Catalase (KatA) and KatA-associated protein (KapA) are essential to persistent colonization in the Helicobacter pylori SS1 mouse model

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Helicobacter pylori infects the human gastric mucosa and elicits an aggressive inflammatory response. Despite the severity of the inflammatory response, the bacterium is able to persist and cause a chronic infection. It is believed that antioxidant defence mechanisms enable this organism to persist. Wild-type H. pylori strain SS1, and KatA- and KapA-deficient mutants, were used to infect C57/BL6 mice to test this hypothesis. Neither KatA nor KapA was essential for the initial colonization of H. pylori SS1 in the murine model of infection. The wild-type SS1 colonized the gastric mucosa at significantly higher levels than both mutants throughout the 24-week experiment. Neither KatA- nor KapA-deficient mutants were able to maintain consistent ongoing colonization for the 24-week period, indicating the necessity of both KapA and KatA in sustaining a long-term infection. At 24 weeks, 5/10 mice inoculated with the KatA mutant and 2/10 mice inoculated with the KapA mutant were colonized, compared with 10/10 of the mice inoculated with the wild-type SS1. An increase in the severity of inflammation in the wild-type-inoculated mice appeared to correlate with the decline in colonization of animals inoculated with the mutants, suggesting that increased oxidative stress militated against continued infection by the mutants. These data indicate that KapA may be of equal or greater importance than KatA in terms of sustained infection on inflamed gastric mucosae.

INTRODUCTION

Helicobacter pylori is a Gram-negative bacterium identified as the aetiological agent of peptic ulcer disease, gastric adenocarcinoma and B-cell mucosal-associated lymphoid tissue (MALT) lymphoma (Enno et al., 1998; Graham et al., 1989; Parsonnet et al., 1991; Uemura et al., 2001; Wotherspoon et al., 1991; Forman et al., 1991). A characteristic feature of the pathogenesis of H. pylori infection is persistence on the inflamed gastric mucosa. The initial inflammatory response elicited by H. pylori infection in humans is characterized by a marked polymorphonuclear neutrophil (PMN) infiltrate (Frommer et al., 1988). The initial PMN infiltrate is followed by an increased number of lymphocytes, monocytes and plasma cells (Crabtree, 1996; Hazell et al., 1987). Upon recruitment, PMNs are activated and release a number of toxic mediators, including reactive oxygen species (ROS). ROS encompass superoxide anions, hydroxyl radical and hydrogen peroxide. It has been proposed that the survival of H. pylori in the presence of the ROS is largely due to its sophisticated antioxidant defence mechanisms, including superoxide dismutase, alkylhydroperoxide reductase, a thiol-linked thiol peroxidase (scavengase) and catalase (Hazell et al., 2001).

Recently Seyler et al. (2001) demonstrated that the superoxide dismutase of H. pylori was essential for colonization of...
the murine gastric mucosa in the \textit{H. pylori} SS1 mouse model of infection. Superoxide dismutase dismutates the superoxide anion into hydrogen peroxide and molecular oxygen. Hydrogen peroxide will react with reduced metallic ions found in biological systems, in particular iron or copper ions, resulting in the formation of the hydroxyl radical (Fenton reaction) (Cadenas, 1989). Hydroxyl radicals are highly reactive and toxic to cells and there is no enzymatic system of detoxification. Therefore it is advantageous for pathogens to encode enzymes such as superoxide dismutase and catalase.

