<td>12.22 ± 5.24</td>
</tr>
<tr>
<td>Apoptosis AI %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antrum</td>
<td>0.39 ± 0.05</td>
<td>1.87 ± 0.45*</td>
<td>0.13 ± 0.04</td>
<td>1.80 ± 0.15†</td>
</tr>
<tr>
<td>Corpus</td>
<td>0.15 ± 0.02</td>
<td>0.51 ± 0.10*</td>
<td>0.15 ± 0.06</td>
<td>0.56 ± 0.30†</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM.

* p < 0.05 and † p < 0.005 compared with gender-matched controls.

at 12 and 36 weeks (p = 0.08) post-infection (Figure 6B). At 36 weeks post-infection, epithelial cell proliferation in the female gerbils with corpus gastritis was significantly higher than in uninfected controls (p < 0.005) and in infected females that had not developed corpus gastritis (p = 0.06). At 36 weeks post-infection, male gerbils with corpus gastritis had significantly higher epithelial cell proliferation than uninfected gender-matched controls (p < 0.05) and those gerbils that had not developed corpus gastritis (p < 0.05).

Epithelial apoptosis in Mongolian gerbils infected with H pylori

In the antral and corpus mucosa of uninfected gerbils, apoptotic epithelial cells were located in the superficial epithelium (Figure 1D), with fewer apoptotic cells being present in the glandular epithelium. Significant age-related decreases in apoptosis in the glandular epithelium of the antrum of uninfected female gerbils were evident between 4 and 12 weeks (p < 0.05) and this was also evident at 36 weeks (p < 0.05) (Figure 6C). In H pylori-infected female gerbils, the level of apoptosis in the superficial epithelium of the antrum was not significantly different from uninfected controls (data not shown). In contrast, apoptosis in the glandular epithelial cells of the antrum (Figure 1E) was significantly increased at both 12 (p < 0.05) and 36 weeks (p < 0.005) post-infection with H pylori compared with uninfected controls (Figure 6C). Apoptosis in the glandular epithelium of the antrum decreased significantly (p < 0.05) from 12 weeks to 36 weeks post-infection with H pylori (Figure 6C).

A significant increase in apoptosis in the glandular epithelium of the corpus was observed in female gerbils infected with H pylori for 36 weeks, compared with uninfected controls (p < 0.005) (Figure 6D). The levels of apoptosis in the glandular epithelial cells of the corpus was significantly increased in the female gerbils with corpus gastritis (n = 4), compared with those animals that had not developed corpus gastritis (n = 2) (p = 0.06) and uninfected controls (n = 8) (p < 0.01). A similar pattern of changes in apoptosis was observed in male gerbils infected with H pylori for 36 weeks (Table 4). In H pylori-infected female and male gerbils, there was no association between apoptotic indices in the antrum, inflammation scores, and IFN-γ transcript levels.

Discussion

The present study confirms previous observations that the H pylori SS1 strain successfully colonizes gerbils [15,16], and further characterizes the mucosal cytokine response and epithelial cell response to H pylori infection in this model. Of note, we have determined that there are marked gender differences in gastric mucosal cytokine responses to H pylori.

Previously, the SS1 strain of H pylori [28] has been used extensively in murine studies on colonization and vaccination. Recently, both short-term [15] and long-term [16] infection studies with this H pylori strain have been carried out in gerbils. In the gerbil model, infection with the SS1 strain results in increased pathology and epithelial proliferative responses, compared with mice [15,16]. In the study of Suzuki et al [15], no distinction between antral and corpus mucosa was made when examining gerbil pathology and epithelial proliferative responses. In the present study, initial inflammation to the SS1 strain was antral-predominant, concurring with earlier studies with other H pylori strains [1,14]. Corpus gastritis was evident in approximately half the gerbils at 36 weeks post-infection, with a trend for increased corpus gastritis in females. Epithelial proliferation was significantly greater in the corpus of animals with corpus gastritis. In addition, in the antrum, a positive correlation between epithelial proliferation in H pylori-infected gerbils and the grade of chronic and active gastritis was observed. Similar observations on epithelial proliferation and gastric inflammation have been described in patients with H pylori infection [9,10]. In contrast to the present study, Peek et al [14] did not observe any association between proliferation and histology in gerbils infected with the H pylori strain G1.1 which, like the SS1 strain, also lacks a functional cag PAI. In addition, infection with G1.1 resulted in no
substantial corpus gastritis or significant epithelial proliferation in the corpus over a similar time period [14]. These differences may relate to other genetic variables between the strains, differing source and gender of the gerbils, and the use of different techniques to determine epithelial cell proliferation.

Consistent with murine studies [12,13,29], the number of apoptotic epithelial cells in the superficial epithelium of the gerbil did not differ between uninfected and infected animals. Apoptotic cells in the surface epithelium are considered to represent normal cell turnover. In contrast, a significant increase in apoptosis was observed in the glandular epithelium of the antrum at 12 and 36 weeks post-infection compared with age-matched controls. A significant decrease in apoptosis was evident in the antrum in infected gerbils between 12 and 36 weeks and this concurs with the earlier observations of Peek et al [14]. However, in the present study, an age-related decrease in apoptosis in glandular epithelial cells of the antrum of uninfected gerbils was also observed, which has not been previously described. In the rat, age-related decreases in apoptosis in colonic epithelial cells have been linked to decreases in caspase activation and inhibition of poly(ADP-ribose) polymerase (PARP) degradation [30]. In contrast to the results with epithelial proliferation, no significant correlation was observed between apoptotic indices in the antrum and histological gastritis scores. Previous studies have documented an inverse association between apoptosis and acute inflammation in the gerbil [14]. However, recent clinical studies also suggest that increases in epithelial apoptosis do not correlate with inflammation [10].

