INFECTION AND IMMUNITY, Nov. 1996, p. 4680–4685 Vol. 64, No. 11
0019-9567/96/$04.00+0

Candida albicans Binding to the Oral Bacterium *Streptococcus gordonii* Involves Multiple Adhesin-Receptor Interactions

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Received 28 May 1996/Returned for modification 13 July 1996/Accepted 15 August 1996

Candida albicans **binds to several species of oral streptococci, in particular *Streptococcus gordonii*, through recognition of a streptococcal cell wall polysaccharide receptor (A. R. Holmes, P. K. Gopal, and H. F. Jenkinson, Infect. Immun. 63:1827–1834, 1995). We now show that isogenic cell surface protein mutants of *S. gordonii* DL1, unaltered in expression of cell wall polysaccharide, are reduced in ability to support adherence of *C. albicans* cells in a solid-phase assay. Inactivation of the *S. gordonii cshA* and *cshB* genes, encoding high-molecular-mass cell surface polypeptides, and inactivation of the *sspA* and *sspB* genes, encoding antigen I/II salivary adhesins, resulted in 40 and 79% reductions, respectively, in adherence of *C. albicans* cells. Inactivation of the *S. gordonii scaA* gene encoding a cell surface lipoprotein had no effect on *C. albicans* adherence. Polyclonal antiserum to streptococcal antigen I/II protein SpaP and antibodies specific to the amino-terminal nonrepetitive (NR) domain of CshA both inhibited adherence of *C. albicans* to *S. gordonii* cells. Conversely antibodies to the amino acid repeat block repetitive (R) domain of CshA, or to ScaA, did not inhibit *C. albicans* adherence. Immobilized recombinant polypeptide fragments of CshA comprising NR domain or R domain sequences both supported adherence of *C. albicans* cells. Expression of *S. gordonii* SspB protein on the surface of *Enterococcus faecalis* conferred on the enterococcal cells the ability to bind *C. albicans*, and this was ablated by antigen I/II antiserum. Collectively the results suggest that interaction of *C. albicans* with *S. gordonii* is mediated by a complement of adhesin-receptor interactions that involves two families of streptococcal multifunctional polypeptide adhesins, bacterial cell wall polysaccharide, and as yet unidentified yeast cell surface components.**

Candida albicans is a common commensal of humans, and long-term colonization with a single strain has been reported (36). Adherence mechanisms are important in the maintenance of colonization by endogenous strains which can go on to cause serious infections when host defenses are compromised (34). *C. albicans* binds to a variety of host cell receptors (reviewed in references 3 and 11) through both lectin (protein-carbohydrate)-like and protein-protein-type interactions (11). Epithelial cell adhesion is correlated with the expression on the yeast cell surface of integrin analogs that bind complement fragments iC3b and C3d (11, 35). Other *C. albicans* adhesins with integrin- or lectin-like properties bind fibronectin (19, 20, 32, 35), laminin (2), and fibrinogen (25). Different adhesins may recognize a common receptor, e.g., fibronectin (19), but bind to discrete domains on that receptor (32). *C. albicans* also adheres to bacterial cells from a variety of genera (12) and in particular to a range of oral streptococci (16). This may be significant in oral cavity colonization by yeast cells, since it is now well established that intermicrobial adhesion contributes to the development, stabilization, and maintenance of oral mixed microbial populations (21, 23).

In a previous study of *C. albicans* binding to oral streptococci, it was found that strains of *Streptococcus gordonii*, *Streptococcus oralis*, and *Streptococcus sanguis* were the best partners for yeast cell adherence (16). Binding of *C. albicans* was attributed to the production by the streptococcal species of structurally related linear cell wall phosphopolysaccharides. When these polysaccharides were extracted from streptococci and immobilized on plastic, they supported the adhesion of *C. albicans* cells (9). The highest-affinity binding was observed between cells of *C. albicans* and *S. gordonii*. The latter organism is a common component of the human oral microbiota and exhibits a wide range of adherence properties (reviewed in references 13, 14, and 23). In particular, *S. gordonii* is a multimediator of intra- and intergeneric bacterial coaggregations with *S. oralis*, *Actinomyces naeslundii*, and *Porphyromonas gingivalis* (4, 23, 24), interactions that have been shown to involve various streptococcal cell surface polypeptides. Thus, cell wall-associated proteins SspA and SspB (previously designated SSP-5 [6]), which are members of the streptococcal antigen I/II family of salivary glycoprotein adhesins (reviewed in reference 13), mediate binding of *S. gordonii* to *A. naeslundii* (7). Additional cell wall-linked proteins CshA and CshB, which are determinants of streptococcal cell surface hydrophobicity (28, 30), are also involved in *Streptococcus-Actinomyces* coadherence (31) and in binding of *S. gordonii* cells to immobilized fibronectin (27). Lastly, ScaA lipoprotein, which is believed to be anchored at the external face of the cytoplasmic membrane, is strongly implicated in the coaggregation of *S. gordonii* and *A. naeslundii* (1, 22).