The catalase of \textit{H. pylori} (KatA) is a homotetrameric protein and is a ’typical’ catalase (lacks peroxidase activity) (Hazell et al., 1991a). Catalase appears to be a cytosolic protein; however, there are data indicating periplasmic and possible surface localization (Hazell et al., 1991a, 2001). The gene, \textit{katA}, has been sequenced and KatA is an excellent vaccine antigen against \textit{H. pylori} (Alm et al., 1999; Manos et al., 1997; Odenbreit et al., 1996; Radcliff et al., 1997; Tomb et al., 1997). Endogenously generated hydrogen peroxide, a by-product of oxygenic metabolism, does not appear to be a significant problem for \textit{H. pylori} as catalase-deficient mutants are viable when cultured \textit{in vitro} (Harris et al., 2002; Hazell et al., 1991b; Manos et al., 1997; Odenbreit et al., 1996; Westblom et al., 1992). We have recently shown that \textit{H. pylori} is resistant to high concentrations (\(\sim 100 \text{ mM}\)) of hydrogen peroxide and that this resistance was abolished in KatA-deficient mutants (Harris et al., 2002). We have also demonstrated that the gene immediately downstream of \textit{katA}, encoding a protein which we have proposed to name KatA-associated protein (KapA) (open reading frame HP0874; Tomb et al., 1997), has a role in the \textit{in vitro} resistance of \textit{H. pylori} to hydrogen peroxide (Harris et al., 2002). Our studies (Harris et al., 2002) and those of Odenbreit et al. (1996) indicate that KapA does not influence catalase activity per se. Thus there is a need to determine if the putative role of KapA in resistance to oxidative damage is trivial or of biological relevance. Further, the importance of \textit{H. pylori} catalase \textit{in vivo} has, until now, never to our knowledge been assessed.

The aims of this study were to investigate the necessity of KatA and KapA in the \textit{H. pylori} SS1 mouse model of infection and to determine whether the absence of these proteins will allow \textit{H. pylori} to colonize and/or maintain infection of the murine gastric mucosa.

\section*{METHODS}

\textbf{Bacteria and culture conditions.} \textit{Helicobacter pylori} SS1 (Sydney strain) was obtained from the culture collection at the School of Microbiology and Immunology, University of New South Wales.

\textit{H. pylori} SS1 KatA-deficient and SS1 KapA-deficient mutants were created by insertional disruption using \textit{aphA3}, encoding kanamycin resistance. Briefly, DNA encoding HP0875 (KatA) and the downstream gene separated by 150 bp, HP0874 (KapA), were cloned from \textit{H. pylori} 26695 into \textit{pGem}. Unique restriction sites were identified into which \textit{aphA3}, encoding kanamycin resistance, was introduced. RT-PCR was used to determine that the mutations were non-polar and that the two genes were not transcriptionally coupled. Specifics of the construction of the isogenic mutants have previously been described (Harris et al., 2002).

Cultures of \textit{H. pylori} SS1 were maintained using Campylobacter selective agar (CSA) consisting of Blood Agar Base no. II (Oxoid), 7% (v/v) horse blood and Skirrow’s selective supplement [5 \(\mu\text{g}\) trimethoprim ml\(^{-1}\), 161.5 \(\mu\text{g}\) polymyxin B ml\(^{-1}\) (Sigma), 10 \(\mu\text{g}\) vancomycin ml\(^{-1}\) (Eli Lilly) and 2.5 \(\mu\text{g}\) amphotericin B ml\(^{-1}\) (E.R. Squibb & Sons) (Skirrow, 1977)]. The isogenic mutants were maintained using CSA supplemented with 20 \(\mu\text{g}\) kanamycin ml\(^{-1}\) (CSAK), as previously described (Harris et al., 2002). The cultures were incubated for 24–48 h in an anaerobic incubator (Forma Scientific) at 37°C in a reduced-oxygen (microaerobic) environment that consisted of 10% CO\(_2\) in air and 95% relative humidity.

Murine gastric tissues were cultured on 7% (v/v) horse blood agar plates containing the Glaxo selective supplement (3-3 \(\mu\text{g}\) polymyxin B ml\(^{-1}\), 20 \(\mu\text{g}\) bacitracin ml\(^{-1}\), 10-7 \(\mu\text{g}\) nalidixic acid ml\(^{-1}\), 10 \(\mu\text{g}\) vancomycin ml\(^{-1}\) and 5 \(\mu\text{g}\) amphotericin B ml\(^{-1}\)) as described by McColm et al. (1995) (GSSA). Kanamycin (20 \(\mu\text{g}\) ml\(^{-1}\)) was used to supplement the medium where appropriate (GSSAK).

