University of Alberta

Characterization and Identification of *Candida albicans* Fimbrial Adhesins and their Receptors on Human Buccal Epithelial Cells

by

Lei Yu

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Department of Medical Microbiology and Immunology

Edmonton, Alberta

Fall, 1996

ISBN 0-612-18137-5

Canada

University of Alberta

Library Release Form

Name of Author: Lei Yu

Title of Thesis: Characterization and Identification of *Candida albicans* Fimbrial Adhesins and their Receptors on Human Buccal Epithelial Cells

Degree: Doctor of Philosophy

Year this Degree Granted: 1996

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly, or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as hereinbefore provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

c/o Dept. of Medical Microbiology and Immunology, University of Alberta,
Edmonton, Alberta, Canada, T6G 2H7

Date: Sept. 25, 1996

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled, "*Characterization and Identification of Candida albicans Fimbrial Adhesins and their Receptors on Human Buccal Epithelial Cells*" submitted by Lei Yu in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Dr. R.T. Irvin (Supervisor)

Dr. R.S. Hodges

Dr. M.S. Peppler

Dr. R.J. Read

Dr. D.Y. Kunimoto

Dr. J.S. Lam
External Examiner

Date: September 20, 1996

Abstract

Candida albicans is the primary etiologic agent of candidiasis, a disease that can vary from superficial mucosal lesions to life-threatening systemic or disseminated diseases. Strains of *C. albicans* have been reported to possess long, thin filamentous protein cell surface appendages termed fimbriae. These fimbriae were fractionated and demonstrated to competitively inhibit yeast cell adherence to human buccal epithelial cells (BECs).

The putative *C. albicans* fimbrial adhesin receptors on BECs were proposed to be glycosphingolipids (asialo-GM_1 and asialo-GM_2) since the fimbrial binding to exfoliated human BECs was inhibited by asialo-GM_1 in *in vitro* binding assays. The fimbriae interact with the glycosphingolipid receptors via the carbohydrate portion of the receptors since the fimbriae were observed to bind to synthetic βGalNAc(1-4)βGal-protein conjugates and the disaccharide was able to inhibit fimbriae binding to BECs in *in vitro* binding assays.

Pseudomonas aeruginosa, a Gram negative bacterial opportunistic pathogen, can bind to the asialo-GM_1 receptor on human BECs that is mediated by its pili. The asialo-GM_1 receptor-binding domain of the *Pseudomonas aeruginosa* strain K (PAK) pilus adhesin is located in the C-terminus of PAK pilin and this region is also the binding epitope of anti-PAK pilin monoclonal antibody, PK99H. *C. albicans* fimbrial adhesin and PAK pilus adhesin share a conserved receptor-binding domain, which is proved by the following evidence that: 1) both the unbiotinylated PAK pilus and fimbrial adhesins inhibited the binding of biotinylated PAK pili and biotinylated *C. albicans* fimbriae to asialo-GM_1 and BECs; 2) both PK99H and Fm16, an anti-*C. albicans* fimbrial monoclonal antibody, blocked fimbriae binding to asialo-GM_1 and BECs and also inhibited *P. aeruginosa* and *C. albicans* whole cell binding to BECs; and 3) the binding of *C. albicans* fimbriae to asialo-GM_1 and BECs and the binding of *C. albicans* to BECs can be blocked by the synthetic peptides that correspond to the whole adhesintope of the PAK pilus (PAK128-144) or part of it (PAK134-140) and their respective anti-peptide antisera.

The results from these studies confirmed that a structurally conserved motif similar to the PAK(128-144) peptide sequence is present in *C. albicans* fimbrial adhesin and that the seven amino acid residues of PAK(134-140) may play an important role in forming an adhesintope for both *P. aeruginosa* PAK pilus and *C. albicans* fimbrial adhesins.

Acknowledgments

First of all, I would like to thank my supervisor, Dr. Randall T. Irvin, for the opportunity to take part in the exciting research that is being carried out in his laboratory. Thanks, Randy, for your generosity, patience, guidance and help over the last five and half years in learning the interaction of the adhesins of microorganism with the host cells and the art of glycoprotein purification, characterization and identification.

I wish to express my gratitude to the supervisory committee, Drs. Robert S. Hodges, Mark S. Peppler, Randy J. Read, Dennis Y. Kunimoto and Joseph S. Lam (external examiner), for their input during the course of my studies and for taking the time in reading and examining my thesis.

I would also like to thank colleagues and friends in Randy's laboratory: Marie Kaplan for preparation of monoclonal antibodies; Hasmukh B. Sheth for βGalNAc(1-4) βGal-BSA conjugates; K.K. Lee, Wah Y. Wong, Daisy Bautista, Dennis Susko and Sastry Parimi for their kindly help.

I would like to thank K. Ens and D. Gowing in Dept. of Botany, Erindale College University of Toronto, Missauga, Ontario for production of anti-*C. albicans* fimbrial monoclonal antibodies. The amino acid analyses carried out by Michael R. Carpenter in Dept. of Biochemistry is gratefully acknowledged. I would like to thank the professors, postdoctoral fellows, graduate students, general office staffs and support staffs in Department of Medical Microbiology and Immunology who gave me countless help.

Finally, the support of the PENCE, CBDN, and the Department of Medical Microbiology and Immunology was greatly appreciated.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	percent
$-NH_2$	an amidated carboxyl terminal
-OH	the α-carboxyl group
A_{405}	absorbance reading taken at 405 nm
A_{490}	absorbance reading taken at 490 nm
Ab	antibody
ABTS	2,2'-azido-di(3-ethylbenzthiazoline sulfonic acid)
Ac	an acetylated N^{α} terminus
AIDS	acquired immunodeficiency syndrome
AP	alkaline phosphatase
APS	ammonium persulfate
Asialo-GM_1	gangliotetraosylceramide
Asialo-GM_2	gangliotriaosylceramide
BCIP	5-bromo-4-chloro-3-indolylphosphate-toulidine
BECs	human buccal epithelial cells
BSA	bovine serum albumin
Bt-	biotinylated
C-terminus	carboxyl-terminus of protein
C3	complement 3
C3d	C3 conversion product
C4	complement 4
CA fimbriae	*Candida albicans* fimbriae
CDH	ceramide dihexosaccharide
CF	crude *Candida albicans* fimbriae
CFU	colony forming unit
cm	centimeter
Con A	Concanavalin A
cpm	counts per minute
CR3	complement receptor 3
CR4	complement receptor 4
CSH	cell surface hydrophobicity
CTH	ceramide trihexosaccharide
Da	daltons

DMSO	dimethyl sulfoxide
EDTA	ethylenediamine tetracetic acid
EF	enriched *Candida. albicans* fimbriae
EM	electron micrography
EP	extracellular polymeric material
Fm16	anti-*Candida albicans* fimbrial monoclonal antibody
Fm34	anti-*Candida albicans* fimbrial monoclonal antibody
g	gram
Gal	galactose
GlcNAc	N-acetyl glucosamine
GM_1	II^3NeuAc-tetraosylceramide
GM_2	II^3NeuAc-triaosylceramide
GM_3	II^3NeuAc-lactosylceramide
GSLs	glycosphingolipids
h	hour
HIV	human immunodeficiency virus
HPLC	high-performance liquid chromatography
I.D.	internal diameter
iC3b	C3 conversion product
IC_{50}	concentration required for 50% inhibition of binding
IgG	immunoglobulin of the G class
IgM	immunoglobulin of the M class
kDa	kiloDaltons
kV	kiloVolts
LCS	lactosylceramide
M	molar
M-GM_1	monosialoganglioside
MAb	monoclonal antibody
mg	milligram
min	minute
ml	milliliter
mM	millimolar
mm	millimeter
M_r	relative mobility
M.W.	molecular weight
N-linked	asparagine linked

NBT	p-nitro blue tetrazolium chloride
ND	not determined
NeuAc	sialic acid; neuraminic acid
NH_2-	amino group
OD	optical density
ox-	a disulfide bond formation between the sulfhydryl groups on the two cysteine residues (residues 129 and 142) on the PAK(128-144) peptide].
PAb	polyclonal antibody
PAK	*Pseudomonas aeruginosa* strain K
PAO	*Pseudomonas aeruginosa* strain O
PBS	sodium phosphate buffered saline
PBST	sodium phosphate buffered saline containing 0.05% Tween 20
PEG	polyethylene glycol
PK99H	anti-*Pseudomonas aeruginosa* strain K pilus monoclonal antibody
PKL1	anti-*Pseudomonas aeruginosa* strain K pilus monoclonal antibody
PMSF	phenylmethylsulfonylfluoride
PVDF	polyvinylidene difluoride
RGD	arginine-glycine-aspartic acid
RPC	reversed-phase chromatography
SAB	Sabouraud
SCID	severe compromised immunodeficienct
SD	standard deviation
SDS-PAGE	sodium dodecylsulfate polyacrylamide gel electrophoresis
SEC	size-exclusion chromatography
TBS	tris buffered saline
TEMED	N,N,N',N'-tetramethylethylenediamine
TFA	trifluoroacetic acid
TLC	thin layer chromatography
Tris	Tris (hydroxymethyl) aminomethane
TSDS-PAGE	tricine sodium dodecylsulfate polyacrylamide gel electrophoresis
TTBS	tris buffered saline containing 0.05% Tween 20
V	volts
v/v	volume per volume
VECs	vaginal epithelial cells

w/v	weight per volume
µg	microgram
µl	microliter
µM	micromolar
µm	micrometer
°C	degrees Celsius

Chapter I Introduction

Candida, which was discovered more than a century ago as a causative organism of oral thrush, has since been demonstrated to infect practically every tissue of the human body (Odds, 1988). Accordingly, the field of *Candida* and candidiasis has grown rapidly. The ever increasing literature on various aspects of infections caused by *Candida* is, in itself, an indication of the concerted effort of leading microbiologists, biochemists and molecular biologists in unraveling the mysteries of its pathogenicity, prevention and treatment (Bodey, 1993; Cutler, 1991; Shepherd, 1991; Kennedy *et al.*, 1992; Calderone, 1993; Odds, 1994; Pendrak and Klotz, 1995).

1. Taxonomy

The genus *Candida* is placed taxonomically with the yeast-like organisms of the Fungi Imperfecti (Saltarelli, 1989). *Candida* belongs to the subfamily of *Cryptococcaceae* (Table I-1). Within the genus *Candida*, *Candida albicans* is found as a natural inhabitant of the skin and mucous membranes of humans. By far, the major human pathogen classified in the genus *Candida* is *C. albicans*. (Douglas, 1987a and b; Calderone and Braun, 1991; Cutler, 1991; Odds, 1992; Bodey, 1993; Rinaldi, 1993).

2. Morphology

2-1 Dimorphism

The vegetative cells of *C. albicans* were first described by Robin in 1853 as having a spherical or broadly oval shape, 2-8.5 x 3.0-14 μm in size (Shepherd, 1991). They reproduce by multilateral budding. *Candida albicans* is a dimorphic fungus which is able to grow both as a yeast and as a mycelium (Shepherd, 1987; Odds, 1988). Dimorphism is defined as an environmentally controlled reversible transition between yeast and mycelial forms (Soll, 1988). The basic element of a mold (mycelium) is the hypha (pl.

Table I-1 One classification system of Candida species of medical significance (Rinaldi, 1993)

SuperKingdom:	Eukaryota
Kingdom:	Fungi (Mycota)
Form-Division:	Fungi Imperfecti
Form-Class:	Blastomycetes
Form-Order:	*Cryptococcales*
Form-Family:	*Cryptococcaceae*
Form-Genus:	Candida Berkhout
Species:	*Candida albicans* (Robin) Berkhout
	Candida albicans variety stellatoidea (Jones et Martin)
	Candida tropicalis (Castellani) Berkhout

hyphae). The hyphal element (filament) grows by elongation with branching occurring at regular intervals. A network of hyphae is known as a mycelium, which constitutes a mold colony (Soll, 1992). Yeasts are unicellular fungi that reproduce by budding or fission (Mackenzie, 1965; Joshi *et al.*, 1973). Conversion of the mold to the yeast form depends on several factors including: (a) increasing the temperature to 35-37°C; (b) presence of an abundance of simple sugars in the medium; (c) presence of sulfhydryl groups, primarily cysteine; and (d) an organic nitrate source (Goodman and Roberts, 1993). *Candida albicans* also produces blastoconidia that do not separate but form a chain of cells termed pseudohyphae. Pseudohyphae arise when the bud remains attached to the mother cell, elongates, and each in turn continues to bud instead of detaching at maturity. They are distinctly different from true hyphae or mycelial cells in appearance and in the composition of their cell walls and septa (Goodman and Roberts, 1993; Soll, 1993 and 1994; Odds, 1994). The phenomenon of dimorphism has been of great interest to medical mycologists, since many dimorphic fungi are pathogenic (Saltarelli, 1989).

2-2 Cell wall of *C. albicans*

The *C. albicans* cell wall is regarded as an important site that influences the organism's virulence (Douglas, 1987a; Cutler, 1991; Kennedy *et al.*, 1992; Calderone, 1993; Odds, 1994). The cell wall surface is a site of adhesion, colonization, and location of secretion of potential virulence factors. It also presents immunomodulatory components to the host, and affects phagocytic events (Calderone, 1993). It offers a target(s) for the development of new antifungal agents that could exhibit selective toxicity by targeting fungal structures such as glucans and chitin, which are not present in mammalian cells (Bodey, 1993). Biochemical studies have demonstrated that the wall is a complex molecular structure, which is composed of proteins, polysaccharides (chitin, glucans, and mannans) and lipids arranged in electron dense layers (Douglas, 1987b). The cell wall appears to be made up of at least five distinct layers (commencing from the plasma

membrane outward): mannoprotein, β-glucan/chitin, β-glucan, mannoprotein, and a fibrillar layer (Calderone and Braun, 1991). However, the number and relative composition of cell wall layers are affected by environmental and physiologic factors (Tronchin *et al.*, 1989; Hostetter, 1994; Pendrak and Klotz, 1995). Mannoproteins are thought to be distributed throughout the layers (Douglas, 1987a; Calderone, 1993). In *C. albicans* there is an outer "fuzzy coat" containing the fibrillar layer (Persi and Burnham, 1981; Tronchin *et al.*, 1981). This fuzzy coat is believed to be important in overall virulence by affecting adherence and phagocytosis (Edwards and Mayer, 1990). Most proposed adhesins to mammalian cells are believed to reside in the outermost fibrillar layer (Douglas, 1987b; Edwards and Mayer, 1990; Calderone and Braun, 1991; Cutler, 1991; Shepherd, 1991; Pendrak and Klotz, 1995). Electron micrographs of *Candida* cells attaching to mammalian cells suggest that the point of contact is at this fibrillar layer (Joshi, *et al.*, 1973 and Joshi and Gavin, 1975; Pugh and Cawson, 1978). Like some Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*, *C. albicans* also expresses long projecting appendages, called fimbriae (Gardiner *et al.*, 1982). Fimbriae are proposed to be one of the major adhesins that mediate the adherence of *C. albicans* to the surface of human buccal epithelial cells (BECs) (Chapter IIIA and IIIB). Another important feature of the *C. albicans* cell surface is the presence of receptors for the complement fragment of C3, iC3b, which binds noncovalently to human polymorphonuclear leukocytes, impairs phagocytic uptake and enhances yeast virulence (Gilmore *et al.*, 1988; Hostetter, 1994).

3. Pathogenesis

3-1 *Candida* infection

C. albicans is best viewed as a commensal fungus; in fact, *C. albicans* is a part of the normal human microbiota (Saltarelli 1989; Calderone and Braun, 1991; Cutler, 1991;

Edwards, 1992; Kennedy, *et al.*, 1992; Odds, 1992). This organism is part of the normal gastrointestinal (Bodey and Sobel, 1993), vaginal (Sobel, 1993) and oral flora of humans (Roseff and Sugar, 1993) and is the leading cause of "opportunistic" fungal disease (Odds, 1994). The same organism may cause intractable disseminated disease in the compromised patient (Shepherd *et al.*, 1985; Rinaldi, 1993). The disease itself generally takes two forms: superficial (mucosal) and invasive (disseminated) (Odds, 1994). *C. albicans* can infect virtually every tissue in the human body (Odds, 1992; Rinaldi, 1993). The spectrum of candidiasis due to this species includes thrush, vaginitis, skin/nail infections, pulmonary disease (including "fungus ball" formation), enteritis, esophagitis, endocarditis, meningitis, brain abscess, arthritis, keratomycosis, pyelonephritis, cystitis, septicemia, chronic mucocutaneous disease, and many other manifestations (Odds, 1994). During recent years the role of *C. albicans* as an etiologic agent of disease has become very important. An increased incidence of candidiasis (both superficial and invasive) in the last three decades is well documented and has been attributed to the widespread use of antibiotics and immunosuppressive agents (Wade, 1993). There is a parallel between the increased incidence and advent of aggressive cancer chemotherapy, greater use of parenteral nutrition, and a more widespread practice of invasive surgical procedures, particularly cardiovascular surgery with its use of prosthetic valves (Ghannoum, 1992). As noted previously, oral/esophageal candidiasis is a major, and often initial, disease occurrence in HIV-positive individuals (Elmets, 1994).

3-2 Virulence factors

The *Candida*-host interplay is more subtle than is depicted in the traditional view (Douglas, 1987a; Ghannoum and Abu-Elteen, 1990; Cutler, 1991; Kennedy *et al.*, 1992; Calderone, 1993; Odds, 1994). The virulence factors expressed or required by *C. albicans* vary, depending on the site and stage of invasion and the nature of the host response. Moreover, the attributes necessary for a *C. albicans* cell to bind to and colonize epithelial

surfaces are not the same as those responsible for epithelial penetration (Odds, 1992). Endothelial colonization and penetration are prerequisites for the final stage of deep tissue invasion, and these processes may require a distinct set of virulence factors (Sherwood *et al.*, 1992). Indeed, a panel of specialized virulence factors may play a role at each stage of the infectious process (Odds, 1994). However, no single *C. albicans* factor accounts for virulence, and not all expressed virulence factors are necessary for a particular stage or site of infection (Klotz and Penn, 1987; Douglas, 1987b; Cutler, 1991; Calderone and Braun, 1991; Hostetter, 1994). Most factors that have been related to fungal virulence are those contributing to candidal persistence (Kennedy *et al.*, 1992; Odds, 1994).

At least eight factors to date may be considered virulence factors for *C. albicans* (Table I-2). Several *C. albicans* factors are absent in other *Candida* species, or they differ qualitatively or quantitatively from those in *C. albicans*, providing a reasonable explanation for the known lower virulence potential of these other yeasts (Odds, 1994). *C. albicans* expresses several potential virulence factors in a variable and somewhat unpredictable manner: surface components in particular are expressed differentially in individual cells within a single environment in addition to differential expression in a variety of environments. This high potential for phenotypic variation from cell to cell should therefore be regarded as an another virulence mechanism which increases the diversity of colonization or virulence factors in cells that are disseminated from one host microniche to another in the course of the infectious process (Kennedy, *et al.*, 1992).

3-2-1 Rapid switching of expressed phenotype

To be successful pathogens, many organisms have developed the capacity to vary phenotype not only by a strict developmental cycle, but also by spontaneously generating variants within infecting populations with phenotypes that appear to have evolved primarily to escape threatening environmental changes such as the development of immunity by the host (Soll, 1992). Prokaryotes like *Salmonella typhimurium* (Glasgwo, *et al.* 1989),

Table I-2 Potential candidal virulence factors

Factor	Reference
1. Rapid switching of expressed phenotype	Soll *et al.*, 1993, 1994
2. Hypha (and pseudohypha) formation	Sobel *et al.*, 1984
3. Thigmotropism	Sherwood *et al.*, 1992
4. Surface hydrophobicity	Hazen and Hazen, 1992, 1993; Glee *et al.*, 1995
5. Surface virulence molecules	
receptors	Mayer *et al.*, 1990; Hostetter, *et al.*, 1990; Eigentler *et al.*, 1989; Alaei *et al.*, 1993
pyrogens	Cutler *et al.*, 1972
immunomodulators	Vecchiarelli *et al.*, 1988; Carrow and Domer, 1985
6. Molecular mimicry	
host-like surface components	Gustafson, *et al.*, 1991
bound host components	Roberts, *et al.*, 1989
7. Lytic enzymes	
proteinase(s)	Tsushima *et al.*, 1994; Monod, *et al.*, 1994; De Bernardis *et al.*, 1996
phospholipase(s)	Ibriham *et al.*, 1995
8. Adhesins	Table I-3

Borrelia hermsii (Barbour, 1989) and *Neisseria gonorrhoeae* (Swanson and Koomey, 1989) and eukaryotes like *Trypanosoma brucei* (Donelson, 1988) have developed switching systems that spontaneously generate antigenic variants in a population associated with an infection in order to adapt to various host microenvironments and to evade the host immune system. The fungal pathogen *C. albicans* is capable of high-frequency, reversible phenotypic switching (Slutsky, *et al.*, 1985). Switching in *C. albicans* can affect antigenicity in a reversible fashion, but it can also affect many other aspects of cellular physiology and morphology, including a number of putative virulence traits (Odds, 1994). *C. albicans* is apparently a permanent diploid organism with no known sexual cycle. The mechanism of phenotypic switching in *C. albicans* involves reversible genetic rearrangement (Saltarelli, 1989; Soll *et al*, 1993).

The substantially high frequency and reversibility of switching, the distinct phenotypes in the two switching systems (yeast/hyphal switching and white/opaque transition), the developmental differences in hyphal formation, and the differences in susceptibility to antifungal agents all suggest that switching systems have a role in the pathogenesis of candidiasis (Slutsky *et al.*, 1985; Soll *et al.*, 1994). Switching may potentiate invasion and proliferation in entirely different body locations/environments, assist in eluding immune defenses by alterations in surface antigenicity, and escaping the effects of antimycotic therapy. Furthermore, switching may selectively enhance adhesion of candidal species to mucosal surfaces, tissue invasion, and secretion of enzymes such as proteinases and phospholipases, which may contribute to virulence in some forms of candidiasis (Soll, 1992; Odds, 1992).

3-2-2 Hypha formation

C. albicans cells can assume a variety of shapes, ranging from spherical, budding yeast cells at one extreme to cylindrical hyphae that develop by continuous apical extension at the other (Saltatelli, 1989; Odds; 1994). The impact of hypha formation on virulence is

possibly the best-known dogma of *Candida* pathogenicity. Hyphae are commonly thought of as the invasive form, whereas yeast forms are thought to be responsible for colonizing epithelia (Cutler, 1991; Douglas, 1987a; Calderone and Braun, 1991). However, the direct histopathological observations show that both yeasts and hyphae are found in infected tissues. A mutant that forms no hyphae at 37°C, although less virulent than its parent, still causes *Candida* vaginitis in rats. *C. albicans* yeast forms may invade some tissues without any hypha formation at all, including isolated vascular endothelium, isolated corneocytes, and gastrointestinal microvilli (Odds, 1992).

3-2-3 Contact sensing (Thigmotropism)

C. albicans also responds to surface topography. When hyphae developing on filters or membranes are placed on an agar medium, they grow through pores and along grooves (Sherwood *et al.*, 1992). This property may aid *C. albicans* hyphae in penetrating some tissues by following surface discontinuities and microscopic breaks (Soll, 1992; Hostteter, 1994).

3-2-4 Surface hydrophobicity

The degree of surface hydrophobicity of *C. albicans* cells contributes to epithelial adherence and to the speed of hyphal germ tube formation (Odds, 1992). Alterations to *C. albicans* cell wall components can lead to changes in cell surface hydrophobicity (CSH), which would then result in a shift in the free energy of interaction of the cell with host tissue (Glee, *et al.*, 1995). CSH shifts are correlated with changes in the length and concentration of fibrils in the exterior layer of the cell wall (Hazen and Hazen, 1992; 1993). CSH shifts are affected by various factors, including temperature (Hazen and Hazen, 1987); physiologic and morphologic factors and environmental conditions also affect expression of cell surface attributes (Hazen and Hazen, 1988; Hazen, 1989). For example, *C. albicans* cells cultured at 25°C are more grossly virulent than are their more hydrophilic

counterparts cultured at 37°C (Antley and Hazen, 1988). Most recently, several hydrophobic proteins on the surface of *C. albicans* cells have been described that are available for hydrophobic interactions with host tissues (Glee, *et al.*, 1995). Hydrophobic cells also exhibited greater adherence to epithelial cells (Hazen, 1989 and 1990; Ener and Douglas, 1992) and extracellular matrix proteins (Hazen and Hazen, 1993), decreased susceptibility to killing by phagocytic cells, and the ability to attach to multiple sites in kidney, spleen, and lymph node tissues (Rotrosen, *et al.*, 1986; Klotz and Penn, 1987).

3-2-5 Molecular mimicry

The ability of a microbe to produce or acquire a surface coat of molecules that mimics host components is a virulence attribute, since such a coating would make the microbe less recognizable as non-self by the host (Hostetter, 1994). There is only indirect evidence for such molecular mimicry as a component of *Candida* virulence (Odds, 1992). *C. albicans* cells circulating in the bloodstream become rapidly coated with host platelets via the fibrinogen-binding ligand. Some of the cell adhesion molecules in *C. albicans* which exhibit antigenic and functional similarities to human complement receptors 3 and 4 (CR3 and CR4) have been identified as integrin analogs. (Tronchin, *et al.*, 1991; Gustafson *et al.*, 1991). The presence of integrin analogs in candidal species gives rise to two important biological correlates. Integrin analogs on yeasts may well mediate adhesion by the same biochemical mechanism because integrins on mammalian cells recognize RGD-containing ligands in extracellular matrices. Evidence of a significant relationship between integrin analogs in yeasts and their counterparts in mammalian cells could provide critical insights into the evolution and function of these complex eukaryotic proteins (Hostetter, 1994).

3-2-6 Lytic enzymes

C. albicans secretes (or expresses at the surface) hydrolases with relatively broad substrate specificities, including a proteinase (Odds, 1988), phospholipase(s) (Ruchel *et al.*, 1990), and lipase(s) (Gozalbo, 1991). Of these, the secreted aspartyl proteinase is the

most thoroughly studied. Only the most virulent *Candida* species (*C. albicans*, *C. tropicalis*, and *C. parapsilosis*) produce proteinases (Ghannoum *et al.*, 1986). Moreover, proteinase-deficient mutants are less virulent than parental strains in mice following intravenous challenge (Macdonald and Odds, 1983). *C. albicans* aspartyl proteinases have been demonstrated to be associated with tissue invasion (Borg and Ruchel, 1988), *C. albicans* adherence (Ray and Payne, 1990) and immunoglobulin degradation (Kaminishi *et al.*, 1995; Ruchel, 1986). They may also play a role in regulating the proteolytic activation of other enzymes and in the regulation of phenotypic switching (Ray and Payne, 1990; Cutler, 1991).

3-2-7 Adhesins

For the great majority of microorganisms, adherence to some kind of surface, animate or inanimate, is a prerequisite to normal life (Law, 1994). Microbial ecology is concerned with the layers of organisms, or biofilms, that cover surfaces in the living world. In the area of disease processes, a microorganism must adhere to its host before it can invade (Westerlund and Korhonen, 1993).

The adherence of *Candida albicans* to host cells is believed to play a critical role in colonization of mucosal surfaces and subsequent infection (Douglas, 1987b; Calderone and Braun, 1991; Edwards and Mayer, 1992; Klotz, 1994; Kennedy *et al.*, 1992; Odds, 1994). *C. albicans* has been demonstrated to bind to human buccal epithelial cells (Kimura and Pearsall, 1978; King *et al.*, 1980; Liljemark and Gibbons, 1973; Staddon *et al.*, 1990; Douglas, 1987a and b; Fukayama and Calderone, 1991), vaginal epithelial cells (Lee and King, 1983a and b; Sobel, *et al.* 1981), human corneocyte cells (Collins *et al.*, 1984; Ray *et al.*, 1984), vascular endothelial cells (Klotz, 1990; Mayer, *et al.*, 1992; Rotrosen, *et al.*, 1985), fibrin-platelet matrix (Leffler and Svanborg-Eden, 1986) and to plastic surfaces (Samaranayake and MacFarlane, 1980; Klotz *et al.*, 1985; Rotrosen, *et al.*, 1986). Adherence of *C. albicans* to mucosal epithelial cell surfaces are influenced by a number of

factors, including temperature (Lee and King, 1983), pH (Persi, *et al.*, 1985), antibiotic treatment (Kennedy, 1990), phenotypic state (Kennedy and Volz, 1985), growth media (McCourtie and Douglas, 1981), commensal bacteria (Centeno, *et al.*, 1983; Kennedy, 1988; Makrides and MacFarlane, 1982), germ tube formation (Kimura and Pearsall, 1980) and tissue sites (Sandin, *et al.*, 1987a and b). *C. albicans* adherence to human buccal epithelial cells (BECs) is somewhat strain dependent (Kearns, *et al.*, 1983; Douglas, 1987a). More extensive reviews of the factors affecting *C. albicans* adherence to epithelial cells *in vivo* and *in vitro* are available (Douglas, 1987b; Kennedy, 1990). The adherence and persistence of *C. albicans* on mucosal surfaces are necessary for initiation of candidiasis-either locally or in widespread, disseminated disease (Kennedy *et al.*, 1992). Adherence to epithelial cells or the oral and vaginal mucosa has been extensively studied and a close correlation is observed between the adhesion of *C. albicans* and their ability to incite disease (Ruchel, 1990).

