PROTEIN-MEDIATED ADHESION OF *STAPHYLOCOCCUS AUREUS* TO SILICONE IMPLANT POLYMER

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SUMMARY. Investigation was made of the role of protein A and clumping factor in the adhesion of *Staphylococcus aureus* to the silicone polymer used for manufacture of cerebrospinal fluid shunting systems. The two proteins were judged to contribute non-specifically to adhesion. *S. aureus* was also shown to be capable of hydrophobic binding, but this was found to be distinct from the demonstrated protein-mediated adhesion.

INTRODUCTION

As is the case with other surgically implanted materials, the useful life of cerebrospinal fluid (CSF) shunting systems is often limited by infection of the device. Staphylococci are the bacteria most commonly responsible for these infections. *Staphylococcus epidermidis* has been responsible for > 50% of reported CSF shunt infections. Several workers have studied the mechanisms whereby this otherwise rarely pathogenic micro-organism is able to colonise synthetic materials; carbohydrate-based and protease-sensitive mechanisms of adhesion have been noted, and the role of hydrophobic binding has also been investigated (Christensen *et al.*, 1982; Høgt *et al.*, 1983). *S. aureus* has been reported from a smaller number of CSF shunt infections (c. 20%) and, perhaps in consequence, its adhesive mechanisms have received less attention. The observation of a trypsin-sensitive mode of attachment to CSF shunt polymer (Barrett, 1983) prompted the present investigation of the adhesive role of two specific *S. aureus* surface proteins (protein A and clumping factor). The ability of *S. aureus* to undergo hydrophobic attachment was also studied.

MATERIALS AND METHODS

Substrates. The substrate for adhesion was polydimethyl and methylvinyl-siloxane (Clean-Grade Elastomer MDX4-4515, Dow Corning, Barry, South Glamorgan). To provide a sufficient surface area for adhesion measurement, this was reduced from the tubular form used for CSF shunts by crushing in liquid nitrogen.

The ability to undergo hydrophobic interactions was assessed with Agarose beads either in the native state, or bearing octyl, decyl, or phenyl hydrocarbon groups (Sepharose, Pharmacia Limited, Milton Keynes, Northants).

Adhesion measurement. Bacteria used in these experiments were grown on nutrient agar

containing D-[1-³H] glucose (Radiochemical Centre, Amersham) at a concentration of 0.025 mCi/ml. After incubation with the substrate, adhesion was assessed by measurement of β -emissions in a scintillation counter, and the degree of adhesion was calculated as the percentage of β -counts in the inoculum which was subsequently found adherent to the substrate after washing.

Selection of bacteria. Strains of *S. aureus* collected from CSF shunt infections were used for studies of hydrophobic attachment. For assessment of the role of clumping factor and protein A, mutant strains lacking these proteins were identified. In the case of clumping factor, such strains were obtained by screening tube-coagulase positive isolates in tests with pooled human plasma. Protein A-deficient strains were identified by a modification of the method described by Winblad and Ericson (1973): strains of *S. aureus* were washed three times in phosphate-buffered saline (PBS, Oxoid BR14a) and finally brought to a just visibly turbid suspension; 50 μ l of each suspension was placed in each of two wells of a microtitration plate. To one well of each pair was added 50 μ l of a 3% suspension of washed sheep red cells in veronal buffer (Oxoid BR16). The other received 50 μ l of identical red cells sensitised with rabbit anti-sheep erythrocyte antiserum (Wellcome Reagents) at a concentration of 2.5 minimum haemagglutinating doses per ml. After mixing, the trays were left at 4°C overnight. Protein A deficient strains were detected by absence of haemagglutination.

Capsule studies. The degree of capsulation was assessed microscopically by Fleming's nigrosin method.

Adhesion experiments. Staphylococci grown overnight on agar containing D-[1-³H] glucose as described above were suspended in PBS and washed twice by centrifugation. The concentration of a visibly turbid suspension of the bacteria was adjusted to give a final E560 value of 0.1, by the addition of more PBS. Each suspension (1 ml) was then incubated with 500 mg of crushed shunt polymer for 2 h at room temperature. The crushed polymer was then washed twice in PBS and placed in a scintillation fluid containing 4 g BBOT (2,5-di[5'-tert-butyl-benzoxazolyl-(2')]thiophen) in 1 L of toluene. A 50- μ l portion of the original bacterial suspension, and 50 μ l of the PBS used to wash the polymer at the end of the experiment were also placed in a scintillant comprising BBOT 4 g, naphthalene 80 g, toluene 600 ml and 2-methoxyethanol 400 ml.