Broth cultures of \textit{H. pylori} were prepared as described previously (Harris et al., 2000). Broth cultures of \textit{H. pylori} were used to infect mice due to the increased motility of the bacteria in liquid. Briefly, 300 ml brain-heart infusion broth (Oxoid), supplemented with Skirrow’s selective supplement and 5% (v/v) horse serum (Oxoid), was inoculated using bacteria from two 36 h CSA cultures of \textit{H. pylori}. The flasks were placed in 3 l anaerobic jars with an appropriate environment generated using an anaerobic gas pack (Oxoid) without the palladium catalyst. The jars were incubated at 37°C for 48 h with moderate agitation. Purity of cultures was determined by phase-contrast microscopy, Gram-stain, rapid urease and catalase tests. Culture densities were adjusted to \(\sim 10^8\) c.f.u. ml\(^{-1}\) prior to infecting the mice. Retrospective colony counts were carried out on the adjusted broth cultures.

\textbf{In vitro sensitivity to hydrogen peroxide.} Sensitivity of \textit{H. pylori} SS1 to hydrogen peroxide was tested as described previously (Harris et al., 2002). Briefly, fresh 24 h cultures of \textit{H. pylori} SS1 wild-type, KatA- and KapA-deficient mutants, grown on CSA or CSAK, were harvested in 0-9% (w/v) NaCl (physiological saline). The cells were washed once and resuspended in physiological saline. The optical densities of the cell suspensions, measured at 600 nm (OD\(_{600}\) 1 cm path length), were adjusted to 0-7 and used immediately. At time zero, 200 \(\mu\text{l}\) of the cell suspension was mixed with 1-8 ml of \(\sim 100\text{ mM}\) hydrogen peroxide. The concentration of the hydrogen peroxide was determined spectroscopically as described previously (Beers & Sizer, 1952; Harris et al., 2002; Hazell et al., 1991a). Immediately after the addition of the cell suspension, 100 \(\mu\text{l}\) was removed and combined with 100 \(\mu\text{l}\) of 1% (w/v) bovine liver catalase (Sigma), to remove residual hydrogen peroxide, if any. Four 1:10 dilutions were made in physiological saline, and were inoculated in 10 \(\mu\text{l}\) spots, in triplicate, onto ‘dry’ CSA plates. Every 3 min the process was repeated, for a total of 21 min. The agar plates were incubated for 5 days at 37°C in a microaerobic environment, after which individual colonies were counted. As a negative control, water was used in place of hydrogen peroxide. The \textit{in vitro} response to hydrogen peroxide was tested twice for each strain.

\textbf{Care and infection of experimental mice.} The animal care and ethics committee of the University of New South Wales approved experiments involving animals. In accordance with animal ethics requirements to minimize animal usage, the number of
animals required was estimated on the basis of the power needed to demonstrate an hypothesized significant reduction of colonization over time in mice infected with KatA-deficient mutants.

One hundred and twenty 6-week-old, female, specific-pathogen-free (SPF) C57/Black6 mice were obtained from the Biological Resource Centre, Little Bay, Sydney. These animals were allowed to adapt to their new environment for 3 weeks prior to commencement of experimentation. The mice were fed autoclaved rat and mouse ration and autoclaved water ad libitum. Mice were divided into four groups of 30 animals and inoculated by oral gavage twice over 3 days with either physiological saline and plated onto GSSA and GSSAK.

