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Adherence of *Pseudomonas aeruginosa* and *Candida albicans* to Glycosphingolipid (Asialo-GM_1) Receptors Is Achieved by a Conserved Receptor-Binding Domain Present on Their Adhesins

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***Pseudomonas aeruginosa*, a gram-negative bacterium, and *Candida albicans*, a dimorphic yeast, are evolutionarily distant microorganisms which can utilize filamentous structures termed pili and fimbriae, respectively, to mediate adherence to glycosphingolipids (asialoganglioside-GM_1) receptors. The mechanism of adherence to glycosphingolipid receptors was investigated in these studies. By using monoclonal antibodies (MAbs) against purified pili of *P. aeruginosa* PAK (PK99H) and monospecific anti-peptide antibodies against the PAK pilin peptides [anti-PAK(128–144) and anti-PAK(134–140)], we demonstrated that these antibodies agglutinated *C. albicans* whole cells and cross-reacted with *C. albicans* fimbriae in immunoblots. A control MAb, PKL1, and anti-PAK(75–84) peptide antibodies failed to agglutinate *C. albicans* whole cells or cross-react with the fimbrial proteins. Conversely, the anti-*C. albicans* fimbrial MAb Fm16, but not Fm34, agglutinated *P. aeruginosa* PAK whole cells and Western blots (immunoblots). The interactions between PK99H and Fm16 and their respective homologous antigens were competitively inhibited by heterologous antigens; this demonstrated that the interactions between the antibodies and the heterologous antigens, i.e., PK99H with *C. albicans* fimbriae and Fm16 with *P. aeruginosa* pili, were highly specific and suggested that both adhesins share a common antigenic determinant. The immunological cross-reactivity between Fm16 and *P. aeruginosa* PAK pilin is localized onto the PAK(134-140) region as shown by a competitive enzyme-linked immunosorbent assay. The PAK(134–140) region of PAK pilin contains the epitope recognized by PK99H and also constitutes part of the receptor-binding domain of the pilus adhesin. Thus, the results from these studies suggest that common cell surface receptors are recognized by the *P. aeruginosa* and *C. albicans* adhesins because of a conserved receptor-binding domain on the adhesins.**

Glycolipids and glycoproteins are ubiquitous macromolecules found on cell surfaces and present good sources of anchor sites or receptors to microorganisms to attach to host cells for colonization and subsequent infection of the host tissues (18). The adherence of microorganisms to host cell surface receptors is a complex process and may involve more than one mechanism of adherence and/or more than a single adhesin. *Pseudomonas aeruginosa*, a gram-negative bacterium, employs a number of adhesins, including alginate (8, 39), pili (15, 40, 43), and other nonpilus adhesins (2, 28, 38, 41), to mediate its adhesion to the epithelial cells of the host. Although the adherence of *Candida albicans*, a dimorphic yeast, to host cells is well established (19, 30), the mechanism is not as well defined as that of *P. aeruginosa*; the available literature suggests that at least four reputed adhesins may be utilized in yeast adherence (4, 6).

We have observed that the same receptors may be utilized by adhesins of a variety of pathogenic species and have thus explored the possibility that these adhesins may have a conserved structural motif or conserved antigenic epitope. *C. albicans* possesses long filamentous fimbriae that act as adhesins in mediating attachment of yeast cells to human buccal epithelial cells (49). The purified *C. albicans* fimbria contains a major fimbrial subunit with a molecular mass of 66 kDa that is highly glycosylated (85% of the molecular mass consists of D-mannose residues). *C. albicans* fimbria-mediated adherence to buccal epithelial cells utilizes asialoganglioside-GM_1 (asialo-GM_1) and asialoganglioside-GM_2 (asialo-GM_2) receptors (50). Baker et al. (1) have shown that *P. aeruginosa* binds to glycosphingolipids isolated from various mammalian sources. *P. aeruginosa* has been demonstrated to utilize asialo-GM_1 as receptors for adhesion to corneal epithelium of mice (12), and we have demonstrated that the *P. aeruginosa* pili bound to asialo-GM_1 receptors in in vitro assays (27).

The structural similarity and similar receptor specificity of the *P. aeruginosa* pilus adhesin and the *C. albicans* fimbrial adhesin led us to explore whether these two adhesins might have a conserved structural feature that would manifest itself as a cross-reactive antigenic epitope. The receptor-binding domain of the *P. aeruginosa* pilus adhesin resides in the carboxy-terminal region of the pilin structural proteins, and a range of antibodies specific for this region of the pilin structural protein is available (7, 15, 25, 26, 27, 45). In this study, we employed anti-*P. aeruginosa* pilus antibodies and anti-*C. albicans* antibodies to demonstrate that a conserved epitope is present on the *Pseudomonas* pilin adhesin and the *Candida* fimbrial adhesin. The ability of anti-fimbrial antibodies to bind

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to the receptor-binding domain of the *Pseudomonas* PAK pilin adhesin suggests that the conserved epitope present on the fimbrial adhesin is involved in fimbrial binding to asialo-GM_1.