Since the Ssp and Csh polypeptides have been shown to be crucial to many of the adherence properties of *S. gordonii*, in that they appear to bind a range of bacterial and human receptors (7, 17, 31), we investigated the role of these polypeptides in the adherence of *C. albicans* to *S. gordonii*. The results in this report demonstrate that the *S. gordonii* cell wall-anchored polypeptide determinants of interbacterial adherence also mediate, in conjunction with streptococcal cell surface polysaccharide, *S. gordonii-C. albicans* coadherence.

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TABLE 1. Bacterial strains used

Organism	Genotype[a]	Reference
S. gordonii		
DL1 Challis	Wild type	33
OB235	*cshA3*::*ermAM*	31
OB271	*cshB2*::*ermAM*	31
OB277	*cshA31*::*cat cshB2*::*ermAM*	31
OB220	*sspA*::*ermAM*	7
OB219	*sspA′sspB′*::*ermAM*	7
OB385	*cshA31*::*cat cshB21*::*aphA-3*[b]	This study
OB392	*cshA31*::*cat cshB21*::*aphA-3 sspA′sspB′*::*ermAM* (OB219 DNA→OB385)[c]	This study
OB470	*scaA2*::*tet*	This study
NCTC 7869 Channon	Wild type	33
E. faecalis		
S161(pAM401)	Wild type (10.4 kb; Cm^r *rep cop*)	5
S161(pAM401EB-5)	Wild type (15.7 kb; Cm^r *SSP-5 rep cop*)[d]	5

[a] Plasmid-encoded phenotype and genotype are given in parentheses.
[b] Similar to OB277 but with *aphA-3* (15) instead of *ermAM* inserted into *cshB* (31).
[c] Donor DNA→recipient strain by transformation.
[d] *SSP-5* gene from *S. gordonii* M5 has been redesignated *sspB* (7).

MATERIALS AND METHODS

Microbial strains and cultivation. Table 1 lists the bacterial strains used in this study. Bacteria were cultured in brain heart infusion medium (Difco Laboratories, Detroit, Mich.) containing 0.5% (wt/vol) yeast extract (BHY). Cultures were inoculated from stock cell suspensions stored at −80°C in BHY medium containing 15% glycerol and were grown at 37°C in closed tubes or bottles without shaking. *C. albicans* ATCC 10261 was grown at 30°C with aeration in a salts-biotin medium containing 1% (wt/vol) glucose (10). These growth conditions were selected to minimize yeast cell aggregation, which is promoted by growth at higher incubation temperatures (9).

Construction of mutants. Methods used for the construction of insertional mutants of *S. gordonii* DL1 have been described in detail elsewhere (15, 31). Strain OB385 was generated by ligating an *aphA-3* gene cassette (15) into a *Hin*dIII site within the *cshB* gene and transformation of strain OB251 *cshA31*::*cat* (31) to kanamycin resistance (200 μg/ml). Strain OB385 cells were then used as recipients in transformation to generate strain OB392 (Table 1). Strain OB470 *scaA*::*tet* was produced as follows. On the basis of the nucleotide sequence of the 933-bp *scaA* gene (GenBank accession number L11577) from *S. gordonii* PK488 (22), two oligonucleotide primers were synthesized (DNA Express, Colorado State University, Fort Collins) in order to amplify by PCR an approximately 760-bp fragment within the *scaA* coding sequence. Sense-strand primer (5′AC GAATTCAATTATCGCAGATAT, incorporating an *Eco*RI recognition sequence [underlined]) and antisense strand primer (5′TGTGGTACCTGTCTCC ATCTTCG, incorporating a *Kpn*I recognition sequence [underlined]) were incubated with *S. gordonii* DL1 DNA (5 ng) and *Taq* in PCR (52°C for 20 s of annealing; total of 34 cycles for extension). The purified product was digested with a combination of *Eco*RI and *Kpn*I and ligated into pBluescript II KS(+) (Stratagene, La Jolla, Calif.) similarly restricted. Almost centrally located within the cloned *scaA* sequence was a unique *Hin*dIII site into which, after filling, was blunt-end ligated a 3.5-kb fragment carrying a tetracycline resistance determinant (TetM) (15). This fragment was transformed into *S. gordonii* DL1 with selection for tetracycline resistance (10 μg/ml). Confirmation that antibiotic resistance marker insertions were at the predicted sites were obtained by blot hybridizations (15, 31).

