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Characterization of Cell Envelope Proteins of *Staphylococcus epidermidis* Cultured in Human Peritoneal Dialysate

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The cell envelope protein profiles of *Staphylococcus epidermidis* cultured in used human peritoneal dialysate (HPD) differed markedly from those of cells cultured in nutrient broth. Compared with broth-grown cells, many cell wall proteins were repressed in HPD, although three proteins of 42, 48, and 54 kDa predominated and an iron-repressible 130-kDa protein was induced. Growth in HPD also resulted in expression of two cell membrane proteins of 32 and 36 kDa which were iron repressible. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblot analysis using monospecific polyclonal antisera raised against the 32- and 36-kDa proteins revealed considerable antigenic and molecular mass homology among 12 *S. epidermidis* isolates from patients with continuous ambulatory peritoneal dialysis-related peritonitis. The 32-kDa antiserum also cross-reacted with a 32-kDa *S. aureus* cell membrane protein. Immunoblots of *S. epidermidis* cell walls and membranes were also probed with normal human serum and serum and HPD from continuous ambulatory peritoneal dialysis patients. While the cell wall proteins of *S. epidermidis* appeared to be relatively poorly immunogenic, the 32- and 36-kDa membrane proteins reacted strongly with antibodies present in each of the body fluids evaluated. These results suggest that the highly conserved 32- and 36-kDa iron-repressible proteins are expressed during growth in vivo and may be involved in iron transport, since all 12 *S. epidermidis* strains examined also produced iron chelators.

An essential factor in any infection is the ability of the infecting microorganism to multiply successfully within the host. During the various stages of infection, a pathogen may be translocated from one body site to another and so must constantly respond to changing environmental conditions. The influence of a host environment on pathogenic bacteria is, however, often overlooked. Investigations of bacterial virulence are usually done with organisms cultured in laboratory media under conditions which do not necessarily reflect those encountered in vivo. Environmental signals such as temperature, pH, osmolarity, gaseous tension, and nutrient availability are all known to influence bacterial physiology, biochemistry, and immunochemistry. Such phenotypic plasticity is frequently manifested in the cell envelope and may thus contribute to both bacterial survival and virulence (2, 23, 33).

Coagulase-negative staphylococci are common skin commensals and are generally regarded as emerging pathogens. Their clinical significance has been increased by the observation of their unique ability to colonize medical implants and prosthetic devices (8, 22). For instance, peritonitis due to coagulase-negative staphylococci, *Staphylococcus epidermidis* in particular, is a major complicating feature in the treatment of end-stage renal failure by continuous ambulatory peritoneal dialysis (CAPD) (1, 24, 28). Although the commercial peritoneal dialysate solutions used in CAPD do not support the growth of coagulase-negative staphylococci, the fluid is modified during dialysis and then becomes capable of supporting bacterial growth (26, 31, 32). At this stage, the immunodeficient environment of the dialyzed peritoneum appears to be suited to the growth of coagulase-negative staphylococci, which probably gain entry to the

peritoneum via the CAPD catheter (24, 28). The ability of coagulase-negative staphylococci to adapt and survive within a dialyzed peritoneum may be related to their capacity to (i) grow in peritoneal fluids (26, 32, 34), (ii) colonize intraperitoneal or plastic catheter surfaces (12, 24, 28), and (iii) avoid phagocytosis or phagocytic killing (17, 27, 28). An additional contributing factor appears to be that the staphylococcal killing capacities of peritoneal macrophages and infiltrating polymorphonuclear leukocytes are impaired in CAPD patients (11, 16).

Although much information on the structure, function, and contribution to virulence of gram-negative bacterial surface proteins is available, there is a relative lack of such data for staphylococci, especially coagulase-negative staphylococci. Whole staphylococcal cell protein profiles have been evaluated as taxonomic and typing tools (5), although in general there have been few attempts to refine such methodology by separating the cell wall from cytoplasmic membrane proteins. In addition, little attention has been given to the influence of culture conditions on the expression of staphylococcal surface antigens which may play a role in bacterial adaptation and survival in vivo. The growth environment is, however, known to influence the staphylococcal cell wall protein profile. The expression of staphylococcal wall proteins has been reported to be influenced by carbon dioxide tension (6) and culture on a surface (3).

In this study, we analyzed the cell envelope protein composition of *S. epidermidis* cultured under conditions designed to mimic those encountered within a dialyzed peritoneum. In particular, staphylococci were cultured in used human peritoneal dialysis fluid (HPD) in an air atmosphere enriched with physiological carbon dioxide levels (5%), since carbon dioxide tension markedly influences both the composition of HPD fluid (32) and staphylococcal surface chemistry (6). We also investigated the presence and

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antigenic homology of two immunodominant iron-repressible cytoplasmic membrane proteins in *S. epidermidis*. These proteins are recognized by antibodies present in human serum and HPD fluid, which implies that they are expressed during growth in vivo.

(This study was previously presented in part [Program Abstr. Annu. Meet. Am. Soc. Microbiol. 1990, D-103, p. 97].)

