Examination of the Morphology of Bacteria Adhering to Peritoneal Dialysis Catheters by Scanning and Transmission Electron Microscopy

THOMAS J. MARRIE,1* MICHAEL A. NOBLE,1 AND J. WILLIAM COSTERTON2

Departments of Medicine and Microbiology, Dalhousie University and the Victoria General Hospital, Halifax, Nova Scotia, Canada B3J 1V8,1 and the Department of Biology, University of Calgary, Calgary, Alberta, Canada T2N 1N42

Received 13 June 1983/Accepted 7 September 1983

We examined Tenckhoff peritoneal catheters by scanning and transmission electron microscopy to study the morphology of bacterial adherence. Two catheters were removed from uninfected patients, three from patients with exit site infections, four from patients with peritonitis, and one from a patient with both exit site infection and peritonitis. Infecting organisms included three of Staphylococcus aureus and one each of Enterobacter sp., Staphylococcus epidermidis, Achromobacter xylosidans, Serratia sp., Klebsiella sp., and Candida albicans. Considerable morphological variation in adherence to the peritoneal dialysis apparatus occurred. No inflammatory cells were ever seen in association with infected cuffs, only two of the five patients with peritonitis had inflammatory cells associated with their catheters. In both instances, these cells tended to occur in clumps and demonstrated no flattening when in contact with the surface. Colonization of the catheter was uneven—bacteria tended to occur in clusters. Extensive matrix formation was evident in several instances, and condensation of this matrix onto the bacteria during the dehydration process rendered clumps of bacterial cells amorphous at times. Bacteria were adherent to the subcutaneous cuff in those patients with exit site infections. Gram-negative bacteria attached to individual dacron fibers of the cuff, often several layers deep. Gram-positive bacteria tended to adhere in clusters.

The development of the Tenckhoff catheter (26) has led to the increasing use of continuous ambulatory peritoneal dialysis as a means of managing patients with renal failure (27). The major limitation to the usefulness of this technique is the high incidence of infection (5, 6, 8, 20, 27). Such infections may involve the site where the catheter exits from the abdominal wall (exit site infection) or the peritoneal cavity (peritonitis) or both (27). The pathogenesis of exit site infections is nearly always contamination from adjacent skin (27) or from carriage sites of pathogenic microorganisms such as the nose (22). Peritonitis can result from breaks in technique, exit site infections, or migration of bacteria across the bowel wall (27). Bacterial adherence to the surfaces of mammalian cells has been studied extensively, and a consensus is developing that such adherence is important in the establishment of infection (7, 21, 25). Recently, bacterial attachment to various devices used in the treatment of patients has been studied (17, 18, 23, 24). Differential adherence is evident—Staphylococcus aureus adheres better to gut sutures than to silk or nylon (24). Surface irregularities are preferential sites of attachment (18), and amorphous extracellular material seems to mediate such bacterial attachment (17, 18). Infected peritoneal catheters seemed to us to be ideal to study the morphology of bacterial attachment to a prosthetic device. We wondered whether attachment to the dacron cuff would be different than to the silicone rubber tubing. The dacron cuff is in a relatively dry environment, whereas the tubing is continuously bathed by the dialysate. Further, many patients received antibiotics for considerable periods of time before removal of the catheter, and we wondered whether this therapy would affect the morphology of the bacteria.

