Salmonella typhimurium and Salmonella enteritidis induce gut growth and increase the polyamine content of the rat small intestine in vivo

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Abstract

The effects of infection by Salmonella enteritidis and S. typhimurium on the small and large intestines, liver, spleen and mesenteric nodules of rats were studied in vivo. Both Salmonella serotypes persisted and proliferated in the gastrointestinal tract and invaded sub-epithelial tissues, mainly the ileum, leading to the systemic distribution of these pathogens. Coincidental with the infection, the rate of crypt cell proliferation increased resulting in substantial growth of the small intestine. The extent of this and the accompanying accumulation of polyamines was particularly dramatic in the ileum where there was also some disruption of the villus epithelium. It is possible that these effects of the infection on the metabolism and morphology of the small bowel, which strongly resembled the changes induced by some plant lectins, may facilitate the colonisation and invasion of the gut by Salmonellae.

Keywords: Salmonella; Gut; Polyamine; Rat

1. Introduction

Salmonella serotypes cause infection in mammals ranging from asymptomatic carriage and localised gastroenteritis to systemic enteric fevers. In recent years the incidence of these infections in developing countries, Europe and the USA has increased rapidly. This has prompted renewed interest in the pathogenesis of disease caused by Salmonella spp. Infections by these bacteria have been studied in rabbits [1–3], guinea pigs [4], rats [5,6], mice [7–9], Rhesus monkeys [10] and chicks [11]. These studies have shown that colonisation and invasion of the gut by salmonellae is associated with major changes affecting the immune system of the gut. As part of the inflammatory response to the infection, there is increased lymphocyte infiltration by mechanisms as yet unknown. However it is not clear whether the attachment to and transport through the gut wall of the bacteria can cause other major changes in the morphology and metabolism of intestinal tissues and, if so, whether these are correlated with the severity.
of the infection. Thus, the main objective of this study was to evaluate the effects of *S. enteritidis* and *S. typhimurium* on gut structure and markers of metabolic and proliferative activity in the intestine, particularly the ileum, using a rat infection model.

2. Materials and methods

2.1. Bacterial strains

*Salmonella typhimurium* serotype 49 (obtained from the Institute for Animal Health, Compton, UK) and *S. enteritidis* 857 (from Central Veterinary Laboratory, (CVL), Addlestone, Surrey, UK) were grown for 48 h under static conditions in nutrient broth (CM1, Unipath, UK). Strains were maintained on Dorset egg slopes at 4°C. Both strains were considered to be smooth because they agglutinated with O-specific sera and did not aggregate spontaneously in phosphate-buffered saline.

2.2. Animal handling and experimental protocol

Eighteen male Hooded-Lister rats (Rowett strain; 19 days old, 40 ± 1 g) individually housed in metabolism cages, within isolators (Moredun Animal Health, Edinburgh, UK) at 21 ± 1°C were pre-fed with a semisynthetic lactalbumin-based control diet [12] for two days (6 g/rat/d; given as two feeds per day). The rats were then fasted overnight and given 1 ml by gavage of nutrient broth containing approximately 1 × 10⁸ CFU/ml of *S. enteritidis* or *S. typhimurium*, or nutrient broth without the bacteria (six rats per treatment group). All animals were fed the control diet 2.5 h later and maintained on this diet for 6 days post-intubation. Water was freely available at all times. At the end of the experiment the rats were killed by anaesthetic overdose of halothane (M&B, UK) and exsanguination. The small intestine, caecum, colon and internal organs were removed aseptically from the abdominal cavity. From the small intestine two pieces (5–7 cm from pylorus and 5–7 cm from the ileo-caecal junction) were taken for histological studies. Further parts (7–17 cm from the pylorus and 7–17 cm from the ileo-caecal junction) were used for estimating bacterial numbers.

Two further pieces (17–27 cm from the pylorus and 17–27 cm from the ileo-caecal junction, respectively) of the small intestine were flushed through with ice-cold saline to remove any contents, freeze-dried, weighed and used for chemical analysis.

2.3. Bacterial counts and identification

The tissues were weighed, homogenised in maximum recovery diluent (Unipath; CM733) with a Janke-Kunkel Ultra-Turrax T25 homogeniser for 10 s at 20,000 rpm. A 10-fold dilution series was prepared from the homogenate to a final dilution of 1 × 10⁷. From each dilution, drops were taken in duplicate by a measured volume pipette (50 drops = 1 ml). Lactose and non-lactose fermenters were isolated on MacConkey Agar No. 3 (Unipath; CM115) at 37°C for 24 h and salmonellae were isolated on XLD (Unipath; CM469) after incubation at 37°C for 24 h. Colonies were counted from the dilution which gave between 10 and 50 colonies. Salmonellae were distinguished from *Proteus spp.* where necessary by the urease test. Confirmation of *Salmonella spp.* was by specific antisera (Murex, UK) and API 20E (BioMerieux, UK).