MATERIALS AND METHODS

Bacterial strains and growth conditions. *S. epidermidis* 901 (previously designated 10 [34]), 11 other *S. epidermidis* strains, and *S. aureus* N and W were isolated from infected dialysis fluid of CAPD patients with peritonitis attending the Renal Unit, The City Hospital, Nottingham, United Kingdom. All strains were fully identified by API Staph (API, Basingstoke, United Kingdom). Bacteria were grown statically for 24 h in 100-ml volumes of pooled (from 25 patients), antibiotic-free, noninfected HPD at 37°C in air enriched with 5% carbon dioxide. For some experiments, bacteria were grown in the same atmospheric conditions in nutrient broth or in HPD supplemented with 20 μM ferric sulfate.

Preparation of cell wall and cytoplasmic membrane proteins. Cell wall and cytoplasmic membrane proteins were prepared as described previously (6) on the basis of a method reported by Cheung and Fischetti (3). Briefly, bacteria harvested from 100 ml of growth medium were washed twice in phosphate-buffered saline (120 mM NaCl, 10 mM sodium phosphate, pH 7.4), suspended in 0.6 ml of digestion buffer (30% [wt/vol] raffinose, 1 mg of benzamidine per ml, 0.5 mg of phenylmethylsulfonyl fluoride per ml in 10 mM Tris hydrochloride, pH 7.4, containing 100 μg of lysostaphin), and incubated for 60 min at 37°C. Protoplasts were removed by centrifugation (11,600 × *g* for 3 min), and the supernatant containing the cell wall proteins was stored frozen at −20°C before electrophoresis. The protoplast pellet was suspended in distilled water and sonicated at 4°C for two 30-s periods. After lysis, cytoplasmic membranes were collected by centrifugation (100,000 × *g* for 30 min).

Surface exposure of cell envelope proteins. Whole cells of *S. epidermidis* 901 were incubated with soluble and immobilized proteolytic enzymes to cleave surface proteins. Trypsin (200 μg/ml in 50 mM Tris hydrochloride, pH 8.0), *S. aureus* V8 protease (200 μg/ml in 50 mM Tris, pH 7.8), or proteinase K (200 μg/ml in 50 mM Tris hydrochloride, pH 7.5) was added to Eppendorf tubes containing 10^{11} bacteria (final volume, 1 ml), and the suspension was incubated for 30 min at 37°C. Alternatively, trypsin immobilized on agarose beads (15 U/ml in 50 mM Tris hydrochloride, pH 7.8) was incubated with whole bacteria as described above for 30, 60, 120, 240, 360, or 480 min or 24 h. After digestion, proteolysis was stopped by addition of phenylmethylsulfonyl fluoride and benzamidine to a final concentration of 500 μg/ml each. Cells were then harvested and washed twice before separation of cell walls and membranes as described above.

SDS-PAGE. Proteins were separated on 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels as described before (34) after being heated in SDS-PAGE sample buffer at 100°C for 5 min before the gels were loaded. For some experiments, proteins were also solubilized at 37 and 60°C in SDS-PAGE sample buffer with or without 2-mercaptoethanol. Approximately 10 μg of protein was loaded per lane. Molecular size standards (Sigma) were run concurrently. After electrophoresis, gels were (i) fixed and stained with Coomassie brilliant blue R250 in 10%

glacial acetic acid–50% methanol and destained in 40% methanol–10% glacial acetic acid, (ii) silver stained with a kit from Bio-Rad Laboratories (Watford, United Kingdom), or (iii) electrophoretically transferred to nitrocellulose for immunoblotting as described by Towbin et al. (25).

Immunoblotting. The nitrocellulose immunoblots were incubated with the following range of probes: (i) monospecific polyclonal rabbit antisera (diluted 1:400) raised as described below against the 32- and 36-kDa iron-repressible staphylococcal cytoplasmic membrane proteins, (ii) pooled normal human serum from 25 people or pooled serum from six CAPD patients diluted 1:20, (iii) 20 ml of sterile HPD from the pool used for bacterial culture, or (iv) goat anti-human immunoglobulin G (IgG) heavy-chain-specific antiserum-peroxidase conjugate. Patients from whom serum and HPD fluid were obtained had no episodes of peritonitis at collection or in the previous 12 months. Secondary detection of both rabbit and human antibodies was performed with a protein A-peroxidase conjugate (Sigma; 10 μg/ml). The reactive bands were visualized with a 25-μg/ml solution of 4-chloronaphthol containing 0.01% H_2O_2. The molecular masses and positions of cross-reacting proteins were then confirmed by counterstaining immunoblots with Ponceau S (0.5% [wt/vol]) in 1% (vol/vol) glacial acetic acid.

Preparation of antisera. Monospecific polyclonal antisera against the iron-repressible 32- and 36-kDa cytoplasmic proteins were raised in rabbits against gel-purified proteins. Membranes prepared from strain 901 cultured in HPD were subjected to preparative SDS-PAGE, and after electrophoresis the gel was washed extensively in distilled water, stained with 0.05% Coomassie brilliant blue R250 in distilled water for 30 min, and destained by extensive washing in distilled water until protein bands were visible. The bands corresponding to the 32- and 36-kDa proteins were excised and lyophilized. Samples were subjected to SDS-PAGE and silver stained to assess purity. The dried gel slices were ground to a powder, and distilled water was added to half of the original gel volume. Following incubation at room temperature, protein concentrations were determined and adjusted to 50 μg/ml. Samples were then mixed 50:50 with Freund incomplete adjuvant, and three 1.2-ml injections were given subcutaneously to rabbits (New Zealand White) at intervals of 21 days. At 14 days after the final injection, blood was removed and serum was separated after clotting and centrifugation.

