Microbial Colonization of Human Ileal Conduits

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Morphological and microbiological techniques were used to locate and identify the microorganisms that colonized the human ileal conduits in 17 different patients from 5 days after surgery up to as many as 16 years of service as a urine conduit. The ecological sequence of this colonization assumes some practical importance because the ascending growth of pathogenic organisms in this essentially open, unvalved urinary tract diversion system leads to the development of life-threatening pyelonephritis. Extensive examination of the microvillus surfaces of the ilea of five accident victims by both transmission and scanning electron microscopy showed that these tissue surfaces were not colonized by bacteria, even in the absence of prophylactic antibiotic therapy, and that these surfaces were not occupied by adherent microorganisms after several years of service as a urine conduit, even when the skin surface stoma and the conduit contents were heavily colonized by bacteria and yeasts. During the initial period (10 days) of postoperative antibiotic therapy, the mucus and urine within the conduit were largely colonized by yeasts. A mixed population of yeasts and gram-positive cocci subsequently developed in the conduit itself, and gram-positive cocci were seen to be avidly adherent to epidermal cells at the stoma. As antibiotic protection was gradually withdrawn, gram-negative organisms became a part of the mixed microbial flora of the conduit contents, and some of the potentially pathogenic organisms of this group (e.g., Escherichia spp., Proteus spp., Pseudomonas spp., etc.) were isolated from patients with pyelonephritis that appeared to come from the ileal conduit. This study of the natural microbial ecology of this surgical urinary tract diversion is intended to provide a rational basis for the manipulation of the ileal conduit to make use of competitive microbial exclusion to protect patients with ileal conduits from ascending infections.

When a bladder is removed because of cancer or neurogenic disease, the urine is diverted from the ureters to the skin surface with a short section of the ileum used to construct an ileal conduit (3, 24). The ureters are connected to the proximal end of a blind tube (6 to 8 in. [ca. 15.2 to 20.3 cm]) constructed of an excised section of the ileum joined to the skin at its distal end. Surgical procedures cannot preclude the reflux of urine from the ileal conduit to the kidneys via the ureters and, because 80% of patients with ileal conduits develop bacteriuria (23), a significant number develop pyelonephritis. Laboratory animals in which ileal conduits were surgically constructed showed an 83% incidence of pyelonephritis (19). In humans, these microbial infections are life threatening (1) and may also indirectly cause stenosis of the conduit (10) or renal deterioration (21) or both.

The autochthonous bacterial population of the ileum is controlled, before surgery, by empirical antibiotic therapy, and the ileal conduit is colonized postsurgically, via the cutaneous opening (stoma), as it is converted from its digestive function into a urine conduit. This ascending colonization is influenced by the postoperative administration of antimicrobial agents, which affect the nature of the organisms entering the conduit. Thus, the organisms that persist in the ileal conduit are influenced by ecological factors and by the selective pressure of antimicrobial therapy.

Because the incidence of bacteriuria and pyelonephritis is high in patients with ileal conduits, despite intermittent antimicrobial therapy, we resolved to study the development and persistence of autochthonous and pathogenic microbial populations in ileal conduits in 17 patients immediately after surgery and up to 16 years after surgery.

MATERIALS AND METHODS

Patients. A total of 17 patients (12 males, 5 females) who had undergone urinary tract diversion within a period of up to 16 years before this investigation were studied, and samples were taken from their conduits as described below. The patients were originally operated on for the treatment of transitional cell carcinoma, bladder cancer, or neurogenic bladder disease. Five accident victims were also included in the investigation as a source of "normal" ileal tissue and were not subjected to antibiotic therapy.

Specimen collection and processing. Urine samples, mucus samples, and cup biopsy specimens of ileal conduit tissues were taken from four patients at 5- to 10-day intervals for up to 40 days after the operation. Another group of 13 patients was examined on one occasion; a swab was taken from the stoma, and urine samples and cup biopsy specimens were taken from superficial and deep areas of the conduit by a double-lumen-catheter technique (2). The mucus and urine samples were cultured by standard microbiological techniques, and organisms were isolated and identified. Mucus samples and cup biopsy specimens were processed for transmission electron microscopy (TEM) by being fixed in 5% glutaraldehyde in cacodylate buffer (0.1 M, pH 7.2) with 0.15% ruthenium red for 2 h at room temperature and then processed as described by Costerton (8) and Chan and Bruce (5). The sections were examined with a Hitachi 500 electron microscope at an accelerating voltage of 60 kV. For scanning electron microscopy (SEM), the specimens were fixed and processed as described by Chan and Bruce (5) and examined with a Hitachi 450 microscope at an accelerating voltage of 20 kV.
TABLE 1. Microbial isolates from stoma and urine samples collected from the ileal conduits of 13 patients over 1 month after surgery, as reported by the Toronto General Hospital Diagnostic Laboratory

