JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1990, p. 195–200
0095-1137/90/020195-06$02.00/0

Vol. 28, No. 2

Serological Response in *Enterococcus faecalis* Endocarditis Determined by Enzyme-Linked Immunosorbent Assay

PATRICIA J. SHORROCK,[1] PETER A. LAMBERT,[1]* EILEEN J. AITCHISON,[1] E. GRACE SMITH,[2] IAN D. FARRELL,[2] AND ERNO GUTSCHIK[3]

Microbiology Research Group, Pharmaceutical Sciences Institute, Aston University, Birmingham B4 7ET,[1] and Public Health Laboratory, East Birmingham Hospital, Birmingham B9 5ST,[2] United Kingdom, and Department of Clinical Microbiology, Bispebjerg Hospital, 2400 Copenhagen, Denmark[3]

Received 26 July 1989/Accepted 16 October 1989

***Enterococcus* (*Streptococcus*) *faecalis* expresses three species-specific surface protein antigens of molecular weights 73,000, 40,000, and 37,000. On Western blotting (immunoblotting), they were detected strongly by immunoglobulin G (IgG) in sera from patients with *E. faecalis* endocarditis, but not in sera from patients with other *E. faecalis* infections or with endocarditis due to other streptococci. We developed an enzyme-linked immunosorbent assay system to measure IgG, IgM, and IgA levels to these antigens and evaluated its potential as a serodiagnostic test for *E. faecalis* endocarditis. The test correctly diagnosed *E. faecalis* endocarditis in 15 of 16 cases. Of 10 cases of endocarditis due to other streptococci and 10 *E. faecalis* infections other than endocarditis, 9 and 8, respectively, gave negative results. The test should prove particularly useful in culture-negative cases, for which choice of appropriate antibiotic therapy for *E. faecalis* endocarditis is vital.**

Infective endocarditis is a difficult condition to diagnose and treat, carrying a mortality of 30% (17). Oral streptococci are the commonest cause (40% of cases), while fecal streptococci (enterococci) are cultured from 10 to 15% of cases (17). *Enterococcus* (*Streptococcus*) *faecalis* is particularly difficult to treat, requiring a combination of penicillins, aminoglycosides, and/or glycopeptide antibiotics (e.g., vancomycin) for several weeks, with attendant side effects (18). The emergence of resistance to these agents is an increasing threat to successful therapy (10, 15). Techniques which might reliably confirm or exclude *E. faecalis* in culture-negative cases and which might be useful in assessing therapeutic response would be of great clinical value. We have shown previously that three surface proteins of molecular weights 73,000, 40,000, and 37,000 are prominant antigens of *E. faecalis*, which are expressed strongly following growth in serum (1, 9). They appear to be specific to *E. faecalis*. Antibodies towards them are found in patients with *E. faecalis* endocarditis, but not in those with endocarditis due to other streptococci or with other *E. faecalis* infections (1). The Western blotting (immunoblotting) methods we used to establish the specificity of the antibodies towards these antigens are not convenient for application as a routine serodiagnostic test for *E. faecalis* endocarditis. We have therefore developed an enzyme-linked immunosorbent assay (ELISA) based on the extracted, partially purified antigens and evaluated its performance in a blind trial. The test can be used to measure levels of specific immunoglobulin G (IgG), IgM, or IgA in serial serum samples and may be useful in monitoring patient response to therapy.

MATERIALS AND METHODS

Organism. The strain used was *E. faecalis* EBH1, an isolate from the blood of a patient with endocarditis at East Birmingham Hospital, Birmingham, United Kingdom (1). Cultures were grown in shake flasks at 37°C for 18 h in a chemically defined medium (CDM; 12), heat-inactivated horse serum (GIBCO Europe, Paisley, United Kingdom), heat-inactivated human serum pooled from >100 healthy volunteers, or CDM supplemented with 1% horse serum. Cells were washed twice in saline before suspension in water to an A_{470} of 5.0. Cells grown in either horse or human serum formed large clumps which were dispersed on washing, but reaggregated on suspension in water. No nutrient supplements were added to the sera and no pH control was used. No antibody to *E. faecalis* was detectable by Western blotting in either the horse or the human sera used for growth of the organism.

