Co-elimination of *mec* and *spa* genes in *Staphylococcus aureus* and the effect of *agr* and protein A production on bacterial adherence to cell monolayers

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Summary. Phenotypic loss of protein A production was tested in six methicillin-resistant (Mc^R) *Staphylococcus aureus* (MRSA) isolates and their isogenic methicillin-sensitive (Mc^S) variants by a radiolabelled IgG-binding assay with washed cells and by Western blotting of supernates prepared from lysed washed cells. Genomic DNA was probed for homology with the protein A gene (*spa*) in *Eco*RI digests and for homology to the methicillin resistance gene (*mec*) in *Hind*III digests. The Mc^S variants had lost homology with *mec*. An isogenic pair of Mc^R and Mc^S strains, and derivatives of *S. aureus* 8325-4 with site-specific mutations of the accessory gene regulator locus (*agr*) and *spa*, were tested for adherence to human peritoneal mesothelial cells in monolayer culture. The isogenic pair were also tested for adherence to HEP-2 and Vero cell monolayers in assays with ³H thymidine-labelled bacteria. Mc^R isolates produced protein A which was absent from three strains that had become Mc^S. This correlated with deletion of the *spa* locus. *Spa* homology, but reduced production of protein A, was retained in one Mc^S strain which also showed reduced adherence to HEP-2, Vero and mesothelial cells ($p < 0.05$) compared with the parent Mc^R strain. A *spa* mutation in strain 8325-4 did not significantly affect adherence to mesothelial cells but mutation in *agr* increased adherence significantly in both Spa⁺ and Spa⁻ strains.

Introduction

Staphylococcus aureus is able to colonise several superficial sites in the body, particularly the nasal mucosa and perineum.^{1,2} Carriers of *S. aureus* have an increased risk of infection of surgical wounds and peritoneal dialysis catheter exit sites.³⁻⁶ It is assumed that bacterial adherence to tissues is required for colonisation to occur.⁷ Adherence studies have used monolayers of continuous cell lines^{7,8} and primary cultures of mesothelial cells which line the peritoneal cavity.⁹⁻¹¹ Although the adherence mechanisms of *S. aureus* are not clearly defined, the inclusion of cell-wall fractions of lipoteichoic or teichoic acid and of protein A in adherence assays have been shown to reduce adherence.¹⁰

S. aureus also has fibronectin-binding protein(s) and it is presumed that these are involved in adherence.¹²⁻¹⁴ Fibronectin is produced by mesothelial and epithelial cells but is not detected on the surface of transformed cells such as HEP-2 or Vero cell monolayers.¹⁵⁻¹⁷ Cellular fibronectin is located in the basement membrane of epithelial cells¹⁷ which may be exposed following trauma. *S. aureus* also binds to fibrinogen.^{18,19}

In *S. aureus*, expression of cell-bound and extracellular proteins is controlled by the accessory gene regulator (*agr*) locus.^{20,21} In assays with a strain deleted for *agr* (*agr*⁻), *S. aureus* proteins have been categorised into four classes (I–IV), based on the amount of protein produced during different phases of growth.²² In *agr*⁺ bacteria, protein A and fibronectin-binding proteins (Class III) are produced maximally during exponential growth whereas the extracellular protein, toxic shock syndrome toxin (TSST-1, Class I), is produced maximally in the post-exponential phase.²² In *agr*⁻ mutants in the post-exponential phase, Class III proteins are produced at high levels and Class I proteins are undetectable. The regulation of exoprotein synthesis by the *agr* locus has characteristics of two-component systems. These involve signal transduction proteins which enable the organism to respond rapidly to environmental change. In *S. aureus*, the AgrB protein provides sensor function and AgrA is involved in the regulation of gene expression, acting as a transcriptional activator.²² As a result, exoprotein gene expression is increased and cell-bound protein is decreased in post-exponential growth.

Methicillin-resistant (Mc^R) *S. aureus* (MRSA) may exhibit spontaneous deletion of the methicillin resistance gene (*mec*).²³ In epidemic Australian and London (EMRSA-1) strains, this is accompanied by

loss of resistance to mercury and cadmium and reduced resistance to tetracycline.²⁴⁻²⁶ Production of protein A also can be co-eliminated with resistance to methicillin and re-introduced by transduction or transformation.²⁷ Introduction of methicillin resistance can also modify levels of protein A expression.²⁷ The protein A gene (*spa*) maps near *mec* on the chromosome of strain 8325.²⁸

In this study, the production of protein A and homology for *spa* in MRSA and isogenic methicillin-sensitive (Mc^S) variants was examined and the adherence of one pair of strains to cell monolayers was compared. Mutant strains for the *spa* and *agr* genes of *S. aureus* 8325-4 were compared for adherence to mesothelial cells.

