Selective translocation of coliform bacteria adhering to caecal epithelium of rats during catabolic stress

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Adult conventional rats were starved for 48 h with or without haemorrhage at 24 h, and translocation of caecal coliforms to mesenteric lymph nodes (MLNs) was measured. Translocation was detected in three of 11 rats without haemorrhage, in 6 of 11 starved and sham-operated rats and in 12 of 22 rats after haemorrhage. In contrast, only one of 13 non-instrumented and fed control rats showed translocation. Translocation was associated with more coliforms adhering to caecal epithelium in rats. Coliform isolates from caecum, caecal epithelium and MLNs were characterised and grouped into different biochemical phenotypes (BPTs) by a biochemical fingerprinting method. Of 291 BPTs detected in the caecum of all rats, 108 were also found on caecal epithelium; 36 BPTs were detected in MLNs, of which 17 were not detected either in the caecum or on the caecal epithelium of the corresponding rats. One isolate from each of these 36 BPTs was selected and compared to the others. Four common (C) BPTs (i.e., C1–C4) were identified among them. Strains of C1 formed the majority of isolates from the caecum (79%), caecal epithelium (71%) and MLNs (91%). In contrast, C2–C4 had a significantly lower incidence both in the caecum and on the caecal epithelium, but not in the MLNs. These findings indicate that not all caecal coliforms adhere to the epithelium during catabolic stress and that for translocation to occur, other bacterial properties besides adhesion are needed. It is also concluded that coliforms with a low incidence in the caecum can translocate with the same efficiency as those with a high incidence.

Introduction

Coliform bacteria often cause infections in patients after surgery [1–3] or trauma [4] or in those who are immuno-compromised [5, 6]. It has been suggested that some of these infections are due to bacteria crossing the intestinal mucosal barrier, a phenomenon called 'bacterial translocation' [7, 8]. Bacterial translocation may occur when the host's immune defence is compromised [9–11], when the gastrointestinal mucosa is physically damaged [12, 13] or when the ecological equilibrium of the indigenous microflora is disturbed resulting in overgrowth of certain bacteria [9, 14]. Therefore, in animals with normal intestinal barrier function bacterial translocation may occur if certain enteric bacteria reach or exceed $10^9$–$10^{10}$ bacteria/g of caecal content or stool [15, 16]. Experimental colonisation of germ-free animals with single strains of bacteria has shown clearly that not all bacteria are able to translocate equally well and that gram-negative enteric bacilli translocate more efficiently to mesenteric lymph nodes (MLNs) than do gram-positive cocci and obligate anaerobes [17, 18]. Little is known about the exact mechanisms by which these bacteria cross the intestinal mucosal barrier or spread to systemic organs and tissues. One likely route is intracellular through the mucosal epithelial cells and then via the lymph to MLNs. As these nodes are normally sterile, the presence of bacteria in MLNs has been used as a marker of bacterial translocation. It is not clear whether strains of bacteria found in MLNs of animal models or in the blood of septic patients with gut-associated bacteraemia are better able to cross the mucosal barrier or whether they have an increased ability to survive in the hostile environment of lymphoid tissues.

For translocation to occur, a crucial initiating event is colonisation of the intestinal mucosa. Gram-negative bacteria are the most common bacteria to adhere to the mucosa and also the most common bacteria to translocate [19]. Increased adherence occurs during catabolic stress and is associated with a permeability
change in coliform translocation after haemorrhagic stress in rats has been demonstrated previously [21]. A biochemical fingerprinting method to characterise these bacteria was used to show that the composition and diversity of the caecal coliform flora are important factors for translocation [22].

The aims of the present study were to characterise caecal coliforms and those adhering to caecal epithelium of rats during catabolic stress and to investigate whether their prevalence in the caecum is important for translocation.