The fungal cell surface, the interface between host and parasite, is a site of important virulence molecules. It is proposed that *C. albicans* cells produce more than one adhesin (Douglas, 1987a and b; Calderone and Braun, 1991; Cutler, 1991; Kennedy *et al.*, 1992; Odds, 1992; Hostetter, 1994; Pendrak and Klotz, 1995). A number of potential *C. albicans* adhesins have been proposed (Table I-3) and include the extracellular polymeric material (EP) which mediates attachment of *C. albicans* to epithelial cells (Brassart *et al.*, 1991; Critchley and Douglas, 1987a and b; Tosh and Douglas 1992), the receptors for complement fragments iC3b and C3d (Alaei, 1993; Calderone, 1988; Gilmore *et al.*, 1988; Gustafson, 1991) and different serum proteins (Nikawa and Hamada, 1990; Casanova *et al.*, 1992b; Page and Odds, 1988). A number of extracellular matrix components such as laminin (Bouchara *et al.*, 1990; Lopez-Ribot *et al.*, 1991), fibronectin (Skerl *et al.*, 1984; Kalo *et al.*, 1988; Klotz, 1991; Negre *et al.*, 1994), collagens (Klotz, 1988 and 1990), entactin (Lopez-Ribot *et al.*, 1994), have been proposed to be *C. albicans* adhesins. Moieties that mediate binding to plastic (Tronchin, *et al.*, 1988), components that are

Table I-3 Proposed *C. albicans* Adhesins

Adhesin	Molecular weight (10^3)	Epithelial ligand	Inhibitor(s)	Reference
Protein-protein				
Integrin analog (iC3b receptor)	130-165	iC3b	MAb, iC3b, RGD peptides	Calderone, 1993 Gustafon, 1991
Fibronectin	60-68	Fibronectin RGD peptides	Fibronectin, proteases	Negre, 1994 Klotz, 1991 Kalo *et al.*, 1988
Laminin	60-68	*ND	Laminin	Bouchara, 1990 Lopez-Ribot, 1991
Fibrinogen-binding protein	60-68	ND	Protease, mercaptoethanol	Lopez-Ribot, 1991 Casanova *et al.*, 1992
Lectin-like				
Fucose-binding protein	ND	Fucose	ND	Tosh and Douglas, 1992
GlcNAc-binding protein	ND	N-Acetylglucosamine	ND	Douglas, 1987a and b
Fimbriae	60-66	Asialo-GM_1 Asialo-GM_2	Asialo-GM_2, Asialo-GM_1, GalNAcGal, MAb,	Chapter IIIB Chapter IIIB Chapter IIIB Chapter IIIC, D
Carbohydrate-glycoprotein				
Mannan	ND	Lectin?	MAb	Han and Cutler, 1995
Partially determined				
Aspartyl proteinase	45	ND	Pepstatin	Ray and Payne, 1990
Factor 6	ND	ND	MAbs, PAbs	Miyakawa *et al.*, 1989

*ND: not determined.

responsible for cell surface hydrophobicity (Hazen, 1992 and 1990; Lopez-Ribot *et al.*, 1991) and oligosaccharides that implicate the binding of *Candida* organism to epithelial cells (Miyakawa *et al.*, 1992) and to macrophages in spleens and lymph nodes (Chaffin, *et al.*, 1993; Han *et al.*, 1993; Kanbe *et al.*, 1993; Li and Cutler, 1993), were also suggested as *C. albicans* adhesins in different environment. Mannans/mannoproteins (Douglas, 1987a and b; Tosh and Douglas, 1992; Calderone, 1993), acid proteinase (Borg and Ruchel, 1988; Ghannoum and Elteen, 1990; Ray and Payne, 1988) and chitin (Sobel, *et al.*, 1981; Segal and Savage, 1986) are also reported as *C. albicans* adhesins. A common feature of these proposed adhesins is that they appear to be mannoproteins (Cutler, 1991; Kennedy *et al.*, 1992; Calderone, 1993; Hostetter, 1994). In addition, *C. albicans* may express hydrophobic molecules that could mediate attachment to cell surfaces through hydrophobic interactions (Hazen and Hazen, 1989; Hazen, 1989; Hazen, 1993; Glee, *et al.*, 1995). Most of these entities have been suggested as virulence factors (Douglas, 1987a; Cutler, 1991, Calderone and Braun, 1991; Kennedy *et al.*, 1992; Klotz, 1992; Odds, 1994; Pendrak and Klotz, 1995). Additionally, a role for *Candida* lipids in adherence has also been proposed by Ghannoum *et al.* (1986). Most recently, *C. albicans* fimbriae have been identified as major adhesins to mediate the attachment of *C. albicans* to BECs (Chapter IIIA and IIIB). The current view of the putative *C. albicans* adhesins has been listed in Table I-3. However, none of these putatitive *C. albicans* adhesins has been completely identified and characterized to date. The nature and the roles of these putative adhesins are described as follows.

I Mannan/mannoprotein

To date, most of the experimental evidence indicates a role for yeast mannoprotein in mediating attachment, at least to buccal epithelial cells (Calderone, 1993). The outermost mannoprotein material on the surface of the fungus is believed to be the most likely candidate for the putative fungal adhesins and receptors. Surface mannoproteins may form

adhesins binding *C. albicans* to epithelial cell surfaces (Pendrak and Klotz. 1995). On the other hand, surface mannoprotein may serve as the ligand for host cell receptors such as the iC3b adhesin or fibronectin adhesin (Hostetter, 1994).

I-1 Extracellular polymeric material (EP)

The presence of a mannoprotein fibrillar or floccular layer on the cell surface of *C. albicans* has been known for some time. A crude mannoprotein preparation obtained from culture supernatants of *C. albicans* grown in medium containing a high concentration of galactose has been demonstrated to inhibit adherence of *C. albicans* to the epithelial cells (Douglas, 1985). This extracellular polymeric material is thought to originate, at least in part, from a surface fibrillar layer (Douglas, 1991). The treatment of *C. albicans* with tunicamycin, an antibiotic which inhibits protein glycosylation, and at low concentrations inhibits synthesis of mannoprotein but not of chitin or glucan, interfered with the adherence of *C. albicans* (Douglas, 1987a and b). It has been observed that the addition of tunicamycin inhibited the formation of the fibrillar layer with a consequential decrease in adherence to BECs (Douglas, 1985). It has been demonstrated that the protein portion of the mannoprotein complex is more important than the carbohydrate moiety in mediating adherence (Tosh and Douglas, 1992). Evidence to support the role of proteins as *C. albicans* adhesins comes from experiments showing that *C. albicans* cell adherence decreases following exposure to heat, various proteolytic enzymes (Sobel *et al.*, 1981; Lee and King, 1983b) or with reducing agents such as β-mercaptoethanol (Lee and King, 1983b). The EP was further purified by a stepwise treatment of EP with N-glycanase, papain, and dilute alkali to cleave the protein and carbohydrate portions of the mannoprotein molecule. The protein fragments were then recovered by affinity adsorption with the trisaccharide determinant of the H (type 2) blood group antigen which terminates in a residue of L-fucose. The purified fucoside-binding protein was devoid of

carbohydrate and inhibited yeast adhesion to buccal epithelial cells 221 times more efficiently than EP did (Tosh and Douglas, 1992).

I-2 *C. albicans* fimbriae

The importance of bacterial fimbriae in adhesion is well documented (Jones and Isaacson, 1990). Freeze-substitution electron microscopy demonstrates the presence of fibrillar-like projections from the cell surface of *C. albicans* (Montes and Wilborn, 1968; Mohamed, 1975; Marrie and Costerton, 1981) and these fibrillar structures have been reported to mediate yeast attachment to exfoliated buccal epithelial cells (Tronchin *et al.*, 1983; Tosh and Douglas, 1992) or vaginal epithelial cells (Lee and King, 1983a; Persi *et al.* 1985), and to renal endothelium (Barnes *et al.*, 1983; Lee and King, 1983a). Most recently, the fibrillar appendages, termed fimbriae, have been isolated and partially characterized both morphologically and biochemically (Chapter IIIA). *C. albicans* fimbriae were initially described by Gardiner *et al.* (1982), but have never been extensively characterized. The role of *C. albicans* fimbriae has been recently demonstrated as the major adhesin mediating the adherence of *C. albicans* to human BECs. The fimbriae receptors on BECs have been further identified (Chapter IIIA and IIIB).

I-3 Mannans

Mannan is one of the dominant components of the *Candida* cell wall and accounts for one-third to one-half of the cell wall dry weight. It is a highly branched polysaccharide with a backbone of α-1,6-linked mannose residues with side chains of mannose residues linked to the backbone by α-1,2 and, with lesser frequency, α-1,3 bonds (Stewart and Ballu, 1968). Maisch and Calderone (1981) showed that cell-surface mannan may play an essential role in the adherence of *C. albicans* to the fibrin-platelet matrices that form on the endocardium of heart valves. When ConA was used for the pretreatment of either the yeast or the epithelial cells, it inhibited adherence (Li and Cutler, 1993). Adherence was restored

by reincubating ConA with a mannose derivative, but not with other sugars. Lectins that do not recognize mannose apparently did not affect adherence (Calderone, 1993). It has been reported that mannans of *C. albicans* are responsible for adherence of yeast forms to spleen and lymph node tissue (Li and Cutler 1993). Most recently, the antibodies directed against mannan antigen(s) have been shown to protect rats against *C. albicans* vaginitis (Cassone *et al.*, 1995) and SCID mice against disseminated candidiasis (Han and Cutler, 1995).

I-4 *Candida* iC3b adhesin

C. albicans rosettes erythrocytes when the cells are coated with complement fragment iC3b. In addition, monoclonal antibodies to the α chain of the human CR3 protein (the iC3b receptor) react directly with *C. albicans* (Mayer *et al.*, 1992) and anti-CR3 antibody blocks *Candida* attachment to iC3b-coated erythrocytes. Several fungal proteins of different molecular masses have been proposed to mediate the binding of iC3b to *C. albicans* (Hostetter *et al.*, 1990; Eigentler *et al.*, 1989; Alaei *et al.*, 1993). The biological importance of binding of iC3b to the fungal surface is unknown, but may decrease phagocytosis of coated cells (Hostetter, 1994).

I-5 The fibronectin adhesin of *Candida*

Fibronectin is a plasma and interstitial tissue glycoprotein to which a number of microorganisms avidly adhere. It has been shown that *Candida* exhibits an affinity for fibronectin (Skerl *et al.*, 1984). The receptors on *C. albicans* appear to function as adhesins to increase adherence of *C. albicans* to human endothelial cells and extracellular matrix (Klotz, 1994). In disseminated candidiasis, the fibronectin adhesin may be responsible for the adherence of the microorganism to intravascular structures such as endothelial cells or to the subendothelial extracellular matrix. In animal models of

disseminated candidiasis, the rabbits can be protected from disseminated disease by an arginine-glycine-aspartic acid (RGD-containing peptide (Klotz and Smith, 1992 and 1995).

II Chitin

A role for chitin in the adhesion of *C. albicans* has been suggested by Segal and Savage (1986). Chitin, its constituent GlcNAc, and chitin-soluble extract have been shown to inhibit adhesion of yeast to vaginal epithelial cells (VECs) (Kahana *et al.*, 1988). This was further confirmed by the effect of chitin and its derivatives on the adhesion of *Candida* to BECs and intestinal tissues of mice (Segal *et al.*, 1988). In an animal model, rats could be protected against vaginitis by chitin (Lehrer *et al.*, 1988).

III Enzyme adhesins

Acid proteinase is the best studied *C. albicans* enzyme that may function as an adhesin (Calderone and Braun, 1991). Multiple isomers of an acid proteinase have been identified in *C. albicans* (Odds, 1992). Acid proteinase is active at low pH, can degrade proteins such as fibronectin (Ray and Payne, 1990) and immunoglobulin (Ruchel *et al.*, 1986; Kaminishi, *et al.*, 1995), and has been localized to the outer layer of *C. albicans* by immunoelectronic microscopy (Pendrak and Klotz, 1995).

IV Lipids

Phospholipids and other lipids, like sterols, are integral hydrophobic architectural components of biological membranes which also provide a functional environment for the proteins and enzymes to express their activities. Lipids extracted from *C. albicans* have been shown to inhibit the adherence of *C. albicans* to BECs (Ghannoum, *et al.*, 1986, 1987). However, the mechanisms of interaction of *C. albicans* lipids with host cells are still unclear.

V C3d receptor and other extracellular matrix protein receptors

C3d binding proteins have been identified on hyphal (60 kDa) and yeast cell (50 kDa) surfaces of *C. albicans*. The role in the infectious process is currently unknown (Calderone and Braun, 1991). A number of plasma and extracellular matrix proteins have been demonstrated to bind to *C. albicans*. These include fibrinogen (Casanova *et al.*, 1992a), vitronectin (Jakab *et al.*, 1993), laminin (Bouchara *et al.*, 1990), and entactin (Lopez-Ribot and Chaffin, 1994). The role of these receptors in the adherence processes remains to be elucidated.

4. Common features of microorganisms in adhesion events

The capacity for adherence to host cell surfaces is a pre-requisite for the process of microbial colonization and invasion (Bliska *et al.*, 1993; Falkow *et al.*, 1992). As a consequence, pathogenic organisms have evolved common structural molecules on their surface (Paranchych *et al.*, 1986; Elleman, 1988; Falkow *et al.*, 1992; Jose *et al.*, 1995) to interact with conserved receptors on the surfaces of host cells (Krivan *et al.*, 1988b) by similar interaction mechanisms. Most recently, it has been demonstrated that both the bacterium, *Pseudomonas aeruginosa* , and the fungus, *Candida albicans*, specifically bind to similar receptors on human BECs by utilizing structurally similar molecules (adhesins) on their surfaces, pili and fimbriae respectively (Chapter IIIA and IIIE; Lee *et al.*, 1994; Sheth *et al.*, 1994).

P. aeruginosa is classified as an opportunistic pathogen and is capable of causing significant morbidity and mortality in patients immunocompromised by severe burns or cancer (Bodey *et al.*, 1983). *P. aeruginosa* is the major etiologic agent responsible for chronic lung infections in cystic fibrosis (CF) patients and is known to be a pathogen in osteomyelitis, ear, eye, and urinary tract infections, and nosocomial pneumonias (Kohler and White, 1979). Corneal degradation as a result of *P. aeruginosa* keratitis can be a serious problem for users of extended wear contact lenses (Hazlett *et al.*, 1993).

Microorganism adherence to host cell surface receptors is a complex process and a number of organisms employ more than one mechanism to adhere to a cell surface (Law, 1994). *Pseudomonas aeruginosa* employs a number of adhesins including alginate (Doig, *et al.*, 1987; Ramphal and Pier, 1985), pili (Irvin *et al.*, 1989; Ramphal and Shands, 1984; Sato and Okinaga, 1987), exoenzyme S (Baker, *et al.*, 1991; Lingwood, *et al.*, 1991) and other non-pilus adhesins (Prince, 1992; Saiman, *et al.*, 1990) to mediate bacterial attachment to host cell receptors.

Pseudomonas aeruginosa is known to produce extracellular polar filaments called pili with a diameter of 5.2 nm and an average length of 2.5 μm (Folkhard *et al.*, 1981). Pili are composed of thousands of 15-kDa pilin monomers helically arranged with a pitch of 4.1 nm and five subunits per turn. The pili of *P. aeruginosa* contribute to its virulence by promoting adherence to epithelial and mucosal cell surfaces (Paranchych *et al.*, 1986; 1990; Irvin *et al.*, 1989). *P. aeruginosa* pili belong to the NMePhe group, or type 4 class, of pili. Type 4 pili are present on many other Gram-negative pathogens such as *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Moraxella bovis*, *Moraxella nonliquifaciens*, *Dichelobacter nodosus* (previously *Bacteroides nodosus*) (Elleman, 1988), *Vibrio cholerae* (Shaw and Taylor, 1990), and the inducible bundle forming pilus of enteropathogenic *Escherichia coli* (Giron *et al.*, 1991). All type 4 pilins possess a conserved hydrophobic N-terminal region comprised of the first 29 amino acids (Paranchych *et al.*, 1986). The crystallographic structure of *N. gonorrhoeae* pilin revealed that the disulfide bond from two C-terminal Cys residues are the most conserved link that forms a hypervariable region or disulfide loop which is composed of a regular β-hairpin followed by a loop connection to the extended C-terminal tail (Parge *et al.*, 1995). *P. aeruginosa* strain K pili (PAK pili) are composed of 15 kDa subunits known as pilin which have a highly conserved N-terminus, a central hypervariable region, and a semiconserved C-terminus. The adherence domain of pilin is located in the C-terminal disulfide loop which is exposed at the tip of the pilus (Lee

et al., 1994). The receptor-binding domain of the *P. aeruginosa* PAK pilin adhesin has been shown to reside in the carboxyl-terminal disulfide-looped region (PAK128-144) of the PAK pilin (Irvin *et al.*, 1989; Lee *et al.*, 1994; Sheth *et al.*, 1994).

5. Cell Surface Receptors

5-1 *P. aeruginosa* PAK pili receptor(s)

Glycolipids and glycoproteins are ubiquitous macromolecules found on cell surfaces and present good sources of anchor sites or receptors for microorganisms to attach to host cells for colonization and subsequent infection of the host tissues (Karlsson, 1989). Assuming that sphingolipids are found only in the outer leaflet of the plasma membrane, they would make up 30%-60% of the lipids in that layer (Yamakawa and Nagai, 1978). Many microorganisms have been demonstrated to bind to carbohydrate sequences on cell surface glycosphingolipids. *E. coli* type 1 pili bind mannose-rich oligosaccharides, whereas pap pili (adherence pili) bind to glycolipids containing the disaccharide Galα(1-4)Gal (Stromberg *et al.*, 1990). The nature of the receptor for *P. aeruginosa* pilus-mediated adherence to the epithelium seems to be sphingolipids. Sphingolipids are well suited to be cell surface receptors because they can be composed of a wide variety of carbohydrates. Oligosaccharide structure of glycosphingolipids are listed in Table I-4. Sugar inhibition data obtained during earlier studies found that sialic acid inhibited adhesion of *P. aeruginosa* to trypsinized BECs when preincubated, but enhanced adhesion to untrypsinized BECs or when not preincubated (McEachran and Irvin, 1985). These findings led Baker *et al.* (1990) to investigate binding of *P. aeruginosa* to sialylated glycolipids. The resulting study demonstrated that *P. aeruginosa* could specifically bind to asialo-GM_1, asialo-GM_2, lactosylceramide, and sialic acid containing glycosphingolipids. The lactosylceramide in this case contained a hydroxylated ceramide. The hydroxylation of ceramide appears to affect the conformation of the sugars and is relevant for bacterial

Table I-4 Oligosaccharide structure of glycosphingolipids

Glycolipid	Oligosaccharide structure
LCS or CDH	Galβ(1-4)Glcβ1-
CTH	Galα(1-4)Galβ(1-4)Glcβ1-
GM_3	NeuAcα(2-3)Galβ(1-4)Glcβ1-
GM_2	GalNAcβ(1-4)[NeuAcα(2-3)]Galβ(1-4)Glcβ1-
GM_1	Galβ(1-4)GalNAcβ(1-4)[NeuAcα2-3]Galβ(1-4)Glcβ1-
Asialo-GM_2	**GalNAcβ(1-4)Gal**β(1-4)Glcβ1-
Asialo-GM_1	Galβ(1-4)**GalNAcβ(1-4)Gal**β(1-4)Glcβ1-

CDH or LCS: ceramide dihexosaccharide or lactosylceramide
CTH: ceramide trihexosaccharide or globotriaosylceramide
GM_3: II^3NeuAc-lactosylceramide
GM_2: II^3NeuAc-triaosylceramide
GM_1: II^3NeuAc-tetraosylceramide
Asialo-GM_2 gangliotriaosylceramide
Asialo-GM_1 gangliotetraosylceramide

binding (Karlsson, 1989). Doig and coworkers (1989) suggested that the pili receptors may be cell surface glycoproteins on BECs because they were able to demonstrate binding of pili to immobilized BEC glycoproteins. Lee and coworkers (1994) found that pili from *P. aeruginosa* strains PAK and PAO bound to the glycolipid asialo-GM_1. There may be more than one receptor on epithelial cells for pili, and there could be receptor variation within the pilin prototypes. These data are consistent with previous studies that demonstrated *Pseudomonas aeruginosa* and other pulmonary pathogens could bind to fucosylasialo-GM_1, asialo-GM_1 and asialo-GM_2, which contain the common disaccharide GalNAcβ(1-4)Gal (Krivan *et al.*, 1988a and 1988b). These organisms did not bind to galactosylceramide, glucosylceramide, lactosylceramide, trihexosylceramide, GM_1, GM_2, GM_3, and several other glycolipids, which suggested that an internal or terminal GalNAcβ(1-4)Gal disaccharide without sialyl residues is the minimum requirement for adherence of these bacteria. TLC overlay assays determined that putative glycolipid receptors for pili are asialo-GM_1 and asialo-GM_2 (MacDonald, 1994). Asialo-GM_1 has been found in substantial amounts in human lung tissue suggesting that asialo-GM_1 may be the receptor for *P. aeruginosa*. Supporting the previous findings, asialo-GM_1 was found to be the receptor for *P. aeruginosa* on the wounded ocular surface of mice (Hazlett *et al.*, 1993).

5-2 *C. albicans* fimbriae receptors

Unlike *P. aeruginosa* where the mechanisms of adherence and potential cell surface receptors have been extensively studied, the adherence mechanisms of *C. albicans* are not well characterized.

The earliest demonstration of the role of lipid components as receptors for microbial adhesins came from the work of Sato and co-workers (Sato, *et al.*, 1974). The total lipids extracted from BECs blocked the adherence of *C. albicans* (Ghannoum *et al.*, 1987).

Among various lipid fractions, sterols, sterol esters and two phospholipids were shown to be highly effective in blocking adherence of *C. albicans* (Ghannoum *et al.*, 1986). These results and those reported from similar experiments with bacterial cells suggest a role for sterols and phospholipids as receptors for both bacteria and yeast. A number of putative surface receptors for *C. albicans* adhesins have been reported (Table I-5).

Critchley and Douglas (1987a, b) have described a *C. albicans* lectin-like adhesin that binds to host-associated carbohydrates which contain fucose or N-acetylglucosamine. Critchley and Douglas (1987a) observed that *C. albicans* adhesion is mediated by more than one receptor. They showed that glycosides containing L-fucose, GlcNAc, and possibly D-mannose can all function as epithelial receptors for different strains of *C. albicans* (Tosh and Douglas, 1992). It has been suggested that fibronectin may function as host cells receptor for *C. albicans* adhesion (Rotrosen *et al.* 1985; Skerl *et al.* 1984). Suzuki and co-workers (Centeno, *et al.*, 1983; Suzuki *et al.*, 1984) have reported that a *C. albicans* cell surface carbohydrate can interact specifically with a host-associated lectin and thus function as an adhesin. *C. albicans* cell surface hydrophobicity may also contribute significantly to adherence to both mucosal epithelial cells and inert surfaces (Collins-Lech, *et al.*, 1984; Critchley and Douglas, 1987a and 1987b). However, none of the putative receptors of *C. albicans* adhesins have been extensively characterized to date.

Recently, evidence has shown that *Cryptococcus neoformans, Candida albicans*, and other yeasts bound specifically to lactosylceramide [(Galα(1-4)Glcβ(1-1)Cer)] (Jimenez-Lucho *et al.*, 1990). Substitution of other sugars for the lactosyl residues (asialo-GM_1 and asialo-GM_1) blocked binding, as did substitution with other glycolipids containing internal lactose sequences. These findings are in contrast with those that used bacteria such as*Neisseria gonorrhoeae* (Stromberg *et al.*, 1988a), *Propionibacterium granulosum* (Stromberg *et al.*, 1988b), and *Bordetella pertussis* (Tuomanen *et al.*, 1988) which bind to glycolipids with internal lactose sequences as well as to lactosylceramide.

Table I-5 Putative *C. albicans* receptors on epithelial cells

Possible receptor	Epithelial cell type	Inhibitors	References
Sugar moieties			
Fucose	Buccal	L-fucose,	Douglas, 1987a, b
			Tosh and Douglas, 1992
		Lectin	Douglas, 1987a, b
	Vaginal	L-fucose	Sobel *et al.*, 1982
Mannose	Buccal	ConA, D-mannose	Sandin *et al.*, 1982
N-acetyl-D-glucosamine	Buccal	N-acetyl-D-glucosamine	Douglas, 1987a, b
Glycoprotein moieties			
Mucins	Intestinal		Kennedy *et al.*, 1987
Fibronectin	Buccal		Skerl *et al.* 1984
Lipid moieties			
Phospholipids	Buccal	Phospholipids	Ghannoum *et al.*, 1986, 1987
Sterols	Buccal	Sterols	Ghannoum *et al.*, 1986, 1987
Glycolipid moieties			
Asialo-GM_1	Buccal	Asialo-GM_1	Chapter IV, V
Asialo-GM_2	Buccal	Asialo-GM_2	Chapter IV, V
		βGalNAc(1-4)Gal	Chapter IV, V

6. The mechanism of interaction of *C. albicans* with host cells

The adherence of microbial pathogens to host cells is an important initial step in most infections and adhesins are significant virulence factors (Beachey, 1981; Farinha *et al.*, 1994). Microbial adherence to a mucosal surface is a complex process that may involve several adherence mechanisms (Hasty *et al.*, 1992; Hoepelman and Tuomanen, 1992).

C. albicans adhesion is a complex biological phenomenon governed by a multiplicity of mechanisms which, depending upon the mucosal surface involved, are partially or wholly functional. However, there is no single factor that makes or permits these organisms to be agent of diseases ranging from superficial through invasive and other fatal diseases (Douglas, 1987a, b; Cutler, 1991; Calderone and Braun, 1991; Klotz; 1987; Odds, 1992; Kennedy *et al.*, 1992; Calderone, 1993; Hostetter, 1994; Pendrak and Klotz, 1995).

Analysis of equilibrium binding of *C. albicans* to human BECs suggested that at least three classes of adhesin-receptor interactions can be resolved (Staddon *et al.*, 1990). As many as four classes of adhesin-receptor interactions (protein-protein; protein-carbohydrate; carbohydrate-carbohydrate; protein-unknown cell receptor hydrophobic interaction) have been suggested by Calderone and Cutler (Calderone and Braun, 1991; Cutler, 1991). Protein moieties of surface glycoproteins on *C. albicans* react with host cell surface protein. This protein moiety recognizes Arg-Gly-Asp (RGD)-containing glycoproteins common to fibronectin (Klotz *et al.*, 1983; Klotz and Penn, 1987; Klotz *et al.*, 1994), vitronectin (Klotz *et al.* 1993), collagens (Klotz, 1990), laminin (Lopez-Ribot *et al.*, 1994; Klotz and Smith, 1995), and other extracellular matrix glycoproteins (Klotz and Smith, 1992). This type of interaction is best exemplified by integrin-mediated adhesion (Hostetter, 1994). This type of protein-carbohydrate interaction is classified as a lectin-like

interaction in which a protein moiety of glycoprotien on the candidal surface recognizes a carbohydrate on the epithelial or endothelial cell. This type of interaction is represented by candidal mannoproteins, which recognize a variety of carbohydrates on blood group antigens on host cells such as fucose-binding protein in extracellular polymeric material (Tosh and Douglas, 1992) and *C. albicans* fimbriae that recognize glycosphingolipids (asialo-GM_1 and/or asialo-GM_2) on human buccal epithelial cells (Chapter IIIA and IIIB). Carbohydrate moieties of *Candida* surface mannoproteins likely bind to unidentified host cell surface lectins (Calderone, 1993; Kanbe *et al.*, 1993; Han and Cutler, 1995). This type of interaction is exemplified by mannans on *C. albicans* , which are responsible for adherence of yeast forms to spleen and lymph node tissue (Kanbe *et al.*, 1993). Surface molecules of *C. albicans* that are responsible for hydrophobic interactions with host cells have also been reported (Hazen and Hazen, 1992, 1993). Most recently, several surface hydrophobic proteins have been identified which mediate the hydrophobic interactions between the yeast and host cell (Glee, *et al.*, 1995).