Materials and methods

Bacterial strains

S. aureus strains used in this study are listed in table I. Six isolates of MRSA and Mc^S variants derived from them have been described previously.²⁶ Isolates GH13, GH25, GH28 and GH31 are EMRSA-1.³⁰ Mc^S variants are designated by the parent strain number with the suffix -01. Strain 8325-4 is a non-lysogenic variant of strain NCTC 8325.³¹ Mutants of strain 8325-4 were derived by allele replacement of the *spa* locus by insertion of an ethidium bromide resistance determinant ($\Delta spa::Eb^r$) and by Tn551 insertion in the *agr* locus as described previously.^{32,33} Strain DU5818 was constructed by transducing *agr::Tn551* into strain DU5723 (8325-4 $\Delta spa::Eb^r$) with bacteriophage 85 and selecting for erythromycin resistance. *S. aureus* strains Wood 46 (ISP740) and Cowan I (ISP393) was provided by Dr P. A. Pattee (Department of Microbiology, Iowa State University, Iowa 50011, USA).

Media

Tryptic Soy Broth (TSB; Oxoid) solidified with agar (Oxoid no. 1) 1% w/v was used for routine culture. Bacteria were grown in IsoSensitest broth (IST; Oxoid) for adherence assays and in TSB for protein A determinations.

Detection of protein A

Cell-bound protein A was detected in washed cells by a liquid phase radiolabelled IgG-binding assay based on the method of Uhlen *et al.*³⁴ Strains were grown overnight in TSB, centrifuged and washed twice in phosphate-buffered saline with Tween 20 0.5% v/v (PBST). Bacteria were resuspended in PBST to OD₅₄₀ 0.4, equivalent to a total count of $c. 5 \times 10^8$ organisms/ml in a Helber counting chamber. Six replicate 1-ml samples were pelleted and resuspended in 200 μ l of ¹²⁵I-labelled rabbit IgG (1 mg/L). After incubation for 1 h with shaking at 37°C, the cells were washed twice with PBST and bound radioactivity was counted in a

gamma counter. Duplicate assays were performed for each strain. Results were calculated as the amount of radiolabelled IgG bound as a percentage of the initial cpm used. The concentration of IgG used in this study was not tested for saturation of protein A and, therefore, in positive strains, higher levels of protein than those detected in the assay may have been present. *S. aureus* Cowan I was included as a known positive strain and Wood 46 as a negative or very low-level producer of protein A.³⁴

Western immunoblotting was used to detect protein A in supernates prepared from lysed washed cells. Cells (1.5 ml) grown to saturation in TSB were centrifuged for 30 s, resuspended and washed in 450 μ l of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). DNAase 1 (Sigma) 10 μ g and pancreatic RNAase (Sigma) 10 μ g were added along with 50 μ g of recombinant lysostaphin (Applied Microbiology Inc., New York) and the mixture was incubated at 37°C for 30 min. The lysate was centrifuged at 11000 *g* for 15 min. The protein concentration of the supernate was measured by the method of Lowry *et al.*³⁵ Between 2 and 10 μ l was boiled with final sample buffer (volume made up to 20 μ l) and applied to a discontinuous SDS-polyacrylamide 12.5% gel.³⁶

Proteins separated by SDS-PAGE were transferred to nitrocellulose membranes (Micron Separations Inc., Westborough, MA, USA) with a semi-dry blotter (BioRad) and 48 mM Tris-HCl, 39 mM glycine (pH 9.2) containing methanol 20% w/v. Filters were blocked with skimmed milk 3% w/v in 10 mM Tris-HCl, NaCl 0.9% w/v, pH 7.4 (TBS). The filters were probed with rabbit anti-protein A serum (Medlabs, Dublin) diluted 1 in 500 in TBS containing skimmed milk 3% w/v and peroxidase-conjugated goat anti-rabbit IgG serum (Sigma) diluted 1 in 250 in TBS containing skimmed milk 3% w/v. The substrate used to develop the bound peroxidase was 4-chloro-1-naphthol 0.005% and H₂O₂ 0.015% w/v in TBS.