Materials and methods

Animals

Fifty-seven male Sprague Dawley rats (F1 hybrid, Microbiological, Bethesda, MD, USA) of average weight 353 (314–395) g were used. The animals were delivered from one breeder (Alab, Sollentuna, Sweden) at least 1 week before the experiments. They were housed in individual cages in a light and temperature controlled environment and were fed a complete rat chow (B & K Standard diet, B & K Universal AB, Sollentuna, Sweden). They were divided into four groups. Thirteen rats (group 1) served as non-instrumented controls and were fed normally until sampling. Eleven rats (group 2) were starved for 48 h before sampling, with free access to water. To assess the effect of catheterisation and handling on the starved rats, 11 rats were starved for 24 h and sham-operated without haemorrhage (group 3). This group was kept for another 24 h with access to water only until sampling. Twenty-two rats (group 4) were subjected to haemorrhage after fasting for 24 h. Animals used in this study were part of a larger study on the effect of glucose supplementation during haemorrhage on bacterial translocation in rats. For this reason, before haemorrhage, 11 rats (group 4a) were supplied with sweetened water (sodium saccharinate solution; Hermes Sweetner, Switzerland; 20 mg/100 ml), while the other 11 rats (group 4b) were given a carbohydrate solution (dextrin 5.5 g, maltose 2.5 g and glucose 2 g/100 ml) and they voluntarily ingested 20–40 ml of the solutions during 12 h. After haemorrhage, the animals were fasted for another 24 h.

Haemorrhage and sampling procedure

Haemorrhage and sampling procedures have been described previously [21]. Briefly, animals in group 4 were subjected to femoral catheterisation after sedation. The femoral catheter was used for bleeding and continuous measurement of mean arterial blood pressure (MAP). The haemorrhage followed a curve with decreasing blood pressure to 55 mmHg MAP, after 60 min. Blood was not re-infused. At the end of haemorrhage, catheters were removed, blood vessels were ligated, wounds were closed and the animals were placed in separate cages. Animals in group 3 underwent the same experimental procedures but were not subjected to haemorrhage. The sampling procedure was the same for all four groups. Before sampling, all animals were anaesthetised as described before [21] and 2 ml of blood were taken for aerobic culture by sterile transthoracic heart puncture. The abdomen was then opened with a midline incision and mesenteric lymph nodes (MLNs) and the whole caecum were excised aseptically and collected for bacteriological analysis. Animals were then killed by a heart incision.

Bacteriological analysis

MLNs were washed free of blood in sterile saline and homogenised in 2 ml of Brain-Heart Infusion Broth (BHI, Difco) with sterile Teflon-coated tissue grinding rods as described previously [21]. Portions (0.5 ml) of tissue homogenate were placed on horse blood 7% and MacConkey agar (Difco) plates. Blood samples were cultured in bottles containing BHI (Bio-Hospital AB, Sweden), incubated for 1 week at 37°C and subcultured on blood and MacConkey agar plates after 48 h and 7 days. Caecal content was collected in 10 ml of Tryptic Soy Broth (TSB, Difco) from which the number of coliform bacteria was estimated after serial dilution in TSB, and calculated as cfu/g of caecal content. Caecal wall was washed in 0.1 M phosphate-buffered saline (pH 7.2) and 1 cm² of that was cut and shaken by vortex mixing for 5 min in 10 ml of TSB. The sample was then transferred to a new TSB tube and again shaken for another 5 min. This procedure was repeated five times on the basis of a pilot study performed on 48-h starved rats, in which the number of coliforms in the spent-wash was estimated after each washing until no coliforms were detected. The caecal wall sample was then homogenised and subjected to quantitative culture analyses in the same way as the MLNs. All microbiological analyses were done in duplicate. Results are expressed as mean values and the Mann-Whitney non-parametric test and Fisher’s exact test were used for statistical analyses.