MATERIALS AND METHODS

Purification of *P. aeruginosa* PAK pili and *C. albicans* fimbriae. The culture conditions of *P. aeruginosa* PAK and the purification of PAK pili have been described previously by Paranchych et al. (35).

Fimbriae were purified from the yeast phase of *C. albicans* 40. *C. albicans* 40 was isolated from the trachea of an intubated intensive care unit patient at Toronto General Hospital, Toronto, Ontario, Canada. Yeast cells were cultured on Sabouraud dextrose agar as described previously by Yu et al. (49). *C. albicans* fimbriae were sheared from the surface of the cell by homogenization and purified by size exclusion high-performance liquid chromatography (49).

Protein concentration. Protein concentrations were determined by the method of Lowry et al. (29) with bovine serum albumin (BSA) as the standard.

Anti-*P. aeruginosa* pilus and anti-*C. albicans* fimbrial antibodies. Monoclonal antibodies (MAbs) were raised against purified *P. aeruginosa* PAK pili in female BALB/c mice (Charles River Breeding Laboratories, Inc.). The immunization protocols, NS1 myeloma cell line, and culture conditions used along with the fusion protocols and hybridoma selection have been described previously (7). Anti-pilus MAbs PK99H and PKL1 used in these studies are of the subtypes immunoglobulin G1(κ) [IgG1(κ)] and IgG3(κ), respectively. PKL1 and PK99H both recognize the 17-residue receptor-binding domain of the PAK pilin. However, while the PKL1 epitope has not been determined, it is different from PK99H. Anti-*C. albicans* fimbrial MAbs were prepared against a purified fraction of the fimbrial preparation by the protocols of Doig et al. (7) as described previously by Yu et al. (49). Anti-fimbrial MAb Fm16 is an IgG2a(κ), and MAb Fm34 is an IgG2(κ).

Rabbit polyclonal anti-PAK(128–144) and anti-PAK(134–140) anti-peptide antibodies were raised against the respective KCTSDQDEQFIPKGCSK-OH and DEQFIPK-NH_2 peptides (amino acid residues are represented by the standard single-letter code; NH_2 represents an amidated carboxyl terminal; OH represents the α-carboxyl group) corresponding to the different regions of the *P. aeruginosa* PAK pilin protein (42). In these studies, the sulfhydryl groups on the two cysteine residues (residues 129 and 142) on the PAK(128–144) peptide have been cyclized to form an intrachain disulfide bond. In the production of anti-peptide antibodies, these peptides were synthesized with a benzoylbenzoyl moiety, a norleucine, and a two-glycine spacer at the amino terminal. The peptides were conjugated onto keyhole limpet hemocyanin protein carriers via the photoreactive benzoylbenzoyl moiety as described previously (26, 37). In competitive enzyme-linked immunosorbent assays (ELISAs) in which synthetic peptides were used, the amino termini of these peptides were acetylated. A control peptide, PAK(75–84), with a GVAADANKLG-NH_2 sequence was included in the competitive ELISA. The syntheses of PAK(75–84), PAK(128–144), and PAK(134–140) were carried out on an Applied Biosystems model 430A automated peptide synthesizer as described previously (48). The purification of these peptides by high-performance liquid chromatography and the characterization and analyses of the peptides have also been described previously (48). The immunization protocols in rabbits to obtain anti-peptide antibodies were performed as described by Lee et al. (26).

Agglutination assays. *C. albicans* was cultured in M9 medium under the conditions described previously by Yu et al. (49). *P. aeruginosa* PAK was cultured on M9 medium by the method of McEachran and Irvin (31). In both cultures, the cells were harvested and washed three times with 10 mM phosphate-buffered saline (pH 7.2) containing 150 mM NaCl (PBS) by centrifugation (10,000 × *g* for 10 min). Cell concentrations were determined on a hemocytometer and adjusted to 2×10^7 cells per ml. Aliquots (50 μl) were added to wells of a 96-well microtiter plate and incubated with equal volumes of serially diluted solutions of antibodies [PK99H, PKL1, Fm16, Fm34, Anti-PAK(128–144), and Anti-PAK(134–140)]. Commercially available affinity-purified normal mouse IgG and normal rabbit IgG (Jackson ImmunoResearch Laboratories) were also employed as controls in these assays. A second set of controls with the bacteria or yeast incubated with 10 mM PBS was included to account for the background in these agglutination analyses. After a 10-min incubation period at 37°C, the cells in the microtiter wells were examined under a phase-contrast microscope, and the results were scored.