Preparation of DNA and Southern blotting

Genomic DNA was prepared by the procedure of Lindberg *et al.*³⁷ and Pattee and Neveln,³⁸ but modified by use of recombinant lysostaphin (60 μ g), by performing the phenol extraction steps at ambient temperature for 45 min on an orbital shaker and by clarifying the phenol extracts by centrifugation at 12000 *g* in siliconised glass Corex tubes.

Standard procedures were employed for DNA manipulations.³⁹ For Southern hybridisation, 5 μ g of genomic DNA was cleaved with *Eco*RI or *Hind*III and the fragments were separated by electrophoresis in an agarose 0.8% gel. The DNA was transferred to Nytran filters (Micron Separations Inc.) and probed with plasmid pSPA721 or pBBB62 DNA that had been labelled by nick translation⁴⁰ with α -³²P dATP (Amersham). The specific activity of the dATP was 3000 Ci/mMol. Plasmid pSPA721 carries the wild-type *spa* gene of *S. aureus* 8325-4 on a 4.3-kb *Eco*RI

Table 1. Characteristics of *S. aureus* strains used

Strain	Relevant characteristics	Reference no.
GH13, GH25, GH28, GH31	EMRSA-1	26
GH19, GH34	MRSA	26
GH1301, GH2501, GH2801	Mc ^S Cd ^S Hg ^S (Tc)	26
GH3101	Mc ^S Cd ^S (Tc)	26
GH1901, GH3401	Mc ^S	26
8325-4	Agr ⁺ Spa ⁺ NCTC 8325 cured of prophage	31
DU5723	Agr ⁺ Spa ⁻ derived from 8325-4	32
DU5818	Agr ⁻ Spa ⁻ derived from DU5723	32
DU5820	Agr ⁻ Spa ⁺ derived from 8325-4	32, 33
Wood46 (ISP740)	Low level or negative Spa production	34
Cowan I	Spa ⁺	34
Newman	Spa ⁺	29

Agr, accessory gene regulator; Spa, protein A; Cd, cadmium; Hg, mercury; Mc, methicillin; (Tc), reduced resistance to tetracycline.

fragment.²⁸ Plasmid pBBB62 is pTZ18R with a 4-kb *Hind*III insert from strain BB270 covering *mecA* (constructed by Drs C. Ryffel and B. Berger-Bächi, University of Zurich; personal communication).

Cell monolayers

HEp-2 and Vero cells were grown to confluence as described previously⁹ in 24-well polystyrene plates. The growth medium was Eagle's Minimum Essential Medium modified with Earle's salts (EMEM; Flow) supplemented with glutamine, non-essential amino acids and fetal calf serum (FCS) 10% v/v. Cell monolayers were washed three times with 1 ml of Earle's balanced salts buffered with HEPES, pH 7.1, (EBS; Flow) before use.

Mesothelial cells were prepared from human omentum.¹¹ Cells were grown in minimum essential medium (MEM) with L-valine substituted by D-valine (MEM-Dval) (Gibco) and supplemented with antibiotics and FCS 10% v/v. Cells were passaged up to three times before being used, the time to confluence varying between 3 days and 1 week. Bacterial adherence was tested within 2–3 days of confluence being reached. Mesothelial cells were identified by their cobblestone appearance when stained by the 'Diff Quick' method (Merz and Dade AG, Switzerland),¹¹ by positive staining for cytokeratin with mouse monoclonal antibody (MAb) to human cytokeratin (clone LP34, Dako Ltd),^{11,41} and by lack of staining with mouse MAb to human von Willebrand factor (clone RF F8:R/1) purchased from the Royal Free Hospital, Hampstead, London.

Cells were stained with rabbit anti-mouse peroxidase conjugate, developed with diaminobenzidine and H₂O₂, and visualised by light microscopy. Materials were obtained from Dako Ltd. Fibronectin was detected in mesothelial cell monolayers by direct

staining with peroxidase-conjugated sheep polyclonal anti-fibronectin antibody (Binding Site Ltd, Birmingham) and developed as described for cytokeratin.