Characterisation of coliform flora

A computerised system for biochemical fingerprinting of bacteria (the PhP system; Biosys inova, Stockholm, Sweden) was used to compare coliforms found in the caecum, on the caecal epithelium and in the MLNs of all rats. This system measures kinetics of bacterial metabolism on highly discriminatory substrates in microtitration plates and yields, for each isolate, a set of quantitative data (biochemical fingerprint) [23–25]. A personal computer programme calculates similarities among tested strains as a dendrogram [25]. The microtitration plates comprise 96 wells with different sets of substrates. The present study used the PhP-RS plates which have been specifically developed for characterisation of coliforms [26]. Reagents used in these plates and the method for comparing bacterial populations have been described
had not been subjected to haemorrhage, in six of 11 translocations were observed in three of 11 rats which contained a gram-positive rod. Among coliforms isolated from each rat, different BPTs were found but in general, the number of these BPTs in the caecum and on the caecal epithelium of the starved and haemorrhaged rats was significantly higher than those of the corresponding samples in the non-instrumented control rats (Table 1). The overall coliform counts in the caeca of starved and haemorrhaged rats were also significantly higher than those in the control group (Table 2). This was associated with more coliforms adhering to caecal epithelium in these animals so their numbers on the caecal epithelium of the starved and haemorrhaged rats were significantly higher than those in the control groups (Table 2). Indeed, the number of coliforms adhering to the caecal epithelium of starved and haemorrhaged rats was up to 30-fold higher than those found in rats that were only starved or sham-operated. However, despite this significant difference, the rate of translocation did not differ significantly among these groups (Table 2).

In all, 303 BPTs were detected among the coliforms isolated from all rats. In the caecum, 291 BPTs were identified of which 108 were also isolated from caecal epithelium and 24 from MLNs (Table 3). Six BPTs were isolated from MLNs and from caecal epithelium, but not from caecum, and six BPTs were isolated only from MLNs (Table 3). To determine whether the same translocating coliform in a rat was also present in other rats, one isolate from each of the 36 BPTs detected in MLNs was selected and compared to each other. Four common (C) (i.e., C1–C4) and 15 single (S) BPTs (i.e., S1–S15) were found among the whole material (Fig. 1). Strains of C1 (n = 6) had a high prevalence in the caecum (79%), on the caecal epithelium (71%) and in MLNs (91%) (Table 4). In contrast, strains of C2 (five isolates) and C4 (eight isolates) had a low prevalence in the caecum (13% and 7%, respectively) and on the caecal epithelium (8% and 27%, respectively) but a high prevalence in MLNs (88% and 73%, respectively), a prevalence not significantly different from that of C1 (Table 4). BPT C2 contained only two isolates from MLNs and was not included in the statistical analysis.

Discussion

The increased rate of translocation after starvation and haemorrhage is in agreement with our previous studies [21, 22] and those reported by others [30–33]. In almost all rats subjected to haemorrhage, the number of caecal coliforms was 100-fold higher than those of the starved animals but, in general, translocation occurred only when the coliform populations in the caecum exceeded $10^9–10^{10}$ cfu/g of caecal content, which is in agreement with those previously reported [15, 16]. It was also found that a high coliform population on caecal epithelium was necessary for translocation which in the present study was $10^4–5$ cfu/cm². Interestingly, the only rat in the non-instrumented control group showing translocation had a similar high
### Table 1. Rate of translocation and the number of BPTs found among the coliforms in caecum, caecal epithelium and MLNs of rats in different experimental groups