Radioactive labeling of yeast cells. Mid-exponential-growth-phase yeast cells of *C. albicans* were metabolically labeled with [^{35}S]methionine (0.62 MBq, 17 μCi, 10^3 Ci/mmol) as previously described (9). The cells were washed and suspended in TNMC buffer (1 mM Tris-HCl [pH 8.0] containing 0.15 M NaCl, 1 mM $MgCl_2$, and 1 mM $CaCl_2$ [4]) at a concentration of 2×10^8 cells per ml (optical density at 540 nm [OD_{540}] = 10.0). Specific radioactivities of between 20 and 10 cells per cpm were obtained.

Solid-phase assay of *C. albicans*-bacterium coadherence. Bacterial cells were immobilized on ScintiStrip (LKB Wallac, Turku, Finland) microtiter wells that were impregnated with scintillant to enable direct counts of bound radioactivity to be made. Remaining binding sites on the plastic were blocked with TNMC buffer containing 0.05% (vol/vol) Tween 20 (TNMC-Tween) as previously described (9). To measure adherence of *C. albicans*, radioactively labeled cells (0.1 ml, 2×10^5 cells) were added to each well, and the plates were incubated with reciprocal shaking (100 rpm) for 1 h at 20°C. Unattached yeast cells in suspension were removed by aspiration, the wells were washed four times with TNMC-Tween (0.1 ml each time), and after aspiration of the final wash fluid, the plates were allowed to dry for 10 min at 20°C. Individual wells were then snap-separated from the plate and counted for radioactivity with a Rack Beta II liquid scintillation counter (LKB). Radioactivity measurements were converted to cell numbers on the basis of the specific radioactivities of the *C. albicans* cell suspensions.

Solid-phase assay of *C. albicans* binding to CshA polypeptide fragments. Segments of the *S. gordonii* DL1 *cshA* gene (31) encoding nonrepetitive (NR) NH_2-terminal (amino acid residues 42 to 886) or amino acid repeat block repetitive (R) COOH-terminal (amino acid residues 1456 to 2263) regions of the CshA polypeptide were isolated following PCR (27). The products were cloned into the glutathione *S*-transferase fusion vector pGEX 5x-3 (Pharmacia Biotech, Uppsala, Sweden), and fusion proteins were purified from induced culture lysates by affinity chromatography followed by cleavage from glutathione *S*-transferase with factor Xa protease as previously described (27). The purified polypeptides in coating buffer (0.02 M Na_2CO_3-$NaHCO_3$ [pH 9.5]) were added to wells (0.05 ml per well; concentration range, 0 to 100 ng) of Scintistrip microtiter plate wells. Nonspecific binding sites were blocked with bovine serum albumin (BSA) (1.0 mg/ml in TNMC for 16 h at 4°C). Adherence of radioactively labeled *C. albicans* cells was then measured as described above.

Antisera and enzyme-linked immunosorbent assay (ELISA). Antibodies were raised in rabbits to a COOH-terminal truncated form of CshA purified from the culture fluid of *S. gordonii* OB186 (30); these antibodies reacted also with CshB polypeptide (31). Antibodies were raised to purified recombinant NR domain or R domain fragments of CshA as recently reported (27). Antiserum to purified cell wall polysaccharide from *S. gordonii* NCTC 7869 was obtained as previously described (9). Antibodies raised in rabbits to the purified P1 (SpaP) antigen I/II protein of *Streptococcus mutans* serotype c were provided by K. W. Knox, Institute of Dental Research, Sydney, New South Wales, Australia. These antibodies are reactive with the *S. gordonii* antigen I/II polypeptides SspA and SspB (6, 7). Rabbit antibodies to recombinant ScaA protein purified from *Escherichia coli* were provided by P. E. Kolenbrander, National Institutes of Health, Bethesda, Md. These antibodies reacted on Western blots (immunoblots) of *S. gordonii* DL1 cell envelope proteins with a single band of approximate molecular mass 35 kDa corresponding to ScaA lipoprotein (1).