Adherence assay

The method was used as described previously.^{9,11} Bacterial strains were grown in IST broth supplemented with 5 µCi/ml [³H] methyl thymidine (Amersham) for 18 h with shaking at 37°C. The specific activity of the tritiated thymidine was 80 or 89 Ci/mmol. Cells were harvested, washed in PBS (pH 7.2) and resuspended to OD₅₄₀ 0.4 in EBS. Bacterial suspension (0.5 ml) at 37°C was added to each well containing a cell monolayer previously washed three times with EBS (37°C). Four, or usually six, replicate wells were prepared for each bacterial culture. After incubation for 1.5 h at 37°C, the suspension was aspirated and the monolayer was washed three times with EBS (0.5 ml). The cell monolayer plus adherent bacteria was then removed by incubation with a solution of trypsin 0.05% w/v and EDTA 0.02% w/v and transferred to scintillation fluid (Optiphase, Pharmacia) for counting. Results were expressed as counts/min (cpm)/monolayer × 100/cpm of the original labelled bacterial suspension. Strains for comparison were tested in the same monolayer plate to reduce the variation which occurs between plates.⁹

Results

Production of protein A in Mc^R strains and Mc^S variants

EMRSA-1 isolates GH13, GH25, GH28 and GH31, and MRSA isolates GH19 and GH34 were tested for protein A. Washed cells were assayed for binding of radiolabelled (¹²⁵I) rabbit IgG, and supernates from lysed bacterial cells were examined by Western immunoblotting. Although the upper limit of detection of protein A was not established, quantitation with the IgG-binding assay, expressed as a percentage of IgG bound, showed that isolate GH19 produced lower levels of protein A (≤ 21.6%) than strain Cowan I (≥ 35.1%) or the other Mc^R isolates (≥ 30.0%). This was consistent with the data from Western immunoblotting for strain GH19 in which five times the amount of protein was loaded for detection of protein A (fig. 1) than was used for other Mc^R strains.

Strain 8325-4 produces a protein A molecule of 53 kDa.⁴² This agrees well with the mol. wt of 55 kDa estimated from fig. 1. The protein A produced by strains GH13, GH25, GH28, GH31 and GH34 was 44 kDa whereas that expressed by strain GH19 was 49 kDa, possibly due to loss of an IgG-binding domain⁴² or repeats from the wall-associated region Xr.⁴³

Protein A was not detected in the Mc^S variant strains GH3101, GH1901 (fig. 1) and GH3401

Fig. 1. Detection of protein A in supernates of washed lysed cells of Mc^R and Mc^S variant strains of *S. aureus* after SDS-PAGE and immunoblotting. Filters were probed with rabbit anti-protein A serum; 8 μ g of protein were loaded for all samples except C, D and E which had 40 μ g of protein. Lanes A, P, strain 8325-4; B, GH13; C, GH1301; D, GH19; E, GH1901; F, GH25; G, GH2501; H, GH25; I, GH2501; J, GH28; K, GH2801; L, GH31; M, GH3101; N, GH34; O, GH3401. Protein A of strain 8325-4 is 53 kDa.⁴² The numbers at the side refer to the mol. wts of the protein A from strain 8325-4 (55 kDa) and strain GH13 (44 kDa).

Fig. 2. Southern blot and hybridisation of genomic DNA of *S. aureus* strains. Lanes A–N, DNA digested with *Eco*RI and probed for *spa*; lanes O–Z, DNA digested with *Hind*III and probed for *mec*. Lane A, strain Newman; B, 8325-4; C, O, GH13; D, P, GH1301; E, Q, GH25; F, R, GH2501; G, S, GH28; H, T, GH2801; I, U, GH19; J, V, GH1901; K, W, GH31; L, X, GH3101; M, Y, GH34; N, Z, GH3401. Less DNA was loaded in lanes C–H.

($\leq 1.3\%$) by the labelled IgG-binding assay. These strains bound IgG to an extent similar to that of Wood 46 ($\leq 2.9\%$) which was treated as a negative control. In strain GH1301, the expression of protein A was markedly reduced in comparison to strain GH13. It was detected in Western immunoblots by increased loading of the gel (five times), but it was not detected with radiolabelled IgG. Protein A production was not reduced in strains GH2501 and GH2801 compared with their parent strains.