Sera. Sera from 36 patients were investigated: 16 with *E. faecalis* endocarditis (confirmed by positive blood cultures); 10 with endocarditis caused by a range of gram-positive organisms, i.e., *Streptococcus faecium* (n = 1), *Streptococcus bovis* (n = 1), nutritionally variant streptococcus (NVS; n = 1), *Streptococcus sanguis* (n = 2), *Streptococcus mitis* (n = 1), *Streptococcus mitior* (n = 1), beta-hemolytic streptococcus (n = 1), *Staphylococcus aureus* (n = 1), and *Staphylococcus epidermidis* (n = 1); and 10 with *E. faecalis* infections other than endocarditis, i.e., drainage site (n = 1), wound swab (n = 1), bronchial washings (n = 1), nephrostomy fluid (n = 1), peritoneal dialysis fluid (n = 2), blood culture (n = 1), urinary tract infection (n = 2), and osteomyelitis (n = 1). Pooled sera from 12 healthy volunteers were used as a control.

Antigen extraction. Cells from a 1-liter culture in horse serum were washed twice in 10 mM Tris hydrochloride buffer, pH 7.4, and suspended in 5 ml of 1% (wt/vol) sodium lauroyl sarcosinate (sarcosyl; Sigma Chemical Co., St. Louis, Mo.) in 10 mM Tris hydrochloride containing 1 mM disodium EDTA. The suspension was incubated at room temperature for 20 min. Whole-cell counts and absorbance measurements showed that no cell lysis occurred during this treatment. The whole cells were removed by centrifugation (10,000 × g, 10 min), and the supernatant was retained as the sarcosyl extract. Precipitated fractions were prepared by sequential addition of solid streptomycin sulfate to 2% (wt/vol) to remove any nucleic acid material and then solid ammonium sulfate to final concentrations of 30, 60, and 90% saturation. After each addition, the solutions were stirred for

* Corresponding author.

30 min at room temperature and the precipitates were deposited by centrifugation (5,000 × *g*, 10 min). The ammonium sulfate-precipitated pellets were suspended in water and dialyzed to remove ammonium sulfate. The supernatant from the final 90% precipitation was also dialyzed. All dialysates were freeze-dried and suspended in water (200 μl).

SDS-PAGE and Western blotting. The gel and blotting systems used were as described previously (1). Two methods of sample preparation were used. Whole-cell suspensions were boiled in sample denaturing buffer for 10 min and then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 12% gels and Western blotting. This procedure released some proteins but did not lyse the cells (cell debris remained in the sample wells at the top of the stacking gel). Alternatively, cell suspensions were first treated with a muramidase, mutanolysin, to lyse the cells (11) and then boiled in denaturing buffer and subjected to SDS-PAGE. This method enabled all cellular proteins to be released and separated. For mutanolysin digestion, 5 μl of *Streptomyces globisporus* enzyme (1,000 U/ml in 0.1 M HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid] buffer, pH 7.2; Sigma) was added to 100 μl of cell suspension in water (absorbance, 5.0). The protease inhibitor phenylmethylsulfonyl fluoride (1 mM) and the preservative sodium azide (0.02%, wt/vol) were added, and the suspension was incubated at 37°C for 18 h. Total counts and absorbance measurements showed that >90% of the cells were lysed. The suspensions were boiled in sample denaturing buffer and subjected to SDS-PAGE and Western blotting.