RESULTS

Comparison of cell wall and membrane proteins of *S. epidermidis* cultured in broth or HPD. Cell wall and cytoplasmic membrane proteins were prepared from *S. epidermidis* 901 grown in nutrient broth or HPD and subjected to SDS-PAGE. Figure 1 compares the wall (lanes A to C) and membrane proteins (lanes D to F) of strain 901 cultured in broth, HPD, or HPD supplemented with 20 μM ferric sulfate. Growth in HPD appeared to result in repression of many cell wall proteins, although three proteins of 42, 48, and 54 kDa were much more prominent in HPD-grown cells (Fig. 1, compare lanes A and B). Differences in the expression of several minor cell wall proteins were also noted following addition of iron to HPD (Fig. 1, compare lanes B and C). In particular, proteins of 130, 36, and 32 kDa which were present in HPD-grown cells (Fig. 1, lane B) were repressed following growth in nutrient broth or HPD containing 20 μM Fe^{3+}.

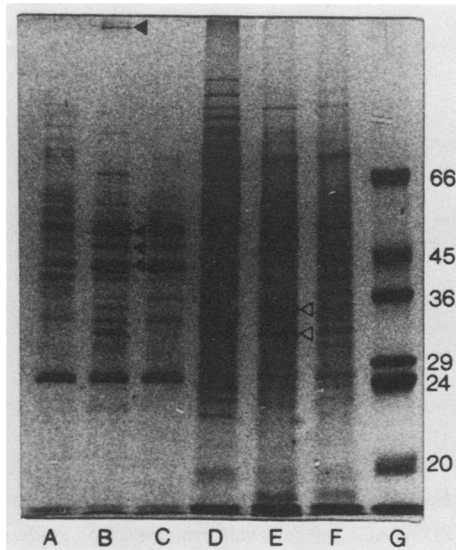The cell membrane protein profiles of strain 901 grown

FIG. 1. SDS-PAGE profiles of cell wall (lanes A to C) and cell membrane (lanes D to F) proteins of *S. epidermidis* 901 prepared from bacteria cultured in nutrient broth (lanes A and D), HPD (lanes B and E), or HPD to which 20 μM Fe^{3+} was added (lanes C and F). Lane G contained molecular mass marker proteins, whose molecular masses are indicated to the right in kilodaltons. The closed arrowheads beside lane B indicate the positions of the 130-, 54-, 48-, and 54-kDa cell wall proteins. The open arrowheads beside lane E indicate the positions of the 36- and 32-kDa membrane proteins. Gels were stained with Coomassie blue.
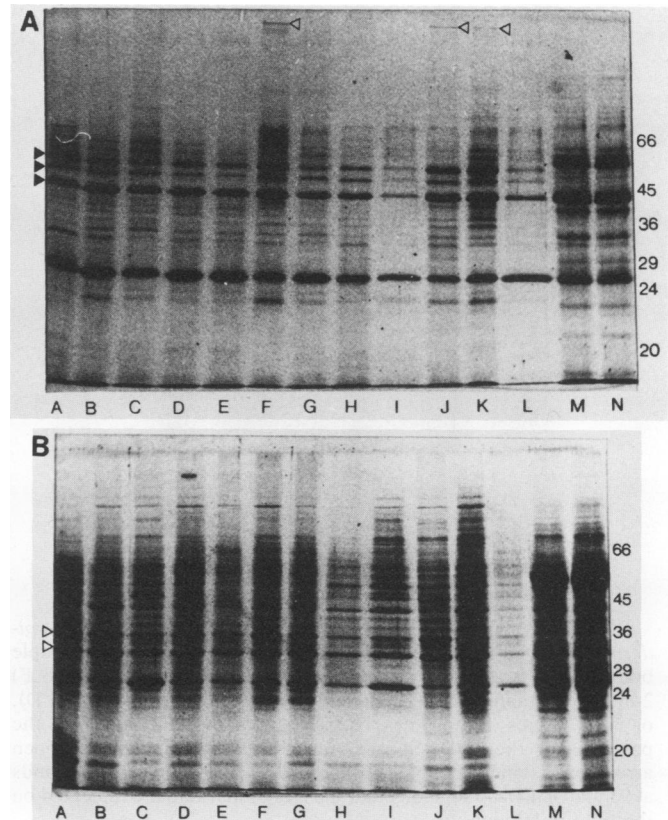

FIG. 2. (A) SDS-PAGE of the cell wall protein profiles of 12 isolates of *S. epidermidis* (lanes A to L) and two *S. aureus* isolates (lanes M and N) from CAPD peritonitis cultured in HPD. The closed arrowheads indicate the positions of the 54-, 48-, and 42-kDa proteins. The open arrowheads beside lanes F, J, and K indicate the positions of the 130-kDa proteins. (B) SDS-PAGE of the cell membrane proteins of *S. epidermidis* and *S. aureus* as in panel A. The open arrowheads mark the positions of the 32- and 36-kDa iron-repressible proteins. In both panels, the molecular masses of protein standards are indicated at the right in kilodaltons.

under the different nutritional conditions also showed marked differences. Many high-molecular-mass proteins present in nutrient broth-grown cells were repressed after culture in HPD. However, two proteins of 32 and 36 kDa, respectively, were induced following growth in HPD (Fig. 1, lane E). Their expression appeared to be dependent on the medium iron content, since addition of 20 μM Fe^{3+} to HPD resulted in their repression (Fig. 1, compare lanes E and F).