Ten mice from each group were killed by CO2 asphyxiation at 8 days, 12 weeks and 24 weeks post initial inoculation. The stomach was removed quickly, bisected along the lesser and greater curves and rinsed in sterile physiological saline. One half of each stomach was placed in 10% neutralized buffered formalin and processed for histology. The other half was weighed and homogenized in 0-1 M phosphate-buffered physiological saline (pH 7-4). The homogenate was serially diluted in physiological saline and plated onto GSSA and GSSAK.

**Histopathology.** Five-micrometre sections of the murine gastric tissue were stained with haematoxylin and eosin for histopathological evaluation or with Steiner’s modified silver stain to grade the bacteriological load. PMN and mononuclear cells in the antrum and body were graded as described by Sutton et al. (2000). Briefly the grading system was as follows: 1, mild multi-focal; 2, mild widespread or moderate multi-focal; 3, mild widespread and moderate multi-focal or severe multi-focal; 4, moderate widespread; 5, moderate widespread and severe multi-focal; and 6, severe widespread. The submucosal inflammation (inflammatory cells under muscularis mucosa or in the non-glandular epithelium) was graded using the following scale: 1, mild multi-focal; 2, mild widespread; 3, moderate multi-focal; 4, moderate widespread; 5, severe multi-focal; and 6, severe widespread. Parietal cell atrophy was graded using the following scale: 1, mild multi-focal; 2, mild widespread; 3, moderate multi-focal; 4, moderate widespread; 5, severe multi-focal; and 6, severe widespread. Parietal cell atrophy was graded using the following scale: 1, mild multi-focal; 2, mild widespread; 3, moderate multi-focal; 4, moderate widespread; 5, severe multi-focal; and 6, severe widespread. The total numbers of lymphoid follicles and glandular abscesses were also counted. The distribution of bacteria was determined using a positive/negative grading scale.

**Statistical analysis.** Statistical significances were determined by Kruskal-Wallis and Tukey’s tests. Where required, chi-square analyses between groups were also carried out. All statistical analyses were performed using SPSS statistical analysis software (SPSS Inc.; ver. 10.0.5).

**RESULTS**

**Response to hydrogen peroxide**

The wild-type and mutants of strain SS1 were tested to ensure they had the same phenotype in terms of resistance to hydrogen peroxide as strain 26695 tested previously (Harris et al., 2002). *H. pylori* SS1 wild-type, and KatA- and KapA-deficient mutants, were exposed to hydrogen peroxide at an initial concentration of ~100 mM for a total of 21 min (Fig. 1). The viability of wild-type SS1 appeared not to be affected by exposure to hydrogen peroxide. Exposing the KatA-deficient mutant to ~100 mM hydrogen peroxide resulted in a rapid drop in viability, reaching the limit of detection between 3 and 6 min of exposure. The KatA-deficient mutant displayed a steady decline in viability after an initial lag period. Over the 21 min time period there was a fivefold decrease in viability. As a negative control, the three isolates of *H. pylori* were exposed to water over the same period of time. There was no apparent drop in viability, nor were there any obvious differences between the three strains over the time period (data not shown).

**Colonization**

If a gene product is essential to primary colonization then the effect of a mutation will be detected early. However, if a gene product is essential to persistence of infection, then longitudinal data are required.

**8 days post-infection.** No *H. pylori* were isolated from the stomachs of the placebo group (negative control) of mice on GSSA or GSSAK (0/10) (Fig. 2A). *H. pylori* SS1 was isolated from 10/10 mice infected with the wild-type SS1 (positive control). Colonies grew on GSSA but not GSSAK, with a median value over all mice of 3·5 × 10^5 cfu. (g stomach)^−1 (Fig. 2A). The KatA-deficient mutant was isolated from 9/10 animals, with comparable results being obtained for the numbers isolated on GSSA and GSSAK. The median value for colonization over all mice by the KatA-deficient mutant was 2·5 × 10^4 cfu. (g stomach)^−1 (Fig. 2A). The KapA-deficient mutants were isolated from the gastric tissue of 9/10 mice, being isolated on both GSSA and GSSAK, again with comparable results. The median colonization for the KapA-deficient mutant over all mice was 1·4 × 10^4 cfu. (g stomach)^−1 (Fig. 2A).