2.4. Chemical analysis

The small intestine tissue (10 cm pieces) was homogenised in 5 ml 10% perchloric acid containing 1.7 diaminohexane (2.6 μg/ml) as internal standard, and centrifuged for 15 min at 10,000 × g at 4°C. The supernatants were removed, diluted with distilled water (1:4 v/v) and analysed for polyamines by HPLC according to the method of Seiler and Kniidgen [14]. The residue was solubilised in 5 ml of 0.3 M NaOH and a portion used for protein estimation by a modified Lowry method [15]. The remainder of the alkaline solution was precipitated by perchloric acid to remove proteins and DNA. The ribose content was determined by the orcinol reaction [16]. The precipitate was resuspended in 5% (w/v) perchloric acid, heated at 80°C for 60 min, centrifuged at 1329 × g at 4°C for 10 min before the deoxyribose content of the supernatant was measured with a diphenylamine reagent [17]. Bovine serum albumin, calf liver RNA and calf thymus DNA, respectively were used as respective standards.
2.5. Histology

Sections of the small intestine were fixed in 4% (w/v) phosphate buffered paraformaldehyde (pH 7), embedded in paraffin wax and sectioned at 3 μm as described earlier [18]. They were stained with haematoxylin and eosin for morphological measurements. Ten properly orientated crypts from the jejunum and ileum were selected at random from each animal and their length measured. Results were calculated as mean ± pooled standard deviations (SED) of six rats per treatment group.

2.6. Statistical analysis

The results were subjected to a one-way analysis of variance by the Minitab computer program (Minitab, State College, PA, USA), and multiple comparisons were done by the Tukey test using the Instat statistical package (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Bacterial counts from rat tissues

Both *S. typhimurium* and *S. enteritidis* colonised the gastrointestinal tract of the rat and were present in all regions of the gut after 6 days with high numbers in the ileum and the caecum, whereas bacterial counts in the jejunum were low (Table 1). Both serotypes were invasive, with relatively large numbers of salmonellae in the mesenteric lymph nodes. The spleen and the liver were also infected, the bacterial numbers in these tissues being significantly increased, especially in rats given *S. typhimurium.*

Table 1

| Culturable salmonellae in samples taken from rats 6 days after oral infection with *Salmonella typhimurium* or *S. enteritidis* | Viable salmonella counts (Log_{10} bacterial counts per g wet tissue) |
|---|---|---|---|
| | Control | *S. enteritidis* | *S. typhimurium* | SED |
| Jejunum a | 1.0 a | 3.5 b | 4.2 b | 0.2 |
| Ileum b | 1.0 a | 5.6 b | 6.5 b | 1.3 |
| Caecum | 1.0 a | 6.9 b | 7.7 b | 1.2 |
| Colon | 1.0 a | 2.5 b | 6.1 b | 2.8 |
| Mesenteric lymph nodes | 1.0 a | 5.8 b | 5.7 b | 1.2 |
| Spleen | 1.0 a | 3.8 b | 4.3 b | 0.5 |
| Liver | 1.0 a | 3.2 b | 3.7 b | 0.4 |

Values represent means for six rats/group and pooled standard deviations (SED).

Values in a horizontal row with different superscripts differ significantly (*P* < 0.05).

a 7–17 cm from pylorus; b 7–17 cm from ileo-caecal junction.
Moreover, the infection did not appear to affect the numbers of lactose fermenters present in the small and large intestines (results not given).

### 3.2. Morphological changes in small intestine

Histological studies indicated that the morphology of the ileum in rats dosed with *S. typhimurium* and *S. enteritidis* was characteristic of a rapidly proliferating tissue (Fig. 1). Thus, the depths of the crypts of Lieberkuhn in the ileal tissue were increased by about 180% in rats infected with either *S. typhimurium* or *S. enteritidis*. However, changes in the jejunum of the infected rats were far less extensive than in the ileum. Rats infected with *S. typhimurium* showed crypt elongation and many mitotic figures. The lamina propria contained an increased number of chronic inflammatory cells with a moderate number of neutrophils. Occasional villi were fused together.

With *S. enteritidis* infection the crypts were also enlarged (Fig. 2) compared with the controls (Fig. 3). The lamina propria showed a marked increase in inflammatory cells including plasma cells and neutrophils. The Peyer’s patches were enlarged and contained clusters of macrophages with very eosinophilic cytoplasm.

The mesenteric lymph nodes and the Peyer’s patches in the distal small intestine were greatly enlarged in all infected rats but especially in those given *S. typhimurium*.