ELISA. Wells of polystyrene microdilution plates (Immulon; rigid flat bottom, unirradiated; Dynatech Laboratories, Inc., Alexandria, Va.) were coated with the specific antigens present in the 90% ammonium sulfate precipitate of a sarcosyl extract of cells grown in horse serum. A 100-μl portion of the suspended dialyzed precipitate was diluted with 80 ml of sodium carbonate buffer (0.05 M; pH 9.6). A 100-μl amount of this diluted antigen solution was added to each well of the microdilution plate and left overnight at 4°C. The wells were washed twice and blocked for 1 h with phosphate-buffered saline containing 0.05% (vol/vol) Tween 20 (PBST). After two further washes in PBST, doubling dilutions of antisera in PBST (100 μl) were added to the wells and the plates were incubated at 37°C for 2 h. The wells were washed twice in PBST, and 100 μl of antibody detection conjugate was added to each well. For IgG detection in the trial of sera from 36 patients, protein A-peroxidase (Sigma) was used at 1.25 μg/ml in PBST. In the longitudinal study of different antibody levels of four patients, goat anti-human IgG-, IgM- or IgA-peroxidase (Sigma), diluted 1:1,000 in PBST, was used. In all cases, the plates were incubated for 2 h at 37°C. After two washes in PBST, 100 μl of chromogenic substrate solution was added to each well. The solution was prepared by adding 10 mg of 3,3',5,5'-tetramethylbenzidine (dissolved in 1 ml of dimethyl sulfoxide) to 100 ml of 0.1 M sodium acetate–citrate buffer, pH 6, followed by 8 μl of hydrogen peroxide (30%, vol/vol). The color reaction was stopped by addition of 35 μl of 2 M sulfuric acid to each well, and the A_{450} was measured. Titers were taken as the reciprocals of the highest dilutions of patient sera giving clear positive values compared with controls for which no primary antibody was used. In the case of the trial measuring IgG levels with protein A-peroxidase conjugate, positive values were recorded for an absorbance of 0.1 or above. For the longitudinal trial measuring IgG, IgM, and IgA levels, positive values were recorded for absorbance values of 0.2 or above. The slightly different endpoints were used because the goat anti-human immunoglobulin-peroxidase conjugates gave higher background readings than the protein A-peroxidase conjugate.

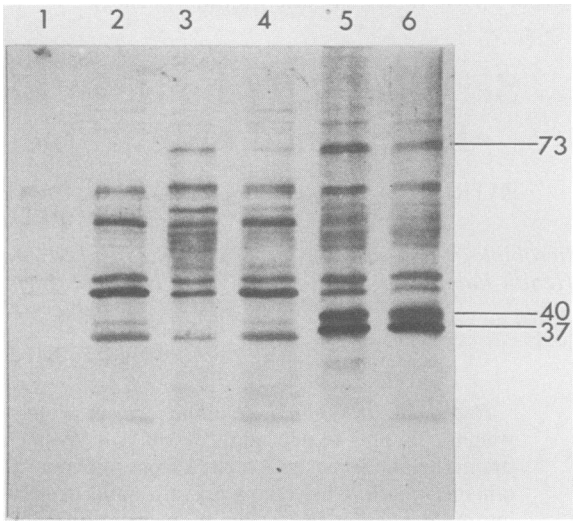

FIG. 1. Western blot of *E. faecalis* whole-cell preparations and mutanolysin digests probed with sera from a high-titer *E. faecalis* endocarditis patient (patient 1 in Table 1) and protein A-peroxidase. Cells were grown in CDM (lanes 1 and 2), CDM plus 1% horse serum (lanes 3 and 4), or horse serum (lanes 5 and 6). Lanes 1, 3, and 5 are whole-cell suspensions boiled in sample denaturing buffer for 10 min; lanes 2, 4, and 6 are mutanolysin digests boiled in sample denaturing buffer for 10 min.

RESULTS

Figure 1 shows the effect of the growth medium on antigen expression by *E. faecalis* EBH1. The 40- and 37-kilodalton (kDa) antigens were strongly expressed by cells grown in horse serum but were barely detectable in cells grown in CDM or CDM plus 1% horse serum. They were present in both the mutanolysin digests and the whole-cell preparations of horse serum-grown cells, indicating effective release from the cells on boiling with denaturing buffer (containing 2% SDS and 5% β-mercaptoethanol). By contrast, CDM-grown cells released hardly any detectable antigen on boiling with denaturing buffer (lane 1), although mutanolysin treatment showed a range of antigens to be present. Addition of 1% horse serum to CDM did not markedly alter the profiles, but the antigens were effectively released on boiling in denaturing buffer. Although cells grown in CDM without and CDM with 1% horse serum produced barely detectable levels of the 37- and 40-kDa antigens, the 73-kDa antigen was expressed at about the same level as in horse serum-grown cells.