Adhesion to hydrophobic surfaces was measured with suspensions of radiolabelled bacteria prepared as above; 2-ml plastic ampoules (Nunc, Roskilde, Denmark) were used containing 1 ml of bacterial suspension and 1 ml of a 20% suspension of Agarose beads. After continuous agitation for 2 h, 300 μ l of the suspension was removed for β -counting, whilst four other 300 μ l volumes were layered on to the surface of 6% dextran (mol. wt 110,000) and centrifuged for 1 min at 600 g to deposit those bacteria bound to the Sepharose beads which were then subjected to β -counting.

Trypsinisation of bacteria. Radiolabelled micro-organisms were treated with trypsin (Sigma type IX) 100 μ g/ml in 0.05 M tris-acetate buffer at pH 8.0 (0.05 M tris [hydroxymethyl] aminomethane adjusted to pH 8.0 with molar acetic acid); control organisms were treated with tris-acetate buffer only. Viable counts confirmed that the bacteria had survived trypsinisation.

RESULTS

The hydrophobic binding to Agarose beads of strains isolated from CSF shunt infections (designated 1-6) was assessed by the above methods and the results are shown in table I. An analysis of variance was done and a comparison of the variance ratio within and between bead types to an F-distribution with three and 41 degrees of freedom yielded a p-value of < 0.025, confirming the tendency for increased binding to increasingly hydrophobic groups. Examination of strains 2,3,5,6 and A and B, all isolates from CSF shunt infections, suggested that these hydrophobic interactions were not influenced by trypsinisation (data not reported here).

To assess the roles of clumping factor and protein A in adhesion to the silicone

TABLE I
Percentage adhesion of *S. aureus* to Sepharose
beads bearing different hydrophobic groups

Strain	Percentage adhesion* in duplicate tests with beads bearing the stated group			
	None	Phenyl	Octyl	Decyl
1	0.0	0.0-38	0.57,0.83	0.98,0.83
2	0.0	2.28,2.88	1.95,...	3.11,6.04
3	0.02,0.06	0.95,1.87	2.88,...	2.72,...
4	1.53,1.86	3.03,2.97	1.88,1.30	4.71,1.72
5	0.27,0.32	2.71,3.09	9.62,4.07	4.41,11.29
6	0.20,0.33	2.57,2.83	5.97,6.51	6.22,7.03
Mean	0.26	2.13	3.54	4.46

* Values represent the number of adherent β -counts expressed as a percentage of those in the inoculum incubated with the polymer substrate (see *Methods*). Most experiments were performed in duplicate; ... = duplicate not done.

polymer, the influence of trypsinisation on adhesion was examined with strains 8-13 (group II), identified as lacking protein A, and strains 14-20 (group III) lacking clumping factor, along with strains A,B,2,3,5 and 6 (group I). Initial experiments had shown that trypsinisation removed the activities of protein A and clumping factor. Table II shows the influence of trypsinisation on the adhesion of selected strains of *S. aureus* to shunt polymer. All strains examined, whether possessing protein A, clumping factor, or both, showed reduced adhesion after treatment with trypsin. Those organisms possessing both proteins showed the greatest adhesion among untrypsinised strains and the greatest reduction in adhesion after treatment with trypsin.

TABLE II
Effect of trypsinisation on percentage adhesion* of *S. aureus* to shunt tubing

Percentage adhesion observed with								
Group I strains possessing clumping factor and protein A			Group II strains lacking protein A			Group III strains lacking clumping factor		
Test strain	untrypsinised	trypsinised	Test strain	untrypsinised	trypsinised	Test strain	untrypsinised	trypsinised
A	15.4,36.3	0.0-3	8	31.7,30.4	10.8,7.2	14	1.6,2.0	0.0
B	7.7,4.8	0.6,0.5	9	19.3,9.7	1.6,2.7	15	13.2,14.7	1.5,2.4
2	8.5,14.1	0.8,1.4	10	20.6,2.0	1.1,0	16	2.4,3.0	0.0
3	49.0,33.3	0.1,1.1	11	1.6,2.3	0.0	17	10.8,0	0.0
5	11.3,12.1	1.0,0	12	6.7,4.1	0.6,2.0	18	7.8,11.3	2.0,1.2
6	4.2,3.5	0.0	13	0.3,0.9	0.0	19	1.0,2.6	0.8,0.8
						20	10.3,14.3	2.7,4.0
Mean	16.7	0.5		10.9	2.2		6.8	1.1

* Values represent the number of adherent β -counts obtained in duplicate experiments and expressed as percentages of the counts in the inoculum incubated with the polymer substrate.