7. Host Defense Mechanisms

As with other fungal diseases, the primary host resistance mechanism that limits candidiasis is cell-mediated immunity (T cells) and nonspecific cellular immunity (i.e., macrophage, NK cells, and neutrophils) (Levitz, 1992). The role of the cell-mediated immune system is to activate the T lymphocytes and macrophages. However, there are contributions made by the humoral systems which have long been neglected (Casadevall, 1995; Han and Cutler, 1995).

In vitro studies demonstrating antibody-mediated killing or enhancement of cellular activity provide supportive evidence for protective antibody immunity. B-cell-depressed mice had greater tissue Candidal populations than controls following cutaneous infection, suggesting a role for antibody immunity (Moser and Domer, 1980). Depletion of murine IgM-bearing B cells affected the generation of protective responses to *C. albicans* infection

(Kuruganti *et al.*, 1988). Administration of immune serum has provided protection against animal candidiasis in some studies (Al-Doory, 1970; Kagaya, *et al.*, 1981; Mourad and Friedman, 1968; Pearsall, *et al.*, 1978). Immune rabbit sera reduced adhesion of *C. albicans* to fibrin-platelet matrices and protected against endocarditis in rabbits (Scheld, *et al.*, 1983). Immune mouse sera reduced pathological lesions caused by *C. albicans* in mice (Ashman and Papdimitriou, 1993). More recently, passive protection was demonstrated with vaginal fluid containing antibodies to mannan constituents and the aspartyl proteinase of *C. albicans* (Cassone, *et al.*, 1995). Han and Cutler (1995) presented strong evidence for the usefulness of antibody immunity against *C. albicans* in which both polyclonal sera and MAbs to a mannan adhesin fraction prolonged mouse survival and reduced Candidal populations in the mouse kidneys. This study established the importance of antibody specificity in mediating protection. In humans, antibodies to a 47-kDa breakdown product of heat shock protein hsp 90 have been associated with recovery from *C. albicans* infections and protection against disseminated disease in patients with AIDS (Matthews *et al*, 1988; Matthews and Burnie, 1992).

Thus, for *C. albicans*, antibody immunity may contribute to host defense by preventing attachment (Epstein, *et al.*, 1982; Vudhichamnong, *et al.*, 1982; Scheld, *et al.*, 1983; Umazume, *et al.*, 1995; Han and Cutler, 1995), providing opsonins for more efficient phagocytosis (Chilgren, *et al.*, 1968), binding to immunomodulating polysaccharides (Fischer, *et al.*, 1978), neutralizing extracellular proteases (Cassone, *et al.*, 1995), and inhibiting the yeast-to-mycelium transition (Casanova, *et al.*, 1990).

The difficulty in establishing the role of antibody immunity in most fungal infections suggests that fungi are either resistant to, escape or neutralize the effects of antibody. In contrast to Gram-negative bacteria, fungi appear to be resistant to complement mediated lysis, presumably because of thick cell walls and limited accessibility to the plasma membrane (Levitz, 1992). *C. albicans* can also produce proteases which can

degrade IgA (Ruchel, 1986) and IgG (Kaminishi, *et al.*, 1995). Although simple in concept, the evaluation of the role of antibody immunity in animal systems involves complex experiments in which the outcome is dependent on multiple variables including antibody quantity, specificity, and isotype composition; inoculum; the timing of infection and antibody administration; route of infection and antibody administration; the virulence of the experimental strain; and the susceptibility of the animal host to infection with organism (Casadevall, 1995). The complexity of antibody testing suggests caution in drawing broad conclusions on the importance of antibody immunity from negative experimental data.

There is now convincing evidence that some antibodies can modify the course of infection to the benefit of the host (Casadevall, 1995). This exciting development suggests that it may be possible to administer or elicit protective antibody immunity in populations at risk for infection despite the continuing uncertainty as to the role of natural immunity in protection (Casadevall, 1995). Some of the contradictory observations against the importance of antibody immunity against *C. albicans* may be explained by the existence of protective, nonprotective, and infection-enhancing antibodies in immune sera, by the antigen variation (Brunham, *et al.*, 1993; Chaffin, *et al.* 1988; Polonelli, *et al.*, 1994a and b) and by the multiple factors involved in the complex experiments (Casadevall, 1995).

8. Treatment

The yeast *Candida albicans*, the most common cause of opportunistic fungal diseases in humans, has increased in significance in recent years (Odds, 1992). In the immunocompromised patient, disseminated candidiasis is a serious disease, often resulting in fatal infection, even in patients who are treated with antifungal agents such as amphotericin B (Anttila *et al.*, 1994; Komshian *et al.*, 1989). As *C. albicans* are eukaryotic cells, it should not be surprising that antifungal agents are associated with substantially more toxicity to humans than antibacterial agents. *Candida* organisms do contain constituents that are uniquely different from those of mammalian cells such as glucans,

mannans and chitins (Cassone and Torosantucci, 1991). However, so far, none of the antifungal drugs are effective in treating disseminated candidiasis. Administration of antibiotics tends to suppress the normal antagonistic flora. This generally results in a superinfection with *C. albicans* that is difficult to treat. Due to the high toxicity to the body of conventional antimycotic therapy (Bodey, 1993), the emergence of resistant *Candida* strains (Fasoli *et al.*, 1990) and the difficulties associated with the diagnosis of disseminated candidiasis (Berenguer, *et al.*, 1993; Reboli, 1993), there is a pressing need for the development of new prevention and treatment strategies. A promising target for prophylaxis is to block the adhesion of the yeast to host surfaces (Ghannoum and Radwan, 1990), which has been proved to be useful to help the host resist disseminated candidiasis. MAbs could have utility as direct antifungal drugs in a manner analogous to the use of antibody therapy against bacterial pathogens. There are possible applications of our investigations to human medicine.

9. The aims of this study

The aims of this study were three-fold: the first was to characterize and identify the *C. albicans* fimbrial adhesin which is responsible for the binding to human BECs; the second was to identify the *C. albicans* fimbrial adhesin's receptors on BECs and their interactions; and the third was to identify and characterize the receptor-binding domain of *C. albicans* fimbrial adhesin.

Chapter II Materials and Methods

1. Strains and culture conditions

C. albicans strain #40 was obtained from the trachea of an intubated intensive care unit patient at Toronto General Hospital. The isolate has been maintained at -70°C in 40% glycerol containing 3% trisodium citrate following the initial isolation and microbiological characterization of the isolate. The isolate was subsequently recovered on Sabouraud-dextrose (SAB) agar (GIBCO) at 37°C for 18 h. *C. albicans* was then recultured on SAB agar plates for 18 h at 37°C and harvested in 3 ml of 10 mM phosphate buffered saline (PBS) pH 7.2 and utilized to inoculate 10 trays (30 cm x 22 cm) of SAB agar which were then incubated for 5 days at 37°C before cells were harvested.

2. Purification of *C. albicans* fimbriae

Fimbriae were purified from the yeast phase of *C. albicans*. *C. albicans* cells were harvested from the agar surface by gentle scraping with a bent glass rod. Harvested cells were suspended in a minimal volume (50 ml/tray) of preparation buffer (10 mM sodium phosphate buffered saline pH 7.2, containing 1 mM $CaCl_2$ and 1 mM phenylmethyl-sulfonyl fluoride). Harvested cells were washed three times with 500 ml of preparation buffer by centrifugation (12,000 x *g* for 20 min at 4°C). Fimbriae were sheared from the cell surface by gentle homogenization (4 x 45 second cycles) using a Brinkmann Homogenizer. The cells were removed by centrifugation (12,000 x *g* for 20 min) and by subsequent filtration of the supernatant through a 0.45 µm filter (Millex-PF, Millipore). The supernatant was concentrated approximately 10-fold with polyethylene glycol 8000 (PEG 8000). The concentrated fimbriae preparation was dialyzed overnight at 4°C against preparation buffer. This material was termed crude fimbriae (CF). The CF preparation was fractionated by size-exclusion high performance liquid chromatography using an isocratic gradient (flow rate = 0.5 ml/min; column = Waters Protein-PAK 300 SW 10 µm)

with preparation buffer as the solvent. The material that was eluted in the void volume was collected, concentrated with PEG and dialyzed against preparation buffer. This material was termed semi-enriched fimbriae and was rechromatographed under identical conditions. The peak which corresponded with the void volume of the column was again collected, concentrated and dialyzed against preparation buffer. This fraction was termed enriched fimbriae (EF).

3. Purification of *P. aeruginosa* PAK pili

The culture conditions of *P. aeruginosa* strain K (PAK) and the purification of PAK pili have been previously described by Paranchych *et al.* (1979). Briefly, PAK/2pfs bacteria were grown on solid medium in large pans and then harvested by scraping the surface of the agar and suspending the cells from 36 trays (about 100 g wet weight) in 1 L SSC buffer (0.15 M NaCl, 0.015 M sodium citrate, pH 7.0). The cells were then stirred with a magnetic stirrer at 5°C for 2 h. Large bits of agar were removed by passing the suspension through a sieve and the pili were removed from the cells by blending in 200 ml portions for 2 min at 2000 rpm with a Sorvall Omnimixer. After removing bacteria by centrifugation at 10,000 x g for 15 min, the NaCl concentration of the supernatant solution was adjusted to 0.5 M. Polyethylene glycol 6000 (PEG 6000) was then added to a final concentration of 1% (w/v), and the solution was allowed to sit for 18 h at 4°C. Both pili and flagella precipitated under these conditions and were removed by centrifugation at 7,000 x g for 20 min. To remove flagella, the pellet was resuspended in a 10% (w/v) $(NH_4)_2SO_4$ solution (pH 4.0) and allowed to stand at 4°C for 2 h. Pili precipitated under these conditions while flagella remained in suspension. Remaining flagella were removed by repeating the ammonium sulfate precipitation step. The final pellet was redissolved in water, dialyzed exhaustively to remove $(NH_4)_2SO_4$, then subjected to a CsCl density gradient centrifugation. The latter procedure involved layering 20 ml of pili solution onto 16 ml of a preformed linear gradient in which the CsCl density ranged from 1.1 to 1.5.

After 20 h of centrifugation at 20,000 rpm in an SW27 rotor using a Beckman L2-65B ultracentrifuge, the pilus band (buoyant density of about 1.3 g/cm^3) was removed, then subjected to a second CsCl density gradient centrifugation step. After recovering the pilus band from the second CsCl gradient and dialyzing to remove CsCl, the pili were resuspended in distilled water and washed by repeated centrifugation for 2 h at 50,000 rpm in a 60-Ti fixed-angle rotor at 4°C. The pili were judged pure when SDS polyacrylamide gel electrophoretic examination of the preparation showed a single protein band of heavily overloaded samples (100 µg/sample).

4. Protein determination of *C. albicans* fimbriae preparations

Protein concentrations of CF and EF were determined using a bicinchoninic acid (BCA) protein assay (Pierce) described by Smith *et al.* (1985) with bovine serum albumin (BSA) employed as the protein standard to generate a standard curve.

5. Carbohydrate compositions of *C. albicans* fimbriae

A phenol-sulfuric acid carbohydrate assay described by Dubois *et al.* (1956) was used to determine the amount of carbohydrate present in the EF preparation. EF was diluted 1:10 with 2 N H_2SO_4. Diluted EF (0.5 ml) was added to 0.5 ml of a 5% (w/v) solution of aqueous phenol and 2.5 ml of H_2SO_4 reagent (2.5 g hydrazine sulfate in 1L of concentrated sulfuric acid) and mixed vigorously before incubation in the dark for 1 h at room temperature. The absorbance at 490 nm of the reaction mixture was recorded. D-mannose (Sigma) was dissolved in 2 N H_2SO_4 and employed as a standard (0 to 100 µg/ml).

Based on the known amount of EF used for the carbohydrate analysis, both the protein and carbohydrate content in the *C. albicans* fimbriae could be used to determine the ratio of carbohydrate and protein.

The carbohydrate composition of the EF was investigated as described by Bryn and Jantzen (1982 and 1986). Briefly, lyophilized carbohydrate samples were methanolyzed with dry 2 M HCl/methanol for 16 h at 85°C. The derivatized sample (2 µl) was used directly. Samples were analyzed with a Varian Vista 6000 equipped with a Varian CDS 401 data station and a flame ionization detector, and employed a J & W DB-5 (95% methyl-polysiloxane, 5% phenylpolysiloxane) 30 cm long x 0.25 mm internal diameter column using helium carrier at a flow rate of 1 ml/min. The column was held isothermally for the initial 4 min at 90°C then rose at 8°C/min to a maximum of 270°C. Authentic carbohydrate samples (Sigma) were derivatized and utilized as standards.

6. SDS-PAGE/TSDS-PAGE and Electrotransfer

6-1 SDS-PAGE

Sodium dodecylsulfate polyacrylamide gel (SDS-PAGE) was performed with polyacrylamide gels in a mini-gel apparatus (Mini-protean^R II Dual Slab Cell, Bio-Rad) as described by Laemmli (1970). Samples were boiled in Laemmli sample buffer for 5 to 10 min and were electrophoresed in electrophoresis buffer (192 mM glycine, 50 mM Tris base and 0.1% SDS) for 50 min at a constant voltage of 200 V with a power supply model 1420A (Bio-Rad Laboratories).

6-2 TSDS-PAGE

Tricine sodium dodecylsulfate polyacrylamide gel (TSDS-PAGE) was performed with three layers of 4%, 10%, and 16.5% polyacrylamide gels in a mini-gel apparatus (Mini-protean^R II Dual Slab Cell, Bio-Rad) as described by Schagger and Von Jagow (1987) and Merril (1990a and 1990b). The protein samples were incubated for 5 min at 100°C in 4% (w/v) SDS, 12% (w/v) glycerol, 50 mM Tris pH 6.8, 2% (v/v) β-mercaptoethanol, and 0.005% (w/v) of bromophenol blue. Using a mini-gel apparatus

(mini-protean^{R} II Dual Slab Cell, Bio-Rad) the samples were electrophoresed for 1 h at a constant voltage of 30 V followed by 2 h at 120 V at room temperature.

6-3 Electroblot

Proteins on the SDS-PAGE or TSDS-PAGE gel were transferred to nitrocellulose membranes or PVDF membranes using the protocol of Towbin *et al.* with a Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad). Briefly, the gels were soaked in electrotransfer buffer (192 mM glycine, 50 mM Tris, pH 8.3 and 20% methanol) for 5 min. The proteins were transferred on 0.5 µm pore size nitrocellulose membrane (Bio-Rad Laboratories, Richmond, CA) which were prewetted in transfer buffer for 10 min or on 0.2 µm pore size PVDF (polyvinylidene difluoride) membranes (Bio-Rad Laboratories) which were prewetted in 100% methanol for 1 min and were subsequently soaked in electrotransfer buffer for 10 min. The transfer was completed after 30 min under constant current of 300 mA (Model 200/2.0 Power supply, Bio-Rad).

7. Amino acid compositions of *C. albicans* fimbrial subunit

Amino acid analysis was performed on *C. albicans* fimbriae electroeluted from a SDS-PAGE gel (described above) that was subsequently purified by reversed-phase chromatography. Crude fimbriae were separated by SDS-PAGE. The band on the gel containing the protein of interest was cut according to the size of the protein (the protein of interest had been previously identified by Western blotting analysis as described below). The gel slices containing the fimbrial subunits were washed (2 x 5 min) with elution buffer (20 mM ammonium bicarbonate) by gently shaking at 50 rpm on a Gyrotory shaker model G2 (New Brunswich Scientific Co.) for 30 min at room temperature. The gel slices were put into dialysis tubing (M.W. cut-off of 6,000 to 8,000) and suspended in water. The proteins were electroeluted from the gel slices in 20 mM ammonium bicarbonate using an electroelution apparatus (Schleicher & Scheull Elutrap^{TM}) by applying a constant voltage

of 200 V for 5 h or 80 V overnight. The eluate was collected and dialyzed against deionized water.

The fimbrial subunits were further purified by reversed-phase HPLC (Aquapore C_4 column 100 x 4.6 mm, I.D. 7) using a linear AB gradient (where solvent A is 0.05% (v/v) aqueous trifluoroacetic acid [TFA] and solvent B is 0.05% (v/v) TFA in acetonitrile) of 2% B/min gradient at a flow rate of 1 ml/min. The eluate was collected and lyophilized. A small amount of the lyophilized fimbriae was hydrolyzed in a glass tube with 200 µl of 6 N HCl, containing 0.1% (w/v) phenol at 110°C for 24 h *in vacuo*. The acid from the hydrolysate was removed by evaporation, resuspended in citrate buffer pH 2.2 and the amino acid content was analyzed with a Beckman Model 6300 amino acid analyzer. No attempt was made to analyze for total 1/2 Cys or Trp, nor were the values for Ser and Thr corrected to take into account losses during hydrolysis.

8. Protein sequencing

Protein sequencing was performed on *C. albicans* fimbrial blots which were electrotransfered from a SDS-PAGE gel (described above). Purified fimbriae (EF) were separated by 10% SDS-PAGE and were subsequently electrotransferred onto 0.2 µm pore size PVDF membrane (Bio-Rad Laboratories, Richmond, CA). The blots were stained with 0.01% (w/v) Coomassie blue (R-250, Bio-Rad) in 45% (v/v) methanol for 1 min and destained in 50% (v/v) methanol until the background was clean. The bands on the air-dried blots containing the protein of interest were cut and were subsequently analyzed by the protein sequencer (Hewlett Packasd G1005A) at Alberta Peptide Institute.

9. Electron microscopy of fimbriae

Fimbriae were diluted 1:100 with 10 mM sodium phosphate buffer pH 7.2. A 20 µl drop of diluted fimbriae solution was placed on a freshly prepared carbon/formvar coated 3 mm 200 mesh copper electron microscope grid (Fisher Scientific). The grid was

blotted with Whatman #1 filter paper, then negatively stained with 1% (w/v) phosphotungstic acid at a pH 7.0 for 10 seconds. The stain was removed by blotting and the sample was examined with a Philips model 410 transmission electron microscope operating at an accelerating potential of 80 kV. Micrographs were recorded on Kodak electron microscope film #4489.

Scanning electron micrographs of fimbriae of yeast phase *C. albicans* bound to human BECs were obtained according to the methods of Murakami *et al.* (1987). Specimens (3 ml) were fixed with a 2.5% (v/v) glutaraldehyde (J.B. EM Services Inc., Point Claire, Dorval Quebec) in 0.1 M phosphate buffer, pH 7.3, and incubated overnight at 4°C. Samples were aliquoted in 1.5 ml eppendorf tubes centrifuged at 120 rpm for 10 min and washed 3 x 20 min with 1.0 ml phosphate buffer pH 7.3. Samples were post-fixed in 2% (w/v) osmium tetroxide in 0.1 M phosphate buffer pH 7.3 for 1 h. The cells were washed by centrifugation as described above. Specimens were then resuspended in 1.0% (w/v) tannic acid in distilled water and incubated for 30 min at room temperature. The solution was removed by aspiration and the cells were washed with water and then resuspended in 2% (w/v) aqueous osmium tetroxide for 1 h and then washed with water. Specimens were then dehydrated in a graded series of ethanol to 100%. Samples were critical point dried and subsequently salted onto a standard Cambridge scanning electron microscope stub pre-coated with a double-sided adhesive tape. Specimens were then directly examined in a Hitachi S 4000 field emission scanning electron microscope operating at an accelerating potential of 2.5 kV.

10. Biotinylation of *P. aeruginosa* PAK pili and *C. albicans* fimbriae

One millilitre of purified *P. aeruginosa* PAK pili (1.76 mg) in 10 mM phosphate buffer pH 7.2 containing 150 mM sodium chloride (PBS) was used for the biotinylation. Freeze dried *C. albicans* fimbriae were resuspended in PBS to a concentration of 1 mg/ml

and a 600 µl aliquot of the fimbriae was removed for biotinylation. Pili and fimbriae in 1.5 ml eppendorf tubes were biotinylated by the addition of 20 µl of biotinamidocaproate N-hydroxysuccinimidyl ester (Sigma Chem. Co., St. Louis, MO) (20 mg/ml in dimethyl-sulfoxide) and incubated at room temperature for 45 min with vigorous agitation. The reaction mixtures were placed into dialysis membranes (M.W. cut-off of 6,000 to 8,000) and dialyzed against 4 L of PBS with 4 changes at 4°C. The biotinylated adhesins were aliquoted and stored at -20°C. PAK pili and *C. albicans* fimbriae were previously demonstrated to bind to asialo-GM_1 (Lee *et al.*, 1994 and Hazlett *et al.*, 1993; Chapter IIIA). Hence, biotinylated PAK pili (Bt-PAK pili) and biotinylated fimbriae (Bt-fimbriae) were assayed for their ability to bind to asialo-GM_1 (Sigma) immobilized onto microtiter wells (0.5 µg/well). The solid-phase binding assays were performed with some modifications, according to the methods of Karlsson and Stromberg (1987) and Lee *et al.* (1994). Biotinylated-adhesins were serially diluted with 10 mM Tris-HCl buffer pH 7.4 containing 150 mM sodium chloride (TBS) and 0.05% BSA (w/v) and added to the pre-coated wells (100 µl per well). After a 2 h incubation at 37°C, the wells were washed five times with TBS containing 0.05% BSA. Streptavidin-alkaline phosphatase conjugate (Jackson ImmunoResearch Laboratory) diluted (1:2500; v/v) with TBS containing 1% (w/v) BSA, was added to the wells (100 µl/well) and incubated for 2 h at 37°C. Both the Bt-PAK pili (1.5 mg/ml) and Bt-fimbriae (0.86 mg/ml) had titers (last dilution where signal binding of the adhesin could be differentiated from controls) of about 10^{-6}.

11. Biotinylation of *P. aeruginosa* and *C. albicans* whole cells

C. albicans whole cells (yeast form) were biotinylated according to the methods of Casanova *et al.*, (1992a) with some modification. *C. albicans* strain 40 was allowed to grow overnight on a Sabouraud agar plate at 37°C. Cells were harvested and suspended in 10 ml of 0.1 M phosphate pH 8.0 containing 150 mM NaCl (PBS). Lyophilized *P. aeruginosa* strain PAK/2pfs was resuspended in 200 µl 10 mM PBS pH 7.4 and plated on

Brain Heart Infusion (BHI) agar (Difco) and cultured overnight at 37°C. A single colony of *P. aeruginosa* strain PAK/2pfs was selected, and re-streaked on BHI plate and incubated overnight at 37°C. *P. aeruginosa* whole cells were harvested and suspended in 10 ml of 0.1 M PBS pH 8.0. *C. albicans* and *P. aeruginosa* whole cells were collected by centrifugation (10 min at 10,000 x *g* at 4°C) and washed with 10 ml of PBS pH 8.0. The cells were collected by a second centrifugation (10 min at 10,000 x *g* at 4°C) and resuspended in 5 ml of 100 mM PBS pH 8.0. Aliquots of 30 µl of biotinamidocaproate N-hydroxysuccimidyl ester (20 mg/ml DMSO) were added to 2 ml of resuspended *C. albicans* or *P. aeruginosa* whole cells diluted with 1 ml of PBS pH 8.0. The mixture was allowed to react for one h in a water-bath shaker (200 rpm) at 22°C. Cells were recovered and washed four times with 10 mM PBS pH 6.0 by centrifugation 10,000 x *g* (for 10 min at 4°C). The biotinylated whole cells were subjected to a final wash with 10 mM PBS pH 7.4 and cell concentrations were adjusted to 10^9 cells per ml by plating serial dilutions of the organisms.

12. Human buccal epithelial cells

12-1 Preparation of BECs for filtration binding assays

Human buccal epithelial cells (BECs) were collected from 10 healthy, non-smoking male volunteers by gentle scraping of the buccal mucosal surface with wooden applicator sticks. These sticks were then agitated in 40 ml of PBS pH 7.2 to remove the BECs. BECs were washed 3 x 10 min with 10 ml of PBS by centrifugation at 2,000 x *g*. Cell clumps were removed by filtration through a 70 µm nylon mesh (Spectrum, Cole-Parmer). The cell concentration was determined directly with a hemocytometer and BECs were resuspended in PBS to a concentration of 2.0×10^5 BECs/ml.

12-2 Immobilized BECs on ELISA plates

BECs were collected and prepared as previously described (see Materials and Methods 12-1). BECs were immobilized onto wells of microtiter plates with some modifications to the methods of Ofek *et al.* (1986) and Sexton and Reen (1992). The wells of the microtiter plate were coated with aliquots (100 µl per well) of poly-L-lysine dissolved in 10 mM PBS pH 7.4. The plate was incubated overnight at 4°C. The cells were washed twice with PBS (200 µl per well) and resuspended in PBS to give a cell concentration of 10^5 cells per ml. Aliquots of BECs were added to the pre-coated wells (100 µl per well). The plate was allowed to incubate at room temperature for 10 min. The cells were then centrifuged for 10 min at 1,000 x *g* on a Sorvall RT6000B Refrigerated Centrifuge (DuPont). The buffer was carefully removed by aspiration using a multi-channel pipettor equipped with electrophoresis Prot/Elec pipet tips (Bio-Rad). The plate was dried overnight in a 37°C incubator. The BECs were fixed with a 0.25% (v/v) solution of glutaraldehyde in 0.1 M phosphate buffer pH 7.3 for 1 h at room temperature. The wells were washed four times with PBS. Unbound sites in the wells were then blocked with aliquots (150 µl per well) of 5% (w/v) BSA in PBS pH 7.4. After a 1 h incubation at 37°C, the cells were washed 3 times with PBS containing 0.05% (w/v) BSA and directly used in adhesion assays.

13. Production of anti-*P. aeruginosa* pili and anti-*C. albicans* fimbrial antibodies

13-1 Anti-fimbriae monoclonal antibodies, Fm16 and Fm34

Anti-*C. albicans* fimbriae monoclonal antibodies were produced by Ens and Gowing[1] with a hybridoma technique previously employed to obtain *P. aeruginosa* pili monoclonal antibodies (Doig *et al.*, 1990). BALB/c female mice (Charles River Breeding

[1] Address: Dept. of Botany, Erindale College, University of Toronto, Missauga, Ontario.

Laboratories Inc.) were immunized on days 1, 8, 15, 32 and 46 with 10 µg EF in a 1% (w/v) $Al(OH)_3$. The EF were first denatured by boiling in a 1% (w/v) SDS and 1 mM β-mercaptoethanol. Animals were exsanguinated and the antibody titers were determined by enzyme-linked immunosorbent assays (ELISA) with the semi-enriched fimbriae (10 µg/ml) as the coating antigens in the microtiter wells (100 µl/well).

Following the development of high titer antibodies, 3 mice were sacrificed and their spleens removed aseptically. The mouse myeloma used for the production of hybridoma clones was NS1. The NS1 cell line was cultured in high-glucose Dulbecco modified Eagle medium supplemented with 2 mM L-glutamine and 10% (v/v) fetal calf serum (GIBCO Laboratories) at 37°C in the presence of 5% CO_2. Cells were passed every 48 h at a split ratio of 1:5 or 1:4. Production and selection of hybridomas were carried out as described by Irvin and Ceri (1985), except that the medium was not supplemented with β-mercaptoethanol. Clones were selected for their ability to synthesize anti-*C. albicans* fimbriae antibodies as determined by ELISA employing EF as the antigens. Positive clones had A_{405} values that were double or greater than control levels. These results were confirmed by Western blots. Positive hybridoma clones were scaled up into 5 ml Dulbecco modified Eagle medium supplemented with 20% (v/v) fetal calf serum, 2 mM L-glutamine, hypoxanthine, aminopterin, and thymidine. Clones were frozen and subsequently, subcloned twice in semisolid agarose (Kennet, 1980a, b). One particular monoclonal antibody, Fm16, was chosen for further analysis. Fm16 is an IgG2aκ based upon isotyping results obtained with the SBA Clonotyping System II (Southern Biotechnology Associates, Inc., Birmingham, USA). Ascites tumors were produced by injecting 10^6 hybridoma cells into pristiane-primed BALB/c male mice (Kroprowski *et al.*, 1977). Ascites fluid was recovered daily with a 25-gauge needle following the development of an ascites tumor. Typically, 15 ml of ascites fluid was collected over a period of 7 to 10 days.

The protocol for production of anti-*C. albicans* fimbriae MAb Fm 34 against a purified fraction of the fimbriae preparation was exactly same as that of Fm16 production. The isotype of Fm34 is IgG2λ.