MATERIALS AND METHODS

Catheters. Tenckhoff peritoneal catheters (Lifemed, Division of Vermonit, Compton, Calif.) were removed from eight patients because of infection: exit site infection, three patients; recurrent peritonitis, four.
TABLE 1. Characteristics of 10 continuous ambulatory peritoneal dialysis patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Duration of catheter in situ (mo)</th>
<th>Type of infection</th>
<th>Organism</th>
<th>Duration of antibiotic treatment before removal of catheter (days)</th>
<th>Organism isolated from catheter or cuff</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>Peritonitis</td>
<td>C. albicans</td>
<td>4 CFU/ml</td>
<td>C. albicans</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>Exit site peritonitis</td>
<td>S. aureus</td>
<td>18</td>
<td>S. aureus, confluent growth Entrobacter sp.</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>Peritonitis</td>
<td>Enterobacter sp.</td>
<td>10 on 2 different occasions</td>
<td>S. epidermidis 0.5 CFU/ml</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>None evident clinically, catheter removed because of poor drainage</td>
<td>S. epidermidis</td>
<td>0 CFU/ml</td>
<td>S. epidermidis</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>Exit site</td>
<td>S. aureus</td>
<td>7</td>
<td>S. aureus (cuff) confluent growth</td>
</tr>
<tr>
<td>6</td>
<td>1.7</td>
<td>Peritonitis</td>
<td>P. aeruginosa, Serratia sp., A. xylosoxidans (latest episode)</td>
<td>7</td>
<td>A. xylosoxidans (catheter + cuff)</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>None—removed at time of transplantation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4.5</td>
<td>Exit site</td>
<td>S. aureus</td>
<td>18</td>
<td>S. aureus 2 CFU</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>Peritonitis second-</td>
<td>Klebsiella sp., L. plantarum, B. fragilis</td>
<td>6</td>
<td>Klebsiella sp.</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>Exit site</td>
<td>Serratia sp.</td>
<td>0 CFU/ml</td>
<td>Serratia sp.</td>
</tr>
</tbody>
</table>

TABLE 2. Observations by scanning electron microscopy on the catheters removed from nine of the dialysis patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Cuff</th>
<th>Inner surface of catheter</th>
<th>Outer surface of catheter</th>
<th>See figure:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>Candida</td>
<td>Amorphous material</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Coccoid bacteria</td>
<td>Occasional clusters of coccoid bacteria</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>No bacteria seen</td>
<td>Occasional clusters of rod-like bacteria</td>
<td>7, top</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>No bacteria seen</td>
<td>Occasional inflammatory cell</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Thick layer cocci</td>
<td>Microcolonies of cocci</td>
<td>6 and 7, bottom</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Marked colonization of individual fibers by rod-shaped bacteria</td>
<td>Sparsely colonized with rod-shaped bacteria, some areas of dense colonization</td>
<td>Sparsely colonized with rod-shaped bacilli, inflammatory cells</td>
<td>4 and 5</td>
</tr>
<tr>
<td>7</td>
<td>No bacteria seen</td>
<td>Considerable accretion, no bacteria seen</td>
<td>Occasional rod-like bacteria</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>Gram positive cocci</td>
<td>Sparse bacterial colonization (rods), many inflammatory cells</td>
<td>Amorphous accretions</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>No bacteria seen</td>
<td>Sparse bacterial colonization (rods), many inflammatory cells</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Observation of the catheter of patient 10 showed nothing unusual.
patients. One patient had both infections, and in two patients the catheters were removed for other reasons. The catheters were immediately cut into several portions and transported to the laboratory.

**Culture technique: catheters.** The cuff portion and a length of the intraperitoneal portion of each catheter (3 to 4 cm) was rolled over the surface of a blood agar plate (Trypticase soy agar [BBL Microbiology Systems, Cockeysville, Md.] containing 5% sheep blood) and a MacConkey agar plate. These plates were incubated aerobically for 18 h. All aerobic isolates were identified by conventional techniques. In addition, the cuff and catheter were rolled over the surface of a prereduced Brucella blood agar plate containing 10 μg of vitamin K₁ per ml, 100 μg of kanamycin per ml, and 7.5 μg of vancomycin per ml. These plates were incubated anaerobically in GasPak jars (BBL) for 48 h. Disposable GasPak hydrogen-carbon dioxide generators were used in the jars to supply a gas mixture containing hydrogen and carbon dioxide. Any anaerobic microorganisms were identified by standard laboratory techniques.

**Peritoneal fluid.** Peritoneal fluid was cultured on all patients by the technique of Vas et al. (28). In brief, 100 ml of fluid was filtered through an 0.22-μm membrane filter (Millipore Corp., Bedford, Mass.), and the

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**FIG. 1.** Scanning electron micrograph of the outer surface of the tip of a Tenchoff catheter removed from patient 1 in Table 1 because of *C. albicans* peritonitis. Tissue (omentum?) was adherent to the tip of the catheter. Note the amorphous background. Many 5-μm spherical structures are embedded in this background. Only *C. albicans* was isolated in culture, and transmission electron microscopy showed typical yeast cells. The bar represents 5 μm.
FIG. 2. Scanning electron micrographs of the inner surface (A) and cuff (B) of a Tenchoff peritoneal catheter removed from patient 2 in Table 1. Sparse colonization of the surface was evident (A). In many instances, it was difficult to be sure that bacteria were present (A, inset). Occasional 1-μm coccoid structures were evident adhering to the cuff material (B, arrows). Bars: inset, 1 μm; the others, 5 μm.
filter was put onto a blood agar plate. Another 100 ml of fluid was treated in a similar manner and cultured anaerobically. This was not carried out in an anaerobic chamber.