Competitive ELISA. Two sets of competitive ELISAs were performed to assess the interactions between the adhesins and the respective cross-reactive antibodies. The first set of competitive ELISAs was carried out to study the interactions between the cross-reacting antibody and whole protein antigens. *Candida* fimbriae (0.5 μg per well) and *Pseudomonas* PAK pili (0.5 μg per well) were coated onto 96-well polystyrene Nunc plates by the addition of 100 μl of the respective antigens in 0.01 M sodium carbonate (pH 9.5) per well. The plates were incubated at room temperature for 6 h and then washed three times with PBS (pH 7.4) supplemented with 0.05% (wt/vol) BSA (buffer A). Excess sites on the microtiter wells were blocked with 5% (wt/vol) BSA in PBS (pH 7.4). After three washes with buffer A, different concentrations of the competing heterologous antigens and the antibody solutions (working titers of 1:1,000 based on the titers of the antibody solutions against their respective homologous antigens in direct ELISA) were premixed prior to addition to precoated microtiter wells containing PAK pili (0.5 μg per well) or *Candida* fimbriae (0.5 μg per well). In the assays with immobilized PAK pili, PK99H or PKL1 was mixed with serially diluted *C. albicans* fimbriae and then added to the microtiter wells (100 μl per well). With *C. albicans* fimbriae as the immobilized antigens, Fm16 or Fm34 was mixed with serially diluted *P. aeruginosa* PAK pili before addition to the microtiter wells. A positive control, consisting of the same working dilution of the antibody incubated with immobilized antigens, was included to assess the reduction in antibody binding in assays containing competing heterologous antigens. The assay mixtures were incubated at 37°C for 2 h. Goat anti-mouse IgG (heavy chain plus light chain) horseradish peroxidase conjugates were added to the wells as secondary antibodies. The wells were washed five times with 250 μl of buffer A per well, and a substrate solution containing 1 mM 2,2′-azino-di-(3-ethylbenzthiazoline) sulfonic acid in 10 mM sodium citrate buffer (pH 4.2; 125 μl per well) was added. The reaction was quenched with sodium azide, and the A_{405} values were determined.

The second set of competitive ELISAs was performed to study the interactions between Fm16 and synthetic peptides corresponding to the receptor-binding region of the *Pseudomonas* PAK pilin adhesin. In these assays, synthetic peptides competed with immobilized *C. albicans* fimbriae for Fm16 binding. Acetylated PAK(75–84) (a control peptide), PAK(128–144), and PAK(134–140) peptides were serially diluted with buffer A and premixed with Fm16 (1:200) before addition to precoated microtiter wells. The assay mixtures in the

TABLE 1. Agglutination of *C. albicans* 40 with antibodies raised against *Candida* fimbriae and *P. aeruginosa* PAK pili

Antibody[a]	Agglutination[b] at antibody dilution of:		
	1:2	1:4	1:8
Fm16	++++	++++	++
Fm34	++++	++++	+++
PK99H	+++	++++	++
PKL1	−	−	−
Anti-PAK(128–144)	++++	+++	++
Anti-PAK(134–140)	++++	+++	++
Normal mouse IgG	−	−	−
Normal rabbit IgG	+	+	+

[a] Fm16 and Fm34 are MAbs raised against *C. albicans* fimbriae (49); PK99H and PKL1 are MAbs raised against *P. aeruginosa* pili from strain PAK (8); anti-PAK(128–144) and anti-PAK(134–140) are anti-peptide antibodies raised against peptides that correspond to the *P. aeruginosa* PAK pilin sequence (25); normal mouse IgG and normal rabbit IgG are commercially available affinity-purified IgG.

[b] Agglutination was assessed qualitatively by phase-contrast microscopy and is reported on a scale of no agglutination (−) to heavy agglutination (++++). A control which consisted of PBS only showed no agglutination.

TABLE 2. Agglutination of *P. aeruginosa* PAK with antibodies raised against *Candida* fimbriae and *P. aeruginosa* PAK pili

Antibody[a]	Agglutination[b] at antibody dilution of:		
	1:2	1:4	1:8
Fm16	++++	+++	++
Fm34	+	+	±
PK99H	++++	++++	+++
PKL1	++++	++++	++
Anti-PAK(128–144)	++++	++++	+++
Anti-PAK(134–140)	++++	++++	+++
Normal mouse IgG	+	+	+
Normal rabbit IgG	ND[c]	ND	ND

[a] Fm16 and Fm34 are MAbs raised against *C. albicans* fimbriae (49); PK99H and PKL1 are MAbs raised against *P. aeruginosa* pili from strain PAK (8); anti-PAK(128–144) and anti-PAK(134–140) are anti-peptide antibodies raised against peptides that correspond to the *P. aeruginosa* PAK pilin sequence (25); normal mouse IgG and normal rabbit IgG are commercially available affinity-purified IgG.