13-2 Anti-*P. aeruginosa* pilin monoclonal antibodies, PK99H and PKL1

Monoclonal antibodies (MAbs) were raised against purified *P. aeruginosa* PAK pili in BALB/c female 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 previously described (Doig *et al.*, 1987). Anti-pili MAbs, PK99H and PKL1 used in these studies, are of the subtype IgG1κ and IgG3κ, respectively. PKL1 and PK99H both recognize the 17-residue receptor-binding domain of the PAK pilin. However, the binding epitope of PKL1 is different from PK99H's.

13-3 Anti-synthetic peptides of PAK pilin antibodies

Synthetic peptides which corresponded to the different regions of the *P. aeruginosa* strain PAK pilin sequence (Sastry *et al.*, 1985) were prepared using an Applied Biosystem Inc. model 430A automated peptide synthesizer by Alberta Peptide Institute. The syntheses, purification and characterization of these peptides have been described previously (Wong, *et al.*, 1992). Ac-PAK(128-144)$_{ox}$-OH (KCTSDQDEQFIPKGCSK) and Ac-PAK(134-140)-NH_2 (DEQFIPK) correspond to the carboxy-terminal of the PAK pilin [amino acid residues are represented by the standard single letter code; Ac- an acetylated N^{α} terminus; -NH_2 denotes an amidated carboxyl terminal; -OH represents the α-carboxyl group; ox- denotes a disulfide bond formation between the sulfhydryl groups on the two cysteine residues (residues 129 and 142) on the PAK(128-144) peptide]. In the production of anti-peptide antibodies, these peptides were synthesized with a

benzoylbenzoyl moiety, a norleucine and two glycine spacer at the amino terminal (instead of an acetylated group). The peptides were conjugated onto keyhole limpet hemocyanin protein carriers via the photoreactive benzoylbenzoyl moiety as previously described (Lee *et al.*, 1989; Parker and Hodges, 1985). The immunization protocols in rabbits to obtain anti-peptide antibodies were performed according to Lee *et al.* (1989). These polyclonal antibodies have been previously described (Sheth *et al.*, 1995, Lee *et al.*, 1989). Anti-PAK(75-84) peptide rabbit polyclonal antibodies and the normal rabbit whole IgG (Jackson ImmunoResearch Laboratory) were be used as controls and PAK(75-84) corresponding to the central part of the PAK pilin with a GVAADANKLG-NH_2 sequence and Ac-TnI(96-139)-NH_2 corres-ponding to the central part of troponin I from rabbit muscle with a sequence consisting of Ac-NQKLFDLRGKFKRPPLRRVRMSADAMLKALLGSSHKVAM DLRAN were included as controls.

14. Immunoblots

14-1 Western blot analysis of *C. albicans* fimbriae preparations

Sodium dodecylsulfate polyacrylamide gel (SDS-PAGE) was performed with 12.5% acrylamide gels in a mini-gel apparatus (Mini-protean^R II Dual Slab Cell, Bio-Rad) as described above. Gels were stained with Coomassie blue (R-250, Bio-Rad) or with silver stain (Tsai and Frasch, 1982).

Proteins on the SDS-PAGE gel were transferred to nitrocellulose membrane described above. Excess binding sites on the membrane were blocked by incubation of the blots overnight at 4°C with a blocking solution consisting of 50 mM tris-hydroxy-methyl aminomethane (Tris) HCl, pH 7.5, 150 mM NaCl, 0.05% (v/v) Nonidet-P40, 0.25% (w/v) gelatin and 3% (w/v) BSA. The membrane was washed twice at room temperature with 10 mM Tris-HCl buffer pH 7.5 containing 0.1% (v/v) Tween-20 and 0.05% (w/v) BSA (TBST). The membrane was cut into 0.5 cm strips and placed into slots in transfer

plates. Mouse anti-EF ascites, Fm16, and normal mouse immunoglobulin G (10.3 mg/ml, Jackson ImmunoResearch Laboratory) were diluted with TBST (1:500) and added to the respective strips and incubated for an hour at 37°C in an incubator shaker (model G25 Gyroshaker, New Brunswick Scientific, New Jersey, USA) set at 100 rpm. The strips were washed three times with TBST. A goat anti-mouse IgG(H+L)-alkaline phosphatase conjugate (Jackson Laboratories) diluted 1:10,000 with TBST was incubated as described above. The strips were washed 3 times with TBST followed by a final wash with Tris-buffered saline. Antibody binding was visualized by the addition of alkaline phosphatase substrates (nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate dissolved in 100 mM Tris-HCl pH 9.5, containing 100 mM NaCl and 5 mM $MgCl_2$). Color development was stopped by rinsing the nitrocellulose strips with deionized water.

14-2 Immunoblotting of pilus and fimbrial adhesins

The discontinuous sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) described by Laemmli and Favre (1973) was used in the chromatography of *C. albicans* fimbriae and *P. aeruginosa* PAK pili and employed 10% gels in a mini-gel apparatus (Mini-protean^R II Dual Slab Cell, Bio-Rad Laboratories). Samples were solubilized by heating at 100°C for 15 min in 60 mM Tris buffer, pH 6.8, containing 2% (w/v) SDS, 5% (v/v) β-mercaptoethanol and 10% (v/v) glycerol. Electrophoresis was conducted under a constant voltage of 200 V using a Bio-Rad model 1420A power supply. Proteins were blotted onto pre-wetted nitrocellulose membrane 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. Pre-stained 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 (Materials and Methods 14-1).

15. ELISA

15-1 Direct Binding Assays

15-1-1 Binding of *C. albicans* (CA) fimbriae to glycosphingolipids (GSLs)

Polystyrene microtiter plates (Nunc) were coated with asialo-GM_1 and ceramide trihexoside (CTH) (Sigma Chem. Co., St. Louis, MO). Aliquots of the GSLs (5 µg/ml resuspended in methanol) were added into the wells (100 µl/well) and the plates were incubated overnight at 4°C. The wells were washed three times with 250 µl/well of 10 mM phosphate buffered saline pH 7.4 containing 150 mM NaCl (PBS) supplemented with 0.05% (w/v) bovine serum albumin (Buffer A). Excess binding sites were blocked by the addition of 200 µl/well of 5% (w/v) BSA in PBS pH 7.4 and incubation at 37°C for 1 h. Wells were washed three times with 250 µl/well of buffer A. Enriched fimbriae ranging from 0 to 40 µg protein/ml in Buffer A were added to the wells (100 µl/well) and incubated for 2 h at 37°C. An aliquot of mouse anti-EF monoclonal antibodies, Fm16 (diluted 1:500), was added to each well (100 µl) and incubated at 37°C for 2 h. Wells were washed 5 times with 250 µl/well of Buffer A. Antibody binding to EF was assessed by the addition of a goat anti-mouse heavy and light chain [IgG(H+L)] immunoglobulin G-peroxidase conjugates (Jackson Laboratories) to each well (100 µl/well) and incubated for 1 h at 37°C. The wells were washed 5 times with 250 µl/well of Buffer A and 125 µl/well of a substrate solution containing 1 mM 2,2'-azido-di-[3-ethylbenzthiazoline sulfonic acid] (ABTS) in 10 mM sodium citrate buffer (pH 4.2) containing 0.03% (v/v) hydrogen peroxide was added. The reaction was stopped by an addition of 125 µl/well of 4 mM sodium azide and the absorbance at 405 nm was recorded.

15-1-2 Binding of *C. albicans* fimbriae to synthetic βGalNAc(1-4)βGal

βGalNAc(1-4)βGal-O$(CH_2)_8COOCH_3$ was synthesized by Dr. O. Hindsgaul group, Department of Biochemistry, University of Alberta, according to the method of Sabesan and Lemieux (1984) and conjugated to BSA by Dr. H. Sheth in 1993, a postdoctoral fellow in Dr. R. Irvin's lab in department of Medical Microbiology and Immunology, University of Alberta. The coupling ratio of disaccharide:BSA was 12:1. The protocol utilized for this assay was similar to that employed for the assessment of *C. albicans* fimbriae binding to GSLs except that the plates were coated with 100 µl/well of a 10 µg/ml solution of synthetic βGalNAc(1-4)βGal-BSA conjugate in 0.01 M carbonate buffer, pH 9.5. The remainder of the protocol was unchanged from that described above.

15-1-3 Binding of *C. albicans* fimbriae to BECs

Human buccal epithelial cells (BECs) were collected according to the method described above. The cell concentration was determined directly with a hemocytometer and BECs were resuspended in PBS to a concentration of 2.0 x 10^5 BECs/ml.

The fimbrial adherence assay was performed using a Manifold filtration apparatus equipped with individual vacuum stopcocks (Model FH 225V, Hoefer Scientific Instruments). Polycarbonate filters, 2.5 cm in diameter, 12 µm pore size (Nucleopore Costar Corp.), were pre-incubated overnight at 4°C with 50 ml of PBS pH 7.2, containing 0.45% (v/v) Tween 20. The pretreated filters were placed into each chamber and washed with 2.5 ml of PBS. One ml samples containing 2.0 x 10^5 BECs in PBS was added to each chamber. Enriched fimbriae (100 µl /chamber, ranging from 0 to 80 µg fimbriae protein /ml) in PBS containing 0.05% (v/v) Tween-20 was added to each chamber and incubated with the BECs for 1.5 h at room temperature. Unbound fimbriae were removed with washes of 2.5 ml of PBS. Mouse anti-*C. albicans* fimbriae monoclonal antibody (ascites fluid diluted 1:3000 with PBS) was added to the BECs (1.2 ml/chamber) and

incubated for 1.5 h at room temperature. BECs were washed five times with 2.5 ml of PBS. Goat anti-mouse IgG(H+L)-peroxidase conjugates (Jackson Laboratories) diluted 1:5000 with PBS were added (1.0 ml/chamber) and incubated for another hour at room temperature. The cells were then washed seven times with 2.5 ml/chamber of PBS. The polycarbonate filters containing BECs were removed from the filtration manifold and placed into glass scintillation vials. The horseradish peroxidase substrate solution (ABTS) was added to each vial (1 ml/vial) and incubated for 30 min at room temperature on a shaker at 100 rpm. The reaction was stopped by the addition of 4 mM sodium azide (200 µl/vial). The substrate solution was pipetted into Eppendorf tubes and centrifuged at 5,000 x *g* for 3 minutes. Aliquots of the supernatants were pipetted into microtiter wells (200 µl/well) and the resulting absorbance at 405 nm was recorded with a Titertek Multiskan Plus microplate recorder.

15-1-4 Binding of biotinylated *C. albicans* to GSLs

The Bt-*C. albicans* whole cells were prepared by the method described above. Polystyrene microtiter plates (Nunc) were coated with asialo-GM_1 and ceramide trihexoside (CTH) (Sigma Chem. Co., St. Louis, MO) Aliquots of the GSLs (5 µg/ml resuspended in methanol) were added into the wells (100 µl/well) and the plates were incubated overnight at 4°C. The wells were washed three times with 250 µl/well of 10 mM phosphate buffered saline pH 7.4 containing 150 mM NaCl (PBS) supplemented with 0.05% (w/v) bovine serum albumin (Buffer A). Excess binding sites were blocked by the addition of 200 µl/well of 5% (w/v) BSA in PBS pH 7.4 and incubation at 37°C for 1 h. Wells were washed three times with 250 µl/well of buffer A. Aliquots (100 µl/well) of serially diluted Bt-*C. albicans* whole cell suspensions (starting from 10^7 CFU/ml) were added to each well and incubated for 2 h at 37°C. The wells were washed three times with TBS containing 0.05% BSA (w/v). Strepavidin-alkaline phosphatase (1:3,000) was added to the wells (100 µl per well) and incubated for 1 h at 37°C. A substrate solution *p*-

nitrophenylphosphate (1 mg per ml of 10% [w/v] aqueous diethanolamine pH 9.6) was added to the wells (100 µl per well). A_{405} readings were recorded following a 45 min incubation at room temperature.

15-1-5 Binding of biotinylated *C. albicans* to BECs

Human buccal epithelial cells (BECs) were collected according to the method described above (see Materials and Methods 12-1). The cell concentration was determined directly with a hemocytometer and BECs were resuspended in PBS to a concentration of 2.0×10^5 BECs/ml. The biotinylated *C. albicans* whole cells were prepared described above (see Materials and Methods 11).

The *C. albicans* adherence assay was performed using a Manifold filtration apparatus equipped with individual vacuum stopcocks (Model FH 225V, Hoefer Scientific Instruments). Polycarbonate filters, 2.5 cm in diameter, 12 µm pore size (Nucleopore Costar Corp.), were pre-incubated overnight at 4 °C with 50 ml of TBS pH 7.2, containing 0.45% (v/v) Tween 20. The pretreated filters were placed into each chamber and washed with 2.5 ml of TBS. 1 ml containing 2.0×10^5 BECs in TBS was added to each chamber. Aliquots (100 µl/well) of serially diluted Bt-*C. albicans* whole cell suspensions (starting from 10^7 CFU/ml) in TBS containing 0.05% (v/v) Tween-20 was added to each chamber and incubated with the BECs for 1.5 h at room temperature. Unbound Bt-*C. albicans* were removed with washes of 2.5 ml of TBS containing 0.05% BSA (w/v). Strepavidin-alkaline phosphatase (1:3000) was added to each vial (1 ml/chamber) and incubated for another hour at room temperature. The cells were then washed seven times with 2.5 ml/chamber of TBS. The polycarbonate filters containing BECs were removed from the filtration manifold and placed into glass scintillation vials. A substrate solution *p*-nitrophenylphosphate (1 mg per ml of 10% [w/v] aqueous diethanolamine pH 9.6) was added to the wells (1 ml per vial) and incubated for 30 min at room temperature on a shaker at 100 rpm. The substrate solution was pipetted into Eppendorf tubes and centrifuged at

5,000 x *g* for 3 minutes. Aliquots of the supernatants were pipetted into microtiter wells (200 µl/well) and the resulting absorbance at 405 nm was recorded with a Titertek Multiskan Plus microplate recorder.

15-2 Inhibition of adhesins binding to Glycosphingolipids

15-2-1 Inhibition of CA fimbriae binding to GSLs with GSLs

The protocol for this assay was similar to that employed for the assessment of *C. albicans* fimbriae binding to GSLs except that *C. albicans* fimbriae were preincubated with asialo-GM_1 and CTH, respectively, for 1 h at 37°C prior to their addition into the wells. A fixed concentration of EF (50 µg/ml) was incubated with varying GSL concentrations (0 to 15 µg/ml) in these assays. The remainder of the protocol was as described above with the direct binding assay (see Materials and Methods 15-1-1).

15-2-2 Inhibition of CA fimbriae binding to GalNAcGal with asialo-GM_1

The ability of asialo-GM_1 to block CA fimbriae was demonstrated using a protocol similar to the above assays with inhibition of CA fimbriae binding to GSLs with GSLs, with the exception that βGalNAc(1-4)Gal-$O(CH_2)_8COOCH_3$ conjugated with BSA was used to coat the plate in place of GSLs.

15-2-3 Inhibition of CA fimbriae binding to GSLs with GalNAcGal

The protocol of this inhibition assay is similar to the inhibition of CA fimbriae binding to GSLs with GSLs, with the exception that βGalNAc(1-4)Gal-$O(CH_2)_8COOCH_3$ was used as a competitor in place of GSLs.

15-2-4 Inhibition of biotinylated adhesins binding to GSLs with unbiotinylated adhesins

The specificities of the Bt-PAK pili and Bt-fimbriae for asialo-GM_1 were demonstrated using a competitive binding assay in which unbiotinylated adhesins were used to inhibit Bt-PAK pili and Bt-fimbriae binding to immobilized asialo-GM_1. Aliquots of Bt-PAK pili (diluted 1:2500 to 0.6 µg/ml with TBS pH 7.4 containing 0.05% BSA) and Bt-fimbriae (1:500 dilution to 1.2 µg/ml) were pre-mixed with equal volumes of serially diluted PAK pili/fimbriae prior to addition into the pre-coated wells containing 5 µg/ml of asialo-GM_1. Unbound Bt-adhesins were removed with five washes of TBS (250µl per well). Streptavidin-alkaline phosphatase was diluted 1:2500 with TBS pH 7.4 containing 1% (w/v) BSA and added to the wells (100 µl per well). The plate was incubated for 2 h at 37°C. The wells were washed five times with TBS (250 µl per well). A substrate solution, ρ-nitrophenylphosphate 1 mg per ml of 10% (w/v) aqueous diethanolamine pH 9.6, was added to the wells (100 µl per well). The A_{405} readings were recorded following a 1 h incubation at room temperature. Bt-PAK pili (1.5 mg/ml) and Bt-fimbriae (0.86 mg/ml) had titers greater than 10^5.

15-2-5 Inhibition of biotinylated adhesins binding to GSLs with synthetic peptides

The methodology for the competitive binding assays where synthetic peptides, the Ac-PAK(128-144)ox-OH, Ac-PAK(134-140)-NH_2, Ac-PAK(75-84)-NH_2 or Ac-TnI(96-139) were used to compete with Bt-PAK pili or Bt-fimbriae binding to asialo-GM_1 is similar to the above inhibition binding assay with anti-peptide antibodies with the exception that the synthetic peptides were used as competitors in place of whole protein adhesins. The concentrations for the Bt-PAK pili and Bt-fimbriae used were the same as above (see Materials and Methods 15-2-4).

15-2-6 Inhibition of biotinylated adhesins binding to asialo-GM_1 with anti-adhesin antibodies

The specificities of the Bt-PAK pili and Bt-fimbriae for asialo-GM_1 were demonstrated using a competitive binding assay in which anti-synthetic peptide antibodies [anti-PAK(128-144), anti-PAK(134-140) and anti-PAK(75-84)] or synthetic peptides, Ac-PAK(128-144)ox-OH, and Ac-PAK(134-140)-NH_2 which correspond to the binding domain of PAK pilin, were used to inhibit Bt-PAK pili/Bt-fimbriae binding to immobilized asialo-GM_1. Normal rabbit whole IgG or synthetic peptide, Ac-PAK(75-84)-NH_2 corresponding to the central region of PAK pili, showed no binding activity to immobilized asialo-GM_1 (Wong *et al.*, 1992). A peptide which corresponds to rabbit skeletal muscle protein troponin I internal sequence [Ac-TnI(96-139)] was used as a control in the inhibition assays. A fixed concentration of the Bt-PAK pili/Bt-fimbriae was selected based on the direct binding assays. Bt-PAK pili were diluted to 0.6 µg/ml and Bt-fimbriae were diluted to 1.2 µg/ml with TBS containing 0.05% BSA.

For antibody inhibition studies, rabbit polyclonal anti-peptide antibodies (2 mg/ml) [anti-PAK(128-144), anti-PAK (134-140), anti-PAK(75-84) and normal rabbit whole IgG] were serially diluted in solutions of Bt-PAK pili and Bt-fimbriae respectively, mixed and incubated for 1 h at 37°C. The aliquots (100 µl) of mixed adhesin-antibody solution were added to the wells which were pre-coated with 0.05 µg/well of asialo-GM_1. The assay mixtures were incubated with the asialo-GM_1 for 1 h at 37°C. The wells were washed three times with TBS containing 0.05% BSA (w/v). Strepavidin-alkaline phosphatase (1:3000) was added to the wells (100 µl per well) and incubated for 1 h at 37°C. A substrate solution *p*-nitrophenylphosphate (1 mg per ml of 10% [w/v] aqueous diethanol-amine pH 9.6) was added to the wells (100 µl per well). A_{405} readings were recorded following a 45 min incubation at room temperature.

15-3 Inhibition of adhesins binding to BECs

15-3-1 Binding of adhesins to immobilized BECs

Bt-PAK pili and Bt-fimbriae were serially diluted with TBS pH 7.4 containing 0.05% (w/v) BSA and added to immobilized BECs (100 µl per well). Biotinylated adhesins were allowed to bind to the BECs for 2 h in a 37°C incubator. Unbound biotinylated adhesins were removed with five washes of TBS (250 µl per well). Streptavidin-alkaline phosphatase was diluted 1:2500 with TBS pH 7.4 containing 1% (w/v) BSA and added to the wells (100 µl per well). The plate was incubated for 2 h at 37°C. The wells were washed five times with TBS (250 µl per well). A substrate solution, *p*-nitrophenylphosphate 1 mg/ml of 10% (w/v) aqueous diethanolamine pH 9.6, was added to the wells (100 µl per well). The A_{405} readings were recorded following a 1 h incubation at room temperature. Bt-PAK pili (1.5 mg/ml) and Bt-fimbriae (0.86 mg/ml) had titers greater than 10^5.

15-3-2 Inhibition of biotinylated-adhesins binding to BECs with unbiotinylated adhesins

Competitive binding assays were set up to demonstrate the specificity of the interactions between the Bt-adhesins and receptors on BECs. A fixed concentration of the Bt-PAK pili/Bt-fimbriae was selected based on the direct binding assays. Aliquots of serially diluted solutions of unbiotinylated adhesins were mixed with equal volumes of Bt-adhesins. The mixtures were added to the BECs (100 µl per well) and allowed to incubate for 2 h at 37°C. The remainder of the protocols was similar to the direct binding assay described above. The ability of the adhesins to interact with BEC receptors was determined as the percent inhibition of Bt-adhesin binding to receptors with respect to the binding in the absence of competing unbiotinylated adhesin.

15-3-3 Inhibition of *C. albicans* binding to BECs with purified fimbriae

C. albicans cells were radiolabelled as previously described by McEachran and Irvin (1985). A loopful of culture from Sabouraud dextrose agar (GIBCO) was used as a source of inoculum for 10 ml of M9 medium supplemented with 0.4% (w/v) glucose. Cultures were incubated at 25°C for 12 h with 150 rpm agitation in G25 Gyrotory shaker (New Brunswick Scientific Co.). Cultures were supplemented with 5 µCi/ml of [^{35}S]-L-methionine (New England Nuclear, Boston, Mass.) after 10 h of incubation. Cells were harvested by centrifugation (12,000 x *g* for 10 min) and washed 3 times with 10 ml of PBS pH 7.2 to remove unincorporated methionine. Washed cells were resuspended in PBS. No clumping was observed during the assay. The amount of [^{35}S]-L-methionine incorporated by the *C. albicans* cells was determined by filtering 1.0 ml of a 1:100 dilution of washed *C. albicans* culture through a 0.2 µm polycarbonate filter (Nucleopore Corp., Pleasanton, CA) in triplicate, washing with 15 ml of PBS, and placing the filter in scintillation vials with 5.0 ml of Aquasol (New England Nuclear, MA). The counts per minute were determined with a Beckman LS-150 liquid-scintillation counter. The specific activity of [^{35}S]-*C. albicans* cells was generally 0.2 cpm/CFU and this remained stably associated with the *C. albicans* cells throughout the assay.

BECs (0.5 ml) were preincubated with EF at varying concentrations (from 0 to 18 µg protein/ml) in polystyrene tubes at 37°C for 1 h (final concentrations: 2.0 x 10^5 BECs/ml). An equal volume of radio-labelled yeast suspended in PBS pH 7.2 was added to the BECs and incubated at 37°C for 2 h, shaking at 300 rpm. Triplicate aliquots were removed after the assay and filtered through 12 µm polycarbonate filters pretreated with 3% (w/v) BSA in PBS. BECs were washed with 15 ml of PBS. The filters were then placed in scintillation vials and the cpms were determined as described above. Yeast binding to BECs was corrected for nonspecific binding of yeast to the 12.0 µm filter (nonspecific

binding was generally less than 15% of the experimental value). The BEC concentration was determined at the end of the assay to correct for cells lost during incubation.

Total and viable cell counts were performed before and after the adhesion assay. Total cell counts were determined using a hemocytometer. Viable counts were determined by serially diluting *C. albicans* in PBS pH 7.2 and plating appropriate dilutions on SAB agar which were incubated at 37°C until visible and countable colonies formed (usually 24 to 48 h).

15-3-4 Inhibition of biotinylated adhesins binding to BECs with synthetic peptides

The specificities of the Bt-PAK pili and Bt-fimbriae for asialo-GM_1 were also demonstrated using a competitive binding assay in which synthetic peptides, Ac-PAK(128-144)ox-OH, and Ac-PAK(134-140)-NH_2 which correspond to the binding domain of PAK pilin, were used to inhibit Bt-PAK pili/Bt-fimbriae binding to immobilized asialo-GM_1. Synthetic peptide, Ac-PAK(75-84)-NH_2 which corresponds to the central region of PAK pili, was used as a control in the inhibition assays. The methodology for the competitive binding assays where synthetic peptides Ac-PAK(128-144)ox-OH, Ac-PAK(134-140)-NH_2, Ac-PAK(75-84)-NH_2 or Ac-TnI(96-139) were used to compete with Bt-PAK pili or Bt-fimbriae binding to asialo-GM_1, is similar to the below inhibition binding assay with anti-peptide antibodies. One exception is that the pre-incubation of adhesins and antibodies for 1 h at 37°C was omitted. Bt-PAK pili were diluted to 0.6 µg/ml and Bt-fimbriae were diluted to 1.2 µg/ml with TBS containing 0.05% BSA.

15-3-5 Inhibition of Bt-adhesins to BECs with antibodies

The specificities of the Bt-PAK pili and Bt-fimbriae for asialo-GM_1 were demonstrated using a competitive binding assay in which anti-adhesin antibodies [Fm16, PK99H, PAK13 and normal mouse IgG] and anti-synthetic peptide antibodies [anti-

PAK(128-144), anti-PAK(134-140) and anti-PAK(75-84)] were used to inhibit Bt-PAK pili/Bt-fimbriae binding to immobilized asialo-GM_1. A fixed concentration of the Bt-PAK pili/Bt-fimbriae was selected based on the direct binding assays. Bt-PAK pili were diluted to 0.6 µg/ml and Bt-fimbriae were diluted to 1.2 µg/ml with TBS containing 0.05% BSA. Mouse monoclonal antibodies and rabbit polyclonal anti-peptide antibodies (2 mg/ml) were serially diluted in solutions of Bt-PAK pili and Bt-fimbriae respectively and mixed completely and incubated for 1 h at 37°C. The aliquots (100 µl) of mixed adhesin-antibody solution were added to the wells which were pre-coated with 0.05 µg/well of asialo-GM_1 or 10^4 cells/well BECs. The assay mixtures were incubated with the BECs for 1 h at 37°C. The wells were washed three times with TBS containing 0.05% BSA (w/v). Strepavidin-alkaline phosphatase (1:3,000) was added to the wells (100 µl per well) and incubated for 1 h at 37°C. A substrate solution *p*-nitrophenylphosphate (1 mg per ml of 10% [w/v] aqueous diethanolamine pH 9.6) was added to the wells (100 µl per well). A_{405} readings were recorded following a 45 min incubation at room temperature.

15-3-6 Inhibition of *C. albicans* fimbriae binding to BECs by GSLs and βGalNac(1-4)βGal

Both GSLs (asialo-GM_1 and CTH) and βGalNAc(1-4)βGal-methylester were used to inhibit *C. albicans* fimbriae binding to BECs. The binding assay was performed using 12 µm polycarbonate filter membranes (Nucleopore Costar Corp.) placed in chambers in a Manifold filtration apparatus equipped with individual vacuum stopcocks (Model FH 225 V, Hoefer Scientific Instruments). The protocols described in Materials and Methods 15-1-1 were employed with some modifications. The fimbriae (50 µg) were preincubated with either βGalNAc(1-4)βGal-methylester, asialo-GM_1, or CTH (total volume of 1 ml PBS pH 7.2 containing 0.05% [v/v] Tween-20) at 37°C for 1 h prior. The mixtures were added to BECs (2.0×10^5 BECs in 1 ml PBS pH 7.2) and incubated at room temperature for 1.5 h.

The remainder of the protocol is as described previously (see Materials and Methods 15-1-1).

15-4 Inhibition of pathogens binding to GSLs

15-4-1 Inhibition of biotinylated pathogens binding to asialo-GM_1 with anti-adhesin Abs and anti-adhesin-peptide Abs

The abilities of anti-peptide antibodies to block whole cell binding to asialo-GM_1 were determined in the following experiments. The Bt-*P. aeruginosa* or Bt-*C. albicans* whole cell suspensions have been diluted in TBS-Mg (50 mM Tris, 100 mM NaCl, 1 mM $MgCl_2$ pH 7.4) to a final cell concentration of 10^9 CFU/ml for Bt-*P.aeruginosa* or 10^5 CFU/ml for Bt-*C. albicans* . The concentration of all the stock anti-peptide antibodies were 2 mg/ml. Serially diluted solutions of monoclonal antibodies (FM16, PK99H, and normal mouse IgG), anti-PAK(128-144), anti-PAK(134-140) or anti-PAK(75-84) rabbit polyclonal anti-peptide antibodies and normal rabbit whole IgG were pre-incubated with biotinylated whole cells at room temperature for 1 h. Aliquots of the antibody/ pathogen cell suspensions were added to the BEC-coated wells (100 µl per well) and incubated for 1.5 h at room temperature. The adherent cells were fixed by 1 h incubation with 0.5% (v/v) formaldehyde in TBS at room temperature. A solution of 3% (w/v) BSA in TBS pH 7.4 was added to the wells (100 µl per well) and incubated for 2 h at 37°C. The wells were washed five times with TBS containing 0.05% BSA (w/v). Streptavidin-alkaline phosphatase diluted 1:2,500 in TBS containing 1% BSA (w/v)was then incubated with the cells for 1 h at 37°C. The wells were washed five times with TBS. A substrate solution *p*-nitrophenylphosphate (1 mg per ml of 10% [w/v] aqueous diethanolamine pH 9.6) was added to the wells (100 µl per well). The A_{405} readings were recorded following a 1 h incubation at room temperature.