Figure 2 shows the antigen profiles of whole cells grown in horse or human serum. Apart from differences in the amount of the antigen band at approximately 65 kDa, the profiles were essentially the same, with the 40- and 37-kDa antigens strongly expressed in both sera. On the basis of this result, we chose horse serum as the more convenient and readily available medium for bulk growth of cells and antigen extraction.

Treatment of horse serum-grown cells with 1% sarcosyl–1 mM EDTA effectively released the major antigens (Fig. 3,

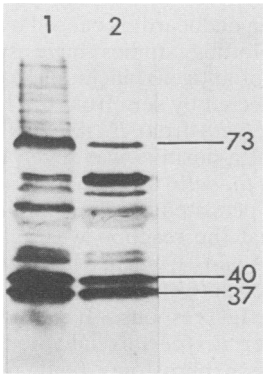

FIG. 2. Western blot of *E. faecalis* whole-cell preparations grown in horse serum (lane 1) and pooled normal human serum (lane 2). Blots were developed as given in the legend to Fig. 1.

lane 1). The 73-, 40-, and 37-kDa antigens were precipitated from the extract by addition of ammonium sulfate to 90% saturation (Fig. 3, lane 4). These partially purified antigens were coated to micro-ELISA plate wells and used to measure IgG levels in patient sera. The results are shown as a scatter plot in Fig. 4. The titers are the reciprocals of the serum dilutions which reduced the color developed to 0.1. This value was chosen because the background color developed for controls with no primary antibody was consistently <0.01. Color developed by a 1:100 dilution of normal human serum pooled from 12 healthy volunteers was 0.01. Of 16 serum samples from *E. faecalis* endocarditis patients, 15 gave positive results, with titers ranging from 1:100 to 1:10,000. Of 10 patients with *E. faecalis* infections other than endocarditis, 2 gave false-positives: one had an osteomyelitis, and the other had a chronic urinary tract infection. Of 10 patients with endocarditis due to organisms other than *E. faecalis*, 1 gave a false-positive; the infecting organism in this case was an NVS. The titers of IgG, IgM, and IgA in a longitudinal study of four *E. faecalis* endocarditis patients are shown in Table 1. For this study, the cutoff point used to

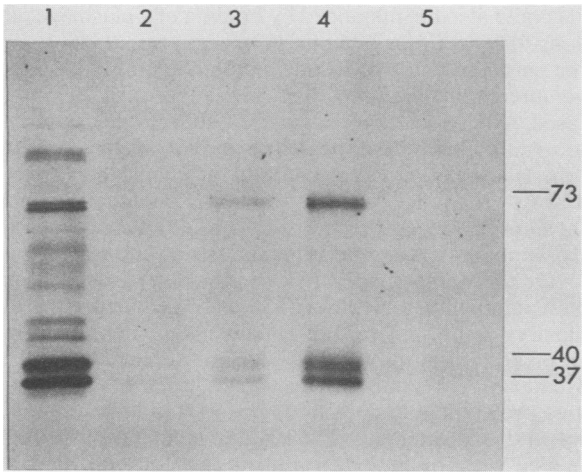

FIG. 3. Western blot of *E. faecalis* antigen preparations revealed as in the legend to Fig. 1. Lanes: 1, sarcosyl extract from whole cells; 2 to 4, ammonium sulfate precipitates from the sarcosyl extract at 30, 60, and 90% saturation, respectively; 5, supernatant remaining after 90% ammonium sulfate precipitation.