**12 weeks post-infection.** After 12 weeks of infection, no *H. pylori* were isolated from the gastric tissue of the non-infected control (placebo) on either GSSA or GSSAK (Fig. 2B). *H. pylori* wild-type SS1 was isolated on GSSA, but not GSSAK, from 9/10 of the animals initially inoculated. The median colonization over all mice inoculated with the wild-type SS1 at this time point was 2·6 × 10^6 cfu. (g stomach)^−1 (Fig. 2B). The KatA-deficient mutant of *H. pylori* SS1 was isolated from 8/10 mice on both
GSSA and GSSAK. The median colonization over all mice inoculated was $2.1 \times 10^3$ c.f.u. (g stomach)$^{-1}$ (Fig. 2B). The KapA-deficient mutant of \textit{H. pylori} SS1 was isolated from 8/10 mice on both GSSA and GSSAK. The median value of colonization over all mice was $6.5 \times 10^4$ c.f.u. (g stomach)$^{-1}$; however, there was a marked variance within this group, with the levels of colonization spanning several orders of magnitude (Fig. 2B).

### 24 weeks post-infection

\textit{H. pylori} was not isolated on GSSA or GSSAK from the gastric tissues of the placebo group (non-infected controls) of mice after 24 weeks of infection (0/10) (Fig. 2C). \textit{H. pylori} wild-type SS1 was isolated on GSSA but not GSSAK from all of the animals initially inoculated (10/10). The median colonization for the wild-type at this time point was $5.4 \times 10^5$ c.f.u. (g stomach)$^{-1}$ (Fig. 2C). KatA-deficient mutants of \textit{H. pylori} SS1 were isolated from 5/10 mice on both GSSA and GSSAK. The median value for colonization over all mice was $1.8 \times 10^5$ c.f.u. (g stomach)$^{-1}$; however, there was a high degree of variance in relation to colonization levels within this group (Fig. 2C). KapA-deficient mutants of \textit{H. pylori} were isolated from the gastric tissue homogenate of only 2/10 mice on both GSSA and GSSAK. The median colonization for the KapA-deficient mutants over all mice was less than 1, due to the limited numbers of animals colonized (Fig. 2C).

### Distribution of colonization

Silver-stained sections for all three groups of mice colonized with \textit{H. pylori} over the three different time points were analysed to determine if the disruption of \textit{kata} or \textit{kapa} had an effect on the distribution of bacteria within the murine stomach. There was no apparent variation of colonization patterns in either of the isogenic mutants. Both isogenic mutants and wild-type SS1 predominantly colonized the antrum and antrum/body border of the stomach, consistent with previous observations regarding \textit{H. pylori} SS1 colonization (Lee et al., 1997).

### Statistical analysis of animal colonization

By chi-square analysis there was a statistically significant decrease in the number of animals colonized ($P<0.05$) at 24 weeks when the mutants were compared to the wild-type SS1. This was not the case at 8 days and 12 weeks. The colonization results were subjected to Kruskal–Wallis analysis and Tukey’s test for the comparison of arithmetic means within time points (comparison of four groups within a given time point) (Table 1). The level of colonization, measured in terms of c.f.u., was significantly lower in the two mutants compared with the wild-type SS1 at 8 days and 12 weeks. At 24 weeks, the \textit{kapa} mutant had significantly lower colonization than wild-type SS1. The level of colonization for the \textit{kata} mutant was intermediate between the wild-type and the \textit{kapa} mutant, reflecting the fact that it was not significantly different from either.

### Histopathology

An increase in inflammation will result in a concomitant increase in the production of ROS. Thus, antioxidant defence systems may be more important to the survival of \textit{H. pylori} in the presence of gastritis than on the naive gastric mucosa.