To determine reactivities of strains with these antisera by ELISA, streptococcal cells were immobilized onto microtiter plate wells (Nunc, Roskilde, Denmark) (9) which were blocked by incubation with TNMC buffer containing 1% (wt/vol) gelatin for 16 h at 4°C. The buffer was aspirated, and doubling dilutions of serum in TNMC containing 0.1% (wt/vol) gelatin (TNMC-gelatin) were added to quadruplicate wells (0.05 ml per well) and incubated for 1 h at 37°C. Wells were washed three times with TNMC-Tween, and antibody binding was detected with peroxidase-conjugated swine immunoglobulins to rabbit immunoglobulins (Dako Corp., Carpenteria, Calif.) diluted 1:1,000 in TNMC-gelatin (0.05 ml). Plates were developed with 1,2-phenylenediamine as the enzyme substrate, and the OD_{492} was measured. Final titers were calculated after subtraction of background binding of the same dilution of preimmune serum to immobilized antigen. Immune sera and preimmune sera showed similar binding when tested against an irrelevant antigen (BSA).

Inhibition of *C. albicans* adherence by antisera. To determine inhibition of adherence by antisera, bacterial cells were immobilized and wells were blocked with TNMC-Tween as described above before a second blocking with 0.5% (wt/vol) BSA in TNMC-Tween for 16 h at 4°C. The wells were then washed twice with TNMC-Tween and preincubated with dilutions of preimmune or immune sera in TNMC buffer for 2 h at 37°C. The wells were again washed twice with TNMC-Tween, and adherence of *C. albicans* cells was determined as described above.

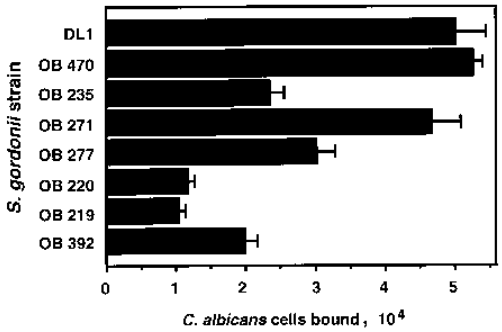

FIG. 1. Adherence of *C. albicans* ATCC 10261 cells to immobilized cells of *S. gordonii* DL1 and isogenic cell surface protein mutants (see Table 1 for details). Input cell number, 2×10^5 yeast cells per well. Values for cells bound are means of quadruplicate determinations from a representative experiment repeated twice. Error bars are standard deviations of the mean ($n = 4$).

RESULTS

Binding of *C. albicans* to cell surface protein mutants of *S. gordonii*. Previous work had identified cell wall polysaccharide extracted with 0.1 M NaOH (29) from *S. gordonii* as a major receptor for binding of *C. albicans* to streptococci (9). Alkali treatment of *S. gordonii* NCTC 7869 cells reduced by >80% the ELISA reactivity of cells with antibodies raised to the polysaccharide; however, binding of *C. albicans* ATCC 10261 cells to the immobilized alkali-treated streptococcal cells was reduced by only <50% (data not shown). Alkali treatment of streptococcal cells did not reduce significantly their reactivities in ELISA with antibodies to the cell wall-associated polypeptides CshA/B and SspA/B (not shown). These observations raised the possibility that *C. albicans* cells were interacting with *S. gordonii* polypeptide adhesins in addition to binding cell wall polysaccharide.

To determine if the *cshA*, *cshB*, *sspA*, and *sspB* gene products were involved in binding of *C. albicans* cells to *S. gordonii*, isogenic mutant strains of *S. gordonii* DL1 with these genes inactivated singly or in various combinations were used in adherence assays. Inactivation of the *cshA* gene reduced by approximately 50% the numbers of *C. albicans* cells bound to streptococcal strains OB235 *cshA3* and OB277 *cshA31 cshB2* (Fig. 1). Inactivation of *cshB* resulted in only slight reduction (<10%) of yeast cell adhesion to *S. gordonii* OB271 *cshB2* cells (Fig. 1). Greater reductions in yeast cell binding were observed for streptococcal strains with inactivated *sspA* and *sspB* genes. Cells of OB220 *sspA* and OB219 *sspA sspB* supported only 23 and 21%, respectively, of the wild-type (DL1) levels of *C. albicans* adherence (Fig. 1). Interestingly, numbers of *C. albicans* cells adhering to *S. gordonii* OB392 in which all four genes were inactivated were greater than those adhering to strain OB219 (Fig. 1). By contrast, inactivation of the *scaA* gene did not affect binding of *C. albicans* cells to *S. gordonii* OB470 *scaA* (Fig. 1).