15-4-2 Inhibition of biotinylated pathogens binding to asialo-GM_1 with adhesin peptides

The competitive binding assays to determine the anti-adhesin properties of synthetic peptides on pathogen whole cell binding to asialo-GM_1 were performed in the same manner as antibody binding assays, with the exception that peptides were used in place of the antibodies and the pre-incubation of adhesins and antibodies for 1 h at 37°C was omitted.

15-5 Inhibition of pathogens binding to BECs

15-5-1 Inhibition of biotinylated pathogens binding to BECs with anti-adhesin and anti-adhesin-peptide antibodies

The ability of anti-adhesin antibodies and anti-adhesin-peptide antibodies to block pathogens binding to BECs were demonstrated using the same protocol as the inhibition binding assays with antibodies inhibiting pathogens adherence to asialo-GM_1, with the exception that human BECs were coated on the plates instead of asialo-GM_1.

15-5-2 Inhibition of biotinylated pathogens binding to BECs by synthetic peptides

The methodology for the competitive binding assays where synthetic peptides, the Ac-PAK(128-144)ox-OH, Ac-PAK(134-140)-NH_2, Ac-PAK(75-84)-NH_2 or Ac-TnI(96-139) were used to compete with Bt-PAK pili or Bt-fimbriae binding to BECs is similar to the above inhibition binding assay with synthetic peptides inhibiting biotinylated pathogens to asialo-GM_1, with the exception that the BECs were used to coat the plates instead of asialo-GM_1.

15-5-3 Inhibition of biotinylated *C. albicans* binding to BECs with GSLs and βGalNAc(1-4)βGal-methylester

The ability of asialo-GM_1 and βGalNAc(1-4)βGal-methylester to block *C. albicans* binding to human BECs was demonstrated using same protocols as synthetic peptide inhibiting *C. albicans* binding to BECs, with the exception that asialo-GM_1 and βGalNAc(1-4)βGal-methylester were used in place of adhesin peptides. All inhibition assays were performed three times.

16. The effect of anti-*C. albicans* fimbrial MAb, Fm16

16-1 MAb Fm16 binding to *C. albicans* fimbriae

Semi-enriched fimbriae (10 µg/ml in 0.01 M carbonate buffer, pH 9.5) were coated onto polystyrene microtiter wells (Nunc) by an overnight incubation at 4°C The wells were washed three times (250 µl/well) with of PBS pH 7.4 supplemented with 0.05% (w/v) bovine serum albumin (Buffer A). Excess binding sites were blocked by incubation at 37°C for 1 h with 5% (w/v) BSA in PBS pH 7.4. Wells were washed three times with 250 µl/well of Buffer A. Aliquots (100 µl/well) of serially diluted mouse anti-EF monoclonal antibodies and anti-fimbriae polyclonal sera (obtained from immunized mice that were sacrificed for fusion of mouse spleen and NS1 cell line) were added to each well and incubated at 37°C for 2 h. A rabbit polyclonal anti-*Ustilago violacea* fimbriae antiserum was a gift from A. Castle (Brock University, St. Catherines, Ontario). Wells were washed 5 times with 250 µl/well of Buffer A. Antibody binding to EF was assessed by adding 100 µl/well (1:5,000) goat anti-mouse or goat anti-rabbit heavy and light chain [IgG(H+L)] immunoglobulin G-peroxidase conjugates (Jackson Laboratories). Following a 1 h incubation at 37°C, the wells were washed 5 times with 250 µl/well of Buffer A and a

substrate solution containing 1 mM 2,2'-azido-di-[3-ethylbenzthiazoline sulfonic acid] (ABTS) in 10 mM sodium citrate buffer pH 4.2, containing 0.03% (v/v) hydrogen peroxide was added (125 µl/well). The reaction was stopped by the addition of 4 mM sodium azide (125 µl/well) and the absorbance at 405 nm was recorded.

16-2 Inhibition of anti-adhesin antibodies binding to adhesins with unbiotinylated heterologous adhesins and synthetic peptides

Two sets of competitive ELISA were performed to assess the interactions between the adhesins with the respective crossreactive antibodies. The first set of competitive ELISAs was carried out to study the interactions between the crossreacting antibody with 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 per well of the respective antigens in 0.01M sodium carbonate pH 9.5. The plates were incubated at room temperature for 6 h and then washed 3 times with PBS pH 7.4 supplemented with 0.05% (w/v) bovine serum albumin (BSA) (buffer A). Excess sites on the microtiter wells were blocked with 5 % (w/v) BSA in PBS pH 7.4. After 3 washes with buffer A, varying 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 pre-mixed prior to addition into 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 into the microtiter wells (100 µl per well). With *C. albicans* fimbriae as the immobilized antigens, Fm16 or Fm34 were mixed with serially diluted *P. aeruginosa* PAK pili before addition into 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 (H+L) horse radish peroxidase conjugates were added into the wells as secondary antibodies. The wells were washed 5 times with 250 µl of buffer A per well and a substrate solution containing 1 mM ABTS in 10 mM sodium citrate buffer pH 4.2 (125 µl per well) was added. The reaction was quenched using sodium azide and the A_{405} was determined.

The second set of competitive ELISAs was performed to study the interactions between Fm16 with synthetic peptides corresponding to the receptor-binding region of the *Pseudomonas* PAK pilin adhesin. In these assays, synthetic peptides were 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 pre-mixed with Fm16 (1:200) before addition into precoated microtiter wells. The assay mixtures in the microtiter plate were incubated at 37°C for 2 h. The remainder of the protocols were performed as the first set of competitive ELISAs described above.

17. Agglutination assays

17-1 Agglutination of *C. albicans* with MAb Fm16

C. albicans yeast were cultured in M9 media as described below except that no radio-labels were added into the media. The cells were fixed with a 1% (v/v) formalin in PBS pH 7.4 by incubation for 1 h at room temperature. The cells were harvested by centrifugation and washed 3 times with PBS. The cell number was determined in a hemocytometer and adjusted to 2 x 10^7 cells/ml. Aliquots (50 µl) were dropped onto microscope glass slides. Aliquots (50 µl) of serially diluted antibodies (Fm16, anti-EF polyclonal antiserum and normal mouse IgG) were added to the *C. albicans* on the slides and incubated for 10 min at 37°C. The agglutination of the yeast were scored by phase contrast microscopy.

17-2 Agglutination assays

C. albicans were cultured in M9 media according to the conditions described above. Briefly, *P. aeruginosa* strain PAK was cultured on M9 media according to the method of McEachran and Irvin (1985). 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 x *g* for 10 min). Cell concentrations were determined on a hemocytometer and adjusted to 2 x 10^7 cells per ml. Aliquots (50 µl) were added into wells in 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.

18. Thin-layer chromatography (TLC) assays

18-1 Binding of *C. albicans* fimbriae to GSLs separated on TLC plate

The thin-layer chromatography (TLC) plate binding assay was performed as described by Baker *et al.* (1991) with minor modifications. Aluminum-backed silica gel Si60 high performance TLC plates (Merck Kieselgel Si60, no fluorescence indicator, E. Merck, Darmstadt, Germany) were cut to produce 8 x 2.5 cm plates which were chromatographed with 100% methanol to the top of the plate to remove impurities and the plates were air dried. Glycosphingolipids (GSLs) (10 µg of each GSL) were loaded 1.0 cm above the base of the plate. The following glycosphingolipids purchased from Sigma Co. (St. Louis, MO) were used: mono-sialoganglioside (M-GM_1), asialoganglioside GM_1

(asialo-GM_1), asialoganglioside GM_2 (asialo-GM_2), lactosylceredroside (LCS), ceramide trihexoside (CTH). GSLs were separated on the TLC plates in chloroform-methanol-water (65:35:8, v/v/v) and air dried. One set of plates was sprayed with 10% sulfuric acid in ethanol and heated at 100-150°C for 5-10 min to char the GSLs for visual detection, and the other set was used for the fimbrial binding assay. The four corners of the plate were bent to 90° and the remainder of the assay was done with the TLC plates inverted in all solutions and at room temperature in an incubator shaker (model G25 Gyroshaker, New Brunswick Scientific, New Jersey, USA) at 20 rpm agitation. The TLC plate was blocked with 50 mM tris-hydroxy-methyl aminomethane pH 7.5 containing 150 mM NaCl (TBS), 0.25% (w/v) gelatin, 3% (w/v) BSA, 5 mM EDTA and 0.05% (v/v) Nonidet P40 in a glass petri dish for 2 h at room temperature. The blocking solution was aspirated and 10 ml of EF (100 µg EF/ml in 100 mM TBS, pH 7.5) was then added. The fimbriae were allowed to bind to GSLs for 2 h at room temperature. The plates were gently washed (2 x 5 min) with 10 ml of 100 mM TBS containing 0.1% (v/v) Tween 20 (TBST). The murine anti-EF monoclonal antibody, Fm16, was diluted 1:200 with TBST and 10 ml was added to the TLC plates. The solution was incubated for 1 h at room temperature. Unbound antibodies were removed by washing the plates with 10 ml of TBST (2 x 5 min). The plate was then incubated with 10 ml of goat anti-mouse immunoglobulin G alkaline phosphatase conjugate (Jackson Laboratories) diluted 1:5,000 with TBST for 1 h at room temperature The plates were washed (2 x 5 min.) with 10 ml of TBST. The alkaline phosphatase activity was localized with nitro blue tetrazolium chloride (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) dissolved in 100 mM Tris buffer, pH 9.5 containing 100 mM NaCl and 5 mM $MgCl_2$. Color development was quenched by rinsing the TLC plate with deionized water and submerging the plate into a 150 mM EDTA solution pH 8.0 for 3-5 min. The plates were air dried, stored in plastic in the dark until they were photographed.

18-2 Analysis of glycosphingolipids from BECs by TLC

The glycosphingolipids (GSLs) from BECs were extracted according to the method described by Hakomori and Siddiqui (1974). Briefly, human BECs were collected from 100 healthy, non-smoking males and washed twice with PBS pH 7.4 by centrifugation (4000 g x 5 min) at 4°C. The cells were subsequently filtered with a nylon mesh filter. The total glycosphingolipids were extracted twice by adding an equal volume of chloroform:methanol (2:1) to the cell pellet (5 g). The two extractions were pooled and clarified with the addition of 1/2 volume of chloroform: methanol (1:2 +3% water). The extract was evaporated and the residue was resuspended in 100% methanol (1 ml). The thin-layer chromatography (TLC) was performed as described above (see Materials and Methods 18-1). A 50 µl aliquot of the BEC glycosphingolipid was subjected to TLC with standards for comparison of 15 µg lactosylceramide, 10 µg asialo-GM_1, 5 µg asialo-GM_2, 10 µg GM_1 and 10 µg GM_2. The glycosphingolipids were visualized by spraying the TLC plate with 10% sulfuric acid in ethanol and charring at 100°C-150°C for 5 to 10 min.

Chapter III Results and discussion

A: Partial characterization of a *Candida albicans* fimbrial adhesin[1]

Results

A-1 Purification of fimbriae from *C. albicans* strain #40

A typical fimbriae preparation from 200 g wet weight of *C. albicans* from 10 trays yielded about 70 mg of crude fimbriae (CF), which then yielded 5 mg of enriched fimbriae (EF). Fimbriae were removed from washed cells by shearing, separated from cells by centrifugation and filtration and then subjected to HPLC size-exclusion chromatography (SEC). The fimbriae eluted as two peaks (the first peak eluting at the void volume of the column) (Figure IIIA-1). Fimbriae were mainly associated with the first peak, as determined by SDS-PAGE (Figure IIIA-2) and electron microscopy (Figure IIIA-3). The first peak was collected and rechromatographed under identical conditions with the fimbriae eluting at the void volume of the column (Figure IIIA-1). The second peak on the rechromatography profile indicated that some of the fimbrial preparations were depolymerized and/or deglycosylated during the purification process, which indicates that *C. albicans* fimbriae are not very stable.

The protein profile of the CF and EF preparations obtained using SDS-PAGE indicated that the molecular weight of the fimbrial subunit was approximately 66 kDa (verified by Western blot analyses, see below) and the putitive deglycosylated fimbrial subunit was 8.7 kDa (Figure IIIA-3). The enriched fimbriae preparation contained almost pure fimbriae (Figure IIIA-2). The 66 kDa proteins were electroeluted from the gel and

---

[1] A version of this section of chapter III has been published: Yu, L., Lee, K.K., Doig, P.C., Carpenter, M.R., Staddon, W., Hodges, R.S., Paranchych, W., and Irvin, R.T. (1994). Infect. Immun. **62**:2834-2842.

subsequently separated by reversed-phase HPLC. The main peak was eluted 30 to 34 min after injection onto the column (Figure IIIA-5).

The data from the agglutination assays using anti-*C. albicans* monoclonal antibody, Fm16 and polyclonal mouse anti-EF antiserum demonstrated that fimbriae were present on the surface of the cells (Table IIIA-1). A negative control using normal mouse IgG failed to agglutinate *C. albicans* yeast. When the negatively-stained EF preparations were examined by transmission electron microscopy, large numbers of fimbrial structures ~8 nm in diameter were observed to mediate *C. albicans* binding to BECs (Figure IIIA-4C). The fimbriae appeared as flexible filaments that frequently aggregated into small bundles of fimbriae (Figure IIIA-4A, 4B). The fimbriae protruding from the surface of the yeast as filamentous structures appeared to be sparsely distributed but appeared to mediate binding to BECs.

A-2 The compositions of the fimbrial subunit

The EF preparation was used to determine the protein and carbohydrate compositions of the *C. albicans* fimbrial subunit. This was achieved by employing a combination of techniques including phenol-sulfuric carbohydrate assay, BCA protein assay and amino acid analysis. The fimbrial subunit was observed to consist of ~85% carbohydrate and ~15% of protein. Gas liquid chromatography of the EF sample indicates that the main carbohydrate moiety of the fimbriae consisted of D-mannose. A number of additional minor components in the EF were not identified (data not shown).

The amino acid compositions of the fimbrial subunits from both 66 kDa and 8.7 kDa protein bands that had been separated by SDS-PAGE and purified by reversed-phase HPLC are shown in Table IIIA-2. Based on compositional analysis, an approximate molecular weight of 8,644 Da was calculated for the protein portion of a fimbrial subunit composed of 79 amino acid residues which is consistent with the molecular weight (8.7 kDa) of the spontaneously deglycosylated *C. albicans* fimbrial subunit on SDS-PAGE

(Figure IIIA-3). These results are also consistent with the quantitative assessment of carbohydrate and protein in the fimbriae as the protein component constitutes ~15% of the fimbrial mass.

A-3 The N-terminal protein sequence of the fimbrial subunit

The N-terminal of protein sequence of *C. albicans* fimbriae determined for the first 10 amino acid residues was NMETPASST/DG.

A-4 Anti-fimbriae antibodies

Fimbriae (EF) were utilized to immunize mice and to produce monoclonal antibodies specific to the fimbriae. ELISA indicated that mouse anti-EF monoclonal antibody, Fm16 and polyclonal antibody (sera obtained during the immunization of the BALB/c mice) had high titres for *C. albicans* fimbriae (10^6 and 10^5 respectively). Western blot analyses indicated that Fm16 recognized a 66 kDa and a 69 kDa band of fimbrial subunit protein (Figure IIIA-6). Rabbit polyclonal anti-*Ustilago violacea* antiserum specific for *U. violacea* fimbriae (Gardiner *et al.*, 1982) was observed to react with the same size protein bands (data not shown).

A-5 The role of *C. albicans* fimbriae in adherence

The role of fimbriae in the mediation of *C. albicans* binding to human buccal epithelial cells was examined. A whole cell ELISA was performed to assess *C. albicans* fimbriae binding to BECs. The data obtained showed that *C. albicans* fimbriae bound to BECs in a saturable and concentration-dependent manner. The binding had almost approached saturation at 50 µg protein/ml of fimbriae (Figure IIIA-7). The concentration of fimbriae required to reach half-maximal binding was determined to be 10 µg/ml, indicating that the binding to cell surface receptors is of high avidity.

Pre-incubation of BECs with purified fimbriae before the addition of *C. albicans* yeast resulted in the inhibition of *Candida* organisms binding to BECs. The fimbrial-mediated inhibition of *C. albicans* binding to BECs was concentration-dependent (Figure IIIA-8). A maximal inhibition of 64% of *C. albicans* binding to BECs was obtained with 30 µg protein/ml of fimbriae (Figure IIIA-9).

Discussion

C. albicans has been found to bind to human BECs (King *et al.*, 1980; Douglas, 1987a and 1987b; Klotz and Penn, 1987; Staddon *et al.*, 1989; Cutler, 1991), but the adhesins which *C. albicans* utilizes to bind to BECs have not been purified and characterized. *C. albicans* possesses fimbriae which are morphologically analogous to bacterial fimbriae (Gardiner and Day, 1985). Although several studies have suggested that fibrous structures, perhaps fimbriae, mediate the adherence of *C. albicans* to BECs (Montes and Wilborn, 1968; Kimura and Pearsall, 1978; Douglas *et al.*, 1981; Marrie and Costerton, 1981; MaCoutie and Douglas, 1984; Tronchin *et al.*, 1984), these fimbriae have not been well characterized. This may be due, in part, to the difficulty of purifying the fimbriae. We have described the isolation and purification of *C. albicans* fimbriae and demonstrated that fimbriae can mediate adherence of these organisms to human buccal epithelial cells.

Fimbriae were easily sheared from the cell surfaces of *C. albicans* when subjected to gentle shearing by homogenization. The purification of fimbriae from the crude extract was achieved by two successive rounds of SEC-HPLC (Figure IIIA-1). *C. albicans* fimbriae were not very stable and appeared to be readily degraded or depolymerized during the purification processes. Optimal preservation of fimbriae was achieved by maintaining the samples on ice and by utilizing PMSF as a protease inhibitor. The addition of calcium appeared to stabilize the fimbrial structure and minimize depolymerization. This is in agreement with the results reported by Gardiner and Day (1985) which indicated that calcium is important in maintaining the structural integrity of fungal fimbriae.

EM analyses revealed the appearance of long filaments in the EF preparation (Figure IIIA-4). SDS-PAGE analyses of CF and EF preparations revealed a major 66 kDa band along with a few faint lower molecular weight bands (Figure IIIA-2). Successive SEC-HPLC removed most of the minor components from the CF preparation, resulting in a

purer EF preparation (Figure IIIA-2). Western blot analyses indicated the presence of two major bands (66 and 69 kDa) (Figure IIIA-6) which were detected with monoclonal antibody, Fm16 which can agglutinate *C. albicans* (Table IIIA-1). The two major bands that were recognized by Fm16 may represent fimbrial subunits with different degrees of glycosylation. The presence of a minor component of 28 kDa (and occasionally a 9 kDa) protein may be a degraded product of the major bands or unglycosylated fimbrial subunits.

Carbohydrate and protein analyses indicated that the fimbrial subunit was composed of approximately 85% carbohydrate and 15% protein by weight. The gas chromatogram of the EF sample revealed that the main component in the carbohydrate moiety of the fimbrial subunit consisted of D-mannose (see Results A-2). Our findings differ from those of Gardiner and Day (1985) in that purified fimbriae from *U. violacea* did not contain any detectable carbohydrate component. Subsequent studies by Castle *et al.* (1992) demonstrated that *U. violacea* fimbriae contained approximately 10% carbohydrate (mannose). Unlike *U. violacea* fimbriae, *C. albicans* fimbrial subunit is normally extensively glycosylated with mannose residues. *C. albicans* yeast forms appeared to be sparsely fimbriated (Figure IIIA-4) whereas *U. violacae* has numerous fimbriae protruding from its cell surface (Poon and Day, 1975a).

Amino acid analysis of the *C. albicans* fimbrial subunit indicated that the most frequent amino acid residues of the protein portion of fimbriae were Val, Asx, Glx, Ser, Thr, Gly, Leu, Ile, Lys and Ala, while little methionine or histidine was detected (Table IIIA-2). The fimbrial subunit has a high proportion of hydrophobic residues (50% of total residues) and a reasonably high level of basic amino acid residues (12.5% of the total residues). The *C. albicans* fimbrial subunit has an amino acid composition that is proportionally similar to that reported for the *U. violacea* fimbriae subunits which has a molecular weight of 74 kDa (Day and Cummins, 1981). The hydrophobicity of the *C. albicans* fimbrial subunit (50% of the total residues) is higher than that of the *U. violacea*

fimbriae subunits (35% of the total residues). Amino acid analysis of the *C. albicans* fimbrial subunit also indicated that the molecular weight of the protein portion of fimbrial subunit was 8,644 Da based on a compositional analysis assuming 79 amino acid residues (~15% of the mass of the fimbrial subunit). This is consistent with the results obtained by both carbohydrate and protein analyses. The fimbrial subunit has an apparent Mr. of approximate 66 kDa as determined by SDS-PAGE (Figure IIIA-2), slightly lower than the Mr. of the *U. violacea* fimbrial subunit. The deglycosylated fimbrial subunit has an apparent Mr. of approximate 8.7 kDa (Figure IIIA-3). N-terminal protein sequencing of the *C. albicans* fimbrial subunit was NMETPASST/DG. The sequencing was unreadable from the 11th amino acid residue. The N-terminal protein sequence data analysis revealed no homology to any known fimbriae/pili sequences. In summary, the major structural subunit of the fimbriae is a glycoprotein which consists of 80%-85% carbohydrate (primarily D-mannose) and 10-15% protein.

Elucidation of the mechanisms which mediate adhesion may allow new therapies to be developed to prevent or treat infection. The role of *C. albicans* fimbriae in the adherence of *C. albicans* to BECs was examined in this paper. First, the direct binding assay indicated that the *C. albicans* fimbriae do bind to BECs. The binding of *C. albicans* fimbriae to BECs was concentration-dependent and saturable at ~50 µg protein /ml of EF (Figure IIIA-7). Second, inhibition assays showed that *C. albicans* fimbriae significantly inhibited the binding of *C. albicans* whole cells to BECs (Figure IIIA-8 and IIIA-9). This implied that the interactions between fimbriae and BECs were specific and involved cell-surface receptors that can recognize and bind to the *C. albicans* fimbrial adhesins. These results indicated that the *C. albicans* fimbriae are functional adhesins. In the case of the *U. violacea*, fimbriae may also have a role in conjugation (Poon and Day, 1975b).

Equilibrium analysis of binding has indicated that the adherence of *C. albicans* to BECs involves multiple adhesin-receptor interactions (Staddon *et al.*, 1990). Several cell-

surface molecules have been identified as possible adhesins, such as CR3-like and β1-integrin like molecules of *C. albicans* (Marcantonio and Hynes, 1988), lectin-like molecules of *C. albicans* (Douglas *et al.*, 1981; Douglas, 1987a and 1987b; Toch and Douglas, 1992), factor 6 moiety of *C. albicans* (Fukazawa *et al.*, 1980; Miyakawa *et al.*, 1989) and the secreted acid proteinase of *C. albicans* (Borg and Ruchel, 1988; Ray and Payne, 1990). Thus far, none of them have been extensively characterized.

We have demonstrated that *C. albicans* fimbriae adhesins mediate the binding of *C. albicans* to BECs *in vitro*, but the role of *C. albicans* fimbriae in mediating the adherence of *C. albicans* to BECs *in vivo* is still unclear. While we have identified the major fimbrial subunit, it is unclear whether there are additional minor components that contribute to the fimbrial structure and function. The role of these fimbriae in pathogenesis remains to be resolved.

Table IIIA-1 Agglutination of *C. albicans* yeast by anti-*C. albicans* MAb Fm16 and polyclonal anti-enriched fimbriae antiserum

	Agglutination[a] by:	
Dilution	Fm16	Anti-EF antiserum
Control (PBS)	-	-
1:1	+++	+++
1:2	++++	++++
1:4	+++	+++
1:8	++	++

[a]Assessed qualitatively by phase-contrast microscopy and reported on a scale from - (no agglutination) to ++++ (heavy agglutination). Normal mouse IgG showed no agglutination with any of the dilutions tested.

Table IIIA-2 Amino acid compositions of fimbrial subunits from C. *albicans* strain #40

Amino acid residues	No. of residues[a] (integer value)/fimbrial subunit	
	66 kDa subunit	8.7 kDa subunit
Asx	8.24 (8)	8.20 (8)
Thr[b]	4.73 (5)	4.85 (5)
Ser[b]	5.73 (6)	5.77 (6)
Glx	8.17 (8)	8.11 (8)
Pro	2.79 (3)	2.80 (3)
Gly	7.02 (7)	7.00 (7)
Ala	6.00 (6)	6.00 (6)
Cys	ND	ND
Val	5.57 (6)	5.66 (6)
Met	0.82 (1)	0.83 (1)
Ile	4.73 (5)	4.75 (5)
Leu	6.82 (7)	6.84 (7)
Tyr	2.55 (3)	2.57 (3)
Phe	3.21 (3)	3.20 (3)
His	1.48 (1)	1.43 (1)
Lys	5.91 (6)	5.94 (6)
Trp	ND	ND
Arg	3.81 (4)	3.87 (4)

[a]Determined experimentally. ND, not determined. The total of 79 residues had an estimated molecular weight of 8,644 Da.

[b]No correction was made for half-Cys and Trp or for the destructive loss of Ser and Thr during hydrolysis.

Figure IIIA-1

Purification of fimbriae from an isolate of *Candida albicans* associated with an extensive pulmonary infection of an intensive care unit patient. Fimbriae were purified by HPLC utilizing successive size exclusion chromatography (SEC) with a Waters Protein-PAK 300 SW 7.8 mm x 30 cm column equilibrated and eluted isocratically at 0.5 ml/min with 10 mM PBS pH 7.2, containing 1 mM calcium chloride and 1 mM PMSF. (A) SEC-HPLC profile of material sheared by gentle homogenization from washed *C. albicans* cells cultured on Sabouraud Dextrose Agar at 37°C for 5 days. The material which was eluted at the void volume (denoted by a solid bar) was collected (termed semi-enriched fimbriae) and rechromatographed under the same conditions. (B) SEC-HPLC profile of rechromatographed fimbriae. Fimbriae which was eluted at the void volume (solid bar) was termed enriched fimbriae (~95% purity). The second peak on the rechromatography profile indicated that some of the fimbrial preparations were depolymerized and/or deglycosylated during the purification process.