To determine whether the 42-, 48-, 54-, and 130-kDa cell wall proteins and the 32- and 36-kDa iron-repressible membrane proteins expressed by bacteria cultured in HPD were strain specific, 12 clinical isolates of *S. epidermidis* and 2 of *S. aureus* from CAPD-induced peritonitis were also examined (Fig. 2A and B). All *S. epidermidis* strains expressed the two iron-repressible membrane proteins (Fig. 2B), although in one strain (lane B) the 36-kDa membrane protein was constitutively expressed (data not shown). The cell wall protein profile of each *S. epidermidis* strain cultured in HPD fluid was very similar to that of strain 901, although only three strains appeared to express the 130-kDa iron-repressible protein (Fig. 2A, lanes F, J, and K).

Since HPD contains serum proteins (mainly albumin, IgG, and transferrin, although at much lower levels than those found in serum [34]), it was important to determine whether any of these serum proteins were present in the *S. epidermidis* cell wall fractions. We have previously reported that IgG, but not transferrin or albumin, from HPD fluid was detected in immunoblots of *S. epidermidis* whole-cell proteins prepared from bacteria cultured in HPD (34). Immunoblots of cell wall proteins were therefore probed with an anti-human IgG heavy-chain–horseradish peroxidase conjugate. A positive reaction confirmed that the 54-kDa protein band observed was, at least in part, the IgG heavy chain (data not shown).

Heat and 2-mercaptoethanol modifiability of cell wall proteins of *S. epidermidis* 901. To characterize further the physical properties of the four major HPD-induced wall proteins, samples were incubated with or without 2-mercaptoethanol at 37, 66, and 100°C. Such experiments may indicate whether any of these proteins were subunits of larger proteins and whether they shared any of the heat modifiability characteristics typically exhibited by the major outer membrane proteins of gram-negative bacteria (15) (Fig. 3). The electrophoretic mobilities of the 42-, 48-, 54-, and 130-kDa proteins were not affected by omission of 2-mercaptoethanol from the SDS-PAGE sample buffer in preparations heated at 100°C (Fig. 3, lane B), suggesting that these proteins are not subunit proteins linked by disulfide bridges. However, new bands at 38 and 93 kDa (Fig. 3, lanes B, D, and F) were observed in each of the samples treated without 2-mercaptoethanol at all temperatures. These proteins were presumably broken down into smaller subunits in the presence of 2-mercaptoethanol. In addition, the solubilization temperature influenced the migration of the 54-kDa protein, the levels of which were considerably reduced in samples incubated at 37°C.
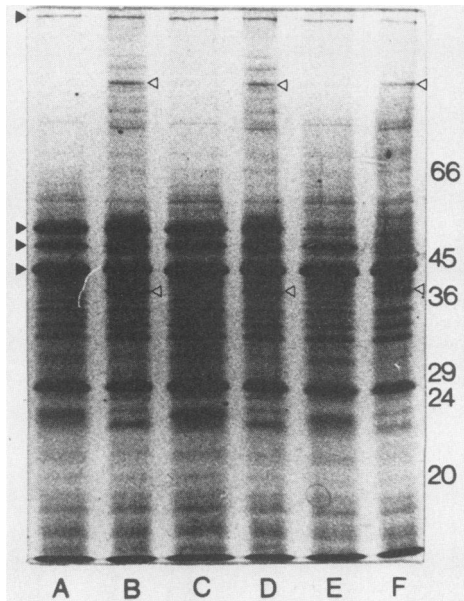
FIG. 3. SDS-PAGE of cell wall proteins prepared from *S. epidermidis* 901 cultured in HPD and incubated in SDS-PAGE sample buffer with (lanes A, C, and E) or without (lanes B, D, and F) 2-mercaptoethanol at 100°C (lanes A and B), 66°C (lanes C and D), or 37°C (lanes E and F). The closed arrowheads indicate the positions of the 130-, 54-, 48-, and 42-kDa proteins. The open arrowheads (lanes B, D, and F) indicate the positions of new bands at 93 and 38 kDa. The molecular masses of markers are indicated on the right in kilodaltons.
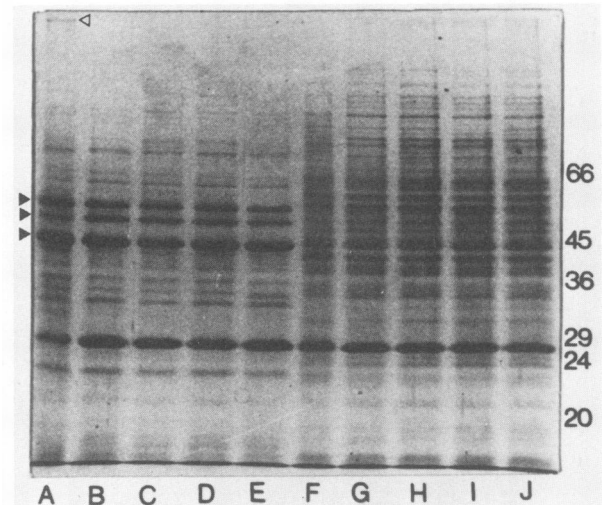

FIG. 4. SDS-PAGE of cell wall proteins of *S. epidermidis* 901 cultured in HPD fluid (lanes A to E) or nutrient broth (lanes F to J) prepared before (lanes A and F) or after (lanes B to E and G to J) treatment with proteases. Lanes: B and G, soluble trypsin; C and H, immobilized trypsin; D and I, *S. aureus* V8 protease; E and J, proteinase K. The closed arrowheads indicate the positions of the 54-, 48-, and 42-kDa wall proteins. The open arrowhead marks the 130-kDa protein. The molecular masses of markers are indicated on the right in kilodaltons.