Cell surface antigen expression by *S. gordonii* DL1 and mutants. One possible explanation for reduced binding of *C. albicans* to *cshA* and *sspA* mutants of *S. gordonii* was that these cells were deficient in cell surface polysaccharide. To test this possibility, reactivities of the wild type and mutants with antiserum against purified polysaccharide were compared by ELISA. As shown in Table 2, reactivities of strain OB277 and OB219 cells with this serum were not significantly different from those of wild-type cells, thus appearing to exclude the above explanation. Cells of strain OB470 *scaA* had approximately 25% reduced ELISA reactivity with polysaccharide antiserum, whereas strain OB392 cells exhibited significantly higher reactivity (Table 2). This increased expression and/or accessibility of surface polysaccharide might therefore account for the greater numbers of *C. albicans* cells adhering to OB392 cells than to OB219 cells (Fig. 1). We also used ELISA to determine the effects of the various insertional mutations on expression of cell surface polypeptides. Cells of strain OB277 and OB392 did not react with antibodies raised to CshA protein, as expected, while cells of OB219, OB470, and DL1 all reacted to similar degrees (Table 2). In the same way, cells of OB219 and OB392 did not react with SpaP (antigen I/II) antibodies, while DL1, OB277, and OB470 all reacted similarly. Cells from all strains except OB470 *scaA* reacted with ScaA antiserum (Table 2). These results demonstrated that the Csh and Ssp (antigen I/II) adhesin families were independently expressed on the cell surface; that is, presentation of SspA and SspB on the cell surface did not require the presence of CshA and CshB polypeptides, and vice versa.

Effects of streptococcal antisera on binding of *C. albicans* to *S. gordonii*. Antibodies reactive with cell surface antigens of *S. gordonii* (Table 2) were tested for their abilities to inhibit adherence of *C. albicans* to *S. gordonii* DL1 cells. Preincubation of immobilized *S. gordonii* cells with SpaP (antigen I/II) serum (1:30 dilution) reduced subsequent binding of *C. albicans* cells by 26% (Fig. 2A). Antibodies to purified CshA from *S. gordonii*, despite showing high-affinity reactivity with streptococcal cells (Table 2), did not inhibit *C. albicans* adherence (data not shown). However, antibodies specific to the NR domain of recombinant CshA, but not antibodies to the R domain, were effective in inhibition of *C. albicans* adherence (maximum inhibition of 16.4% at 1:30 serum) (Fig. 2B). These results showed that the NR domain of CshA was accessible on the streptococcal cell surface for interaction with *C. albicans* cells. Antipolysaccharide serum at 1:10 dilution inhibited bind-

TABLE 2. Surface expression of polysaccharide and polypeptide antigens by *S. gordonii* DL1 and isogenic mutants as determined by ELISA

S. gordonii strain (phenotype)	ELISA (OD_{492})[a] reactivities (mean ± SD [$n = 4$]) with antiserum to:			
	S. gordonii polysaccharide (1:50)	*S. gordonii* CshA protein (1:1,000)	*S. mutans* SpaP (antigen I/II) protein (1:2,000)	Recombinant *S. gordonii* ScaA protein (1:500)
DL1 (wild type)	0.062 ± 0.005	0.484 ± 0.036	0.619 ± 0.033	0.192 ± 0.010
OB277 ($CshA^-$ $CshB^-$)	0.068 ± 0.004	0.000 ± 0.000	0.599 ± 0.032	0.247 ± 0.021
OB219 ($SspA^-$ $SspB^-$)	0.065 ± 0.007	0.481 ± 0.039	0.003 ± 0.000	0.230 ± 0.017
OB392 ($CshA^-$ $CshB^-$ $SspA^-$ $SspB^-$)	0.090 ± 0.005	0.008 ± 0.000	0.000 ± 0.000	0.212 ± 0.009
OB470 ($ScaA^-$)	0.046 ± 0.004	0.447 ± 0.020	0.574 ± 0.050	0.000 ± 0.000

[a] A total of 2×10^7 streptococcal cells were immobilized, and primary antisera were diluted as indicated. ELISA values were corrected for values obtained with preimmune sera (at equivalent dilutions).