<table>
<thead>
<tr>
<th>Patient</th>
<th>Time after surgery</th>
<th>Stoma samples</th>
<th>Bacteria found in</th>
<th>Deep and superficial urine samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 mo</td>
<td>Proteus mirabilis, E. coli 1, E. coli 2, enterococci, Candida albicans, Staphylococcus aureus, Enterobacter cloacae, Morganella spp., Citrobacter spp., Klebsiella oxytoca, Pseudomonas aeruginosa</td>
<td>E. coli 1, E. coli 2, P. mirabilis, C. albicans</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4 mo</td>
<td>P. vulgaris, E. coli, K. oxytoca, Morganella morganii, enterococci, Streptococcus viridans</td>
<td>P. rettgeri, M. morganii, enterococci, K. oxytoca, P. vulgaris</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4 mo</td>
<td>Streptococcus spp.</td>
<td>Enterococci, gram-positive and gram-negative bacilli, C. albicans</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4 mo</td>
<td>E. coli, K. pneumoniae, enterococci, S. epidermidis</td>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6 mo</td>
<td>E. coli</td>
<td>E. coli, gram-positive bacilli</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6 mo</td>
<td>Staphylococcus spp.</td>
<td>Staphylococcus spp.</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7 mo</td>
<td>Staphylococcus spp.</td>
<td>Staphylococcus spp.</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>17 mo</td>
<td>Staphylococcus spp., Streptococcus spp., gram-positive bacilli</td>
<td>E. coli 1, E. coli 2, K. pneumoniae, enterococci</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>20 mo</td>
<td>E. coli, P. mirabilis, enterococci</td>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>33 mo</td>
<td>M. morganii, P. aeruginosa, K. pneumoniae, enterococci</td>
<td>K. pneumoniae, K. oxytoca</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>43 mo</td>
<td>E. coli, K. oxytoca, Providencia rettgeri, P. aeruginosa, enterococci</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>10 yr</td>
<td>E. coli</td>
<td>E. coli</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>16 yr</td>
<td>E. coli, gram-positive cocci</td>
<td>E. coli, gram-positive cocci</td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

Ileal tissues were obtained from five accident victims who had no recent history of exposure to antibiotics and from seven patients who had undergone urinary tract bypass surgery and had been given preoperative antibiotic therapy. Examination of very large areas (at least 4 mm² in each of the five accident victims) of the surfaces of these ileal tissues by SEM showed intact villus structures and extensive remnants of the mucus blanket (Fig. 1), but no bacterial cells could be discerned either on the tissue surface or embedded in the mucus. Similarly, TEM of these ileal tissues showed intact, well-developed microvilli and a partial retention of the mucus blanket, but no bacteria or other microorganisms could be seen either on the tissue surface or within the mucus (Fig. 2). At least $6.0 \times 10^3 \mu m$ of the tissue surface was examined in this study. TEM of a biopsy specimen taken from the wall of an ileal conduit 40 days after surgery showed that the microvilli were truncated and degenerated (Fig. 3), but this histological change is not universal when the ileum becomes a urine conduit; well-developed microvilli were often seen in ileal conduits 2 to 16 years after surgery.