Susceptibility of cell wall proteins to proteolytic cleavage. To determine which, if any, of the cell wall proteins were exposed at the cell surface, three proteases were used. After growth, whole bacteria were incubated with soluble or insoluble trypsin, proteinase K, or *S. aureus* V8 protease before separation of cell walls and membranes. Since the gram-positive cell wall is a relatively open structure (33), pretreatments with soluble and insoluble trypsin were compared because insoluble trypsin was unlikely to penetrate very deeply into the cell envelope matrix whereas most of the cell envelope proteins should be accessible to soluble trypsin.

Apart from the 130-kDa iron-repressible protein, none of the major cell wall proteins present in HPD-grown cells were digested after 30 min of incubation with either soluble or insoluble protease (Fig. 4). In addition, no further changes were apparent after 24 h of incubation with immobilized trypsin, although all of the cell wall proteins were digested with soluble trypsin (data not shown). Interestingly, the 54-kDa cell wall protein, which immunoblotting experiments showed, at least in part, to be the IgG heavy chain, was only slightly reduced in staining intensity after proteolytic digestion of whole cells with protease. Immunoblots of cell walls isolated from staphylococci incubated with immobilized trypsin for 30 min to 24 h were probed with the anti-human IgG heavy chain. No reactive bands were observed, suggesting that the surface-bound IgG had been digested and that a 54-kDa *S. epidermidis* cell wall protein had comigrated with the IgG heavy chain (data not shown).

These experiments with proteolytic enzymes suggest that among the major HPD-induced *S. epidermidis* wall proteins, the 130-kDa protein is surface exposed but the 42-, 48-, and 54-kDa proteins are not.

Antigenic homology of iron-repressible cytoplasmic membrane proteins. Preparative gel electrophoresis was used to purify the 32- and 36-kDa cytoplasmic membrane proteins from strain 901. Bands excised from SDS-PAGE gels were reelectrophoresed on SDS-PAGE gels and silver stained to confirm the presence of a single protein before monospecific polyclonal antisera were raised in rabbits. On immunoblots, the 32-kDa serum reacted with the 32-kDa protein in strain 901 and the 11 other *S. epidermidis* isolates examined (Fig. 5A) and also cross-reacted with a cell membrane protein of the same mass from both *S. aureus* strains. Antisera raised against the 36-kDa protein reacted with the homologous protein in each of the *S. epidermidis* strains examined (Fig. 5B) but not with *S. aureus* membrane proteins.

Immune responses of CAPD patients to *S. epidermidis* wall and envelope proteins. Immunoblotting experiments were done to determine whether any of the cell wall and membrane proteins expressed by *S. epidermidis* cultured in HPD were recognized by antibodies in serum or HPD from CAPD patients or in pooled normal human serum. Proteins from 12 *S. epidermidis* and 2 *S. aureus* isolates cultured in HPD fluid were separated by SDS-PAGE, transferred to nitrocellulose membranes, and probed with either HPD fluid or serum, followed by a protein A-horseradish peroxidase conjugate to detect bound immunoglobulins.