Homology for *mec* and *spa* in Mc^R and Mc^S variant strains

Southern blots of genomic DNA digested with *Hind*III were probed with the 4-kb *Hind*III fragment of pBBB62 containing *mec*-specific DNA. Each MRSA strain had a 4-kb fragment which hybridised with the *mec* probes (fig. 2, lanes O–Z). The Mc^S variants GH2501, GH2801 and GH1901 lacked homology with *mec*, and the lack of homology previously reported for strains GH1301, GH3101 and GH3401²⁶ was confirmed (fig. 2).

For the detection of *spa*, genomic DNA was digested with *Eco*RI and Southern blots were probed with a 4.3-kb fragment from strain 8325-4 which carries the intact *spa* gene (fig. 2, lanes A–N). The weaker signals in lanes C–H (fig. 2) are due to lower levels of DNA loaded as shown by ethidium bromide staining of the gel (results not shown). Each of the MRSA strains showed homology with *spa*. With the exception of strain GH19, which had a fragment size of 6.8 kb, the fragment size was 5.9 kb (fig. 2). Lanes A and B show the 4.3-kb fragment of the control strain Newman and the probe strain 8325-4 respectively. Hybridisation with *spa* established that deletion of the *spa* gene in strains GH3101, GH1901 and GH3401 was associated with the phenotypic loss of protein A but that strain GH1301, which retained homology with *spa* on the same size fragment as the parent strain GH13, expressed reduced levels of protein A. To detect whether major alterations had occurred in the *spa* fragment of this variant strain, two further probes representing 5' and 3' terminal sequences of the *spa* locus of strain 8325-4 were used to hybridise with *Eco*RI digests of strains GH13 and GH1301. The resulting blot profile

Table II. Adherence of *S. aureus* strains GH13 and GH1301 to cell monolayers

Cell monolayer	Percentage adherence (SD) of strain		Mann-Whitney U Test p value
	GH13	GH1301	
HEp-2	9.7 (0.6)	4.1 (1.3)	0.024
Vero	9.1 (1.8)	1.0 (0.1)	0.0022
Mesothelial	6.7 (0.55)	1.9 (0.23)	0.0022

Table III. Adherence to mesothelial cells of *agr* and *spa* mutants of *S. aureus* strain 8325-4

Strain	Phenotype	Percentage adherence (SD)	Mann-Whitney U test* p value
8325-4	Agr ⁺ Spa ⁺	5.43 (0.49)	—
DU5723	Agr ⁺ Spa ⁻	5.13 (0.34)	0.7
DU5818	Agr ⁻ Spa ⁻	12.43 (0.29)	0.03
DU5820	Agr ⁻ Spa ⁺	13.6 (0.18)	0.03

* Comparison with strain 8325-4.

for strain GH1301 was unchanged compared with strain GH13 (not shown).

Adherence of strains GH13 and GH1301 to cell monolayers and the role of spa and agr in adherence of strain 8325-4

With HEp-2, Vero and mesothelial cell monolayers, strain GH13 adhered at levels which were consistently and significantly greater than those for strain GH1301, which produced low levels of protein A (table II). Similar results were obtained in repeat experiments. To test the apparent correlation between low level production of protein A and reduced adherence to cell monolayers, mutants of strain 8325-4 for *spa* and *agr* were compared with strain 8325-4 for adherence to mesothelial cells. The *spa* mutation in strain DU5723 had no effect on adherence compared with strain 8325-4 (table III). Strain 8325-4 produced low levels of protein A which was considerably increased in the *agr* mutant (DU5820). Mutation at the *agr* locus in both the *spa*⁻ strain DU5818 and in the *spa*⁺ strain DU5820 resulted in a significant increase in the levels of adherence compared with strains 8325-4 and DU5723 (table III). Similar results were obtained in repeat experiments.

Discussion

It has been known for some time that protein A production in *S. aureus* is affected by gain or loss of methicillin resistance. Cohen and Sweeney²⁷ showed that protein A production could be co-eliminated and co-transduced with methicillin resistance, suggesting

that the *mec* and *spa* loci are linked. By pulse-field gel electrophoresis and transformation linkage analysis, the *spa* gene has been shown to map near to *mec* on the chromosome of strain 8325-4 in the *Sma*I G fragment.^{28,44}