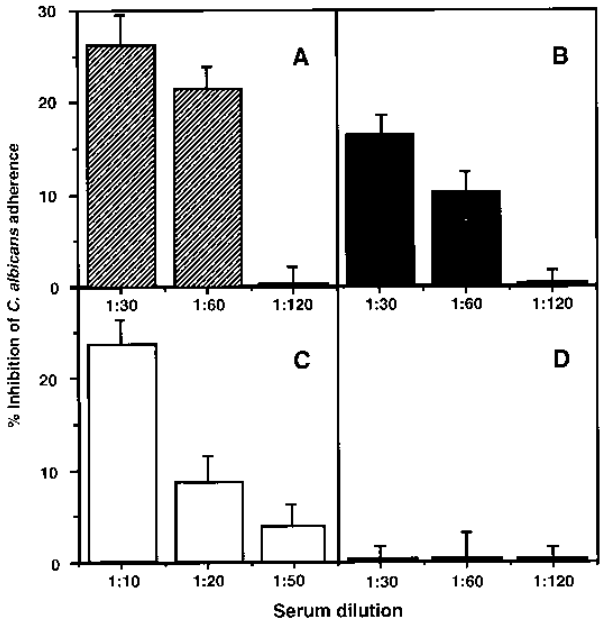

FIG. 2. Effects of antibodies to streptococcal cell surface antigens on adherence of *C. albicans* cells to immobilized cells of *S. gordonii* DL1. Values are percent inhibition of adherence of *C. albicans* (input, 2×10^5 cells per well) compared with adherence to *S. gordonii* cells treated with an irrelevant rabbit antiserum at equivalent dilution. (A) SpaP (antigen I/II) antiserum; (B) antibodies to the NR domain of recombinant CshA; (C) *S. gordonii* NCTC 7869 cell surface polysaccharide antiserum; (D) recombinant ScaA antiserum. Values are the means of quadruplicate determinations from a representative experiment repeated twice. Error bars are standard deviations of the mean ($n = 4$).

ing of *C. albicans* cells by 23% (Fig. 2C), confirming previous results (9). For each of these sera, degree of inhibition of *C. albicans* adherence was inversely related to serum dilution (Fig. 2). Although antibodies to ScaA protein reacted with the *S. gordonii* cell surface (Table 2), no inhibition of *C. albicans* adherence to streptococci was observed by ScaA antiserum at 1:30 dilution (Fig. 2D).

Adherence of *C. albicans* to domains of CshA. Polypeptide fragments comprising the NR or R domain of recombinant CshA were immobilized on microtiter plate wells. Binding of these polypeptides to the well surfaces was confirmed by ELISA with domain-specific antisera. Antibodies to the COOH-terminal truncated form of CshA purified from *S. gordonii* were 10-fold more reactive in ELISA with the R domain fragment than with the NR domain fragment.

The numbers of *C. albicans* cells binding to NR or R domain fragments of CshA were, in each case, proportional to the amount of polypeptide immobilized, with a maximum of 1.26×10^5 cells (63% input) or 9.6×10^4 cells (48% input), respectively, bound to 50 ng of NR or R domain polypeptide (Fig. 3). Nonspecific binding to immobilized BSA or glutathione *S*-transferase was <10% input yeast cells (not shown).

Adherence of *C. albicans* to antigen I/II polypeptide. To demonstrate that *C. albicans* cells bound directly to streptococcal antigen I/II proteins as well as to CshA, we used recombinant *Enterococcus faecalis* S161(pAM401EB-5) carrying the cloned *sspB* gene from *S. gordonii* M5 (5). Cells of the recombinant strain, but not cells of the parental strain S161(pAM401), produced cell surface SspB antigen, as confirmed by ELISA using SpaP antiserum (Table 3). Binding of *C. albicans* to *E. faecalis* S161 cells expressing SspB polypeptide was more than threefold greater than binding to the control (parental) strain,

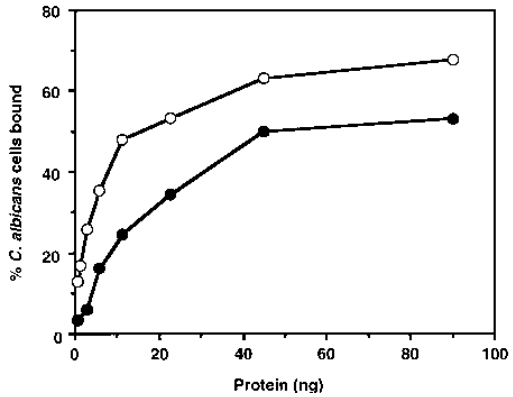

FIG. 3. Adherence of *C. albicans* to purified NR domain (○) or R domain (●) fragments of recombinant CshA polypeptide immobilized onto microtiter wells. Points are the means of quadruplicate determinations from a representative experiment repeated twice. Errors did not exceed 10% of the means.

and adherence was ablated by preincubation of enterococci with SpaP antiserum (Table 3). Neither strain of *E. faecalis* reacted with antibodies raised to *S. gordonii* polysaccharide.