(i) Cell membrane antigens. The antigenic profiles of cell membranes prepared from *S. epidermidis* and *S. aureus* cultured in HPD and probed with HPD from CAPD patients or with normal human serum are shown in Fig. 6A and B. *S. epidermidis* and *S. aureus* showed distinct profiles, and many more reactive bands were observed in the latter. The major 32- and 36-kDa *S. epidermidis* iron-repressible membrane proteins were highly immunogenic and recognized by antibodies in both HPD and normal human serum. Pooled sera from CAPD patients, however, reacted very weakly with these proteins and all other proteins (data not shown).
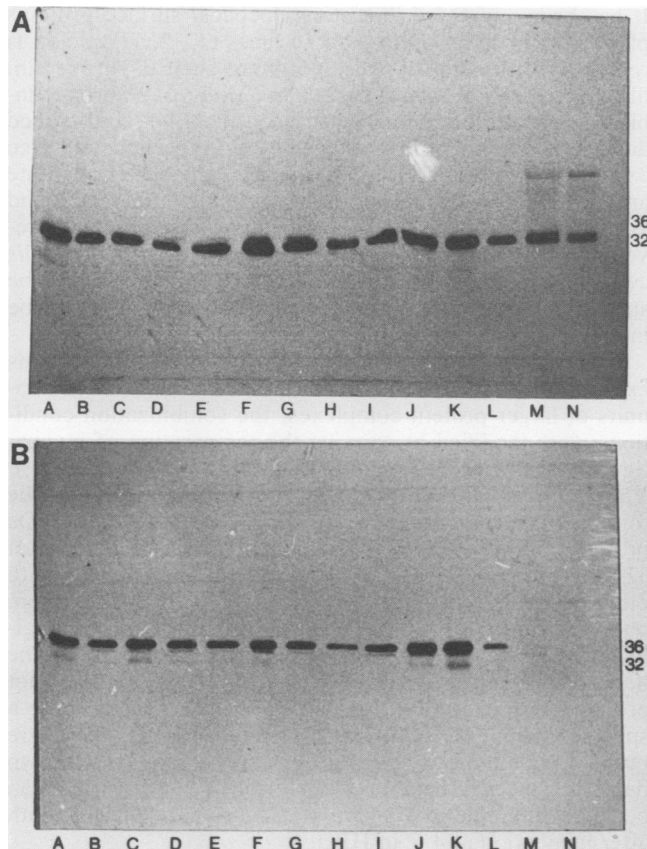
FIG. 5. (A) Immunoblot showing the reactions of a monospecific polyclonal antiserum raised against the purified 32-kDa iron-repressible membrane protein from *S. epidermidis* 901 with cell membranes prepared from 12 *S. epidermidis* strains (lanes A to L) and two *S. aureus* strains (lanes M and N) cultured in HPD. Strain 901 was in lane J. (B) Immunoblot showing the reactions of a monospecific polyclonal antiserum raised against the purified 36-kDa iron-repressible membrane protein from *S. epidermidis* 901 with cell membranes prepared from 12 *S. epidermidis* strains (lanes A to L) and 2 *S. aureus* strains (lanes M and N) cultured in HPD. Strain 901 was in lane J. In both panels, the molecular masses of markers are indicated on the right in kilodaltons.
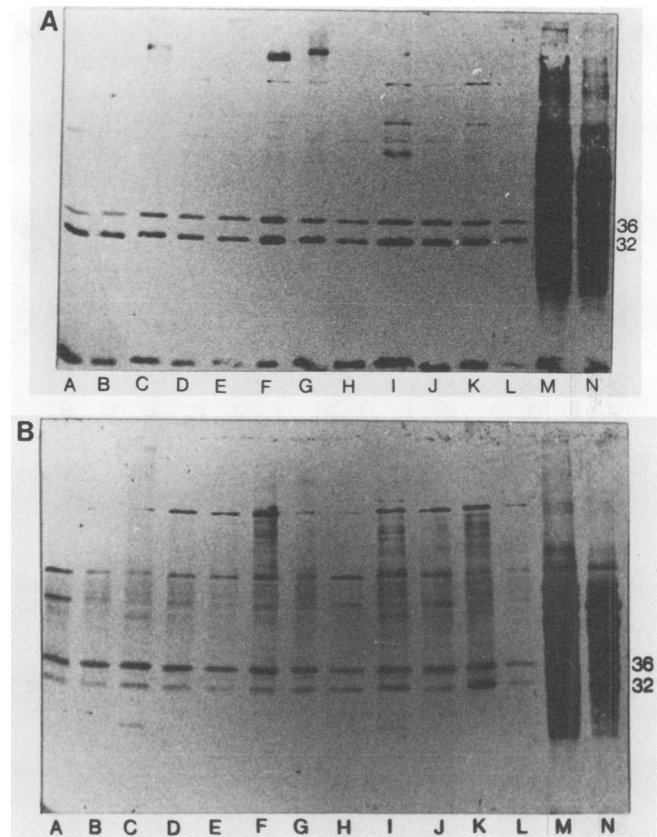

FIG. 6. Immunoblot showing the reactions of antibodies in pooled HPD with cell membrane proteins from 12 *S. epidermidis* (lanes A to L) and 2 *S. aureus* (lanes M and N) strains cultured in HPD. (B) Immunoblot showing the reactions of antibodies in pooled normal human serum with cell membrane proteins from 12 *S. epidermidis* (lanes A to L) and 2 *S. aureus* (lanes M and N) strains cultured in HPD. In both panels, the molecular masses of markers are indicated on the right in kilodaltons.

In addition, many other membrane proteins were recognized by antibodies present in normal human serum (Fig. 6B).

(ii) Cell wall antigens. Comparison of the antigenic patterns observed after *S. epidermidis* cell wall proteins were probed with HPD or normal human serum revealed several differences. In particular, one or two high-molecular-mass (100 kDa) antigens which were not visible in Coomassie blue-stained gels were recognized by antibodies in HPD (Fig. 7A) but not by antibodies in pooled normal human serum (Fig. 7B) or pooled serum from CAPD patients (data not shown).

For most strains, only weak reactions between the *S. epidermidis* 42-, 48-, and 54-kDa proteins with antibodies in pooled HPD (Fig. 7A), pooled normal human serum (Fig. 7B), and serum from CAPD patients (data not shown) were noted, although several other strongly reactive bands were observed in three strains (Fig. 7A and B, lanes A, H, and J). Some reactivity between antibodies to the 32- and 36-kDa iron-repressible membrane proteins was also revealed following incubation of cell wall proteins with HPD (Fig. 7A). Since small amounts of the 32- and 36-kDa proteins were

observed in the cell wall protein preparation shown in Fig. 1 (lane B) and since these membrane proteins are highly immunogenic, two possible explanations arise. Either the wall preparations used were contaminated with low levels of these proteins or a fraction of their population is cell wall rather than cell membrane associated. Similar results were obtained with wall proteins probed with normal human serum, although interestingly, the 36-kDa protein was more strongly reactive than the 32-kDa protein (Fig. 7B).