Gender differences in the epithelial and histological response to gastric H felis infection are evident in mice, with female C57/BL6 mice having increased pathology, epithelial proliferation, and apoptotic changes with long-term infection [13]. In the present study, no significant differences in epithelial proliferation or apoptosis were evident between long-term infected male and female gerbils.

The analysis of mucosal responses to H pylori and the inter-relationship between epithelial and inflammatory responses has been impeded by the lack of genomic data on gerbil inflammatory mediators. Given the importance of Th1 cytokines in gastric responses to H pylori or H felis [17–19,31–33] in other species, we amplified RNA transcripts for IFN-γ and IL-12p40 in the gerbil by RT-PCR and verified them by sequencing. Infection with H pylori was associated with marked up-regulation of the levels of transcripts coding for both IFN-γ and IL-12p40 in the gerbil gastric mucosa. Thus, the gerbil, in common with humans [31], other primates [32], and mice [33], has a Th1-polarized response to gastric H pylori infection.

IL-12p40 can be a component of both IL-12 and IL-23 by binding the p35 subunit of IL-12 or the p19 subunit of IL-23 [34]. Both IL-12 and IL-23 will stimulate Th1 responses and IFN-γ-secreting T cells [34]. IL-12p40 transcript levels are increased in the gastric mucosa of patients with H pylori infection [35]. H pylori preferentially induces IL-12, but not IL-10, secretion by dendritic cells [36]. There was no evidence of increased IL-10 transcript levels in the gerbil gastric mucosa in response to H pylori infection. However, up-regulation of IL-10, an important immunoregulatory cytokine that protects against Th1 mucosal inflammation [37], has been observed in patients with cag PAI-positive H pylori infection [35].

The levels of gastric IFN-γ transcripts in H pylori-infected gerbils increased with age. Age-related changes in intestinal cytokine responses to enteric infections have been observed in mice, with ageing animals having increased Th1 cytokine responses [38]. Additionally, in the present study, significant differences were observed between the IFN-γ and IL-12p40 transcript levels in H pylori-infected male and female gerbils at 36 weeks post-infection. This, to our knowledge, is the first observation of gender differences in cytokine responses to H pylori infection. Whether these differences occur earlier in infection requires further investigation. Sex differences in cytokine production are considered to contribute to gender variation in patterns of infectious and autoimmune diseases. Interestingly, gender differences in the susceptibility of mice to experimental autoimmune disease have been attributed to males producing less IL-12 and IFN-γ than females [39,40]. Increased IL-12 responses in females have been associated with oestrogen activation of STAT4 [39]. Further studies are required to characterize gender-related differences in mucosal cytokine responses to H pylori in other species.

In C57BL/6 mice, gender differences are apparent in both the histological and the epithelial response to gastric H felis infection [13], with females having more severe pathology. However, no gender differences were observed in the development of gastric cancer in C57BL/6 mice following 13–15 months of H felis infection [41]. In contrast, in hypergastrinaemic INS-GAS mice (on an FVB/N background), H pylori infection results in gastric adenocarcinoma in males, but not in females [41,42]. There are marked strain differences in the parietal cell life span between FVB/N and C57BL/6 mice [43], with parietal cells in the former having a considerably reduced life span. Whether gender differences in the parietal cell life span in FVB/N mice contribute to male restricted gastric cancer development in the INS-GAS model is unknown. The mechanisms by which gender influences disease in Helicobacter-infected rodent models may differ between in-bred mouse strains and Mongolian gerbils.

In addition to IL-10, TGF-β has a major role in reducing the severity of mucosal inflammation [37]. IL-10 facilitates the up-regulation of TGF-β [37], which inhibits T-cell function by blocking proliferation and differentiation [44]. In the present study, constitutive expression of TGF-β was observed in the gerbil gastric mucosa and an age-related increase in...
expression was observed in both uninfected controls and _H. pylori_-infected gerbils. No data are available on age-related changes in gastric TGF-β expression in other species, but in humans plasma TGF-β levels are significantly elevated in the elderly [45]. Furthermore, TGF-β increases the expression of markers of cellular senescence in fibroblasts [46]. TGF-β has a modulatory effect on gastric patholgy in BALB/c mice with thymectomy-induced autoimmune gastritisis [44]. In this experimental model, _H. pylori_ infection inhibits the development of autoimmune gastritis and protection was associated with _H. pylori_-induced up-regulation of gastric TGF-β expression [47]. The absence of any bacteria-associated increase in either IL-10 or TGF-β in the gerbil with _H. pylori_ infection may account for the severe pathology in this model.

In conclusion, the mucosal response to _H. pylori_ in female gerbils is characterized by a marked Th1 response and an absence of a down-regulatory response. Differences in cytokine production may be responsible for the increased susceptibility of gerbils to _H. pylori_ infection. This may be an important factor in the more severe gastritis observed with _H. pylori_ infection in gerbils than in mice. Future studies with gerbil-specific cytokine antisera will facilitate further exploration of gastric inflammatory responses to _H. pylori_.

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**References**