In the Australian epidemic strain ANS46²⁵ and the closely related UK epidemic strain EMRSA-1,²⁶ resistance to cadmium, mercury and tetracycline (due to a chromosomal insert of pT181) are co-eliminated with *mec* although not all deletions are identical.⁴⁵ From the results of our experiments, the *spa* gene can be added to the list of genes shown to be linked to the *mec* region in EMRSA-1 isolates and in other MRSA. We have shown that the Mc^S derivative, GH1301, which retains mercury resistance and homology for a *merA* probe,²⁶ has lost homology for *spa* in this *mec*-region deletion. Similarly, the MRSA derivatives, GH1901 and GH3401 lost homology for *spa* along with homology for *mec*. In comparison, the EMRSA-1 derivatives GH2501, GH2801 and GH1301, which have lost resistance to Cd, Hg and Tc, retained homology for *spa*. The marked reduction of protein A produced by strain GH1301 but the unchanged pattern of hybridisation with the 4.3-kb *Eco*RI probe and with probes for the sequences 3' and 5' to *spa*, indicates that the change in level of expression is not due to a gross DNA re-arrangement. It suggests either that control of expression of protein A has been effected by the deletion somewhere in the *mec* region or that a point mutation in *spa* required for production of the protein has been affected. Cohen and Sweeney²⁷ found that, with certain donor strains, transduction of *mec* into strain 8325 would increase or reduce the level of protein A produced, suggesting that the *mec* region can affect expression of protein A. The *agr* locus, which represses protein A production in the post-exponential growth phase, maps at a site distant from *mec* and *spa*⁴⁴ so it is unlikely that the observed changes in level of expression of protein A involve this locus directly. Cohen and Sweeney²⁷ found that mutations to high-level protein A production in strain 8325, which are presumably in *agr*, were unaffected by introduction of *mec*.

The MRSA strains have larger *spa* fragments than strain 8325-4 (4.3 kb). The differences in fragment size between strain GH19 (6.7 kb) and the EMRSA-1 and GH34 strains (5.9 kb) suggest that strain GH19 is less closely related. Strains GH19 and GH34 are distinct from each other and from the EMRSA-1 isolates used in this study, both in phage type and in the range and location of antimicrobial resistances.²⁶ The smaller size of protein A in the clinical isolates compared to strain 8325-4 could be because their *spa* gene lacks one of the five major repeats present in that of strain 8325-4.⁴²

The *mec* deletion in strain GH1301, which showed reduced production of protein A and reduced adherence, indicated that protein A might be involved in adherence, as has been suggested in other studies.¹⁰ From experiments with *agr* and *spa* mutations of strain

8325-4 our results show that protein A does not significantly affect adherence to mesothelial cells although there is a significant increase in adherence in *agr*⁻ mutants compared with *agr*⁺ strains. This suggests that a cellular product in exponential growth is involved. The advantage of using site-specific mutations avoids the problems of other mutations which might be present after chemical mutagenesis or spontaneous mutation.⁴⁶ If the results with strain 8325-4 are applicable to the MRSA strains, a factor other than reduced production of protein A in strain GH1301 is needed to explain the reduction in adherence and suggests that a structural gene involved in adherence or a gene that regulates its expression resides in the *mec* region. Retention of *spa* gene homology may not affect this interpretation, as similar results for adherence to HEp-2 and mesothelial cells were obtained with strain GH31 and strain GH3101, which is *spa*⁻ (unpublished observations).

Fibronectin has been detected on the surface of human mesothelial cells in some^{11,47} but not in other studies.⁴⁸ Growth conditions affect the amount of

fibronectin produced by cells.⁴⁷ Mesothelial cells grown in MEM-Dval, as in this study, produce some surface fibronectin which is increased when cells are grown in standard media with L-valine.¹¹ However, as transformed cells may not contain fibronectin,¹⁶ unlike primary cell cultures, the similar results for adherence of strains GH13 and GH1301 to HEp-2, Vero and mesothelial cells in this study suggest that a binding site(s) other than, or in addition to, fibronectin is involved.¹⁰ It has been shown⁴⁹ that anti-fibronectin antibody reduced adherence of *S. aureus* to fibronectin to a greater extent than to mesothelial cells, suggesting an additional receptor site on mesothelial cells. The increase in adherence in *agr* mutants independent of *spa* suggests that *agr*, which controls expression of a wide range of cell-bound components and extracellular products, controls expression of a locus involved in adhesion. The correlation between levels of protein A produced and levels of adherence to cells¹⁰ could also be explained if another surface component, which is similarly under *agr* control, was responsible for adherence.

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