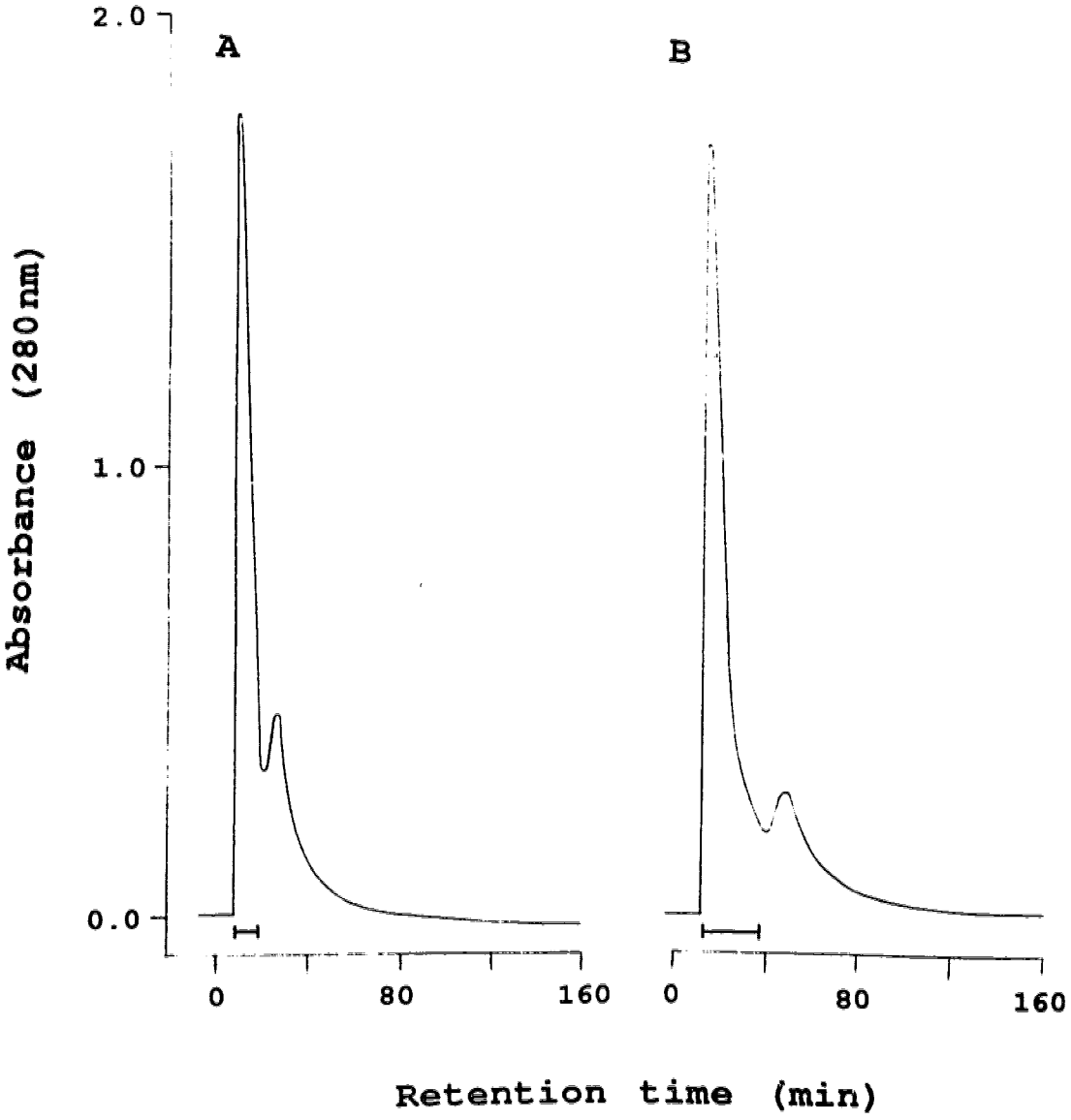

2.0
1.0
0.0
A
B
0
80
160
0
80
160
Absorbance (280nm)
Retention time (min)

Figure IIIA-2

Sodium dodecylsulfate polyacrylamide gel electrophoresis analyses of *C. albicans* fimbriae. A 12.5% (acrylamide:bisacrylamide, 30:1) cross-linked mini-protein gel was used. The following fimbriae preparations were loaded into the wells of the gel: lanes a and b: 5 µg of enriched fimbriae; lanes c and d: 5 µg of semi-enriched fimbriae and lanes e and f: 10 µg of crude fimbriae. Gel electrophoresis was carried out with a Mini-Protean vertical electrophoresis cell (Bio-Rad) under constant voltage of 200 V for 50 min. Silver staining technique was used to visualize protein bands.

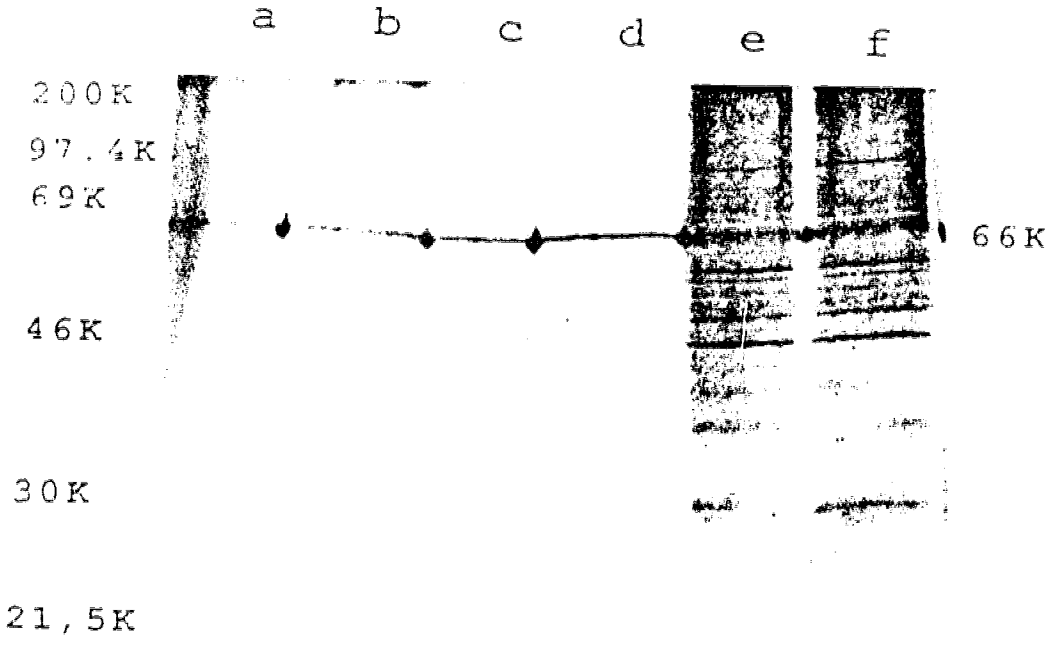

a
b
c
d
e
f
200K
97.4K
69K
46K
30K
21,5K
66K

Figure IIIA-3

SDS-PAGE of the spontaneously deglycosylated *C. albicans* fimbriae. Lane A, B (duplicate) and Lane C, D (duplicate) represent different preparation of the fimbriae from different *C. albicans* colonies. Coomassie brilliant blue staining technique was used to visualize protein bands.

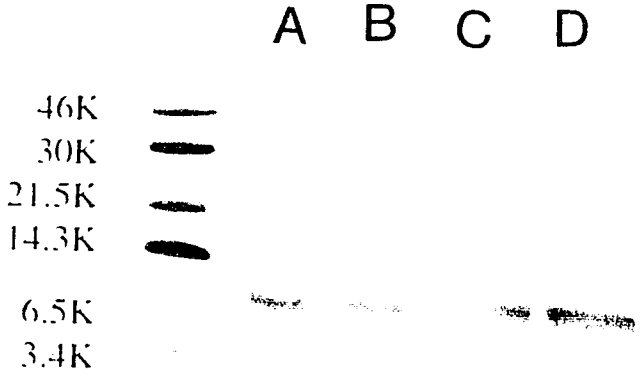

Mol. Weight
A
B
C
D
46K
30K
21.5K
14.3K
6.5K
3.4K

Figure IIIA-4

Negative stain electron micrographs of enriched *C. albicans* fimbriae taken by Irvin in 1993 in Department of Medical Microbiology and Immunology, University of Alberta. (A) Fimbriae frequently aggregated to form loosely associated bundles of filaments. The solid line represents 1 µm. (B) The fimbriae (highlighted by arrows) are frequently decorated with material and appear to be flexible. (C) An scanning electron micrograph of *C. albicans* fimbriae taken by Irvin in 1993 in Department of Medical Microbiology and Immunology, University of Alberta. Glutaraldehyde-fixed *C. albicans* [the arrow indicates fimbria involved in *C. albicans* binding to BEC(s)] bound to human BECs was postfixed with 2% (w/v) osmonium tetroxide in 0.1 M phosphate buffer (pH 7.3) and 1.0% (w/v) aqueous tannic acid, critical point dried, and then salted onto a scanning electron microscope stub and examined in a Hitachi S4000 field emission scanning electron microscope operating with an accelerating potential of 2.5 kV. The bars represent 1 µm.

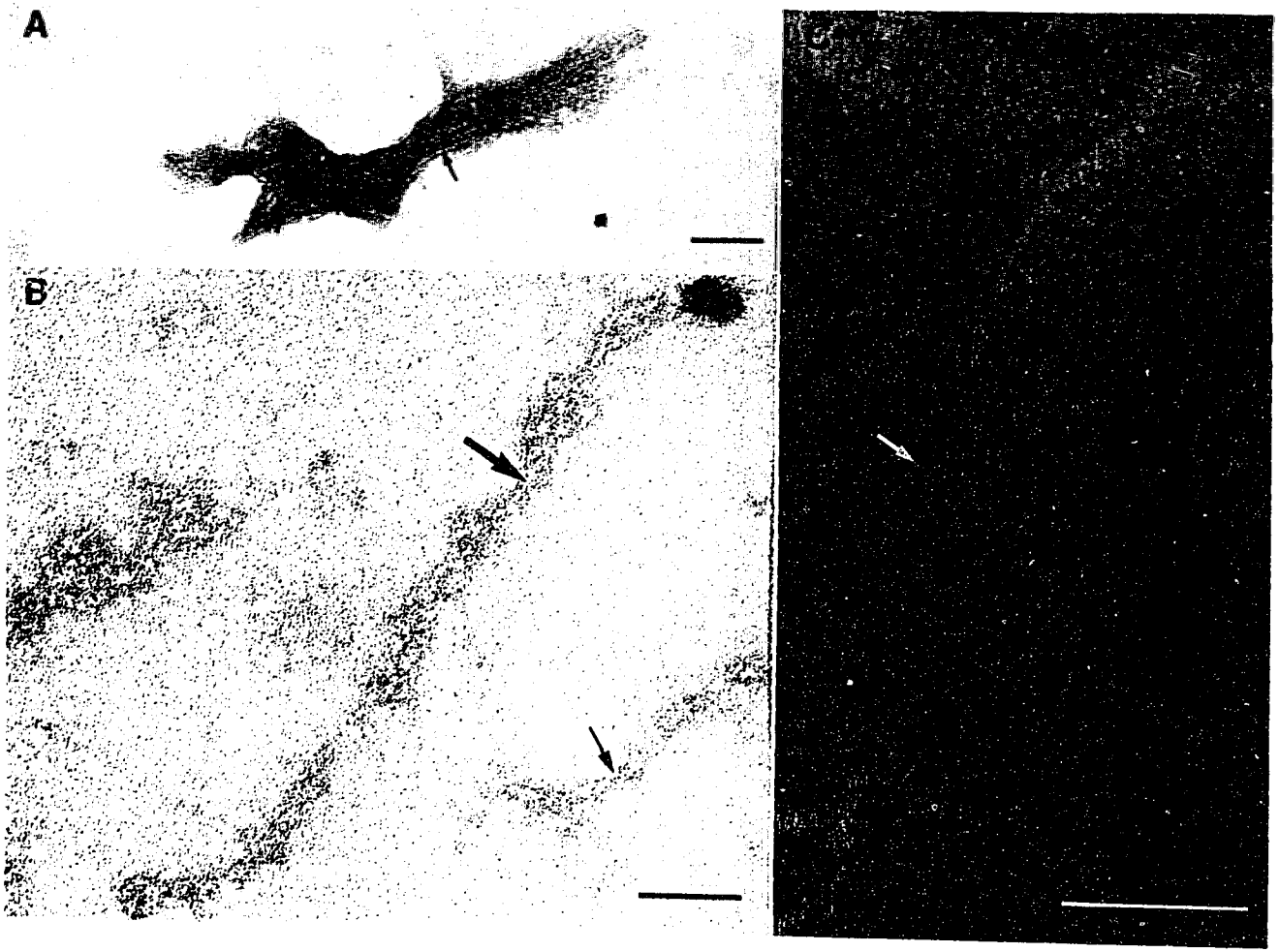

A
B
C

Figure IIIA-5

Reversed-phase HPLC chromatogram of electroeluted fimbrial protein. Crude fimbriae were subjected to SDS-PAGE and the fimbrial band on the gel was removed by electroelution. Lyophilized fimbriae were further purified by RP-HPLC on a Aquapore C_4 100 x 4.6 mm column of 7 µm pore size (Pierce) with a linear AB gradient of 2% B/min (where solvent A is 0.05% aqueous trifluoroacetic acid and solvent B is 0.05% trifluoroacetic acid in acetonitrile) at a flow rate of 1 ml/min.

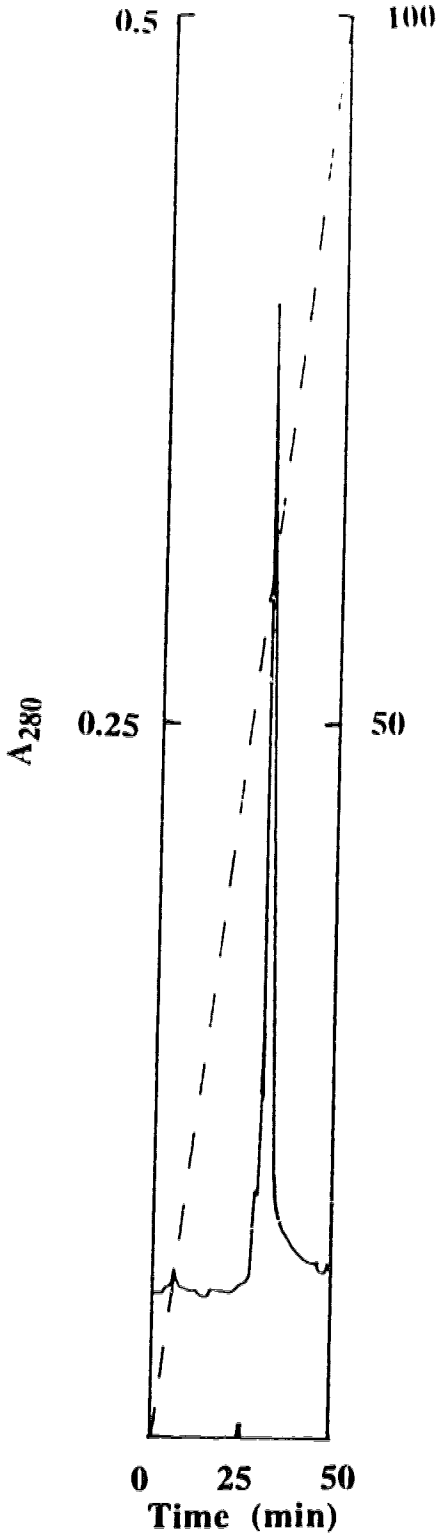

0.5
100
A_{280}
0.25
50
0
25
50
Time (min)

Figure IIIA-6

Western blot analyses of enriched *C. albicans* fimbriae. Fimbriae were loaded into the wells (30 µg/well) in a 12.5% cross-linked running gel, separated by SDS-PAGE and transblotted onto nitrocellulose membrane as described in Methods and Materials. Lane a shows the molecular weight markers. Fimbriae in lanes b and c were probed with a murine ascites containing anti-fimbriae monoclonal antibody, Fm 16 (diluted 1:500), and lanes d and e were probed with an affinity-purified commercial normal mouse IgG (diluted 1:500). A goat anti-mouse IgG-alkaline phosphatase conjugate was used as the secondary antibody. The purplish bands were obtained using nitro blue tetrazolium and 5-bromo-4-chloro-3-indoyl phosphate as the alkaline phosphatase substrates.

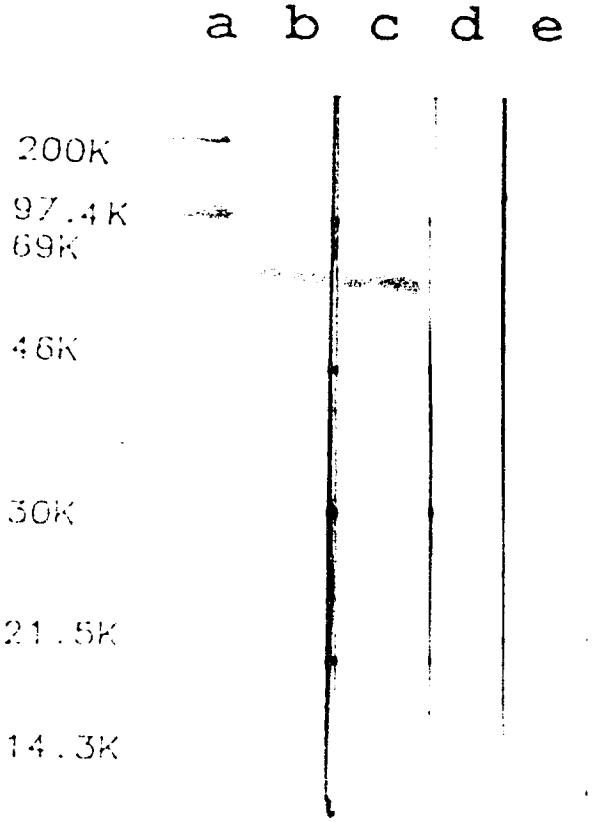

a b c d e
200K
97.4K
69K
46K
30K
21.5K
14.3K

Figure IIIA-7

Direct binding of *C. albicans* fimbriae to human buccal epithelial cells (BECs). A whole cell ELISA assay was employed to assay for the adherence of fimbriae to BECs (collected from 10 healthy, non-smoking male volunteers). *C. albicans* fimbriae (EF) were incubated with BECs for 1.5 h at room temperature in PBS pH 7.2. A murine anti-*C. albicans* fimbrial monoclonal antibody, Fm16, and an anti-mouse IgG-horseradish peroxidase conjugate were employed to quantitate EF binding to BECs which were collected on the surface of 12 μm pore size polycarbonate filters (Nucleopore). The binding of fimbriae to BECs was represented as the A_{405} observed in relation to the concentration of fimbriae used.

Figure IIIA-7

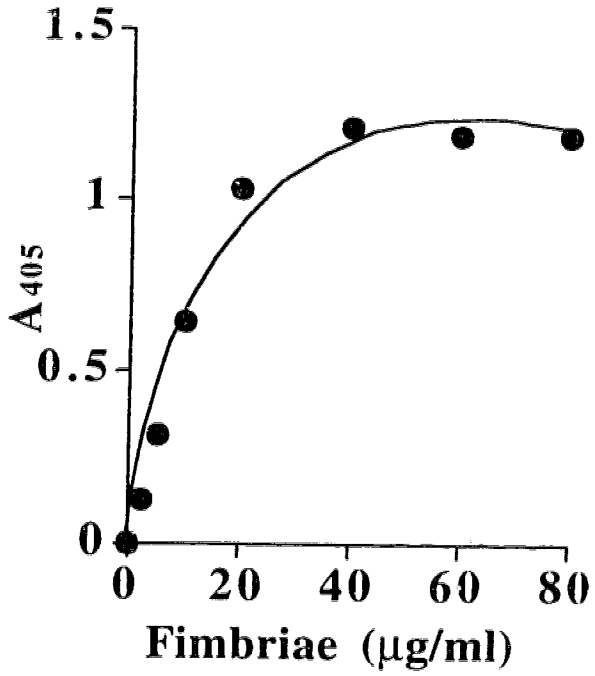


Figure IIIA-8

Inhibition of *C. albicans* whole cell binding to human BECs by direct competition with enriched fimbriae. The bars represent ± 1 S.D. The adherence assay of Staddon *et al.* (1990) which involved metabolically [^{35}S]-labelled *C. albicans* was used. BECs (2 x 10^5 cells/ml PBS pH 7.2) were pre-incubated with varying concentrations of enriched fimbriae (0 to 20 µg protein/ml) for 1 h at 37°C prior to their addition to radio-labelled yeast (ratio of 100 yeast to 1 BEC). The assay mixture was incubated for 2 h at 37°C, shaking at 300 rpm on a gyrotary shaker. Washed BECs with adherent *C. albicans* were transferred to glass scintillation vials and the counts per minute were recorded.

Figure IIIA-8

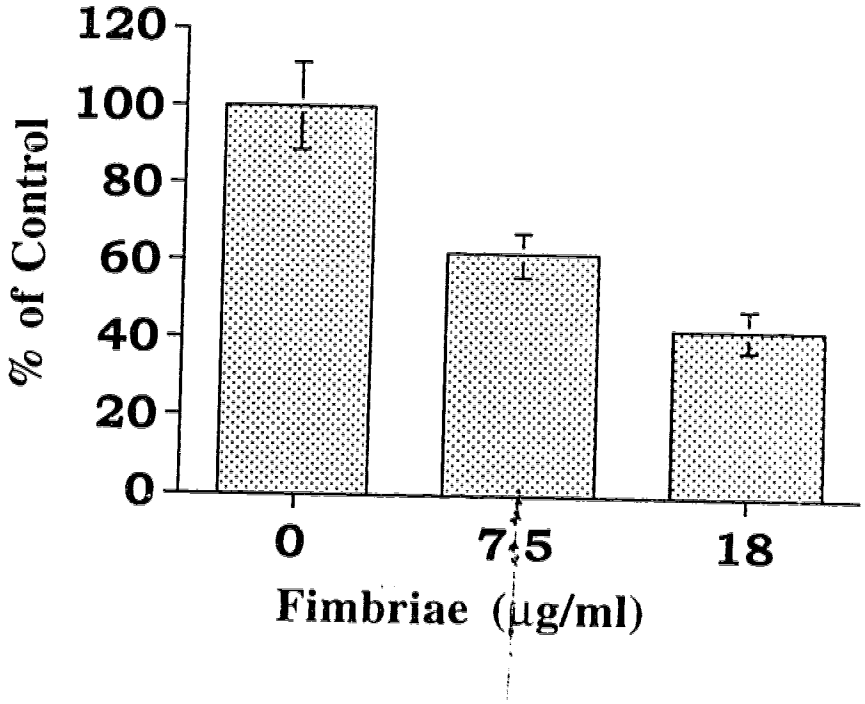
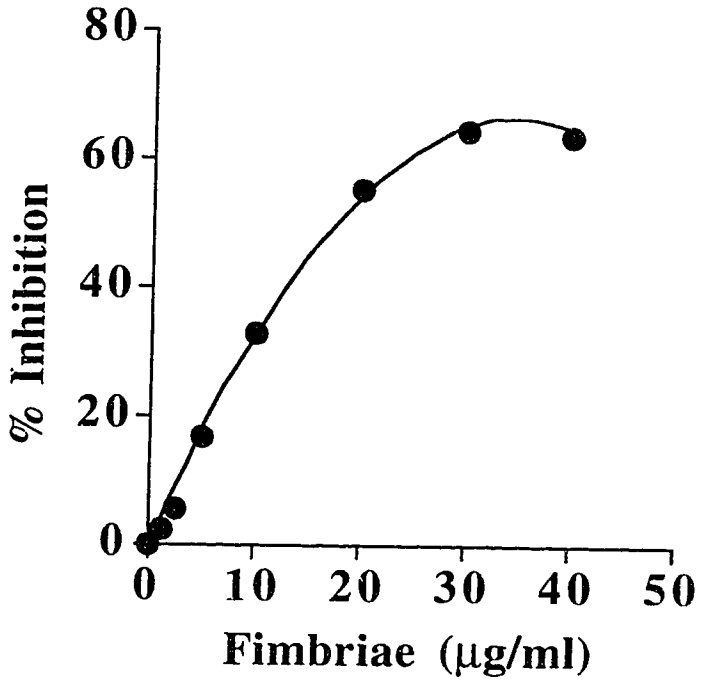


Figure IIIA-9

Inhibition of the binding of biotinylated *C. albicans* whole cell to human BECs. The binding of Bt-*C. albicans* immobilized BECs was determined in the presence of the purified fimbriae (EF). The enriched fimbriae mediated inhibition of biotinylated *C. albicans* whole cells to human BECs is presented as the percent inhibition of whole cell binding to human BECs with respect to the level of whole cell binding to human BECs in the absence of any fimbriae.

Figure IIIA-9



B. Fimbria-mediated adherence of *Candida albicans* to glycosphingolipid receptors on human buccal epithelial cells[2]

Results

B-1 Binding of *C. albicans* fimbriae to glycosphingolipids on TLC

A number of glycolipids, separated on thin layer chromatography (TLC) plates, were examined for their ability to be recognized by fimbriae purified from *C. albicans* (Figure IIIB-1). Bound fimbriae on the TLC plates were visualized with a Protein G affinity-purified anti-*C. albicans* fimbriae monoclonal antibody, Fm16, obtained from ascites tumor in BALB/c mice as previously described (Materials and Methods 13-1), and a goat anti-mouse IgG alkaline phosphatase. Fm16 was produced by immunizing mice with *C. albicans* fimbriae and isolated from a hybridoma cell line that expressed a $IgG2_a\kappa$ which bound to the fimbrial structural subunit. Fm16 was observed to bind to the cell surface of *C. albicans* (Table IIIA-1) and inhibit fimbriae binding to human buccal epithelial cells (Figure IIID-3). Normal mouse IgG does not bind to *C. albicans* fimbriae. In this study, Fm16 was utilized to detect *C. albicans* fimbrial binding to TLC separated glycosphingolipids and affinity-purified normal mouse IgG was employed as a control. The results in Figure IIIB-1 showed that *C. albicans* fimbriae bound specifically to asialo-GM_1 [gangliotetraosylceramide: βGal(1-3)βGalNAc(1-4)βGal(1-4)βGlc(1-1)Cer] and asialo-GM_2 [gangliotriosylceramide: βGalNAc(1-4)βGal(1-4)βGlc(1-1)Cer]. A normal mouse IgG control which was used instead of Fm16 failed to detect fimbrial binding to asialo-GM_1 and asialo-GM_2 (Figure IIIB-1, Lanes 3 and 4) as the normal mouse IgG does

[2] A version of this section of chapter III has been published: Yu, L., Lee, K.K., Sheth, H.B., Srivastava, G., Hindsgaul, O., Paranchych, W., Hodges, R.S., and Irvin, R.T. (1994). Infect. Immun. **62**:2843-2848.

not bind to fimbriae. Fimbriae failed to bind to M-GM_1, lactosylceramide (LCS) or ceramide trihexoside (CTH) [αGal(1-4)βGal(1-4)βGlc(1-1)Cer].

B-2 Glycosphingolipids on human BECs

Total GSLs extracted from human BECs contain asialo-GM_1, asialo-GM_2 and LCS along with a variety of other glycosphingolipids visualized on the TLC plate (Figure IIIB-2). A comparison of standard GSLs with GSLs extracted from BECs yielded almost identical reference factor (Rf) values [LCS (0.7), asialo-GM_2 (0.475) and asialo-GM_1 (0.325)] (Figure IIIB-2). The densities of the charred bands reflect the amount of the GSLs. LCS and asialo-GM_1 appear to be more abundant than asialo-GM_2 in the total GSLs from BECs.

B-3 Specificity of *C. albicans* fimbriae binding to GSL receptors

The binding of *C. albicans* fimbriae to glycosphingolipid (GSL) receptors was ascertained using glycosphingolipids immobilized on microtiter plates (Figure IIIB-3). We have used asialo-GM_1 and CTH (as a negative control) in solid-phase binding assays and found that *C. albicans* fimbriae bound to asialo-GM_1 in a saturable, concentration-dependent manner (Figure IIIB-3A). The concentration of fimbrial protein required for half-maximal binding is 8 μg/ml under the assay conditions, indicating a reasonably high affinity of fimbriae for asialo-GM_1. Fimbriae failed to bind to CTH. The specificities of *C. albicans* fimbriae for glycosphingolipids were verified with competitive binding assays. GSLs were suspended in phosphate buffered saline (PBS) at a sufficiently low concentration to prevent micellar formation. When homologous competitors were used in inhibition assays, *C. albicans* fimbriae binding to the respective immobilized asialo-GM_1 was reduced by 78% (Figure IIIB-3B). The concentration of asialo-GM_1 required for half-maximal inhibition is 1.44 μg/ml. As expected, no competition of fimbriae binding to CTH was observed as fimbriae do not bind to this glycolipid (Figure IIIB-3A).

B-4 Binding of *C. albicans* fimbriae to synthetic βGalNAc(1-4)βGal disaccharides

Krivan *et al.* (1988a and b) have proposed that the minimal receptor structural requirement of a number of asialo-GM_1-specific adhesins is the disaccharide βGalNAc(1-4)βGal. This disaccharide sequence was synthesized by Dr. O. Hindsgaul group, Department of Chemistry, University of Alberta and conjugated to bovine serum albumin (BSA) by Dr. H. Sheth (a postdoctoral fellow in Dr. R. Irvin's lab) to determine if the *C. albicans* fimbriae would bind to this disaccharide. When βGalNAc(1-4)βGal-BSA conjugates were immobilized onto microtiter plates, *C. albicans* fimbriae were observed to bind to these disaccharides in a saturable, concentration-dependent manner (data not shown). The interactions between fimbriae and the disaccharide were specific as the binding of *C. albicans* fimbriae to the βGalNAc(1-4)βGal-BSA conjugates was competitively inhibited by solution-phase βGalNAc(1-4)βGal-methylester (Figure IIIB-4A). The half-maximal inhibition was attained at 8.2 μM of βGalNAc(1-4)βGal-methylester suggesting a highly specific interaction. Furthermore, when asialo-GM_1 was employed as the competitor, the ganglioside also competitively inhibited *C. albicans* fimbriae binding to βGalNAc(1-4)βGal-BSA conjugates (Figure IIIB-4B). The concentration of asialo-GM_1 required for half-maximal inhibition was 7.5 μg/ml.