DISCUSSION

The linear cell wall polysaccharides extracted from *S. gordonii* or *S. oralis* cells with dilute alkali or by heating act as receptors for binding of *C. albicans* (9). The polysaccharides characteristically contain a combination of glucose, galactose, rhamnose, *N*-acetyl-D-glucosamine, and *N*-acetyl-D-galactosamine (9, 23). The ligand structure within these polysaccharides that is recognized by the yeast cell adhesin(s) is not known. Given the similarity of glycosyl linkages in these bacterial polysaccharides with those found on eukaryotic cell surface oligosaccharide receptors, it seems likely that the *Candida* adhesin(s) mediating binding to epithelial and/or endothelial cells (see the introduction) may also be involved in streptococcal cell binding. Two lines of evidence suggested, however, that binding to streptococcal polysaccharide was not the sole mechanism involved in *C. albicans* adherence to *S. gordonii*. First, alkali treatment removed >80% surface polysaccharide from streptococcal cells, but these cells still supported adhesion of *C. albicans* in the solid-phase assay. Second, antibodies raised to the polysaccharide never inhibited by more than 25% the adherence of *C. albicans* to streptococci (Fig. 2C).

The idea that *C. albicans-S. gordonii* coadherence resulted from interactions in addition to the *Candida* lectin-streptococcal carbohydrate reaction was confirmed by experiments with

TABLE 3. Binding of *C. albicans* to cell surface-expressed SspB (antigen I/II) polypeptide in *E. faecalis*

E. faecalis strain	ELISA reactivity with SpaP antiserum (1:2,000) ($OD_{492} \pm$ SD [$n = 4$])	*C. albicans* cells bound (10^4) ± SD ($n = 4$) in the presence of[a]:	
		Irrelevant rabbit serum	SpaP (antigen I/II) antiserum
S161(pAM401)	0.00 ± 0.00	0.56 ± 0.09	0.50 ± 0.04
S161(pAM401EB-5)	0.51 ± 0.02	1.9 ± 0.20	0.54 ± 0.04

[a] Immobilized *E. faecalis* cells were incubated with serum (0.05 ml of 1:50 dilution in TNMC buffer) for 2 h at 37°C before addition of *C. albicans* cells (2×10^5) and measurement of cells bound as described in Materials and Methods.

isogenic mutants of *S. gordonii* altered in expression of specific cell surface polypeptides. Thus, mutants in which the *cshA* or *sspA* gene was inactivated were much reduced in ability to support yeast cell adherence, *sspA* mutants being more reduced in this capacity than *cshA* mutants. These observations are similar in trend to those made for *S. gordonii-A. naeslundii* coadherence, in which case loss of CshA (259 kDa) resulted in 50 to 70% reduction in adherence of streptococci to actinomyces (27, 31), depending on the *A. naeslundii* strain used, while loss of SspA (171 kDa) resulted in up to 90% reduction in adherence to actinomyces (7, 17). On the other hand, a role (if any) for CshB (approximate molecular mass, 245 kDa) in mediating *C. albicans* adhesion is not clear. Although inactivation of *cshB* led to a small reduction in *C. albicans* cell adherence, we have shown recently, using CshA-specific antibodies, that *cshB* mutants of *S. gordonii* have reduced cell surface expression of CshA (27). These data, taken together with the results indicating that the NR domain of CshA, which is not present in CshB (27), carries the major adhesin determinants for *A. naeslundii* (27) and for *C. albicans* (this report), suggest that CshB may play a supporting role for CshA-mediated adherence reactions. Confirmation of this possibility might be obtained following purification of the CshB polypeptide and an analysis of its binding properties.

Delineation of the relative functional properties of the SspA and SspB proteins is also not yet possible. These polypeptides are 82% identical in amino acid sequence across their lengths, and the cell adhesion-mediating sequences for salivary glycoproteins (18) within the COOH-terminal halves of the polypeptides are identical (7). There is evidence to suggest that both SspA and SspB polypeptides are adhesins. First, inactivation of the *sspA* gene resulted in 50% or greater reduction in adherence of *S. gordonii* cells to salivary agglutinin glycoprotein (17), *A. naeslundii* (7, 17), and *C. albicans* (Fig. 1). Second, purified SspB protein has been shown to bind salivary agglutinin glycoprotein in vitro (8), and expression of SspB on the *E. faecalis* cell surface confers on these cells the ability to bind agglutinin (5) as well as *C. albicans* (Table 3). Currently then, one possibility is that the SspA and SspB are adhesins that function most effectively on the cell surface as a heterodimer or as some other adhesin complex. Clarification of the relative levels of expression and functions of SspA and SspB polypeptides awaits further genetic analyses, comparison of the binding properties of the purified polypeptides, and the generation of monospecific antisera to these proteins.