Many more *S. aureus* than *S. epidermidis* proteins reacted with antibodies in both HPD and serum (Fig. 7A and B), although sera from CAPD patients reacted much more weakly than normal human serum or HPD fluid (data not shown).

DISCUSSION

Bacterial peritonitis due primarily to infection by coagulase-negative staphylococci, in particular, *S. epidermidis*, is a major complicating factor in the management of patients with end-stage renal failure by CAPD (1, 24). As part of our investigation of the adaptation and survival of coagulase-negative staphylococci within a dialyzed peritoneum, we examined the influence of growth under physiological conditions relevant to CAPD (i.e., in HPD fluid in an air
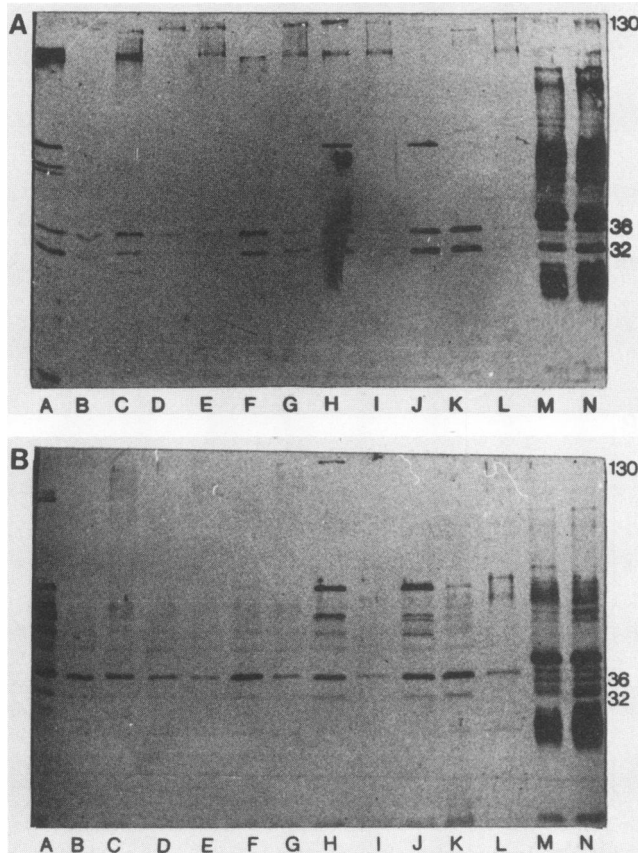
FIG. 7. (A) Immunoblot showing the reactions of antibodies in pooled HPD with cell wall proteins from 12 *S. epidermidis* (lanes A to L) and 2 *S. aureus* (lanes M and N) strains cultured in HPD. (B) Immunoblot showing the reactions of antibodies in pooled normal human serum with cell wall proteins from 12 *S. epidermidis* (lanes A to L) and 2 *S. aureus* (lanes M and N) strains cultured in HPD. In both panels, the molecular masses of markers are indicated on the right in kilodaltons.

atmosphere enriched with 5% carbon dioxide; 32) on the expression of cell envelope proteins in *S. epidermidis*. Compared with nutrient broth-grown cells, many cell wall proteins were repressed in the walls of HPD-grown *S. epidermidis* and a characteristic pattern of three major wall proteins of 42, 48, and 54 kDa was observed in all 12 *S. epidermidis* isolates. This protein profile differed markedly from that exhibited by *S. aureus*. In addition, an iron-repressible cell wall protein with a molecular mass of 130 kDa was also observed in some *S. epidermidis* strains.

The marked phenotypic changes observed in *S. epidermidis* cell wall protein profiles following growth in HPD may reflect the response of the organism to growth in a stressful environment. The environmental signal responsible for this response has not been elucidated, and we were unable to reproduce the changes in protein profile by exposing staphylococci growing in nutrient broth to extremes of temperature, pH, or osmolarity or by addition of synthetic iron chelators such as ethylenediamine di-*o*-(hydroxyphenylacetic acid) (EDDA) (unpublished data). However, since many of the proteins present in the walls of nutrient broth-grown *S. epidermidis* are repressed in HPD, they are presumably not essential for cell viability. The functions of the major HPD-induced *S. epidermidis* cell wall proteins are unknown.

It has been suggested that staphylococcal surface proteins play a role in adhesion to inert surfaces (4, 12, 13) and/or as receptors for binding of plasma proteins such as vitronectin, fibronectin, and collagen (14, 21). In *S. aureus*, a fibronectin-binding protein of 210 kDa has been identified and cloned and shown to exist in both cell wall-associated and free extracellular forms (14). Such proteins appear to constitute an important mechanism for host tissue adherence and virulence. However, our experiments using proteases revealed that of the four major HPD-induced *S. epidermidis* cell wall proteins, only the 130-kDa protein appeared to be surface exposed and, therefore, have the potential to be involved in any such interactions.