B-5 Binding of *C. albicans* fimbriae to GSL receptors on human BECs

We have previously shown that the fimbriae of *C. albicans* were able to bind to buccal epithelial cells (BECs) (Chapter IIIA). Purified fimbriae were able to block *C. albicans* whole cell binding to BECs (Chapter IIIA). The receptors which mediated the binding to *Candida* fimbriae were not identified. In these experiments, fimbriae were

preincubated with βGalNAc(1-4)βGal-methylesters, asialo-GM_1 or CTH prior to their addition to BECs. The results showed that βGalNAc(1-4)βGal-methylester (Figure IIIB-5A) and asialo-GM_1 (Figure IIIB-5B) inhibited *C. albicans* fimbriae binding to BECs by 70% and 80%, respectively, suggesting that glyco-conjugates present on the cell surfaces were interacting with fimbriae. CTH failed to inhibit *C. albicans* fimbriae binding to BECs.

Discussion

C albicans is an opportunistic fungal pathogen that appears to be able to employ several adhesins to mediate attachment of the organism to cell surface receptors (Calderone and Braun, 1991; Kennedy *et al.*, 1992; Culter and Kanbe, 1993). We have established that *C. albicans* produces a fimbrial adhesin that can mediate the adherence of the organism to exfoliated human BECs in *in vitro* binding assays (Chapter IIIA). Fungal fimbriae have been previously described in other yeasts (Poon and Day, 1975a and b; Gardiner *et al.*, 1982; Gardiner and Day, 1985) and the *C. albicans* fimbriae appear to be similar to those structures. Fimbrial-mediated adherence of bacterial organisms is a common theme (Sharon, 1986; Finlay and Falkow, 1989; Paranchych and Frost, 1988; Irvin, 1993) but such an adherence mechanism has not been previously described in yeast.

There has been a tremendous amount of literature supporting the role of carbohydrate receptors on glycolipids or glycoproteins in mediating attachment to bacterial adhesins (Leffler and Svanborg-Eden, 1986; Krivan *et al.*, 1988a and 1988b; Karlsson, 1989). The initial observation of *C. albicans* binding to glycolipid receptors were made by Jimenez-Lucho *et al.* (1990). We report here that *C. albicans* can utilize glycosphingolipids as receptors and that the yeast express fimbrial structures which mediate this interaction. The glycosphingolipids extracted from BECs show that these cells contain asialo-GM_1 and asialo-GM_2 (Figure IIIB-2). The data further supported the hypothesis that the binding of *C. albicans* fimbriae to BECs is mediated by asialo-GM_1-like receptors on BECs. *C. albicans* fimbriae bound specifically to asialo-GM_1 and asialo-GM_2 (Figures IIIB-1, and IIIB-3). Synthetic disaccharides with the βGalNAc(1-4)βGal sequence found on the carbohydrate moiety of these gangliosides were sufficient for interaction with the *C. albicans* fimbriae (Figure IIIB-4A and unpublished data). This interaction could be inhibited by asialo-GM_1 (Figure IIIB-4B). We have previously demonstrated that *C. albicans* fimbriae bind to BECs and the fimbriae inhibit yeast binding to BECs (Chapter

IIIA). In these present studies, we showed that synthetic βGalNAc(1-4)βGal and asialo-GM_1 inhibited *C. albicans* fimbriae binding to BECs (Figure IIIB-5), suggesting that there are glyco-conjugates on BECs that could interact specifically with fimbriae. Significantly, the ability of the synthetic disaccharide to inhibit fimbrial binding to BEC receptors indicates that the receptors likely contain a βGalNAc(1-4)βGal sequence. These observations support the hypothesis that the minimal carbohydrate sequence required for binding of microorganisms to similar series of gangliosides is a βGalNAc(1-4)βGal sequence (Krivan *et al.*, 1988a and 1988b).

Unlike the findings of Jimenez-Lucho *et al.* (1990) that described the binding of *C. albicans* to the glycosphingolipid, LCS, our data indicates that *C. albicans* fimbriae bind to asialo-GM_1. The *C. albicans* strain #40, a clinical isolate, used in the preparation of fimbriae in these studies was different from the strain employed by Jimenez-Lucho *et al.* (1990). Our culture and study conditions also differed from those employed by Jimenez-Lucho *et al.* (1990). Thus, the two strains may express different adhesins requiring distinct receptors. In addition, the binding assay conditions used were also different. In our assays, 0.05% (v/v) Tween-20 was present in the buffers. We have observed that the absence of detergent in the buffers resulted in non-specific binding to LCS as well as M-GM_1 and phospholipids (unpublished observations).

In these studies, we have identified an adhesin for *C. albicans* that recognized glycosphingolipid receptors on buccal epithelial cell surfaces. We have not investigated whether the fimbrial adhesin is expressed in the mycelial form. Immunological data suggest that fimbriae are widely distributed amongst strains of *C. albicans* (unpublished observations). The fimbrial adhesin appears to differ from previously reported adhesins due to its receptor specificity (Klotz and Smith, 1991; Gustafson *et al.*, 1991; Cutler, 1991; Calderone and Braun, 1991; Tosh and Douglas, 1992; Casanova, 1992b). We are

attempting the isolation and purification of *C. albicans* fimbrial receptors from epithelial cells to determine whether glycosphingolipids represent *in situ* receptors with biological relevance to yeast adherence. Work is in progress in mouse infection models to evaluate the role of fimbriae in *C. albicans* virulence.

Figure IIIB-1

The binding of *C. albicans* fimbriae to glycosphingolipids (GSLs) separated on thin layer chromatography (TLC) plates. Glycosphingolipids (10 μg) were chromatographed on TLC plates with a mobile phase that consisted of chloroform, methanol, and water (65:35:8, v:v:v). Lane 1: charred GSL standards; ceramide trihexoside (CTH); lactosylceramide (LCS); asialoganglioside-GM_2 (Asialo-GM_2); asialoganglioside-GM_1 (Asialo-GM_1); mono-sialoganglioside-GM_1 (M-GM_1); Lane 2: the same set of GSL as Lane 1; Lane 3: asialo-GM_1; Lane 4: M-GM_1 and asialo-GM_2; and Lane 5: LCS and CTH. The TLC binding assay is described in detail in the Materials and Methods (section 18-1) where the GSLs were first incubated with *C. albicans* fimbriae. Fm16 was used to probe fimbrial binding in Lane 2 while a normal mouse IgG was used in Lanes 3, 4 and 5 as a control. A goat anti-mouse IgG alkaline phosphatase conjugate was utilized to detect bound fimbriae on the TLC plates. Color development was obtained using nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indoyl phosphate dissolved in 100 mM Tris buffer, pH 9.5, containing 100 mM NaCl and 5 mM $MgCl_2$.

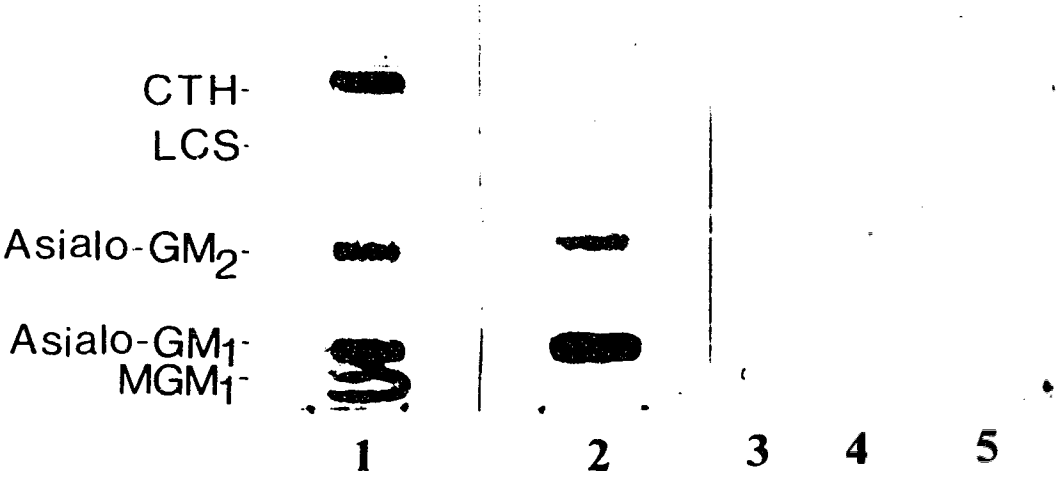

CTH-
LCS-
Asialo-GM2-
Asialo-GM1-
MGM1-
1
2
3
4
5

Figure IIIB-2

Analysis of total glycosphingolipids from BEC by thin layer chromatography. The GSLs were chromatographed on a TLC plate with a mobile phase of chloroform-methanol-water (65:35:8, v:v:v). Lane A is the charred GSL standards [LCS (15 μg), asialo-GM_2 (5 μg), asialo-GM_1 (10 μg), M-GM_2 (10 μg) and M-GM_1 (10 μg)]. Lane B is the charred total GSLs (50 μl) extracted from BECs of 100 healthy, non-smoking males.

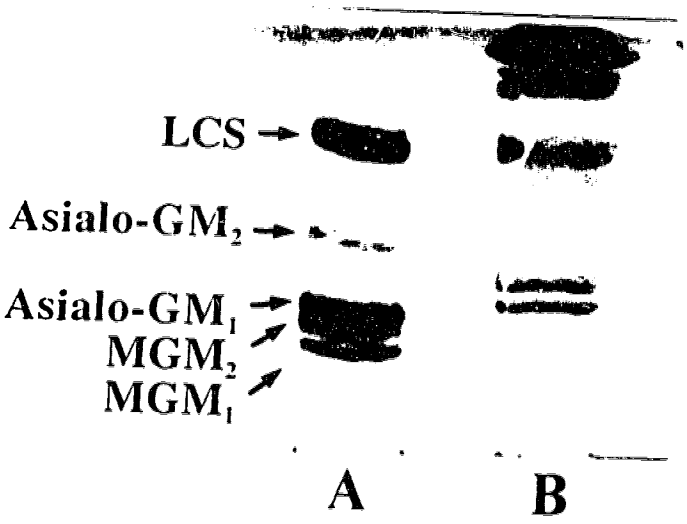

LCS
Asialo-GM2
Asialo-GM1
MGM2
MGM1
A
B

Figure IIIB-3

The binding of *C. albicans* fimbrial adhesin to immobilized glycosphingolipid receptors. A) Binding of fimbriae to asialo-GM_1 (◆) and CTH (■) immobilized on microtiter wells (0.5 μg/well). The respective GSLs were incubated with enriched fimbriae (ranging from 0 to 4 μg/well) for an hour at 37°C. ELISA protocols utilizing anti-*C. albicans* fimbrial monoclonal antibody, Fm 16, as the primary antibody and a goat anti-mouse IgG alkaline phosphatase conjugate as the secondary antibody were employed to quantitate fimbrial binding to asialo-GM_1 and CTH. The amount of fimbriae bound is represented as the absorbance values measured at 405 nm. B) Inhibition of *C. albicans* fimbrial binding to asialo-GM_1 (◆) and CTH (■) with the respective homologous GSLs. The protocols were similar to those used for (A) except that the *C. albicans* fimbriae (12 μg/ml) were preincubated with asialo-GM_1 and CTH at 37°C for an hour prior to their addition into the precoated microtiter wells. The % inhibition is the inhibition of fimbrial binding in the presence of the competing antigens with respect to the binding in the absence of any competitors under identical conditions.

Figure IIIB-3

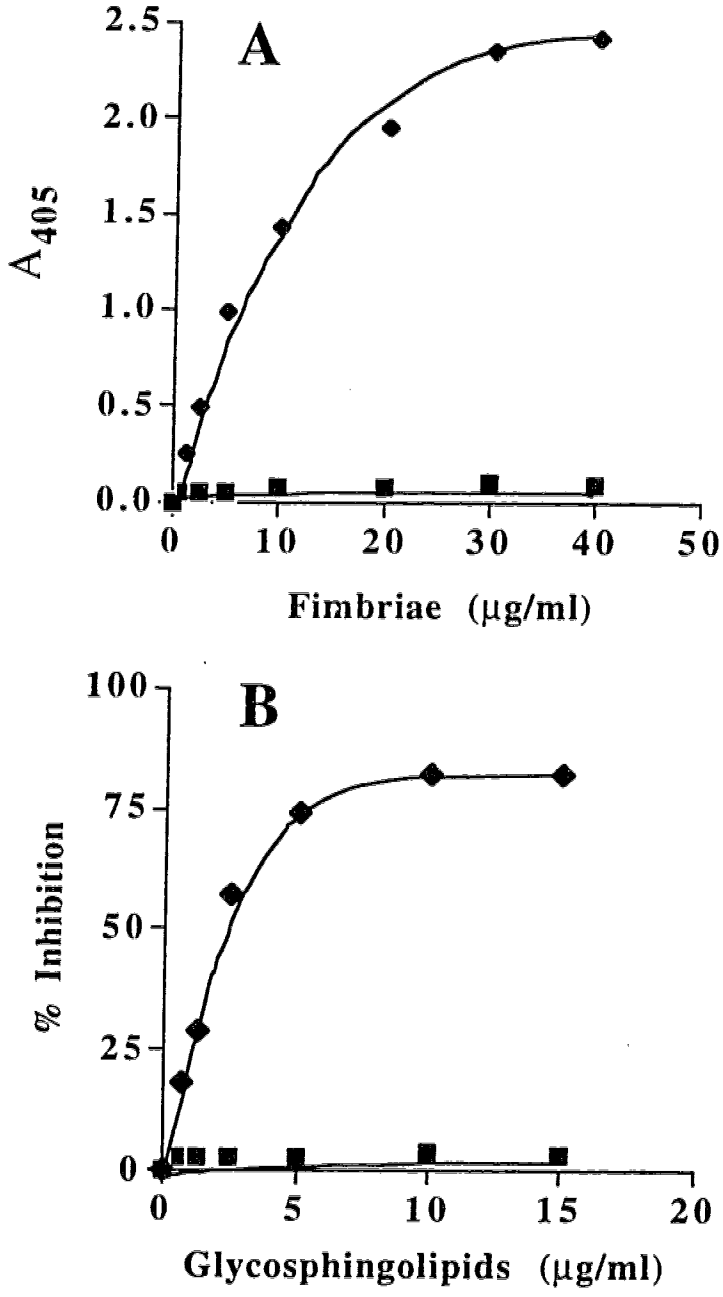


Figure IIIB-4

C. albicans fimbrial adhesin binds to the minimal disaccharide sequence of βGalNAc(1-4)βGal. A) Inhibition of fimbriae binding to βGalNAc(1-4)βGal-BSA conjugates. βGalNAc(1-4)βGal-BSA conjugates were immobilized onto microtiter wells (0.5 μg/well). These conjugates were incubated with enriched fimbriae (12 μg/ml) that had been preincubated with varying concentrations of βGalNAc(1-4)βGal-methylester. The binding of fimbriae to the disaccharide-conjugates was quantitated using the same ELISA protocols as in Figure IIIB-2. B) Inhibition of fimbriae binding to βGalNAc(1-4)βGal-BSA with asialo-GM_1. The conditions for these inhibition assays are similar to those used in Figure IIIB-3A. The % inhibition is the inhibition of fimbrial binding in the presence of the competing antigens with respect to the binding in the absence of any competitors under identical conditions.

Figure IIIB-4

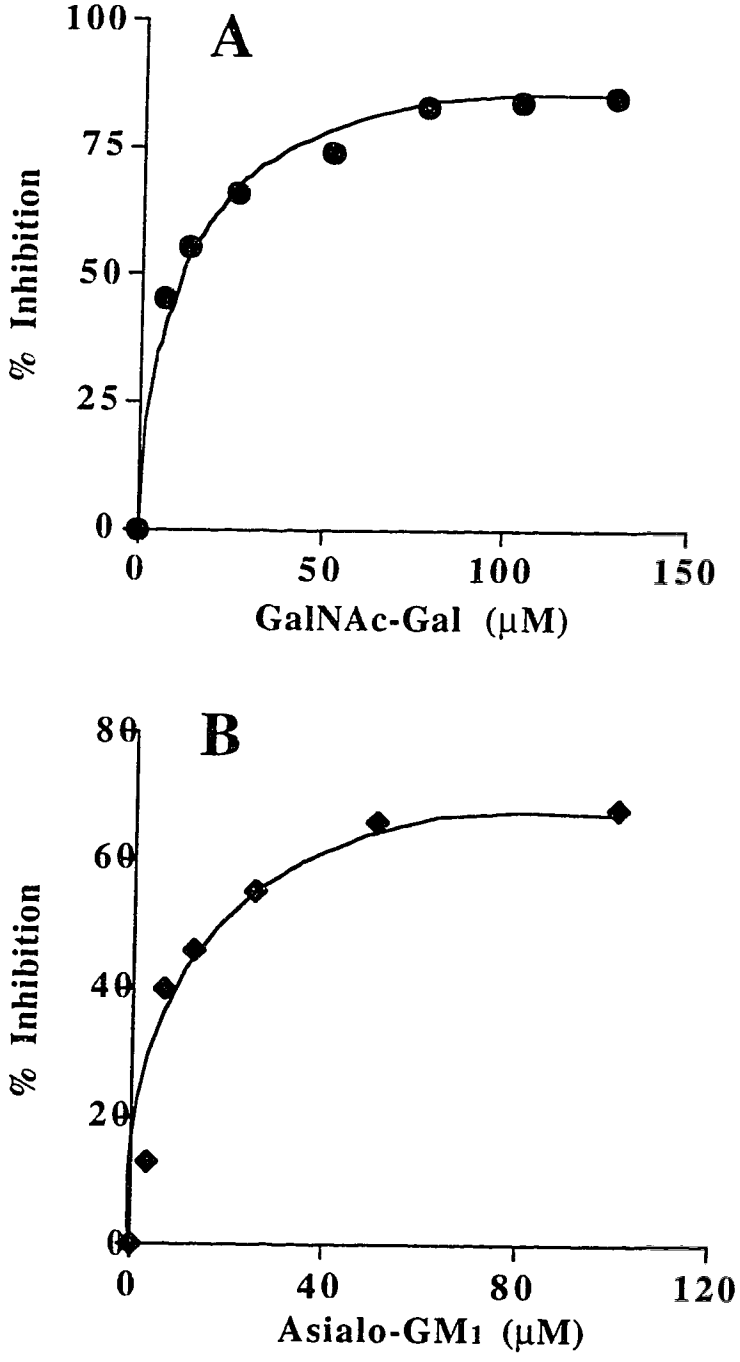
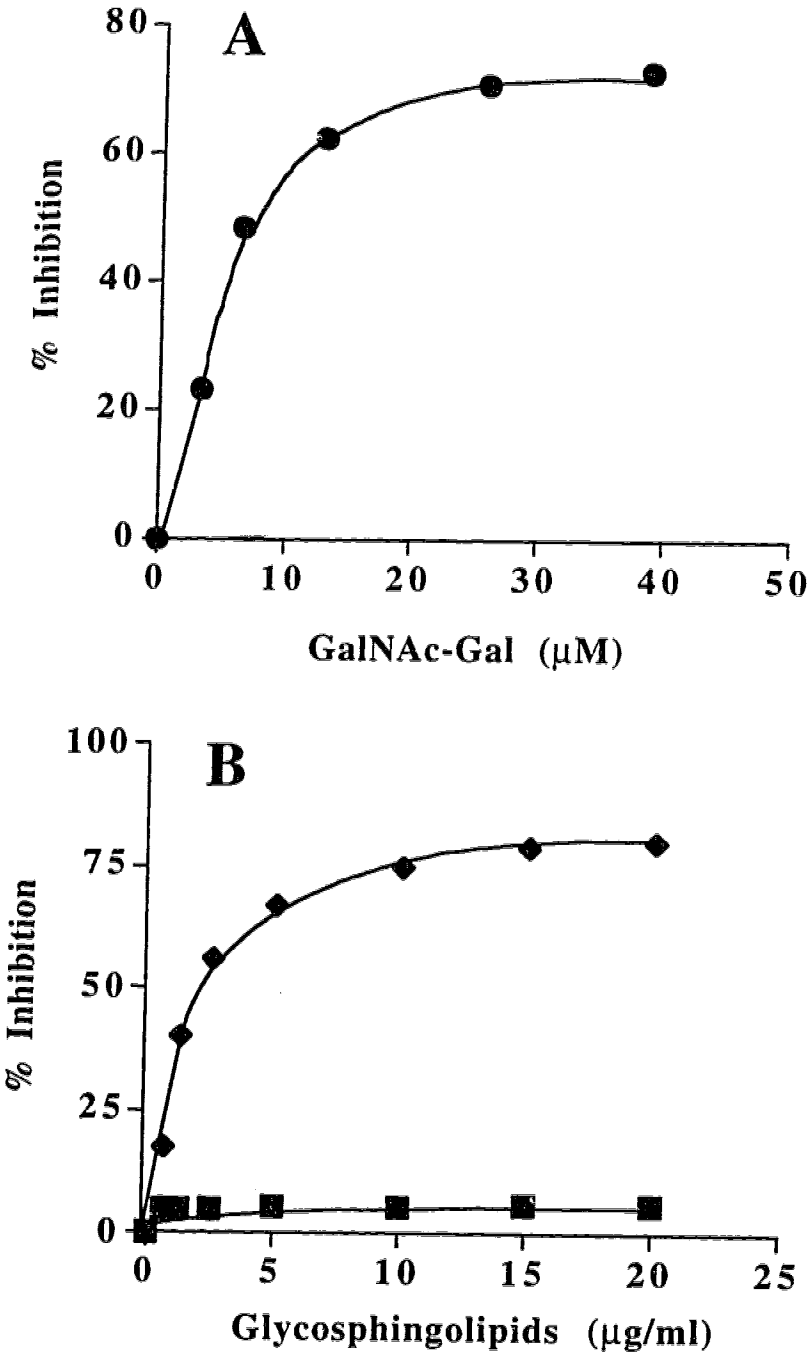


Figure IIIB-5

Inhibition of *C. albicans* fimbrial binding to glycoconjugate receptors on human buccal epithelial cells (BECs) with synthetic βGalNAc(1-4)βGal-methylester (A) and GSLs (B). BECs were collected from non-smoking healthy male volunteers as described in Materials and Methods. These assays were performed using a manifold filtration apparatus equipped with individual vacuum stopcocks (model FH 225V, Hoefer Scientific Instruments). The assay mixture consists of 2.0 x 10^5 BECs, fimbriae (50 μg) and varying concentrations of competitors in a total volume of 1.0 ml of 10 mM phosphate buffer, pH 7.2, containing 150 mM NaCl. *C. albicans* fimbriae were preincubated with either βGalNAc(1-4)βGal-methylester (●, panel A), asialo-GM_1 (◆, panel B) or CTH (■, panel B) for an hour at 37°C prior to addition to the BECs. The % of inhibition is the inhibition of binding in the presence of the competing antigens with respect to the binding in the absence of any competitors.

Figure IIIB-5



C. 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[3]

Results

C-1 Agglutination of *C. albicans* with anti-*P. aeruginosa* pilus adhesin antibodies

C. albicans expresses fimbrial structures on its cell surfaces as previously demonstrated by electron microscopy (Montes and Wilborn, 1968; Mohamed, 1975; Marrie and Costerton, 1981; Tokunaga *et al.*, 1990; Chapter IIIA). Monoclonal antibodies (MAbs) have been raised against the purified *C. albicans* fimbriae (Chapter IIIA) and two of these MAbs, Fm16 and Fm34, were used in these agglutination assays. *C. albicans* strain #40, a clinical isolate, was agglutinated by Fm16 and Fm34 as shown in Table IIIC-1. When MAbs (PK99H and PKL1) (Doig *et al.*, 1987) raised against the *P. aeruginosa* PAK pilus adhesin 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 IIIC-1). However, neither PKL1 nor commercial normal mouse IgG was able to agglutinate *C. albicans*. The epitope of PK99H is a linear epitope (residues 134-140) located within the disulfide-looped carboxy-terminal region of the 144-amino acid residue long PAK pilus subunit (Wong *et al.*, 1992). 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 IIIC-1). The PAK(128-144) peptide represents the 17 residues that encompass the entire disulfide loop at the carboxy-terminal of PAK

[3] A version of this section of chapter III has been published: Yu, L., Lee, K.K., Hodges, R.S., Paranchych, W., and Irvin, R.T. (1994) Infect. Immun. **62**:5213-5219.

pilin (Sastry *et al.*, 1985; Lee *et al.*, 1989). 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* strain PAK recognized by PK99H and the two anti-PAK peptide antibodies.

C-2 Agglutination of *P. aeruginosa* with anti-*C. albicans* fimbriae antibodies

Reciprocal assays were carried out to assess the abilities of anti-*C. albicans* fimbriae antibodies to agglutinate *P. aeruginosa* PAK whole cells. As expected, MAbs against *P. aeruginosa* PAK pilus (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 IIIC-2). Anti-*C. albicans* fimbriae Fm16 MAb also resulted in agglutination of *P. aeruginosa*. The ability of Fm34 to agglutinate *P. aeruginosa* was not significantly different from the control normal mouse IgG. This demonstrated that Fm16, but not Fm34, recognizes a surface antigen that is present on *P. aeruginosa*.

C-3 Western blot analyses

The ability of anti-*C. albicans* fimbriae antibodies and anti-*P. aeruginosa* pili antibodies to cross react with heterologous antigens was demonstrated by Western blot analyses. A concentrated crude *C. albicans* fimbriae preparation was used in these assays. Aliquots containing equal amounts of fimbriae were separated by SDS-PAGE, blotted onto nitrocellulose membrane and probed with different antibodies (Figure IIIC-1A). Fm16 and Fm34 (Lanes 1 and 2) bound to a 66 kDa band as expected. Additional bands at 47 kDa (Fm16 and Fm34), 54 kDa (Fm16 only) and 39 kDa (Fm34 only) were also observed. As the fimbrial subunit is heavily glycosylated as mentioned in Chapter IIIA, the presence of more than one major band may have resulted from varying degrees of glycosylation of the fimbrial subunit. Fm16 and Fm34 probably recognize different epitopes as the intensities

of the 66 kDa and 47 kDa bands and the banding patterns are markedly different; these observations could be due to the varying degrees of exposure of the epitopes arising from differences in glycosylation. 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 (Figure IIIC-1A) of the Western blot with the rabbit polyclonal anti-PAK (134-140) peptide antibody reflects a slightly higher antibody concentration relative to the other monoclonal antibodies. When anti-*Pseudomonas* pilin antibodies were utilized in the Western analyses to probe for fimbrial binding, PK99H and anti-PAK(128-144) (Lanes 3 and 4) also bound to the 66 kDa and 47 kDa bands. Anti-PAK(134-140) (Lane 5) antibodies had strong affinities only for the 66 kDa band. Interestingly, Anti-PAK(128-144) also recognized the 54 kDa and 39 kDa bands observed individually with Fm16 and Fm34. Control antibodies consisting of Anti-PAK(75-84), normal rabbit IgG and normal mouse IgG (lanes 6, 7 and 8) did not bind to the 66 kDa or 47 kDa bands. In addition, there is a small molecular weight 13 kDa band (may be a highly deglycosylated fimbrial subunit) observed with Fm16, Fm34, PK99H and Anti-PAK(128-144) (Lanes 1-4).

The reciprocal studies using *P. aeruginosa* PAK pili that have been electrophoresed, blotted onto nitrocellulose membrane and probed with the respective antibodies were conducted (Figure IIIC-1B). All the anti-*Pseudomonas* pilin antibodies, PK99H, Anti-PAK(128-144) and Anti-PAK(134-140) bound to the 15 kDa PAK pilin band (Lanes 2, 3 and 4). Fm16 also bound to the 15 kDa 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 monoclonal antibodies raised against antigens prepared from different species of organisms, share a common antigenic epitope.

C-4 Competitive ELISA 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 (Figure IIIC-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 crossreacts with fimbriae, PKL1 does not bind the yeast antigen (data not shown). Hence, the binding of PKL1 to immobilized PAK pili was not greatly affected by the addition of *C. albicans* fimbriae (Figure IIIC-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 (Figure IIIC-2B). PAK pili were effective in reducing Fm16, 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% as compared with the control where no competitors were present. These data demonstrated that the interactions between the monoclonal antibodies with heterologous antigens were specific and of high affinity.