[b] Agglutination was assessed qualitatively by phase-contrast microscopy and is reported on a scale of no agglutination (−) to heavy agglutination (++++). A control which consisted of PBS only showed no agglutination.

[c] ND, not done.

microtiter plate were incubated at 37°C for 2 h. The remainder of the protocols were done like the first set of competitive ELISAs described above.

SDS-PAGE and immunoblotting of pilus and fimbrial adhesins. The discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) described by Laemmli and Favre (23) was used in the chromatography of *C. albicans* fimbriae and *P. aeruginosa* PAK pili and involved the use of 10% gels in a mini-gel apparatus (Mini-Protean II dual slab cell; Bio-Rad Laboratories). Samples were solubilized by heating at 100°C for 15 min in 0.06 M Tris buffer (pH 6.8) containing 2% (wt/vol) SDS, 5% (vol/vol) β-mercaptoethanol, and 10% (vol/vol) glycerol. Electrophoresis was conducted under a constant voltage of 200 V from a Bio-Rad model 1420A power supply. Proteins were blotted onto prewetted nitrocellulose membrane by using a Mini Trans-Blot electrophoretic transfer cell (Bio-Rad) under a constant current of 300 mA (model 200/2.0 power supply; Bio-Rad) for 30 min. Prestained molecular weight markers were used to ensure the transfer of proteins from the gel to the nitrocellulose membrane. The remainder of the immunoblotting procedure was carried out as described previously by Yu et al. (49).

RESULTS

Agglutination of *C. albicans* with anti-*P. aeruginosa* pilus adhesin antibodies. *C. albicans* expresses fimbrial structures on its cell surfaces as demonstrated previously by electron microscopy (49). MAbs have been raised against the purified *C. albicans* fimbriae (49), and two of these MAbs, Fm16 and Fm34, were used in these agglutination assays. *C. albicans* 40, a clinical isolate, was agglutinated by Fm16 and Fm34 as shown in Table 1. When MAbs raised against the *P. aeruginosa* PAK pilus adhesin (PK99H and PKL1) (see reference 7) were utilized in the agglutination assay, it was demonstrated that the PK99H MAb raised against a bacterial adhesin was also effective in the agglutination of the yeast (Table 1). However, neither PKL1 nor commercial normal mouse IgG was able to agglutinate *C. albicans*. The epitope of PK99H is a linear epitope (residues 134 to 140) located within the disulfide-looped carboxy-terminal region of the 144-amino-acid-residue PAK pilus subunit (48). When anti-PAK(128–144) and anti-PAK(134–140) rabbit polyclonal anti-peptide antibodies were employed, they were both able to agglutinate *C. albicans* whole cells (Table 1). The PAK(128–144) peptide represents the 17 residues that encompass the entire disulfide loop at the carboxy terminus of PAK pilin (26, 42). A normal rabbit IgG used as a control resulted in very limited clumping of *C. albicans* whole cells. These results suggested that a conserved antigenic epitope present on the surface of the *C. albicans* whole cell is similar to that on the pilin molecule of *P. aeruginosa* PAK recognized by PK99H and the two anti-PAK peptide antibodies.

Agglutination of *P. aeruginosa* with anti-*C. albicans* fimbrial antibodies. Reciprocal assays were carried out to assess the abilities of anti-*C. albicans* fimbrial antibodies to agglutinate *P. aeruginosa* PAK whole cells. As expected, MAbs against *P. aeruginosa* PAK pili (PK99H and PKL1) and anti-PAK peptide antibodies [PAK(128–144) and PAK(134–140)] were able to agglutinate *P. aeruginosa* PAK whole cells (Table 2). Anti-*C. albicans* fimbrial MAb Fm16 also resulted in agglutination of *P. aeruginosa*. The ability of Fm34 to agglutinate *P. aeruginosa* was not significantly different from that of the control normal mouse IgG. This demonstrated that Fm16 recognized a surface antigen that is present on *P. aeruginosa*.