The statistical significance of the different histopathological grades observed between the mice that were in the placebo group, infected with the wild-type SS1, infected with the Kata-deficient mutants and infected with the KapA-deficient mutants of \textit{H. pylori} SS1 were compared...
Table 1. Statistical analysis of the levels of colonization in the murine stomach obtained by *H. pylori* SS1 wild-type, KatA- and KapA-deficient mutants and a placebo

The analyses were carried out using Kruskal–Wallis analysis and Tukey’s statistical tests for colonization. Tukey’s test indicates significance at a confidence level of 5% ($P \leq 0.05$) as denoted by a ‘*’ in the table, allowing comparisons between groups in terms of levels of colonization at a given time point.

<table>
<thead>
<tr>
<th></th>
<th>8 days post-inoculation</th>
<th>12 weeks post-inoculation</th>
<th>24 weeks post-inoculation</th>
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<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>SS1</td>
<td>KatA</td>
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<tr>
<td>Placebo</td>
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<td>SS1</td>
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<td>KatA</td>
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<td>KapA</td>
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</table>

within time points (different groups within the same time point) and within groups (same groups at different time points).

Table 2 shows the arithmetic mean of each group at each of the three different time points for each of the histopathological results. The inflammatory response was characterized by a mild multifocal infiltration of PMNs and mononuclear cells in both antrum and body.

There were no statistically significant differences between any of the groups graded for the presence of PMNs in the antrum, lymphoid aggregates or gland abscesses.

For the mice infected with the wild-type SS1 the increase in PMNs and lymphocytes/plasma cells in the body of the stomach over time were statistically significant ($P < 0.05$). The differences in grades of the PMNs and lymphocytes in the body of the stomach, over time, of mice infected with the KatA- or KapA-deficient mutants or the placebo mice were not statistically significant.

The increases in severity over time for submucosal inflammation and parietal cell atrophy were also statistically significant ($P < 0.05$) for mice infected with the wild-type SS1. The differences over time for submucosal inflammation and parietal cell atrophy of mice infected with

Table 2. Table of the arithmetic mean of the grading for the different histopathological events for each of the groups at the three different time points

Antrum and body PMN and LP (lymphocytes and plasma cells) and submucosal inflammation (Sub. inflam.) were graded between 0 and 6 (6 being the most severe). Parietal cell atrophy (PC atrophy) was graded between 0 and 3 (3 being the most severe). The lymphoid aggregates (Lymph. aggr.) and gland abscesses are actual numbers observed. Observations relate to the murine stomach inoculated with *H. pylori* SS1 wild-type, KatA- and KapA-deficient mutants or a placebo.

<table>
<thead>
<tr>
<th>No. of mice colonized</th>
<th>Antrum</th>
<th>Body</th>
<th>Sub. inflam.</th>
<th>PC atrophy</th>
<th>Gland abscess</th>
<th>Lymph. aggr.</th>
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<tr>
<td></td>
<td>PMN</td>
<td>LP</td>
<td>PMN</td>
<td>LP</td>
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<tr>
<td>Placebo</td>
<td>0/10</td>
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<td>0.4</td>
<td>0.0</td>
<td>0.2</td>
<td>0.0</td>
</tr>
<tr>
<td>SS1</td>
<td>10/10</td>
<td>0.2</td>
<td>0.0</td>
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</tr>
<tr>
<td>SS1 KatA</td>
<td>9/10</td>
<td>0.0</td>
<td>0.4</td>
<td>0.5</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>SS1 KapA</td>
<td>9/10</td>
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<td>0.0</td>
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<td>12 weeks</td>
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<tr>
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<td>0.7</td>
<td>0.4</td>
<td>0.7</td>
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<td>0.5</td>
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<td>0.4</td>
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<td>SS1 KapA</td>
<td>8/10</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.3</td>
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<td>24 weeks</td>
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<tr>
<td>Placebo</td>
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<td>0.9</td>
<td>1.1</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>SS1</td>
<td>10/10</td>
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<td>1.6</td>
<td>2.3</td>
<td>1.4</td>
<td>7.0</td>
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<tr>
<td>SS1 KatA</td>
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<tr>
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<td>2/10</td>
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<td>1.2</td>
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<td>0.3</td>
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the KapA- and KatA-deficient mutants, or mice in the placebo group, were not statistically significant.