Scanning electron microscopy. Cuffs and pieces of the intraperitoneal portion of the catheter were placed in a fixative solution consisting of 5% glutaraldehyde buffer (0.067 M, pH 6.2) with 0.15% ruthenium red for 1 h at room temperature =22°C. The preparations were washed three times in the buffer and then “metalized” by using osmium tetroxide and thiocarbohydrazide (13). This was followed by dehydration in ethanol and Freon 113 before critical point drying (3). The specimens were then examined with a Hitachi S450 (Hitachi, Rexdale, Ontario) scanning electron microscope.

Transmission electron microscopy. Small pieces were cut from some of the cuffs and material was scraped from the surface of some of the catheters. This material was fixed as outlined above, then washed five times in the buffer, and dehydrated through a graded acetone series. All of the solutions used in processing these specimens except the 90 and 100% acetone solutions were made up to contain 0.5% ruthenium red. After further dehydrations in propylene oxide, the specimens were embedded in Vestopal (R) (Ladd Industries, Burlington, Vt.), sectioned, stained with uranyl acetate and lead citrate (19) reinforced with evaporated carbon, and examined with a model 801 electron microscope (Associated Electronic Industries, Harlow New Town, England) at an accelerating voltage of 60 kV.

RESULTS

Table 1 shows the duration that each peritoneal catheter was in place, the site of infection, the microorganisms isolated, and the duration of antibiotic therapy before removal of the catheter. In Table 2, the scanning electron microscopy findings are summarized. The morphology of adherence of the various microorganisms to the catheters and cuffs varied considerably. Only one patient had fungal peritonitis (patient 1 in Table 1). The Candida cells (Fig. 1) were embedded in an amorphous background. This probably represented the tissue that was adherent to the tip of the catheter at the time of the removal. The morphology of bacterial adherence to the catheter cuffs is shown in Fig. 2B, 3, and 4. Rod-like bacteria were seen adherent to individual dacron fibers in Fig. 4B. The bacteria were adherent in layers. Bacterial adherence to the inner and outer surfaces of the catheters varied considerably from one area to another (Fig. 5A and B). In general, inflammatory cells were uncommonly observed; however, when present they often occurred in large clumps such as in Fig. 5 (B). Some microorganisms were associated with an extensive matrix (Fig. 6B). All the sites of this catheter that were colonized showed bacteria embedded in a matrix. This matrix condensed down around bacteria during the dehydration process, often rendering identification of adherent structures as clumps of microorganisms difficult (Fig. 7A and inset). Figure 7B is a dramatic illustration of this phenomenon: matrix enclosed cells and bare cells are shown side by side.

One catheter from a patient who had no

FIG. 3. Transmission electron micrograph of material shaved from the cuff of a peritoneal catheter removed from a patient (patient 8, Table 1) who had a S. aureus exit site infection. S. aureus was isolated from the cuff. Note the typical gram-positive cell wall of the two bacteria shown. The bar represents 0.1 μm.
clinical or bacteriological evidence of infection had a few rod-like bacteria adherent to a clean catheter surface. These structures varied in size and shape (Fig. 8). No obvious effects of antibiotic therapy were noted (Fig. 3 through 6).

DISCUSSION

In vitro studies of the adherence of coagulase-negative staphylococci to the surfaces of intravenous catheters have shown that within a short time (5 to 30 min), single bacterial cells attach.

FIG. 4. Scanning electron micrographs of the cuff of a Tenchoff peritoneal catheter removed from patient 6 in Table 1. Achromobacter xylosoxidans was isolated from this material. (A) A low power view showing the fibrous nature of dacron cuff. (B) Many rod-shaped structures adhering to an individual fiber. It is evident that the bacteria are piled in layers and interconnected by a fibrous matrix. Bar (A), 500 μm; bar (B), 5 μm.
and microcolonies are evident after 40 to 60 min, culminating in heavy colonization by 6 to 12 h (18). Subsequent events (48 to 96 h) include erosion of layers of the catheter surface and encasement of the bacterial cells by a slimy material (18). Production of this slime-like material correlates with "clinical significance" of such isolates and is enhanced by casamino acids and glucose (2).