### 3.3. Changes in small bowel metabolism

Metabolism of the rat small intestine was appreciably altered following infection (Table 2), with significant increases in the weight of the small intes-
Chemical and histological analyses of 10 cm of ileum taken from rats 6 days after oral infection with *Salmonella typhimurium* or *S. enteritidis*

<table>
<thead>
<tr>
<th>Mucosa†</th>
<th>Control</th>
<th><em>S. enteritidis</em></th>
<th><em>S. typhimurium</em></th>
<th>SED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry weight (g/100 g DBW † † )</td>
<td>0.12 a</td>
<td>0.14 b</td>
<td>0.17 c</td>
<td>0.01</td>
</tr>
<tr>
<td>Crypt length ( μm )</td>
<td>61 a</td>
<td>115 b</td>
<td>108 c</td>
<td>2.0</td>
</tr>
<tr>
<td>Protein (mg)</td>
<td>14.5 a</td>
<td>24.7 b</td>
<td>27.9 b</td>
<td>3.7</td>
</tr>
<tr>
<td>RNA (mg)</td>
<td>1.5 a</td>
<td>4.4 b</td>
<td>5.2 b</td>
<td>0.8</td>
</tr>
<tr>
<td>DNA (mg)</td>
<td>0.25 a</td>
<td>0.41 b</td>
<td>0.45 c</td>
<td>0.03</td>
</tr>
<tr>
<td>Putrescine (nmol)</td>
<td>40 a</td>
<td>55 b</td>
<td>138 c</td>
<td>6.0</td>
</tr>
<tr>
<td>Spermidine (nmol)</td>
<td>224 a</td>
<td>267 b</td>
<td>279 b</td>
<td>18.0</td>
</tr>
<tr>
<td>Spermine (nmol)</td>
<td>154 a</td>
<td>200 b</td>
<td>215 b</td>
<td>19.0</td>
</tr>
</tbody>
</table>

Values represent means for six rats/group and pooled standard deviations (SED).

Values in a horizontal row with different superscripts differ significantly (P < 0.05).

† 17–27 cm from ileo-caecal junction; † † DBW, dry body weight.

As previously observed [20], the Peyer’s patches of the distal small intestine were highly enlarged in infected rats. This suggests that invasion and translocation of salmonella to the lymph nodes may have occurred primarily via the terminal ileum. Additionally, the mesenteric lymph nodes of infected rats were also greatly enlarged. These findings are compatible with the view that facultative pathogens such as salmonellae translocate via the regional lymph nodes to the spleen and liver [19]. However, the possibility of invasion through the caecal epithelium cannot as yet be fully excluded.

A major finding in this work was that colonisation/invasion by both *Salmonella* serotypes induced considerable enlargement of the small intestine, particularly the ileum. Although this increase was due to some extent to lymphocyte/macroage infiltration, the hyperplastic growth of the ileum induced by the bacteria as indicated by the deepening of the proliferative compartment, the near doubling of the crypts of Lieberkuhn and the correspondingly large increase in DNA content of ileal tissue (Table 2) made a substantial contribution to the enlargement. Furthermore, the process of repairing the damage caused to the epithelial surface of ileal villi by the presence of bacteria could have, as a proliferative signal contributed to the growth of the intestine. Since all these structural changes are likely to have an adverse effect on the integrity of the epithelial surface, they may facilitate the colonisation and invasion of the gut by the pathogens.
Coincident with the morphological changes, the polyamine content, particularly spermidine and spermine, of the ileum of rats infected by salmonellae was significantly increased. This is a known prerequisite for cellular growth and differentiation because polyamines are involved in protein, RNA and DNA syntheses [21,22]. Furthermore, the accumulation of spermidine and spermine in the gut may also serve as an indicator of increased metabolic activity in this tissue [18,23]. Thus, the elevated spermidine and spermine content of the ileum in infected rats was consistent with hyperplastic growth. This suggests that the intestinal growth induced by salmonellae colonising the gut may occur via a polyamine-dependent mechanism.

The metabolic changes of the small intestine in salmonella infected rats resembled those characteristic of lectin induced gut growth [24]. Thus, polyamine-dependent crypt cell hyperplasia, interference with cellular metabolism and disruption of the small intestine epithelium occur in rats fed diets containing relatively large amounts of some purified plant [25–27] or bacterial [28,29] lectins. This is not really surprising as under some conditions most Salmonella serotypes express a number of fimbrial and other types of adhesins/lectins [30] and lectin-like components. Although it has been proposed that these are involved in the infection process by pathogens [31–34], definite proof is still lacking. However, the similarity between the dramatic morphological and compositional changes and the hyperplastic growth of the intestines induced in rats by dietary lectins and infection by salmonellae give considerable, albeit indirect, support for the involvement of bacterial lectins in the infection process. Accordingly, bacterial lectins may not only be responsible for some of the changes in the glycosylation of surface receptors [35] and interference with gut metabolism, but may also, through lectin-mediated bacterial adherence, help pathogenesis and facilitate the infection and invasion processes.

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