**DISCUSSION**

Our understanding of pathogenesis has changed and developed over the last decade. It is recognized that primary pathogens are well adapted to the host they infect. This adaptation goes beyond recognized major virulence factors. A well-adapted pathogen has evolved a metabolism and physiology that is matched to the host. Furthermore, the pathogen maintains a range of aggressive and defensive factors, each important to the process of infection and disease.

Resistance to oxidative stress is critical to the maintenance of infection, as one of the host’s primary defences against bacterial infection is the release of ROS. *H. pylori* possesses antioxidant defence mechanisms, including superoxide dismutase, catalase, alkylhydroperoxide reductase, thiol peroxidase and potentially KapA (Harris et al., 2002; Hazell et al., 2001). The aim of the current investigation was to examine the role of both KatA and KapA in the pathogenesis of *H. pylori* SS1 in the murine model of infection (Lee et al., 1997).

It has previously been shown that catalase is not essential for the survival of *H. pylori* in vitro (Harris et al., 2002, Hazell et al., 1991a, b; Manos et al., 1997; Odenbreit et al., 1996; Westblom et al., 1992). We have proposed that catalase is a crucial component in the evasion of the host immune response (Hazell et al., 2001). This theory was supported by the findings of Ramarao et al. (2000) that *H. pylori* induces the release of ROS upon adherence to professional phagocytes in vitro, and catalase-deficient mutants did not survive the respiratory burst. These data are supported by our study showing that catalase facilitates survival of *H. pylori* upon exposure to high (~100 mM) concentrations of hydrogen peroxide (Harris et al., 2002).

We have previously shown that a protein encoded by a gene downstream of KatA, KapA, facilitates in vitro resistance to hydrogen peroxide in *H. pylori* 26695 (Harris et al., 2002). Despite the presence of catalase activity, KapA-deficient mutants of *H. pylori* 26695 were sensitive to killing by hydrogen peroxide. In *vitro* it was apparent that both KatA and KapA play a role in the survival of the bacterium in the presence of hydrogen peroxide. Although a role for KapA has been identified, further characterization is required, particularly relevant to human disease.

Not all strains of *H. pylori* colonize mice. Exposing *H. pylori* SS1 wild-type to ~100 mM hydrogen peroxide had no apparent effect on the viability of the cells. KatA-deficient strains of *H. pylori* SS1 were hypersensitive to hydrogen peroxide. The KapA-deficient mutant retained viability for a short period (lag) before sustaining a fivefold decrease in viability over 21 min (Fig. 1). These findings are consistent with those previously described for *H. pylori* 26695 wild-type, KatA- and KapA-deficient strains (Harris et al., 2002).

The initial time point in the animal experiments was to assess the role of KatA and KapA in primary colonization. Eight days post-inoculation, mice infected with the KatA-deficient and KapA-deficient mutants were colonized at a level approximately one order of magnitude lower than the mice infected with the wild-type SS1. These data demonstrated that neither KapA nor KatA was essential for the colonization of the murine gastric mucosa by *H. pylori* SS1, but suggested an advantage in having functional KatA and KapA.

After 8 days of infection, little had developed in terms of the inflammatory response. Infection appeared to be localized to the antrum and to a lesser extent, the antrum/body border for the wild-type SS1 and the KatA and KapA-deficient mutants. The predominance of antral colonization by *H. pylori* SS1 is in accord with the findings of Lee’s group (Lee et al., 1997; Lee, 2000). It was further determined that the distribution of colonization in infected mice did not change throughout the course of infection.