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Number of rats tested</th>
<th>Number showing translocation</th>
<th>Number of colonies tested/rat</th>
<th>Mean number of BPTs found (range)</th>
<th>Mean number of colonies tested/rat (range)</th>
<th>Mean number of BPTs found (range)</th>
<th>Mean number of BPTs found (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-instrumented control (group 1)</td>
<td>13</td>
<td>1†</td>
<td>32</td>
<td>2.9 (1–6)‡</td>
<td>5 (0–24)</td>
<td>1.5 (0–6)$§$</td>
<td>24</td>
</tr>
<tr>
<td>Starved (48 h) (group 2)</td>
<td>11</td>
<td>3</td>
<td>32</td>
<td>6.6 (2–13)</td>
<td>25 (24–32)</td>
<td>3.7 (1–8)</td>
<td>9 (4–16)</td>
</tr>
<tr>
<td>Starved (48 h) and sham-operated (group 3)</td>
<td>11</td>
<td>6</td>
<td>32</td>
<td>5.0 (2–8)</td>
<td>24</td>
<td>4.3 (2–8)</td>
<td>9 (4–16)</td>
</tr>
<tr>
<td>Starved (48 h) and haemorrhaged (55 mmHg) (group 4):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4a: sweet water</td>
<td>11</td>
<td>6</td>
<td>32</td>
<td>6.2 (2–11)</td>
<td>26 (24–32)</td>
<td>4.0 (1–8)</td>
<td>21 (8–24)</td>
</tr>
<tr>
<td>4b: carbohydrate solution</td>
<td>11</td>
<td>6</td>
<td>32</td>
<td>5.2 (3–9)</td>
<td>26 (24–32)</td>
<td>3.4 (1–7)</td>
<td>21 (8–24)</td>
</tr>
</tbody>
</table>

*Number of BPTs were calculated only from positive MLN cultures.
†p = 0.023 for group 1 versus group 3 and p = 0.0098 for group 1 versus group 4 (4a and 4b).
‡p = 0.0021 for group 1 versus groups 2, 3 and p = 0.0053 for group 1 versus group 4 (4a and 4b).
§p = 0.0067 for group 1 versus groups 2, 3 and p = 0.0003 for group 1 versus group 4 (4a and 4b).
$\ddagger$One positive culture was not investigated.

### Table 2. Number and range of coliforms found in the caecum, on caecal epithelium and in MLNs of rats in different experimental groups

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Mean number (cfu/g)</th>
<th>Range</th>
<th>Mean number (cfu/cm²)</th>
<th>Range</th>
<th>Mean number (cfu/MLN)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-instrumented control (group 1)</td>
<td>$1.7 \times 10^7$</td>
<td>(8.9 × 10⁶)–(1.6 × 10⁸)</td>
<td>$3.0 \times 10^5$</td>
<td>(0–(3.1 × 10⁴)</td>
<td>13</td>
<td>0–168</td>
</tr>
<tr>
<td>Starved (48 h) (group 2)</td>
<td>$4.8 \times 10^4$</td>
<td>(4.3 × 10⁴)–(2.4 × 10⁷)</td>
<td>$1.3 \times 10^3$</td>
<td>(0.4 × 10¹)–(1.0 × 10⁷)</td>
<td>41</td>
<td>0–324</td>
</tr>
<tr>
<td>Starved (48 h) and sham-operated (group 3)</td>
<td>$1.8 \times 10^1$</td>
<td>(2.0 × 10⁶)–(4.9 × 10¹)</td>
<td>$2.0 \times 10^1$</td>
<td>(3.5 × 10⁶)–(5.1 × 10¹)</td>
<td>103</td>
<td>0–960</td>
</tr>
<tr>
<td>Starved (48 h) and haemorrhaged (55 mmHg) (group 4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4a: sweet water</td>
<td>$9.9 \times 10^0$</td>
<td>(4.7 × 10⁰)–(4.0 × 10¹)</td>
<td>$6.1 \times 10^0$</td>
<td>(2.1 × 10⁰)–(2.8 × 10⁰)</td>
<td>87</td>
<td>0–312</td>
</tr>
<tr>
<td>4b: carbohydrate solution</td>
<td>$1.4 \times 10^1$</td>
<td>(6.7 × 10⁰)–(4.7 × 10¹)</td>
<td>$4.4 \times 10^0$</td>
<td>(3.6 × 10⁰)–(2.2 × 10⁰)</td>
<td>92</td>
<td>0–480</td>
</tr>
</tbody>
</table>

*p = 0.001 for group 1 versus each of the groups 2, 3 and 4 (4a and 4b).
number of coliforms on the caecal epithelium, although their numbers in the caecum were as low as in the other control rats. This finding suggests that the population of coliforms on the caecal epithelium might be even more important for translocation than that in the caecum alone.