Western blot (immunoblot) analyses. The abilities of anti-*C. albicans* fimbrial antibodies and anti-*P. aeruginosa* pilus antibodies to cross-react with heterologous antigens were demonstrated by Western blot analyses. A concentrated crude *C. albicans* fimbrial preparation was used in these assays. Aliquots containing equal amounts of fimbriae were separated by SDS-PAGE, blotted onto a nitrocellulose membrane, and probed with different antibodies (Fig. 1A). Fm16 and Fm34 (Fig. 1A, lanes 1 and 2) bound to a 66,000-molecular-weight (66K) band as expected. Additional bands at 47K (Fm16 and Fm34), 54K (Fm16 only), and 39K (Fm34 only) were also observed. Since the fimbrial subunit is heavily glycosylated, as noted by Yu et al. (49), the presence of more than one major band may have resulted from different degrees of glycosylation of the fimbrial subunit. Fm16 and Fm34 probably recognize different epitopes since the intensities of the 66K and 47K bands and the banding patterns are markedly different; these observations could be due to the different degrees of exposure of the epitopes arising from differences in glycosylation or the inherent differences in the affinities of the two anti-fimbrial

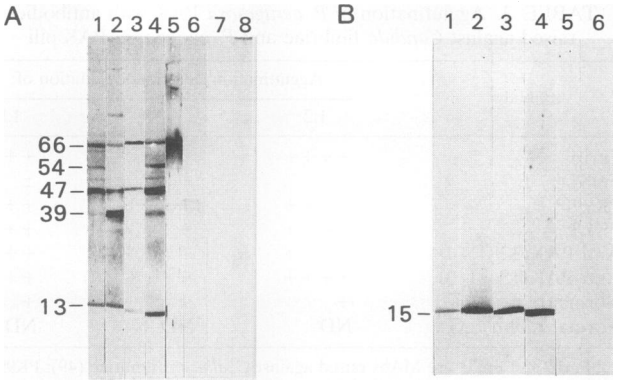

FIG. 1. Binding of anti-*P. aeruginosa* pilin adhesin antibodies and anti-*C. albicans* fimbrial adhesin antibodies to homologous and heterologous antigens in Western blot analyses. Proteins were separated by SDS-PAGE on 10% cross-linked gels and blotted onto nitrocellulose membranes. (A) *C. albicans* fimbrial proteins (10 μg per lane) were probed with Fm16, Fm34, PK99H, anti-PAK(128–144), anti-PAK (134–140), anti-PAK(75–84), normal rabbit IgG, and normal mouse IgG (lanes 1 to 8, respectively). (B) *P. aeruginosa* PAK pilin proteins (3 μg per lane) were probed with Fm16, PK99H, anti-PAK(128–144), anti-PAK(134–140), Fm34, and normal mouse IgG (lanes 1 to 6, respectively). The appropriate goat anti-mouse IgG-alkaline phosphatase or goat anti-rabbit IgG-alkaline phosphatase conjugates were employed as secondary antibodies. Bands were obtained with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate as substrates.

MAbs for fimbrial binding. Different degrees or patterns of glycosylation of the fimbrial adhesin likely result in multiple reactive species in the Western blots. The more-diffuse staining pattern observed in lane 5 (Fig. 1A) of the Western blot with the rabbit polyclonal anti-PAK(134–140) peptide antibody reflects a slightly higher antibody concentration relative to those of the other MAbs. When anti-*Pseudomonas* pilin antibodies were utilized in the Western analyses to probe for fimbrial binding, PK99H and anti-PAK(128–144) (Fig. 1A, lanes 3 and 4) also bound to the 66K and 47K bands. Anti-PAK(134–140) (lane 5) antibodies had strong affinities only for the 66K band. Interestingly, anti-PAK(128–144) also recognized the 54K and 39K bands observed individually with Fm16 and Fm34. Control antibodies consisting of anti-PAK(75–84) and normal rabbit IgG and normal mouse IgG (lanes 6 to 8) did not bind to the 66K or 47K bands. In addition, there is a low-molecular-weight 13K band (which may be a deglycosylated fimbrial subunit) observed with Fm16, Fm34, PK99H, and anti-PAK(128–144) (lanes 1 to 4).

Reciprocal studies with *P. aeruginosa* PAK pili that have been electrophoresed, blotted onto a nitrocellulose membrane, and probed with the respective antibodies were conducted (Fig. 1B). All of the anti-*Pseudomonas* pilin antibodies, PK99H, anti-PAK(128–144), and anti-PAK(134–140), bound to the 15K PAK pilin band (lanes 2 to 4). Fm16 also bound to the 15K PAK pilin band (lane 1), but Fm34 did not recognize the *Pseudomonas* antigen (lane 5). A control normal mouse IgG did not react with the PAK pilin (lane 6). These observations suggested that the two adhesins found in the bacterium *P. aeruginosa* and the yeast *C. albicans* share a common antigenic determinant which allowed recognition by heterologous antibodies and that PK99H and Fm16, two MAbs raised against antigens prepared from different species of organisms, share a common antigenic epitope.