The evidence presented suggests that streptococcal Csh and Ssp wall-associated proteins, but not ScaA lipoprotein, are involved in *C. albicans-S. gordonii* coadherence, in addition to streptococcal cell surface polysaccharide. It is clear that at least for CshA and SspB, these proteins may bind directly to *C. albicans* cells. The regions of SspB protein involved in binding are under investigation. For CshA, the major adhesion determinants may reside within the NR domain of CshA, as suggested by the observation that antibodies raised specifically to the NR domain were effective in inhibiting *C. albicans* adherence to *S. gordonii*. Although *C. albicans* cells bound to NR domain and to R domain recombinant polypeptide fragments (Fig. 3), the available evidence argues against the R region of CshA as carrying major adhesive determinants on the streptococcal cell surface. Thus, despite exhibiting high affinities of binding to streptococcal cells, neither antibodies raised to the R domain fragment nor antibodies raised to the truncated CshA polypeptide (which were chiefly immunoreactive with the R domain) were effective at inhibiting *C. albicans* adhesion to *S. gordonii* cells. Presumably, therefore, recognition of the NR domain is sufficient for CshA-mediated *C. albicans* cell adherence to streptococci. This interpretation of the relative functional activities of the NR and R domains of CshA is also consistent with evidence that the latter is associated with determining streptococcal cell surface hydrophobicity (28).

S. gordonii has acquired adhesins which recognize a range of prokaryotic and eukaryotic receptors in the oral environment, as well as a polysaccharide receptor that has been shown thus far to be recognized by *C. albicans*. The availability of multiple adhesin-receptor interactions might present a colonization advantage to organisms by increasing the strength of attachment to substrates and the range of available attachment sites. The presence of multiple adhesin-receptor interactions between *S. gordonii* and *C. albicans* may act to stabilize the intermicrobial association. However, it is difficult to assess precisely the relative contributions of Csh proteins, Ssp proteins, and polysaccharide to the overall adherence of yeast to streptococcal cells. In fluid-phase coaggregation of *S. gordonii* and *C. albicans* cells, the recognition by yeast cells of the streptococcal polysaccharide may be a key interaction, since coaggregation is completely blocked by microgram quantities of exogenously added polysaccharide (9). The polysaccharide is arguably a significant determinant in the solid-phase interaction between *C. albicans* and *S. gordonii*. This possibility is suggested by previous data showing that levels of *C. albicans* binding to a range of streptococcal strains correlated well with levels of yeast cell adherence to isolated cell wall polysaccharides extracted from the respective strains (9). However, the Csh and Ssp proteins also contribute significantly to coadherence, as demonstrated by antibody inhibition experiments and by the dramatic reduction in ability of mutants disrupted in *csh* and *ssp* genes to support *C. albicans* cell binding. Thus, it appears that within the complex macromolecular environment of the streptococcal cell surface, the favored oligosaccharide receptor for yeast cells must be present in conjunction with the Ssp and Csh proteins in order to achieve maximal levels of *C. albicans* cell adherence. *S. gordonii*, *S. oralis*, and *S. sanguis* all produce Csh-like antigens (28), antigen I/II polypeptides (26), and linear cell wall polysaccharides (9, 23), which may explain why strains of these species support the highest-affinity binding of yeast cells (9, 16). On the other hand, organisms such as *S. mutans* and *E. faecalis* do not present the necessary cell surface architecture to support *C. albicans* adherence (9, 16). Multiple adhesin-receptor interactions are now recognized as being important in the establishment of oral bacterial communities, and in particular it may be envisaged that they are crucial in permitting less prolific microorganisms, such as *C. albicans*, to be retained within the highly competitive oral microbial ecosystem.

ACKNOWLEDGMENTS

We thank D. R. Demuth, K. W. Knox, and P. E. Kolenbrander for the gifts of strains and antisera, and we thank P. E. Kolenbrander for communication of data prior to publication.

This research was supported by the Health Research Council of New Zealand. H.F.J. gratefully acknowledges receipt of a Commonwealth Medical Fellowship.

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Editor: T. R. Kozel