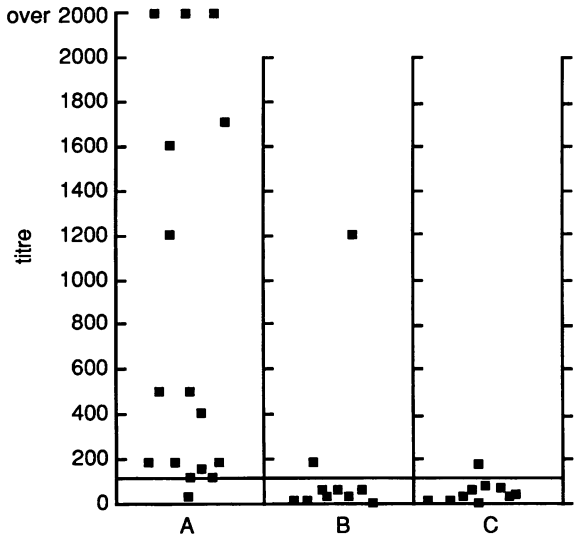

FIG. 4. Levels of IgG to *E. faecalis* specific antigens in sera from patients with: A, *E. faecalis* endocarditis; B, *E. faecalis* infections other than endocarditis; and C, endocarditis caused by organisms other than *E. faecalis*.

determine the titer was an absorbance of 0.2. The goat anti-human conjugates gave background absorbances of around 0.02 with no primary antibody.

DISCUSSION

Expression of the 73-, 40-, and 37-kDa antigens was strongly influenced by the growth medium. Cells grown in CDM, investigated for possible large-scale culture, gave barely detectable levels of the 40- and 37-kDa antigens. They were also very resistant to boiling in SDS; virtually no antigen was released unless the cells were digested with muramidase first. Addition of 1% horse serum to the CDM rendered the cells susceptible to release of antigen with SDS, indicating a change in the physical properties of the cell wall. The presence of 1% horse serum in CDM slightly increased expression of the 73-kDa antigen and produced other minor changes in the antigenic profile, but did not induce expression of the 40- and 37-kDa antigens. Because our previous Western blotting studies had identified the 40- and 37-kDa antigens as the most prominent in *E. faecalis* endocarditis patients (1), we required a medium which induced their expression. Strong expression was achieved by growth in either horse or human serum. We used horse serum for convenience. It is readily available in bulk from commercial sources, and we have found negligible variation in the growth of *E. faecalis* and antigen expression with different batches. The antigens were easily released from serum-grown cells by boiling with SDS, the profiles being identical with those from muramidase-digested cells.

We found that efficient antigen extraction could be achieved by treatment of serum-grown cells with 1% sarcosyl–1 mM EDTA at room temperature, a method used by Jenkinson for release of proteins from cell walls of *Streptococcus sanguis* (8). The 73-, 40-, and 37-kDa proteins were then precipitated by addition of ammonium sulfate and coated to the wells of polystyrene microdilution plates for measuring antibody levels in patient sera by ELISA. In establishing the optimum conditions for the assay, we chose an absorbance of 0.1 as the cutoff point for the lowest

TABLE 1. IgG, IgM, and IgA levels in sera from *E. faecalis* endocarditis patients at intervals after diagnosis of infection[a]

Patient	Mo/day	ELISA titer[b]		
		IgG	IgM	IgA
1	1/6	6,000	400	125
	1/7	6,000	250	60
	7/3	6,500	360	85
	7/6	5,500	300	150
	7/11	6,000	300	150
	7/15	7,500	200	100
	7/22	6,000	200	100
	7/24	6,500	200	110
	9/12	2,000	30	75
	25/8	1,600	30	150
2	1/11	700	120	20
	2/3	750	90	25
	3/26	800	75	75
	8/14	10,000	500	420
	9/3	8,000	500	500
	9/7	9,000	350	350
	10/3	7,000	200	220
3	1/17	3,500	650	900
	2/7	4,000	3,200	1,200
	5/16	1,200	800	600
	11/5	800	550	800
4	1/30	3,000	85	0
	6/26	1,200	65	0
	10/30	3,000	85	0
	19/31	1,500	25	0

[a] All patients were treated with gentamicin and ampicillin. Serum samples were taken after administration of the antibiotics on the dates shown to monitor gentamicin levels. The timing of the serum samples does not reflect the complete antibiotic course given to each patient.

[b] Titers are the reciprocals of the dilutions of sera required to reduce the color to an A_{450} of 0.2.

positive result when protein A-peroxidase was used to measure IgG levels in patient sera. This value was increased to 0.2 when goat anti-human immunoglobulin-peroxidase conjugates were used for IgG, IgM, and IgA levels. These values were chosen as 10-fold higher than the absorbance produced by controls with no primary antibody. The sensitivity of the assay compares favorably with that of other ELISA systems described for detection of antibodies to gram-positive bacteria. Van de Rijn et al. (16) used a cutoff value of 0.1 in their ELISA for detection of antibodies to NVS in patients with endocarditis, and Jacob et al. (7) used a value of 0.2 in an ELISA for antibodies to *Staphylococcus aureus* in experimental osteomyelitis.