We undertook an intensive study of 17 patients, ranging from 1 day to 16 years after urinary tract diversion, to determine the extent of microbial colonization of their ileal conduits. Swab samples were taken at the stoma, and urine and cup biopsies were taken from a superficial site 3 cm inside the stoma and from a deep site near the conduit ureter junction. The keratinized epithelial cells obtained from stoma samples taken from patients 2 weeks after surgery were heavily colonized by gram-positive cocci (Fig. 4); samples collected over 1 month after surgery contained a great variety of gram-positive and gram-negative bacteria (Table 1). Detailed TEM examination of large areas of the surfaces of the deep and superficial biopsy specimens from all 17 patients showed the complete absence of adherent bacteria on the surfaces of the microvilli of the ileal conduit tissues (Fig. 3). Mucus collected from the deep and superfi-

FIG. 1. SEM of a critical-point-dried, thioacarbohydrazide-treated preparation of ileal tissue from an accident victim with no immediate past history of antibiotic usage. The villi can be clearly seen, as can remnants of the mucus blanket, and careful examination of very large areas (at least 4 mm² in each of the five accident victims) of the surfaces of the microvilli of this tissue at a higher magnification did not reveal any bacteria or protozoa. Bar, 50 µm.

FIG. 2. TEM of a section of the same ruthenium red-stained preparation seen in Fig. 3 showing the long and well-developed microvilli and the extensive fibrous glycocalyx that surrounds these finger-like projections. Bar, 0.1 µm.

FIG. 3. TEM of a section of a ruthenium red-stained preparation of a biopsy taken from an ileal conduit 40 days after surgery. The relocation of this tissue and its use as a urine conduit altered the surface structure of the tissue; the microvilli became shorter and degenerated in their organization. This effect was seen throughout this particular sample, but was not seen in all patients. Bar, 0.1 µm.
FIG. 4. TEM of a section of a ruthenium red-stained preparation of a stoma sample taken 2 weeks after surgery. During this period of intensive postoperative antibiotic therapy, most of the surfaces of keratinized epidermal cells were very heavily colonized by gram-positive cocci. Bar, 1 μm.

FIG. 5. TEM of a section of a ruthenium red-stained preparation of mucus recovered with urine from a deep site near the conduit-ureter junction of an ileal conduit 13 days after surgery. At this stage in postoperative antibiotic therapy, this material was heavily colonized by a mixed population of yeasts and gram-positive cocci, and this colonization followed an earlier stage in which only yeasts were found in the mucus from the deep sampling site. Bar, 1 μm.
FIG. 6. SEM of a critical-point-dried, thiocarbohydrazide-treated preparation of a deep-site biopsy of an ileal conduit 40 days after surgery. A huge ball of microbes occupied the mucus at the tissue surface, and this mass was composed of large numbers of spherical bacterial cells (arrows), smaller numbers of large, spherical yeast cells, and occasional hyphal elements of the colonizing yeasts. Careful examination of the tissue surface (T) at a higher magnification revealed no adherent bacterial or fungal cells. Bar, 5 μm.

FIG. 7. TEM of a section of a ruthenium red-stained preparation of mucus recovered with urine from a deep site in the ileal conduit of a patient who had suffered six incidents of pyelonephritis in the 16 years after his surgery. Note that both gram-positive and gram-negative bacterial cells grew in glycocalyx-enclosed microcolonies within this mucus. Bar, 1 μm.
cial areas of the conduit during the first 10 to 13 days after surgery was heavily colonized, initially by yeast cells and then by a mixture of yeasts and gram-positive cocci (Fig. 5). SEM of mucus specimens showed extensive microcolonies of both yeasts and cocci. SEM of deep and superficial biopsy specimens often showed huge aggregates of bacteria and yeasts in mucus separated from the tissue surfaces (Fig. 6), although areas not covered by mucus were entirely devoid of adherent bacteria (Fig. 6). The very large microbial aggregates seen in the mucus were composed of both coccolid bacteria (ca. 1 μm) and spherical and hyphal yeast cells (ca. 3 μm). Thus, both TEM and SEM showed that the tissue surfaces of the ileal conduit are not colonized by bacteria or yeasts in patients examined from 1 day to 16 years after surgery.

As time after surgery increased and as patients were removed from post-surgical antibiotic regimens, a large variety of pathogenic bacteria (Table 1) was commonly found at >10⁷ cells per ml of urine. A large proportion of these pathogenic organisms were gram-negative species (Table 1), and direct examination of urine specimens by TEM showed large numbers of gram-negative bacteria in glycocalyx-enclosed microcolonies in the fibrous mucus matrix. One patient (number 13 in Table 1) had had six incidents of pyelonephritis, caused by Escherichia coli, which had been controlled by extensive antibiotic therapy, and the urine specimens taken from his ileal conduit 16 years after surgery still showed a preponderance of gram-negative bacteria in extensive microcolonies in the fibrous mucus matrix and a smaller number of gram-positive cocci (Fig. 7).