DISCUSSION

It is generally impossible to decide whether the microbial contamination responsible for infection of an implanted device has occurred during or after its insertion, although it appears likely that either possibility may account for some prosthetic infections (Downes, 1977; Lidwell *et al.*, 1983). These two possible modes of infection have implications for operative techniques and for surgical prophylaxis and treatment of subsequent infections. In terms of the implanted device, different considerations must also be applied to microbial contamination of the native material during manipulation before implantation in the tissues, and to colonisation of materials occurring after implantation, because, in the latter state, the material presents a different surface for bacterial attachment as a result of the rapid adsorption of host tissue components (Feijen *et al.*, 1979). The different surface characteristics result in different affinities for bacteria. For example, *S. epidermidis* adheres more strongly to native silicone polymer from which CSF shunts are manufactured than to the same material coated with plasma proteins; perhaps, in this case, infection is more likely to occur at the time of surgery. In contrast, *S. aureus* adheres equally well to either surface (Barrett, 1983).

In common with several other studies (Hogt *et al.*, 1983; Ashkanazi, 1984; Botta *et al.*, 1984) the present work has investigated infection of implant materials in the native (i.e., pre-implantation state). Silicone polymers in this condition present extremely hydrophobic surfaces (Dow Corning, 1963) and it was considered possible that colonisation with bacteria might occur by virtue of hydrophobic interactions. Binding of bacteria to increasingly hydrophobic surfaces has been demonstrated by Fletcher and Loeb (1979) working with a marine pseudomonad, and the work of Hogt *et al.* (1983) suggested that *S. epidermidis* is also capable of hydrophobic interaction. The general tendency of proteins to bind to increasingly hydrophobic surfaces was noted by MacRitchie (1972) and raises the possibility of bacterial surface proteins being responsible for hydrophobic attachment. Support for this view is found in the work of Rutter and Abbott (1978), where the adhesion of a strain of a viridans streptococcus to a hydrophobic (polystyrene) surface was more markedly reduced by the action of trypsin than was adhesion to a hydrophilic (glass) surface. Hogt *et al.* (1983) noted the pepsin sensitivity of adhesion of *S. epidermidis* to a hydrophobic surface, although in this case trypsin had no effect on the organism's adhesive property.

The present study has shown that *S. aureus* is capable of interacting increasingly strongly with surfaces of increasing hydrophobicity, although the extent of this interaction is considerably less than in the case of many bacteria such as the gonococcus (Trust *et al.*, 1980). As the hydrophobic binding of *S. aureus* was not sensitive to the action of trypsin, the trypsin-sensitive adhesion of *S. aureus* to CSF shunt polymer shown by Barrett (1983) is presumably not based on hydrophobic interactions.

In the present study, the nature of the trypsin-sensitive mediator of *S. aureus* adhesion to shunt polymer was investigated by studying the adhesion of mutants lacking the surface proteins, clumping factor and protein A. The findings do not suggest any specific interaction between these proteins and the CSF shunt material. Strains of *S. aureus* possessing both clumping factor and protein A (group A, table II) showed intrinsically greater adhesion to CSF shunt polymer than did those lacking either protein (groups B and C, table II). After treatment with trypsin, the strains in

groups B and C both displayed reduced adhesion to the polymer, suggesting that clumping factor and protein A both played a part in mediating adhesion. The proportionately greater adhesion of those strains in group A may result from the cumulative action of the two proteins. However, it is also possible that an adhesive role is played by other trypsin-sensitive factors not expressed by the strains in groups B and C. This could be explained if the defective strains lacked surface proteins additional to the protein A and clumping factor noted in this study, resulting in a quantitatively greater degree of protein-substrate interaction in the case of the control strains. For example, certain strains of capsulate *S. aureus* have been found to display protein A activity whilst the activity of other surface antigens may remain masked (King and Wilkinson, 1981). This is, however, unlikely to account for the results obtained in the present study, since none of the test strains was found to be capsulate. It appears likely, therefore, that the adhesion of *S. aureus* to untreated shunt polymer (i.e., before implantation) is mediated by non-specific interactions with several bacterial surface proteins, and that this adhesion is not hydrophobic in nature.

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