Results of a blind trial, using the ELISA system, confirm the serodiagnostic potential of the antigens in *E. faecalis* endocarditis, with a 94% success rate (15 of 16) for positives and a 91% success rate (10 of 11) for negatives (endocarditis due to other organisms). The *E. faecalis* endocarditis patient who failed to give a positive result had transitional cell carcinoma of the bladder. This malignancy or therapy with adriamycin or both could have contributed to the poor immune response to infection. Of the three false-positives (15% of negative cases), the highest titer (1:1,200) was found in serum from a patient with *E. faecalis* osteomyelitis. This site of infection and its protracted nature are analogous to the conditions of infective endocarditis, in which prolonged release of antigen from the infection site could be responsible for the high IgG response of the patient. The second false-positive was an endocarditis caused by an NVS (titer of 1:180). Western blotting studies have shown that NVS produce a number of antigens, including bands of 73 and 40 kDa, which are detected by sera from the NVS endocarditis patient, but not by sera from *E. faecalis* endocarditis patients. The third false-positive was given by serum from a patient with an *E. faecalis* urinary tract infection (titer of 1:180) which had persisted for several months. Western blotting showed that the reaction was against the 73- and 37-kDa antigens. Again, it seems likely that in this case prolonged release of antigen from the site of infection had elicited a detectable IgG response. It should be noted that no sera from the other *E. faecalis* infections (drainage site, bronchial washings, nephrostomy fluid, peritoneal dialysis fluid, urinary tract, wound swab, and blood culture) gave positive titers of 1:100 or above. The blood culture was obtained from a patient with multiple stones in the common bile duct who had undergone a recent colocystectomy. Either these infections were of too short a duration to elicit an IgG response, or the antigens are produced particularly strongly by organisms when they infect specific sites. No detectable response was obtained with control sera from healthy individuals. We used pooled sera from 12 individuals in this study, which gave an absorbance of 0.01 at a dilution of 1:100. Western blotting studies on separate sera from over 100 healthy individuals have confirmed that no detectable antibody to the *E. faecalis* antigens is present.

All strains we have examined to date, from whatever source, are capable of expressing the antigens provided they are grown in horse serum. We have found no major differences in Western blot antigen profiles between *E. faecalis* strains from endocarditis and those from other infections. Guzman et al. (5) have recently reported significant differences in adhesive properties between endocarditis strains of *E. faecalis* and those from urinary tract infections. The former adhered better to Girardi heart cells than did strains from urinary tract infections, but associated less efficiently with human neutrophils. Guzman et al. speculate that different adhesins are involved and report that growth in serum makes all strains express galactose-containing adhesins which might be involved in adherence to cardiac cells (5). A functional role of the 40- and 37-kDa antigens in endocarditis remains to be established. Adhesion of *E. faecalis* to heart tissue could also be influenced by binding of a plasma protein such as fibronectin or albumin (13).

The serial titers of different immunoglobulin types show some interesting features. The long exposure to antigen released from a cardiac vegetation during the course of endocarditis would be expected to induce an IgG response and the levels of IgG were generally far higher than those of IgM or IgA. Samples taken earlier in the course of infection might show higher IgM responses. The serum samples in this study were taken after clinical diagnosis of endocarditis and the start of antibiotic therapy. In the four cases shown in Table 1, ampicillin and gentamicin were used from the date of the first serum sample. For patient 1, IgG levels remained high for several months, with low, but detectable levels of IgM and IgA. This patient had had *E. faecalis* endocarditis on two previous occasions and appeared to have retained a high IgG titer throughout. The reductions in months 9 and 25 coincided with the patient's clinical deterioration. Patient 2 showed a dramatic peak in IgG with corresponding rises in IgM and IgA around month 8 after diagnosis of *E. faecalis* endocarditis. This might be due to a major release of antigen from the site of infection, with subsequent immune response. Patient 3 produced a significant IgM response in