In marine environments, growth and division of bacteria at a solid-liquid interface occurs quickly and at nutrient concentrations too low to permit growth in the aqueous phase (10). Such a finding explains the earlier observations of Zobell (30) of the pronounced tendency of bacteria to grow in close association with surfaces in aquatic environments.

In this and in other studies (14–16), we have observed that bacteria adhere to various foreign devices in the human body in a manner similar to that shown in both in vitro experiments (2, 18) and in aquatic environments (10). The infected

FIG. 5. Scanning electron micrographs of two portions of the peritoneal catheter from the same patient as in Fig. 4. (A) Outer aspect of the surface. Note the colonization by rod-shaped bacteria. In places, the outline of the bacterial cell is obscured, and towards the upper left hand corner the bacteria are covered completely by an amorphous matrix. Only inflammatory cells are seen covering the inner aspect of another portion of the catheter (B). Both bars represent 5 μm.
FIG. 6. Scanning electron micrographs of the outer surface of the subcutaneous portion of a peritoneal catheter removed from patient 5 in Table 1. S. aureus was isolated. A low power view (A) shows that the surface of the catheter is obscured. At higher magnification (B) myriads of cocci are evident. An amorphous matrix covers many of these cells. Bar (A), 5 μm; bar (B), 5 μm.
peritoneal catheters have a relatively dry surface (cuff) and an aquatic environment (intraperitoneal portion of the catheter). We did not observe any major morphological differences in the adherence of bacteria to these two components of the catheter nor did we observe any differences between the exterior and interior surfaces of the catheters.

It is known that *S. aureus* (27) and *Pseudomonas aeruginosa* (8, 11) infections complicating peritoneal dialysis are associated with a low cure rate and frequently necessitate removal of the catheter. The apparent mechanisms are that *S. aureus* predisposes to intraabdominal abscesses (27), and *P. aeruginosa* infection is frequently associated with sinus tract infection (11). Whether differences in exopolymer production by microorganisms adherent to such foreign bodies are also important in this regard remains to be determined. That such differences exist is evident from an examination of Fig. 4 through 8.

Host defense factors are impaired in the pres-

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**FIG. 7.** Scanning electron micrographs of portions of peritoneal catheters removed from patient 3 in Table 1 (A) and patient 5 in Table 1 (B). These photomicrographs illustrate the effect of condensation of bacterial matrix during the dehydration process. The clump (A, arrows) which is shown at higher magnification in inset is recognizable as bacterial cells, with difficulty. The bottom micrograph (B) shows naked cells side by side with ones covered by condensed matrix. The bars represent 5 μm.
ence of a foreign body. It appears that opsonization is inadequate and that polymorphonuclear leukocytes degranulate on contact with some foreign materials leading to a decrease in their phagocytic function (29). In addition, human monocytes remain spherical when in contact with foreign materials, a factor which may have some effect on their efficiency as phagocytic cells (12). The decreased pH and increased osmolality of the solutions used in peritoneal dialysis result in a decrease in activity of blood leukocytes (4). We observed inflammatory cells on only three catheters (patients 4, 6, and 9; Table 1 and Fig. 5). The appearance of the inflammatory cells in Fig. 5B is representative of those seen in patients 4 and 9 as well. Note the rounded appearance of these cells. Confluent layers of bacteria or fungi were frequently seen and not one inflammatory cell was evident (Fig. 1, 5A, and 6). Inflammatory cells were never observed on cuffs (Fig. 4). Certainly inflammation of the exit site was evident in several of these patients, and all the patients with peritonitis had ≥500 leukocytes per mm$^3$ of peritoneal effluent. In other studies of such patients with peritonitis, the leukocyte counts have ranged from 720 to 3,290 per mm$^3$ (9). More than 50% of these cells were monocytes or macrophages (9).

An alternate approach to the problem of infection in continuous ambulatory peritoneal dialysis patients would be to identify the mechanism of binding of bacterial exopolymers to the surface and then alter the surface to prevent adherence. Incorporation of isothiazalone biocides, which penetrate the polymeric matrix of bacteria, into the wall of the catheter may be another approach (1) to the prevention of bacterial adherence to such prosthetic devices. 

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