As part of the experimental design we hypothesized that as the inflammatory response developed over time, the importance of KatA and potentially KapA would become apparent. After 12 weeks of infection, the level of colonization exhibited by mice infected with the wild-type SS1 increased by an order of magnitude, in accord with the findings of Lee et al. (1997). Mice infected with the KapA-deficient strain of *H. pylori* showed a slight increase in the median level of colonization. However, mice infected with KatA-deficient strains experienced a decrease in levels of colonization.

The bacterial load of mice infected with the wild-type SS1 was slightly, but not significantly, reduced when the level of colonization at 24 weeks of infection was compared to the levels observed at 12 weeks infection. Lee et al. (1997) also observed a fall in the level of colonization in long-term infections, probably due to an increase in the host immune response. One of the shortcomings of the murine model of infection is the limited inflammatory response observed (Lee et al., 1997). The general trend for the mice infected with the wild-type SS1 was to display more severe pathology over time. The change in inflammatory response is perhaps best demonstrated by the increase in severity of submucosal inflammation and incidence of parietal cell atrophy between 12 and 24 weeks for mice infected by wild-type SS1 (Table 2). These observations suggest that the bacterial load modulates the immune response of the host.

The KatA-deficient mutant of SS1 colonized significantly fewer mice (5/10), and at lower levels, than the wild-type SS1 at 24 weeks post inoculation. The level of inflammation and severity of infiltrate observed in the mice still colonized by the KatA-deficient mutants at 24 weeks was not as aggressive as that seen in the mice infected with the wild-type SS1.
Animals that had cleared infection had limited inflammation (data not shown). Thus, the KatA-deficient mutant was less able to sustain long-term infection, presumably due to hypersensitivity to exogenous hydrogen peroxide within the inflamed gastric mucosa. This is likely to have been exacerbated by the trend towards the development of more severe gastritis seen in the mice infected with the wild-type SS1.

Significantly, after 24 weeks of infection, only 2/10 mice inoculated with the KapA-deficient mutants were colonized. These results demonstrate that KapA is important to sustain long-term infection in the murine gastric mucosa. Our data suggest that KapA may be of equal or greater importance than KatA in terms of in vivo survival. Thus, determining the precise activity of KapA is of paramount importance, not only for its potential as a therapeutic target, but also to enhance the understanding of the bacteria–host interaction and the response to oxidative stress by *H. pylori*.

In our previous study, we reported that KapA appeared to be unique (Harris *et al.*, 2002). Further analysis has also eliminated the small periplasmic ankyrin-like protein (AnkB), which is critical for optimal resistance to hydrogen peroxide, encoded by a gene immediately downstream of katB in *Pseudomonas aeruginosa* (Howell *et al.*, 2000). The increase in severity of parietal cell atrophy over the period of infection in the mice infected with the wild-type SS1 was significant. Comparatively low levels of parietal cell atrophy were observed in the stomachs of the mice infected with the isogenic mutants over the 24-week period, suggesting that parietal cell atrophy was also related to the bacterial load and perhaps oxidative stress, as a consequence of inflammation in the body of the stomach in this animal model; this observation parallels the development of corpus atrophy in humans (Dixon, 2001).

Animal models of infection vary in the extent to which they replicate human disease. The presence of PMNs is an important component of the human inflammatory response to *H. pylori*. As shown here, in the murine model of infection, inflammation is not as severe as in humans and PMN inflammation is limited. These differences are likely to be significant when investigating mechanisms of host-response evasion (Lee, 2000). Notwithstanding the differences between the mouse and human, KapA and KatA are important to the long-term survival of *H. pylori* in the inflamed gastric environment. This probably relates to the increased mucosal concentration of ROS, particularly hydrogen peroxide.

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**REFERENCES**