month 2 and a slightly lower IgA response without a correspondingly large increase in IgG. This contrasts with patient 4, who had very low IgM and no detectable IgA. There are no obvious explanations for the different patterns of antibody response in the four patients studied. Presumably the duration and extent of the infections and the outcome of antibiotic therapies have a major influence on the amount of antigens released into the circulation from the site of infection. The strong IgA response in patient 3 is notable. We are not aware of previous reports of IgA responses to infecting organisms in endocarditis. The widely varying levels of IgA found in the four patients might reflect different patterns of tissue damage at the site of infection. A local IgA response on the tissue surface around the site of infection followed by release of the antibody during therapy could be responsible for the high levels of IgA in the serum of patient 3. There was no clear-cut relationship between the duration of illness and the antibody titers in the patients we studied. Further longitudinal studies are needed to correlate antibody levels with patient response to therapy. It would also be useful to measure antigen levels or immune complexes in the sera. Espersen et al. (4) have shown that detection of staphylococcal antigen in urine offers a rapid method for diagnosis of *Staphylococcus aureus* endocarditis.

The 73-, 40-, and 37-kDa antigens are clearly prominant in *E. faecalis* endocarditis, but their identity and function are unknown at present. Using monospecific antisera to the 40- and 37-kDa antigens and indirect immunofluorescence, we have shown that they are exposed on the cell surface. Handley and Jacob (6) have reported the presence of fimbrial structures on the surface of *E. faecalis*, but electron microscopy with immunogold detection shows that the fimbrial structures are not labeled with the monospecific antiserum (P. J. Shorrock, P. A. Lambert, and P. S. Handley, unpublished results). Possibly the antigens are anchored in the cytoplasmic membrane and protrude through the wall to the cell surface. A membrane location would explain their extraction from whole cells with sarcosyl. Further studies are in hand to determine their location in the cell wall or membrane or both.

A pheromone-mediated mechanism of plasmid transfer operates in *E. faecalis* (3). The conjugal transfer of DNA occurs when donor and recipient cells aggregate. The recipient cells produce a low-molecular-weight peptide pheromone (clumping inducing agent) which induces adhesive protein antigens of 130 and 73 kDa on the surface of the donor cells, resulting in aggregation (14). It is possible that the 73-kDa antigen we have described is the same as the 73-kDa protein which is involved in clumping. In this respect, it is interesting to note that serum-grown cells, which express the specific antigens most strongly, autoagglutinate.

Independent studies on enterococcal endocarditis by Burnie et al. have identified a number of other *E. faecalis* protein antigens which show promise for exploitation in serodiagnosis (2). They found strong IgM responses in endocarditis patients to bands of 112, 88 to 90, and 45 to 47 kDa and strong IgG responses to the 88- to 90-kDa and 45- to 47-kDa antigens. The relationship of these antigens to our 73-, 40-, and 37-kDa antigens remains to be established. Allowing for different estimates of molecular sizes, the 45- to 47-kDa bands might be equivalent to our 40- and 37-kDa bands. The 112- and 88- to 90-kDa bands found by Burnie et al. are not detected in our system, possibly reflecting the different growth conditions used.

In summary, our results clearly show that an ELISA based on the 40- and 37-kDa *E. faecalis*-specific antigens has a positive predictive value in *E. faecalis* endocarditis. By using protein A-peroxidase conjugate with tetramethylbenzidine as chromogenic substrate to detect IgG in patient serum, a cutoff absorbance value of 0.1 indicates a positive reaction. This value is 10 times higher than the background color obtained with no primary antibody. With serum dilutions of 1:100, the test gave a positive result in 94% of patients with *E. faecalis* endocarditis and a negative result in 91% of cases with endocarditis due to other organisms. The test therefore shows considerable promise for the accurate positive diagnosis or exclusion of *E. faecalis* infection in endocarditis. The preliminary longitudinal studies on four patients also suggest an application as a guide to response to therapy.

ACKNOWLEDGMENT

This work was supported by a research studentship to P.J.S. from the Science and Engineering Research Council, United Kingdom, which is gratefully acknowledged.