Gut translocation has been recognised for many years, but its pathological significance remains unclear. It may occur randomly in a healthy host with no adverse consequence. However, recent studies implicate bac-

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**Table 3.** Number of BPTs found in the caecum, on caecal epithelium and in MLNs of rats of all experimental groups

<table>
<thead>
<tr>
<th>Caecum</th>
<th>Caecal epithelium</th>
<th>MLN</th>
<th>Number of BPTs</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>−</td>
<td>−</td>
<td>178</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>89</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>19</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>+</td>
<td>5</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>+</td>
<td>6</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>+</td>
<td>6</td>
</tr>
</tbody>
</table>

*One sample was not investigated.*

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**Fig. 1.** UPGMA dendrogram derived from comparison of 36 strains, each representing a BPT found in MLNs of rats. Each strain is represented by a horizontal line. Different strains are connected with vertical lines at the similarity level they show to each other. Strains with similarities above the assigned identity (ID) level are regarded as common (C) BPTs and those below the ID-level are regarded as single (S) BPTs. From caecum and caecal epithelium, up to 32 colonies and from MLNs, up to 24 colonies were tested. The percentage of strains belonging to each BPT is given in front of the strains. Treatment groups were: 1, non-instrumented control rats; 2, starved (48 h) rats; 3, starved and sham-operated rats; 4, starved and haemorrhaged rats supplemented with sweetened water (4a) and carbohydrate solution (4b).
terial translocation as a possibly important factor in the pathogenesis of sepsis and multiple organ failure [34–36]. Although the intestinal tract serves as an important source of infection in these patients, direct evidence on the origin of translocating strains has not always been provided. With the use of a specific method for fingerprinting of gut flora, the present study not only verified the presence of translocating strains in both the caecum and on the caecal epithelium, but also estimated their prevalence in each sample. With the same fingerprinting technique, a previous study showed that a few BPTs existed in the caecum of healthy non-instrumented rats and that the number of BPTs increased when the animals were stressed [22]. A similar pattern was also found in the present study. However, it was also found that only some of several BPTs present in the caecum adhered to caecal epithelium and even fewer translocated. A significant difference between the number of translocating strains adhering to caecal epithelium before translocation and those that did not was also found. These findings indicate that adherence to caecal epithelium is a preliminary step and probably a prerequisite for translocation of coliforms in rats. However, the finding that not all adhering bacteria translocated suggests that other bacterial properties besides adhesiveness are needed for translocation to occur.

Coliforms are the most common gram-negative bacteria to adhere to the terminal ileum and caecum. Animal experiments have shown that this adhesion is enhanced by nutrient deprivation [19] or when the animals are fed diets that are completely absorbed in the proximal intestine [37]. In the present study, starvation of rats resulted in an increased number of coliforms adhering to caecal epithelium. When starvation was followed by haemorrhage, there was a further increase in the population of coliforms in both the caecum and on the caecal epithelium, but the rate of translocation did not increase significantly. A previous study showed that a brief starvation period before hypotension caused by non-lethal haemorrhage was associated with a significantly greater increase in bacterial translocation than in normally fed rats [38]. It has been also shown that pre-treatment of rats fasted for 24 h with glucose infusion before haemorrhage improved their survival after an otherwise fatal blood loss [39]. The present study did not find any differences in the rate of translocation or the number of coliforms in the caecum and on the caecal epithelium of haemorrhaged rats supplemented with either sweetened water or carbohydrate solution.