DISCUSSION

Patients who have undergone cystectomy for bladder cancer are at a very high risk of acquiring upper urinary tract infections, because the microbiological barrier function of the autochthonously colonized urethra (11, 12) and the defense mechanisms of the normal bladder (9) are no longer protective and because ileal conduits cannot be constructed with valves to prevent reflux through the ureters to the kidneys (22). Surgeons note (22) that ileal conduits of an optimum length (6 to 8 in. [ca. 15.2 to 20.3 cm]) conduct urine satisfactorily and offer some protection against ascending colonization and infection. Nevertheless, the majority of patients with urinary tract diversion develop bacteriuria and, often, obstruction of the stoma (21), loop stenosis (10), pyelonephritis (13), and renal deterioration (14).

It is known that the human ileum is not heavily colonized by bacteria, and this observation was confirmed by our direct electron microscopic observation of very large areas of the surfaces of ileal samples from patients either treated or not treated with antibiotics. Although the mucus blanket was largely retained by novel handling (20) of these specimens, neither bacteria nor protozoa were seen on the tissues or in the mucus. This lack of an autochthonous microbial flora contrasts with the heavily colonized ilea of ruminants (7) and rodents (20). Because natural urine conduits such as the human urethra are colonized by autochthonous bacteria (12) and because these organisms are avidly adherent to uroepithelial cells (4, 5, 11, 18), we anticipated that bacteria entering the stoma would readily colonize the surfaces of the microvilli of the ileal conduit. However, a very thorough examination of the ileal tissue surfaces obtained from patients 1 day to 16 years after surgery showed that they were virtually devoid of adherent microorganisms. The flowing urine, on the other hand, contained large numbers of yeast cells during the initial 1- to 10-day period of intensive postoperative antibiotic therapy and a mixed population of yeasts and gram-positive cocci during the 10- to 13-day postoperative period. Despite the presence of this vigorous luminal population of yeasts and gram-positive cocci and despite the avid adhesion of gram-positive cocci to epithelial cells at the stoma, these organisms did not develop an adherent microbial population on the tissue surfaces of the ileal conduit.

The mucus present in the conduit is produced primarily by the ileal goblet cells, although it is likely that the Tamm-Horsfall glycoprotein secreted by renal tubular cells is also present. The latter has been shown to adhere to uropathogenic bacteria which have a mannose-sensitive adhesin and to block the adherence of these organisms to uroepithelial cells (16, 17). However, bacteria which have a mannose-resistant adhesin have been found to adhere to uroepithelial cells regardless of the presence of the Tamm-Horsfall urinary mucus (17) or of mucopolysaccharides which coat the surfaces of uroepithelial cells (18). In the present study, a large variety of uropathogenic bacteria, some of which contained mannose-resistant adhesins or mannose-sensitive adhesins or both, adhered to mucus within the conduit and developed into dense microcolonies even in patients who had been treated with antibiotic therapy for various periods of time. An earlier study by Needham et al. (15) also demonstrated the presence of large numbers of uropathogens in the urine of patients with ileal conduits.

Because the urinary tract diversion patient has an open urinary tract system, with reflux between the conduit and the kidneys, we would expect a rate of pyelonephritis even higher than that observed. The outward flow of the mucus, coupled with its capacity to bind bacterial cells, may contribute to this apparent protection and may account for the failure of luminal microorganisms to colonize the ileal tissue surfaces. Other factors, such as urine pH, the lack of specific receptor sites, and local immune factors, may enhance this putative protective function of the mucus.

This pattern of microbial colonization of the ileal conduit suggests that the principle of competitive exclusion (6) could be used to develop a vigorous autochthonous bacterial population both in the mucus-filled lumen and on the tissue surfaces of the ileal conduit and thus to preclude ascending pathogenic colonization. We shall examine the early postoperative inoculation of the ileal conduit with autochthonous bacteria (e.g., Lactobacillus spp. of vaginal origin) in an animal model to assess the efficacy of a vigorous adherent nonpathogenic bacterial population in the prevention of pathogenic bacterial invasions of the upper urinary tract.

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LITERATURE CITED

4. Bruce, A. W., R. C. Y. Chan, D. Pinkerton, A. Morales, and P.