LITERATURE CITED

1. **Aitchison, E. J., P. A. Lambert, E. G. Smith, and I. D. Farrell.** 1987. Serodiagnosis of *Streptococcus faecalis* endocarditis by immunoblotting of surface protein antigens. J. Clin. Microbiol. **25**:211–215.
2. **Burnie, J. P., M. Holland, R. C. Matthews, and W. Lees.** 1987. Role of immunoblotting in the diagnosis of culture negative and enterococcal endocarditis. J. Clin. Pathol. **40**:1149–1158.
3. **Dunny, G. M., C. Funk, and J. Adsit.** 1981. Direct stimulation of the transfer of antibiotic resistance by sex pheromones in *Streptococcus faecalis*. Plasmid **6**:270–278.
4. **Espersen, F., J. Wheat, R. B. Kohler, and J. White.** 1988. Detection of staphylococcal antigen in urine from patients with severe *Staphylococcus aureus* infections. Serodiagn. Immunother. Infect. Dis. **2**:357–363.
5. **Guzman, C. A., C. Pruzzo, G. LiPira, and L. Calegari.** 1989. Role of adherence in pathogenesis of *Enterococcus faecalis* urinary tract infection and endocarditis. Infect. Immun. **57**: 1834–1838.
6. **Handley, P. S., and A. E. Jacob.** 1981. Some structural and physiological properties of fimbriae of *Streptococcus faecalis*. J. Gen. Microbiol. **127**:289–293.
7. **Jacob, E., D. M. Arendt, I. Brooke, L. C. Durham, M. C. Falk, and J. J. Schaberg.** 1985. Enzyme-linked immunosorbent assay for the detection of antibodies to *Staphylococcus aureus* cell walls in experimental osteomyelitis. J. Clin. Microbiol. **22**: 547–552.
8. **Jenkinson, H. F.** 1986. Cell-surface proteins of *Streptococcus sanguis* associated with cell hydrophobicity and coaggregation properties. J. Gen. Microbiol. **132**:1575–1589.
9. **Lambert, P. A., E. J. Aitchison, E. G. Smith, and I. D. Farrell.** 1986. Serodiagnosis of *Streptococcus faecalis* endocarditis. J. Infect. **13**:309–311.
10. **Mederski-Samoraj, B. D., and B. E. Murray.** 1983. High-level resistance to gentamicin in clinical isolates of enterococci. J. Infect. Dis. **147**:751–757.
11. **Morris, E. J., N. Ganeshkumar, and B. C. McBride.** 1985. Cell surface components of *Streptococcus sanguis*: relationship to aggregation, adherence, and hydrophobicity. J. Bacteriol. **164**: 255–262.
12. **Shockman, G. D., M. J. Conover, J. J. Kolb, L. S. Riley, and G. Tonnies.** 1961. Nutritional requirements for bacterial cell wall synthesis. J. Bacteriol. **81**:44–50.
13. **Shorrock, P. J., and P. A. Lambert.** 1989. Binding of fibronectin and albumin to *Enterococcus (Streptococcus) faecalis*. Microb. Pathog. **6**:61–67.
14. **Tortorello, M. L., and G. M. Dunny.** 1985. Identification of multiple cell surface antigens associated with the sex phero-

mone response of *Streptococcus faecalis*. J. Bacteriol. **162:** 131–137.
15. **Uttley, A. H., C. H. Collins, J. Naidoo, and R. C. George.** 1988. Vancomycin-resistant enterococci. Lancet **i:**57–58.
16. **Van de Rijn, I., M. George, A. Bouvet, and R. B. Robert.** 1986. ELISA for the detection of antibodies to nutritionally variant streptococci in patients with endocarditis. J. Infect. Dis. **153:** 116–121.
17. **Wilson, W. R., and J. E. Geraci.** 1983. Antibiotic treatment of infective endocarditis. Annu. Rev. Med. **34:**413–427.
18. **Wilson, W. R., and J. E. Geraci.** 1985. Treatment of streptococcal infective endocarditis. Am. J. Med. **78**(Suppl. 6B):128–137.