Some translocating coliforms were also found in other rats. With the same model of haemorrhagic stress in rats, previous studies showed that the strains best able to translocate belonged to two common BPTs and that rats not carrying any of these two BPTs did not show translocation [21, 22]. The present study extended that observation by analysing the population size of each translocating BPT in the caecum and on caecal epithelium. It was found that while some common BPTs had a high prevalence in the caecum and on the epithelium (i.e., BPT C1, Fig. 1), some appeared only in low numbers. Interestingly, the prevalence of these BPTs in MLNs did not differ significantly from each other, suggesting that while translocation of some coliform strains is facilitated by their prevalence in the caecum or on the caecal epithelium, or both, low prevalence strains also adhere to the epithelial cells and translocate with the same efficiency. Alternatively, this could also indicate that among the strains with translocating ability, some compete well in the intestine and grow to high numbers while others cannot. In this study a few of the translocating strains were not found on either caecal epithelium or in the corresponding caecal samples. These strains were present in low numbers in both samples and most probably escaped detection. However, it is also possible that they had originated from other parts of the intestinal tract. Also, a number of BPTs were found only on caecal epithelium, and could not be detected in the caecum or MLNs. Some of these BPTs had a high similarity to other BPTs in the same material. A previous study showed that some of the biotype characters of *Escherichia coli* change slightly upon subculturing and storage [29]. Changes have also been observed in some biochemical reactions of *E. coli* strains after oral inoculation of rats and subsequent recovery from faeces.

<table>
<thead>
<tr>
<th>Common BPTs</th>
<th>Caecum</th>
<th>Caecal epithelium</th>
<th>MLN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of colonies tested</td>
<td>Number (%) of BPTs found</td>
<td>Number of colonies tested</td>
</tr>
<tr>
<td>C1</td>
<td>192</td>
<td>151 (79%)</td>
<td>160</td>
</tr>
<tr>
<td>C2</td>
<td>160</td>
<td>21 (13)</td>
<td>160</td>
</tr>
<tr>
<td>C3</td>
<td>64</td>
<td>21 (33)</td>
<td>64</td>
</tr>
<tr>
<td>C4</td>
<td>256</td>
<td>18 (7)</td>
<td>256</td>
</tr>
</tbody>
</table>

*C3 contained only two strains and was not included in the statistical analysis.

*tp < 0.00001 for C1 versus C2 and C4.

*Not significant for C1 versus C2 or C4.

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Table 4. Prevalence of different BPTs found among translocating coliforms in the caecum, on caecal epithelium and in MLNs of rats in all experimental groups.
of the animals (unpublished observations). In view of these findings we postulate that the strains showing high similarity to other BPTs in the present study might be also phenotypic variants of those BPTs. However, six strains from five rats were found only in MLNs. These strains belonged to unique BPTs with no phenotypic similarities to other translocating strains and there is a high probability that they did not originate from the gut.

A previous study showed that starvation similar to that used in the present model causes an increase in the number of coliforms and their BPTs in the caecum of rats [22]. Similar results were also found in the present study. The higher number of BPTs observed in the caecum of the starved rats appears to be a direct effect of starvation on the stability of the caecal flora. Factors such as competition for the limited nutrients could lead to a great shift in the composition of the flora so that the strains present in small numbers grow profusely and adhere to the caecal epithelium, translocating when haemorrhage is additionally imposed. Bacterial translocation involves complex interactions between host defence mechanisms and the abilities of bacteria to translocate across the mucosal barrier and survive in the hostile environment of the lymphoid tissues. Therefore, failure to find certain BPTs in MLNs, although present in high numbers on caecal epithelium, might be due to their rapid elimination from the host tissues. If this be the case, the true determinant of translocation might be the ability of the bacteria to resist the host immune system and not their ability to cross the epithelial barrier. Nonetheless, the fact that certain BPTs with a low prevalence on caecal epithelium translocated and survived in MLNs with the same efficiency as those having higher incidence on caecal epithelium, suggests that the ability of a bacterial strain to adhere and cross the epithelial barrier may be as important as its survival in MLNs.

In conclusion, it was found that both the number and types of coliforms increased in the caecum during catabolic stress, but not all different types adhered to the epithelium. Translocation of the adhering coliforms was associated with an increased number on the caecal epithelium. However, it was also found that for translocation to occur, other bacterial properties besides adhesion are necessary. Furthermore, the prevalence of a particular type of coliform in the caecum or on the caecal epithelium is not a critical factor for translocation in rats during catabolic stress.

References

25. Möllby R, Kühn I, Katouli M. Computerized biochemical