To determine whether the major HPD-induced proteins exist in oligomeric forms or as disulfide bridge-linked subunits of larger protein complexes, the solubilization conditions were modified by varying the temperature of incubation in the presence or absence of 2-mercaptoethanol. Varying the solubilization conditions had little effect on the four major *S. epidermidis* wall proteins, although the 54-kDa protein appeared to be absent from samples incubated at 37°C and so may possibly be complexed noncovalently with other cell wall components. In addition, the 54-kDa protein observed on SDS-PAGE gels appeared to be two comigrating proteins, an *S. epidermidis* cell wall protein and the human IgG heavy chain. Since *S. epidermidis* does not produce protein A (20), this binding of IgG is likely to be a specific immune reaction to cell surface antigens. These are probably common to both nutrient broth- and HPD-grown bacteria, since we have previously shown (34) that IgG can be detected bound to *S. epidermidis* grown in nutrient broth after incubation at 4°C in HPD.

In gram-negative bacteria, such as *Escherichia coli*, the apparent molecular masses of the major outer membrane proteins are influenced by the temperature of solubilization (15). OmpA, which plays a role in maintaining cell envelope integrity, for example, has apparent molecular masses of 35 kDa when solubilized at 100°C and 28 kDa when solubilized at 37°C (15). Such unusual electrophoretic behavior was not shown by any of the major HPD-induced *S. epidermidis* cell wall proteins; this may merely reflect the fundamental differences between the architecture of gram-positive cell walls and gram-negative outer membranes and the functions of their constituent proteins.

Growth of *S. epidermidis* in HPD also resulted in expression of two immunodominant cytoplasmic membrane proteins of 32 and 36 kDa, respectively. These proteins were poorly expressed in broth-grown cells and were repressed following addition of excess iron to HPD. They can, however, be induced in nutrient broth after chelation of the available iron by addition of compounds such as EDDA (unpublished data). They therefore appear to be regulated by the medium iron content. HPD contains not only a very low level of iron (<0.5 μM Fe^{3+}; 29, 34) but also the serum iron-binding glycoprotein transferrin (34). Thus, the availability of iron as a staphylococcal nutrient is severely limited. In response to iron deprivation, many gram-negative bacteria are known to derepress high-affinity iron-sequestering systems on the basis of either (i) low-molecular-mass iron chelators (siderophores) and their corresponding outer membrane receptors (9) or (ii) direct interaction between a bacterial cell surface receptor and the host iron-binding glycoprotein (19). *S. epidermidis* does not appear to bind the transferrin present in HPD (34). The 32- and 36-kDa proteins may conceivably function as siderophore receptors, since the relatively open structure of the gram-positive cell wall

would not present a barrier to the passage of low-molecular-mass siderophores (33). Recently, the isolation and characterization of a staphylococcal siderophore of the carboxylate type, called staphyloferrin A, has been described by Meiwes et al. (18). In addition, we have previously reported that the *S. epidermidis* strains examined in this study synthesize siderophores (7), although they have not been chemically characterized. Further work is under way to determine whether the 32- and 36-kDa proteins function as siderophore receptors and whether expression of siderophores and the 32- and 36-kDa proteins is coordinately controlled. In addition, the possible role of the 130-kDa iron-repressible *S. epidermidis* wall protein in iron metabolism is also under investigation. Furthermore, previous reports have indicated that similar high-molecular-mass surface proteins in *S. aureus* appear to be involved in adherence (3, 4, 14). The *S. epidermidis* 130-kDa surface protein may similarly act as an adhesin, and its role as such is under investigation.

The molecular masses and antigenic homology of the iron-repressible cytoplasmic membrane proteins appeared to be highly conserved in all of the *S. epidermidis* strains examined. A protein cross-reactive with the 32-kDa protein was also present in two *S. aureus* strains. Interestingly, the siderophore staphyloferrin A is produced by both *S. aureus* and many coagulase-negative species, including *S. epidermidis* (18), pointing to the existence of a common receptor among the staphylococci. Further evidence that the 32- and 36-kDa *S. epidermidis* antigens are expressed during growth in vivo was obtained from immunoblotting experiments in which antibodies to these proteins were found in both normal human serum and serum and HPD fluid from CAPD patients. Antibodies against the iron-repressible outer membrane proteins of gram-negative bacteria, such as *E. coli* (10) and *Pseudomonas aeruginosa* (30), have been found in both normal human sera and convalescent sera. However, their role in protection, perhaps by blocking siderophore-mediated iron transport and therefore multiplication in iron-restricted environments, has yet to be established.

Antibodies present in pooled HPD and human serum reacted with the 54- and 48-kDa but not the 42-kDa *S. epidermidis* cell wall proteins in only 3 of 12 strains examined. This suggests that there is antigenic variation among these staphylococcal wall proteins. HPD, but not normal human serum, also contained antibodies to several high-molecular-weight antigens which were not visible in Coomassie blue-stained gels. Serum from uninfected CAPD patients reacted with fewer cell wall and cell membrane proteins than HPD or normal serum. This may reflect the lowered immunological status reported previously in CAPD patients (28), which may contribute to the development of peritonitis in these patients. Thus, the variation in the expression of cell envelope proteins observed in *S. epidermidis* cultured in vitro in HPD is reflected in the antibody responses of patients undergoing treatment by CAPD. The role of these antibodies, if any, in protecting patients from staphylococcal peritonitis, as well as the role of surface proteins in the virulence of coagulase-negative staphylococci, remains to be evaluated.

ACKNOWLEDGMENTS

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