Competitive ELISAs using heterologous antigens. The specificity of the interactions between the antibodies with heterologous antigens could be demonstrated by competitive ELISAs. *P. aeruginosa* PAK pili were immobilized onto microtiter wells, and *C. albicans* fimbriae were employed as the competing antigens (Fig. 2A). The binding of PK99H to immobilized PAK pili was inhibited by increasing amounts of *C. albicans* fimbriae. *C. albicans* fimbriae were able to reduce PK99H binding to PAK pili by greater than 60%. Unlike PK99H, which cross-reacts with fimbriae, PKL1 does not bind the yeast antigen (data not shown). Hence, the binding of PKL1 to immobilized PAK pili was not affected greatly by the addition of *C. albicans* fimbriae (Fig. 2A). When *C. albicans* fimbriae were employed as the immobilized antigens, PAK pili were used as competitors to inhibit Fm16 and Fm34 binding to the fimbriae (Fig. 2B). PAK pili were effective in reducing Fm16

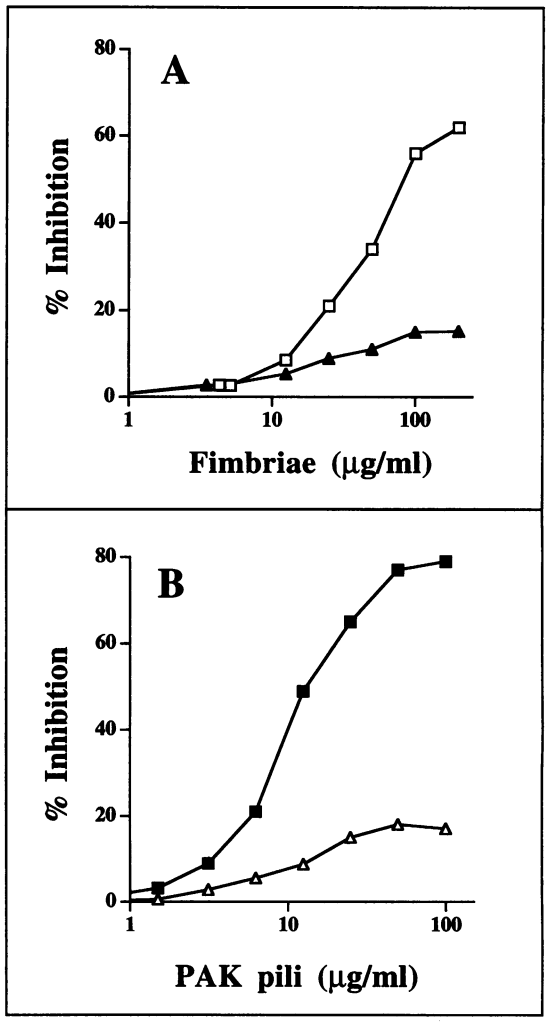

FIG. 2. Specificities of the interactions between anti-adhesin MAbs and heterologous antigens. (A) *P. aeruginosa* PAK pili were employed as immobilized antigens (0.5 μg per well), and inhibition of the binding of PK99H (□) and PKL1 (▲) to pili by competing *C. albicans* fimbriae was determined. (B) *C. albicans* fimbriae were employed as immobilized antigens (0.5 μg per well), and inhibition of the binding of Fm16 (■) and Fm34 (△) to fimbriae by competing *P. aeruginosa* PAK pili was determined. The specificities of the interactions between competing heterologous antigens and the respective MAbs are represented as the percent inhibition of the antibody binding to its own antigen as a result of the competitor.

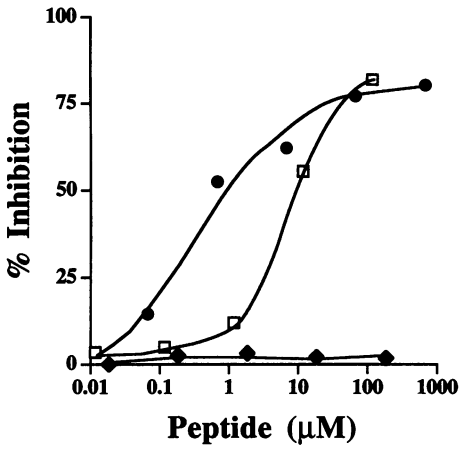

FIG. 3. Interactions between anti-*C. albicans* fimbrial MAb Fm16 and synthetic peptides that correspond to the *P. aeruginosa* PAK pilin adhesin. *C. albicans* fimbriae were immobilized onto the wells of a microtiter plate, and PAK(75–84) (◆), PAK(128–144) (●), and PAK(134–140) (□) peptides were used as competitors of Fm16 binding to fimbriae. The abilities of the *Pseudomonas* PAK pilin peptides to interact specifically with Fm16 are represented by the percent inhibition of Fm16 binding to immobilized *C. albicans* fimbriae due to the peptides with respect to Fm16 binding in the absence of peptide competitors.

but not Fm34 binding to immobilized fimbriae. Under the experimental conditions used in these assays, PAK pili inhibited Fm16 binding to immobilized *C. albicans* fimbriae by 80% compared with the control, where no competitors were present. These data demonstrated that the interactions between the MAbs and heterologous antigens were specific and of high affinities.

Similarity between the epitopes of Fm16 and PK99H. The specificities of the interactions between Fm16 and synthetic peptides could be assessed by using competitive ELISAs. In these assays, *C. albicans* fimbriae were immobilized onto microtiter wells, and synthetic peptides were utilized as competitors of Fm16 binding to fimbriae. The 17-mer peptide PAK(128–144), which spans the disulfide-bonded carboxy terminus of the *Pseudomonas* PAK pilin, inhibited Fm16 binding to immobilized fimbriae by about 80% (Fig. 3). The shorter 7-mer peptide PAK(134–140), which corresponds to the epitope of PK99H, was less effective than PAK(128–144) in the inhibition of Fm16 binding to immobilized *C. albicans* fimbriae. The 50% inhibitory concentrations for PAK(128–144) and PAK(134–140) are approximately 0.45 and 6.5 μM, respectively. A control peptide, PAK(75–84), which corresponds to the central region of the *Pseudomonas* pilin molecule, had no effect on Fm16 binding to immobilized fimbriae. The same experiment was performed with Fm34, and PAK(128–144) and PAK(134–140) had no significant effect on Fm34 binding to *C. albicans* fimbriae (49a). These results suggested that the interactions between Fm16 and the peptides were highly specific and that the fimbrial subunit has an antigenic determinant that is similar or identical to the DEQFIPK antigenic epitope sequence found on the *Pseudomonas* PAK pilin.

DISCUSSION

P. aeruginosa is a gram-negative opportunistic bacterial pathogen, while *C. albicans* is an opportunistic fungal pathogen. Although they are different from an evolutionary standpoint, both of these organisms are capable of binding to similar receptors (12, 20, 27, 45, 50). Our studies have shown that *P. aeruginosa* pili bind to glycosphingolipid (asialo-GM_1) receptors (27, 45) and that the receptor-binding domain resides in the carboxy-terminal disulfide-looped region of the PAK pilin (15, 24, 27, 36). *C. albicans* and other fungi have been shown previously to bind to glycosphingolipids (16). Recent in vitro studies have demonstrated that *C. albicans* expresses fimbrial structures that mediate yeast adherence to human buccal epithelial cells via asialo-GM_1 receptors (49, 50). The ability of *P. aeruginosa* pili and *C. albicans* fimbriae to bind to the same receptors could be achieved through structurally similar receptor-binding domains of these two adhesins. If this is true, antibodies raised against the defined receptor-binding domain of the *Pseudomonas* PAK pilin adhesin may recognize the conserved antigenic determinant found on the *C. albicans* fimbrial adhesin.

The first indication of a conserved epitope in *C. albicans* was demonstrated by the abilities of antibodies raised against *P. aeruginosa* whole pilin (PK99H) or synthetic peptides [anti-PAK(128–144) and anti-PAK(134–140)] to bind to fimbriae and cause agglutination of the yeast (Table 1). *C. albicans* 40 is a clinical isolate obtained from a patient in Toronto General Hospital. Dot blot analyses using yeast whole cells with PK99H suggest that the antigen is present on a wide range of clinical isolates of *C. albicans* (14a). Conversely, an anti-fimbrial MAb, Fm16, was able to recognize a conserved epitope in *P. aeruginosa* PAK which enabled the antibody to agglutinate bacterial whole cells (Table 2). The presence of a structurally conserved antigenic determinant on these adhesins was evident from Western analyses. The abilities of heterologous antibodies to bind to the respective immobilized antigens in direct ELISAs (data not shown) and Western blot assays (Fig. 1) were demonstrated by the binding of anti-*P. aeruginosa* PAK pilin antibodies to *C. albicans* fimbrial adhesins and the binding of anti-*C. albicans* fimbrial antibodies to *Pseudomonas* pilus adhesins. The immunological cross-reactions of Fm16 and PK99H with PAK pili and fimbriae, respectively, were highly specific since Fm34 and PKL1 showed little or insignificant levels of cross-reactivity with the respective heterologous antigens in these assays. Furthermore, the interactions between Fm16 and PK99H with their respective fimbriae and PAK pili antigens could be specifically inhibited by the addition of heterologous competing antigens (Fig. 2). This demonstrated that both antigens contain structurally similar epitopes that competed for binding to the MAbs that were used in the competitive ELISAs (PK99H and Fm16). However, Fm34 and PKL1 recognize different epitopes on the respective fimbrial and PAK pilus antigens than their corresponding counterparts and were not affected by the presence of competing heterologous antigens. The binding of PK99H to PAK pili occurs at the receptor-binding domain of the *Pseudomonas* pilin adhesin, and the MAb is effective in blocking bacterial adherence to human buccal epithelial cells (7). PK99H may be binding to or near the receptor-binding domain of the *C. albicans* fimbriae since the addition of this antibody could block yeast adherence to buccal epithelial cells (14a). It is interesting that while both PK99H and PKL1 bound to the PAK(128–144) 17-mer peptide (Fig. 3), PKL1 failed to agglutinate *C. albicans* whole cells (Table 1). The epitope present on the 17-residue peptide that is recognized by PKL1 differs from the epitope recognized by PK99H because PKL1 does not bind to the PAK(134–140) 7-mer peptide (data not shown).

Since the binding of Fm16 and PK99H to fimbriae and PAK pili could be competitively inhibited by heterologous antigens, they share a common epitope. This was suggested by the ability

of Fm16 to bind to PAK(128–144) and PAK(134–140) peptides in direct ELISAs (data not shown) and by the abilities of anti-PAK(128–144) and anti-PAK(134–140) anti-peptide antibodies to agglutinate *C. albicans* (Table 1). The PK99H epitope has also been determined and consists of a linear 7-amino-acid-residue sequence, DEQFIPK, located in the disulfide-looped region at the carboxy terminus of the PAK pilin molecule (48). The *C. albicans* fimbrial subunit is a 66K glycoprotein, but the amino acid sequence of the protein has yet to be determined (49). Hence, the epitope of Fm16 could not be determined because the sequence of the fimbrial subunit is unknown. However, the effectiveness of PAK(128–144) and PAK(134–140) in inhibiting the binding of Fm16 to fimbriae suggests that the epitope of Fm16 is similar to that recognized by PK99H. Thus, a homolog of the DEQFIPK sequence found in the *P. aeruginosa* PAK pilin appears to be present in the *C. albicans* fimbrial subunit.

In addition to the *C. albicans* fimbriae, a number of the cell surface components, including mannoproteins (4, 9), mannans (17, 32), lipids (10), and chitins (44, 46), have been described as possible adhesin candidates. Most of the proposed *C. albicans* adhesins to date are mannoproteins (13). *C. albicans* fimbria is similar to many of the candidate adhesins in that it is a mannoprotein (49, 50). *C. albicans* may employ multiple mechanisms in its interactions with host cells. These interactions have been categorized into three types: protein-protein, protein-sugar, and sugar-unknown host receptors (13, 33). The *C. albicans* fimbrial adhesin-receptor interactions are likely to involve the protein moiety of the fimbria with the carbohydrate portion of the glycosphingolipids (asialo-GM_1 and asialo-GM_2) on the epithelial cells because the conserved epitope on the fimbrial subunit is similar to the receptor-binding domain on the *Pseudomonas* pilin protein. The ability of synthetic βGalNAc(1-4)βGal to inhibit fimbrial binding to epithelial cells also supports this contention (50). Fimbrial adhesins differ from the integrin-analog adhesin and the fibronectin-binding protein in terms of their molecular weights and their interactions with their receptors (protein-protein). The integrin analog (iC3b receptor) is a 130- to 165-kDa mannoprotein and recognizes iC3b or RGD peptides on host surfaces (3, 11, 14, 34). A 58-kDa mannoprotein has been identified recently as a fibronectin-binding protein and specifically interacts with fibronectin on endothelial cell surfaces (5). The fimbria has a lectin-like activity, and the *C. albicans* fimbrial adhesin-receptor interactions are likely to involve the binding of the protein moiety of fimbriae to βGalNAc(1-4)βGal contained in the glycosphinogolipids (asialo-GM_1 and asialo-GM_2) on the epithelial cells (50). Fimbrial adhesins also differ from the extracellular polymeric material of *C. albicans* which was described as putative adhesins by Douglas and coworkers (9, 47). The receptor specificity of the *C. albicans* fimbrial adhesin described here differs from that described for the extracellular polymeric material adhesin of Tosh and Douglas (47), which appears to recognize fucose and *N*-acetylglucosamine. Work is in progress to further characterize the fimbrial adhesin receptors that are present on epithelial cell surfaces.

The occurrence of conserved receptor-binding domains on adhesins of different species of microorganisms could be possible since these adhesins can recognize and utilize similar cell surface receptors. Krivan et al. (21, 22) have observed that many pulmonary pathogens, including *P. aeruginosa*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Klebsiella pneumoniae*, are able to utilize the minimal disaccharide sequence βGalNAc(1-4)βGal present on glycolipids as receptors. It remains to be determined whether the *P. aeruginosa* pilus adhesin and the *C. albicans* fimbrial adhesin are part of a large family of adhesins containing a conserved structural motif. Work is in progress to determine the amino acid sequence of the *C. albicans* fimbrial subunit and to locate the receptor (asialo-GM_1)-binding domain of the fimbrial adhesin.

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