C-5 Similarity between the epitopes of Fm16 and PK99H

The specificities of the interactions between Fm16 and synthetic peptides could be assessed 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-terminal of the *Pseudomonas* PAK pilin inhibited Fm16 binding to immobilized fimbriae by about 80% (Figure IIIC-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

IC_{50} for PAK(128-144) and PAK(134-140) are approximately 0.45 μM and 6.5 μM, respectively. A control peptide, PAK(75-84) which correspond 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 (Yu, Lee and Irvin, data not shown). These results suggest 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 *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 these organisms are capable of binding to similar receptors (Hazelett *et al.*, 1993; Krivan *et al.*, 1991; Lee *et al.*, 1994; Sheth *et al.*, 1994; Chapter IIIB). Our studies have shown that *P. aeruginosa* pili bind to glycosphingolipid (asialo-GM_1) receptors (Lee *et al.*, 1994; Sheth *et al.*, 1994) and that the receptor-binding domain resides in the carboxy-terminal disulfide-looped region of the PAK pilin (Irvin *et al.*, 1989; Lee *et al.*, 1989). *C. albicans* and other fungi have been previously shown to bind to glycosphingolipids (Jimenez-Lucho *et al.*, 1990). 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 (Chapter IIIA and IIIB). 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 IIIC-1). *C. albicans* strain #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* (Irvin, unpublished data). 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 IIIC-2). The presence of a structurally conserved antigenic determinant

on these adhesins was evident from the Western blot analyses. The abilities of heterologous antibodies to bind to the respective immobilized antigens in direct ELISA (data not shown) and in Western blot assays (Figure IIIC-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* fimbriae antibodies to *Pseudomonas* pili adhesins. The immunocross-reactions of Fm16 and PK99H with PAK pili and fimbriae, respectively, were highly specific as Fm34 and PKL1 showed little or insignificant levels of crossreactivities 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 (Figure IIIC-2). This demonstrated that both antigens contain structurally similar epitopes that competed for binding to the monoclonal antibodies (PK99H and Fm16) that were used in the competitive ELISAs. However, Fm34 and PKL1 recognize different epitopes on the respective fimbriae and PAK pili 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 BECs (Doig *et al.*, 1987). PK99H may be binding to or near the receptor-binding domain of the *C. albicans* fimbriae as the addition of this antibody could block yeast adherence to BECs (Irvin, Unpublished data). It is interesting to note that while both PK99H and PKL1 bound to the PAK(128-144) 17-mer peptide (Figure IIIC-3), PKL1 failed to agglutinate *C. albicans* whole cells (Table IIIC-1). The epitope present on the 17-residue peptide that is recognized by PKL1 differs from the epitope recognized by PK99H as PKL1 does not bind to the PAK(134-140) 7-mer peptide (data not shown).

Fm16 and PK99H may share a common epitope since the binding of Fm16 and PK99H to fimbriae and PAK pili could be competitively inhibited by heterologous antigens (Figure IIIC-2). This was suggested by the ability of Fm16 to bind to PAK(128-144) and

PAK(134-140) peptides in direct ELISA (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 IIIC-1). The PK99H epitope has also been determined and consists of a linear seven amino acid residue sequence, LEQFIPK, located in the disulfide-looped region at the carboxy-terminal of the PAK pilin molecule (Wong *et al.*, 1992). The *C. albicans* fimbrial subunit is a glycoprotein of molecular weight of 66 kDa but the protein sequence of the fimbriae has yet to be completely determined (Chapter IIIA). Hence, the epitope of Fm16 could not be determined as 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 homologue 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 (Ahrens *et al.*, 1983; Sundstrom and Kenny, 1984 and 1985; Ponton and Jones, 1986a and 1986b; Sundstrom *et al.*, 1987, 1988; Casanova *et al.*, 1989; Leusch, 1989; Ollert and Calderone, 1990; Tosh and Douglas, 1992), mannans (Miyakawa *et al.*, 1992; Kanbe *et al.*, 1993), lipids (Ghannoum *et al.*, 1986) and chitins (Suzuki *et al.*, 1984; Segal and Savage, 1986) have been described as possible adhesin candidates. Most *C. albicans* adhesins proposed to date are mannoproteins (Hostetter, 1994). *C. albicans* fimbriae are similar to many of the candidate adhesins in that they are mannoproteins (Chapter IIIA and IIIB). However this may just reflect the normal glycosylation pattern that *C. albicans* utilizes for extracellular proteins. *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 (Hostetter *et al.*, 1990; Odds, 1994). The *C. albicans* fimbrial adhesin-receptor interactions are likely to involve the protein moiety of fimbriae with the carbohydrate portion of the glycosphingolipids (asialo-GM_1 and asialo-GM_2) on the epithelial cells as the conserved

epitope on the fimbrial subunit is similar to the receptor-binding domain on the *Pseudomonas* pilin protein. The ability of synthetic GalNAc-Gal to inhibit fimbrial binding to epithelial cells also supports this contention (Chapter IIIA and IIIB). Fimbrial adhesins differ from the integrin-analog adhesin and the fibronectin-binding protein in terms of their molecular weight and their interactions with their receptors (protein-protein). The integrin-analog (iC3b receptor) are 130-165 kDa mannoproteins and recognize iC3b or RGD peptides on host surfaces (Bandel and Hostetter, 1993; Gilmore *et al.*, 1988; Hostetter, 1994; Ollert *et al.*, 1990). A 58 kDa mannoprotein has recently been identified as an fibronectin-binding protein; it interacts specifically with fibronectin on endothelial cell surfaces (Casanova *et al.*, 1992b). The fimbriae have a lectin-like activity and the *C. albicans* fimbrial adhesin-receptor interaction is likely to involve the binding of the protein moiety of fimbriae to GalNAc-Gal contained in the glycosphingolipids (asialo-GM_1 and asialo-GM_2) on the epithelial cells (Chapter IIIB). Fimbrial adhesins also differ from the extracellular polymeric material of *C. albicans* which was described as a putative adhesin by Douglas and coworkers (Douglas, 1991; Tosh and Douglas, 1992). The receptor specificity of the *C. albicans* fimbrial adhesin described here differs from that described for the EP adhesins of Douglas and coworkers which appears to recognize fucose and N-acetylglucosamine (Tosh and Douglas, 1992).

The occurrence of conserved receptor-binding domains on adhesins of different species of microorganism could be possible since these adhesins can recognize and utilize similar cell-surface receptors. Krivan *et al.* (1988a, 1988b and 1991) have observed that many pulmonary pathogens, including *Pseudomonas aeruginosa, Haemophilus influenzae, Staphylococus aureus, Streptococcus pneumoniae* and *Klebsiella pneumoniae*, are able to utilize the minimal disaccharide sequence, βGalNAc(1-4)Gal, present on glycolipids as receptors. It remains to be determined if the *P. aeruginosa* pilus adhesin and the *C.*

albicans fimbrial adhesin are part of a large family of adhesins containing a conserved structural motif.

Table IIIC-1

The agglutination of *C. albicans* strain #40 with antibodies raised against *Candida* fimbriae and *P. aeruginosa* PAK pili

	Antibody dilution			
Antibody*	**1:2**	**1:4**	**1:8**	**Control****
Fm16	++++	++++	++	-
Fm34	++++	++++	+++	-
PK99H	+++	++++	++	-
PKL1	-	-	-	-
Anti-PAK(128-144)	++++	+++	++	-
Anti-PAK(134-140)	++++	+++	++	-
Normal mouse IgG	-	-	-	-
Normal rabbit IgG	+	+	+	-

* Fm16 and Fm34 are monoclonal antibodies raised against *C. albicans* fimbriae (see Chapter IIIA); PK99H and PKL1 are monoclonal antibodies raised against *P. aeruginosa* pili from strain PAK (Doig *et al.*, 1990); 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 (Lee *et al.*, 1990); normal mouse IgG and normal rabbit IgG are commercial available affinity-purified immunoglobulin G.

** The control consisted of PBS only.

The symbols "+" and "-" indicate the degrees of the agglutination: ++++, easily observed by naked eyes; +++, clearly observed by naked eyes; ++, observable by naked eye but clearly seen under phase contrast microscope; +, observed only by phase contrast microscope; -, no agglutination was observed.

Table IIIC-2

The agglutination of *P. aeruginosa* strain PAK with antibodies raised against *Candida* fimbriae and *P. aeruginosa* PAK pili

	Antibody dilution			
Antibody*	**1:2**	**1:4**	**1:8**	**Control****
Fm16	++++	+++	++	-
Fm34	+	+	±	-
PK99H	++++	++++	+++	-
PKL1	++++	++++	++	-
Anti-PAK(128-144)	++++	++++	+++	-
Anti-PAK(134-140)	++++	++++	+++	-
Normal mouse IgG	+	+	+	-
Normal rabbit IgG	n.d.	n.d.	n.d.	n.d.***

* Fm16 and Fm34 are monoclonal antibodies raised against *C. albicans* fimbriae (see Chapter IIIA); PK99H and PKL1 are monoclonal antibodies raised against *P. aeruginosa* pili from strain PAK (Doig *et al.*, 1990); 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 (Lee *et al.*, 1990); normal mouse IgG and normal rabbit IgG are commercial available affinity-purified immunoglobulin G.

** The control consisted of PBS only, and *** n.d. denotes "not done"

The symbols "+" and "-" indicate the degrees of the agglutination: ++++, easily observed by naked eyes; +++, clearly observed by naked eyes; ++, observable by naked eye but clearly seen under phase contrast microscope; +, observed only by phase contrast microscope; -, no agglutination was observed.

Figure IIIC-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 lane 1) Fm16, lane 2) Fm34, lane 3) PK99H, lane 4) Anti-PAK(128-144), lane 5) Anti-PAK(134-140), lane 6) Anti-PAK(75-84), lane 7) normal rabbit IgG and lane 8) normal mouse IgG. (B) *P. aeruginosa* PAK pilin proteins (3 μg per lane) were probed with lane 1) Fm16, lane 2) PK99H, lane 3) Anti-PAK(128-144), lane 4) Anti-PAK(134-140), lane 5) Fm34 and lane 6) normal mouse IgG. The appropriate goat anti-mouse IgG-alkaline phosphatase or goat anti-rabbit IgG-alkaline phosphatase conjugates were employed as secondary antibodies. Bands were obtained using nitroblue tetrazolium and 5-bromo-4-chloro-3-indoylphosphate as substrates.

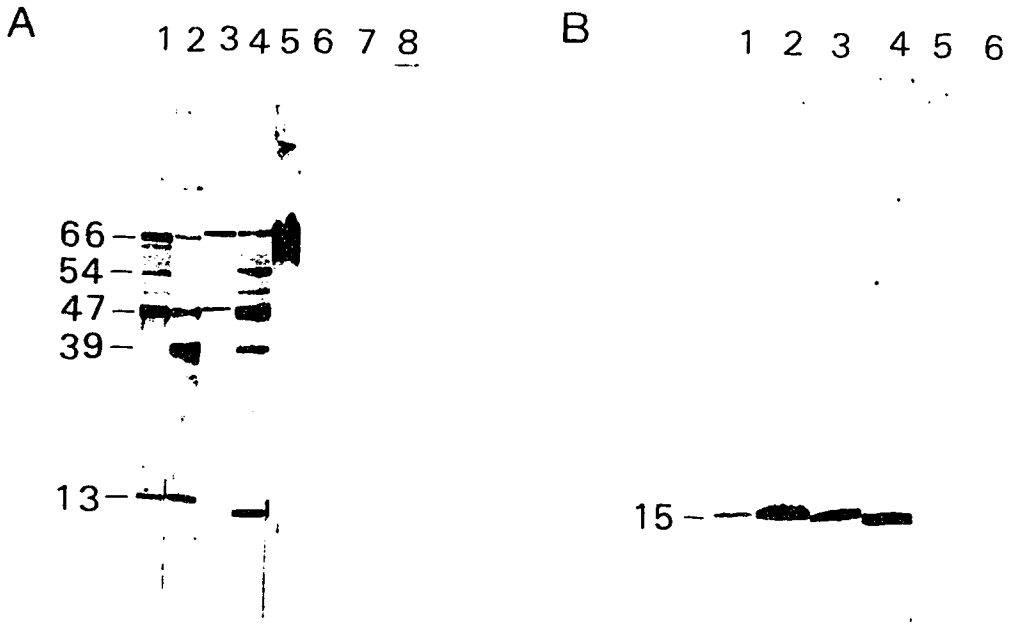
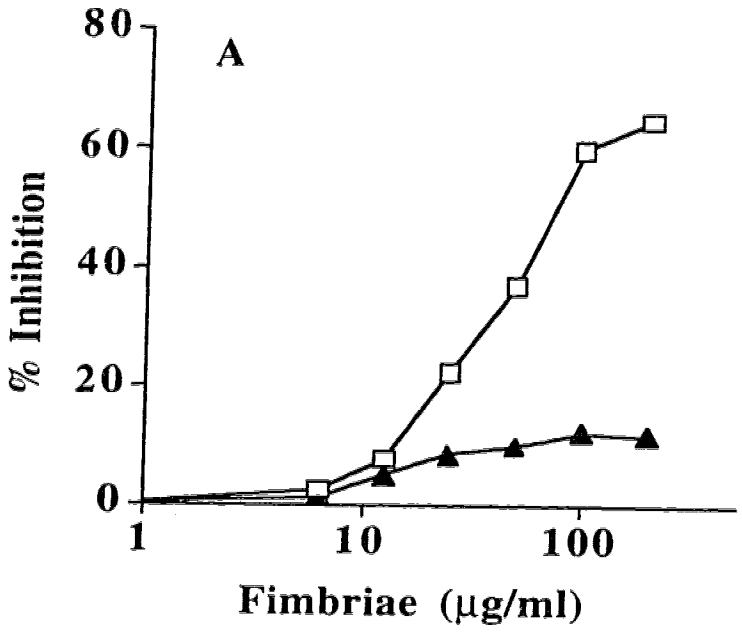

A
1 2 3 4 5 6 7 8
66
54
47
39
13
B
1 2 3 4 5 6
15

Figure IIIC-2

Specificities of the interactions between anti-adhesin monoclonal antibodies with heterologous antigens. (A) *P. aeruginosa* PAK pili were employed as immobilized antigens (0.5 μg per well) and the binding of PK99H (□) and PKL1 (▲) to pili were competed with *C. albicans* fimbriae. (B) *C. albicans* fimbriae were employed as immobilized antigens (0.5 μg per well) and the binding of Fm16 (■) and Fm34 (Δ) to fimbriae were competed with *P. aeruginosa* PAK pili. The specificities of the interactions between competing heterologous antigens with the respective monoclonal antibodies are represented as the % inhibition in the antibody binding to its own antigen as a result of the competitor.

Figure IIIC-2



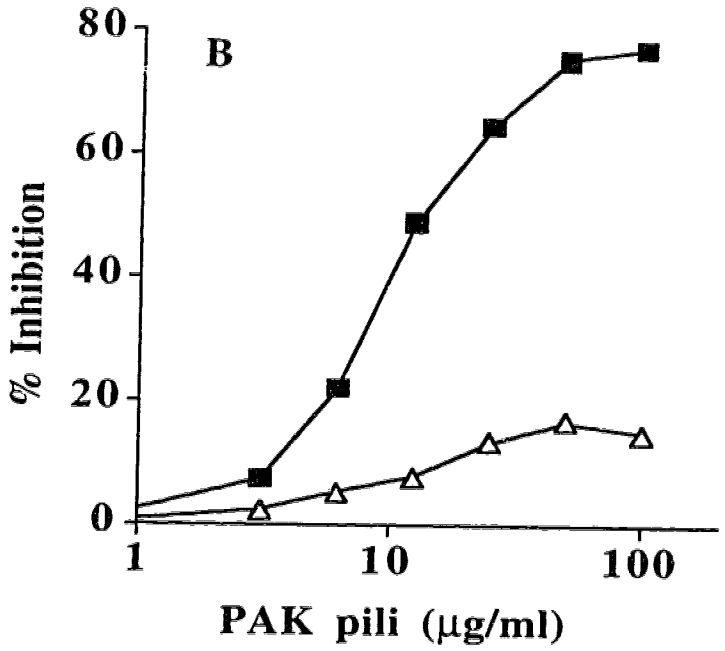
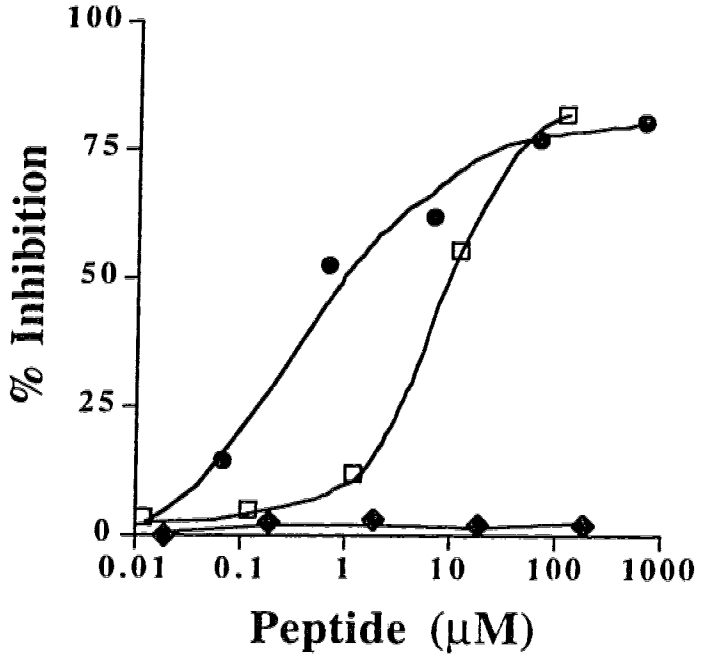


Figure IIIC-3

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

Figure IIIC-3



D. Anti-adhesin antibodies that recognize a receptor-binding motif (adhesintope) inhibit pilus/fimbrial-mediated adherence of *Pseudomonas aeruginosa* and *Candida albicans* to asialo-GM_1 receptors and to human buccal epithelial cell surface receptors.[4]

Results

D-1 *P. aeruginosa* pilus and *C. albicans* fimbrial adhesins bind to asialo-GM_1

We have demonstrated previously that *P. aeruginosa* pilus and *C. albicans* fimbrial adhesins are able to bind to the glycosphingolipid asialo-GM_1 (Lee *et al.*, 1994; Chapter IIIC). In these experiments, the two adhesins were assayed for their abilities to compete with each other for binding to immobilized asialo-GM_1. Biotinylated PAK pili (Bt-PAK pili) and biotinylated *C. albicans* fimbriae (Bt-fimbriae) were employed in these assays. As shown in Figure IIID-1A, unbiotinylated *C. albicans* fimbriae and PAK pili were equally effective in blocking biotinylated *P. aeruginosa* PAK pili binding to asialo-GM_1 (Ka = 0.156 ml/μg and 0.35 ml/μg respectively). Conversely, *P. aeruginosa* PAK pili also inhibited Bt-fimbriae binding to asialo-GM_1 (Ka = 0.012 ml/μg) (Figure IIID-1B). As expected, unbiotinylated *C. albicans* fimbriae also blocked Bt-fimbriae binding to asialo-GM_1 (Ka = 0.025 ml/μg). These observations suggest that *P. aeruginosa* pilus and *C. albicans* fimbrial adhesins may recognize the same receptor epitopes on the glycosphingolipid.

---

[4] A version of this section of chapter III has been published: Lee, K.K., Yu, L.,. Mackonald, D.L., Paranchych, W., Hodges, R.S., and Irvin, R.T. (1996). Can. J. Microbiol. **42**:479-486.

D-2 Anti-adhesin antibodies block *C. albicans* fimbriae binding to asialo-GM_1

MAbs directed against *C. albicans* fimbriae have been prepared previously (Chapter IIIA) and their abilities to block fimbriae binding to asialo-GM_1 were determined. The epitope recognized by one of these MAbs, Fm16, is believed to constitute a conserved structural motif that is also recognized by MAb PK99H, an anti-*P. aeruginosa* PAK pilus antibody (Chapter IIIA). The structural motif on the fimbrial subunit has been suggested to be associated with the adhesin function of the fimbrial adhesin (Chapter IIIB). Fm16 competitively inhibited Bt-fimbriae binding to asialo-GM_1 (Figure IIID-2). Normal mouse immunoglobulin G did not affect Bt-fimbriae binding to asialo-GM_1.

The epitope of MAb PK99H is located within the asialo-GM_1-binding domain in the C-terminal disulfide loop region of PAK pilin (Lee *et al.*, 1994) and this antibody has been shown to cross-react with the *C. albicans* fimbriae (Chapter IIIB). PK99H has also been demonstrated to inhibit PAK pili binding to asialo-GM_1 (Lee *et al.*, 1994). When PK99H was added to Bt-fimbriae in the binding assay, this MAb inhibited Bt-fimbrial adhesin binding to asialo-GM_1 by 90% (Figure IIID-2). A second anti-*P. aeruginosa* PAK pilin MAb, PAK-13, which also binds to the C-terminal disulfide-looped region of PAK pilin was also able to reduce Bt-fimbriae binding to asialo-GM_1 (Figure IIID-2). The abilities of PK99H and Fm16 to inhibit *C. albicans* fimbriae binding to asialo-GM_1 confirmed our hypothesis that the conserved epitope on the fimbrial adhesin recognized by these two MAbs is required for binding to receptors.

D-3 Anti-adhesin antibodies block *C. albicans* fimbriae binding to BECs

Irvin and co-workers (Chapter IIIA and IIIB) have shown that *C. albicans* fimbriae can bind to BECs and that this binding is inhibited by asialo-GM_1. The antibodies that were demonstrated to block *C. albicans* fimbriae binding to asialo-GM_1 (Figure IIID-2) were assayed for their abilities to inhibit fimbrial adhesin binding to receptors present on

the surfaces of BECs. BECs obtained from male volunteers were immobilized onto microtiter wells and the binding of Bt-fimbriae to these cells was performed in the presence of anti-adhesin antibodies. Anti-*C. albicans* fimbriae MAb Fm16 inhibited Bt-fimbriae binding to BECs on the microtiter wells as shown in Figure IIID-3. Anti-*P. aeruginosa* pili MAb PK99H and PAK-13 inhibited Bt-fimbriae binding by 80% and 53%, respectively. However, a normal mouse IgG had little effect on Bt-fimbriae binding to BECs. The data obtained with PK99H and Fm16 demonstrated that not only glycosphingolipid receptor asialo-GM_1, but also *in situ* receptors present on BECs, are recognized by the conserved structural motif of the fimbrial adhesin.

D-4 Heterologous adhesins compete for binding to receptors on BECs

P. aeruginosa pilus and *C. albicans* fimbrial adhesins have been previously demonstrated to bind to cell surface receptors on BECs (Lee *et al.*, 1994; Chapter IIIC). If similar BEC receptors were utilized by these two adhesins, they will be able to compete against one another in a competitive assay. When the competitive assays were performed, it was shown that fimbriae were as effective as PAK pili in the inhibition of Bt-PAK pili binding to BECs (Figure IIID-4A). The IC_{50} value for *C. albicans* fimbriae is about 2 logs lower than that for PAK pili (Ka = 1.39 ml/μg and 0.017 ml/g respectively). A control which consisted of F-pili from *Escherichia coli* had no effect on Bt-PAK pili binding to BECs (data not shown). In a reciprocal assay, Bt-fimbriae binding to BECs was reduced by 50% of control levels in the presence of competing heterologous PAK pili adhesins (Ka = 0.0168 ml/μg) (Figure IIID-4B). PAK pili were not as effective as fimbriae in the inhibition of Bt-fimbriae binding to BEC receptors (Ka < 10^{-3} ml/μg). These data demonstrated that the *P. aeruginosa* pilus and *C. albicans* fimbrial adhesins recognized similar *in situ* receptors present on the cell surface of BECs. The data also suggested that the interactions between *P. aeruginosa* PAK pili and BEC receptors did not have as high an affinity as those between *C. albicans* fimbriae and the same receptors.

D-5 Anti-adhesin antibodies block whole cell binding to BECs

Whole organisms were biotinylated and anti-adhesin antibodies were assayed for their abilities to block whole cell binding to BECs. As shown in Figure IIID-5A, PK99H reduced Bt-*P. aeruginosa* whole cell binding to BECs by 60-70%. Anti-*C. albicans* fimbria MAb Fm16 also inhibited *P. aeruginosa* whole cell binding to BECs by about 65% of control levels (Figure IIID-5A). In a reciprocal assay, Bt-*C. albicans* whole cell binding was effectively reduced by heterologous anti-PAK pilus MAb PK99H (Figure IIID-5B). Normal mouse IgG had no effect on either biotinylated *P. aeruginosa* or *C. albicans* adherence to BECs.

Discussion

Microbial adhesion is an important step in successful colonization of the host and microorganisms have developed effective means to attach to cell surface receptors. Interestingly, some organisms appear to utilize the same or similar receptors. Although evolutionarily distant, *P. aeruginosa*, a Gram-negative bacterium, and *C. albicans*, a dimorphic fungus, have evolved adhesins that enable them to adhere to the glycosphingolipid asialo-GM_1 (Lee *et al.*, 1994; Chapter IIIB). The *P. aeruginosa* pilus is a long filament consisting of a vectorial assembly of many subunits (Frost and Paranchych, 1977; Folkhard *et al.*, 1981). The binding domain of the pilus adhesin lies in the disulfide-loop region at the carboxy-terminal of the pilin, the structural subunit of the pilus (Irvin *et al.*, 1989; Lee *et al.*, 1989). The receptor-binding domain has been shown to be exposed only at the distal tip of the pilus (Irvin, 1990; Lee *et al.*, 1994). Like the pilus adhesin, the binding domain of the fimbrial adhesin of *C. albicans* is also the structural subunit of the long filamentous fimbriae (Chapter IIIA). The ability of these two adhesins to bind to the same receptors led us to investigate the basis of this adhesion phenomenon.

The interactions between *P. aeruginosa* pili and *C. albicans* fimbriae with asialo-GM_1 could be mediated by different epitopes on the carbohydrate moiety of asialo-GM_1. The data from the competitive binding assay indicated that the pili and the fimbriae compete with each other for binding to immobilized asialo-GM_1 (Figure IIID-1). This suggested that the same receptor epitope was recognized by the two adhesins. This observation is in agreement with previous studies which demonstrated that the disaccharide sequence βGalNAc(1-4)βGal was sufficient for both pili and fimbriae binding (Sheth *et al.*, 1994; Chapter IIIB). Previous studies have also shown that asialo-GM_1 blocked the fimbrial adhesin binding to glycosphingolipid receptors and to human BECs (Lee *et al.*, 1994; Chapter IIIB), although we have been unable to demonstrate asialo-GM_1-mediated inhibition of pili binding to BECs. Asialo-GM_1-like receptors present on human buccal

epithelial cells appear to be utilized by the *P. aeruginosa* pilus and *C. albicans* fimbria adhesins as demonstrated by the inhibition of biotinylated adhesin binding in the presence of unbiotinylated homologous and heterologous adhesins (Figure IIID-4). The pilus and fimbrial adhesins have similar avidity for asialo-GM_1 (Figure IIID-1). However, the avidity for *in situ* receptors on BECs was higher for fimbriae than for PAK pili (Figure IIID-4). The identity of the *in situ* receptors on epithelial cells that are recognized by these adhesins is not known. Preliminary studies have indicated that the receptors for the *P. aeruginosa* pilus adhesin may be glycoproteins (Doig *et al.*, 1990). It is possible that a carbohydrate sequence similar to asialo-GM_1 (possibly βGalNAc(1-4)βGal) may be present on various glycoproteins. The glycosphingolipids extracted from BECs show that these cells contain asialo-GM_1 and asialo-GM_2 (Figure IIIB-2A). The data further support the hypothesis that the binding of *C. albicans* fimbriae and PAK pili to BECs is mediated by asialo-GM_1 like receptors on BECs.

The receptor-binding domain on the pilus adhesin of *P. aeruginosa* strain PAK resides in the disulfide-loop carboxy-terminal region (Irvin *et al.*, 1989: Lee *et al.*, 1989: Lee *et al.*, 1994). MAb PK99H, which binds to the receptor-binding region of the *P. aeruginosa* pilus adhesin, has been shown to be cross-reactive with the fimbrial subunit of *C. albicans* fimbriae (Chapter IIIC). Conversely, an anti-*C. albicans* fimbriae MAb Fm16 was also cross-reactive with *P. aeruginosa* PAK pilin and binds to the carboxy-terminus of the pilus adhesin (Chapter IIIC). These observations suggested that the conserved epitope recognized by PK99H and Fm16 represents the asialo-GM_1-like receptor-binding domain. When these two antibodies were added to the binding assays, Bt-fimbriae binding to asialo-GM_1 and to BECs was greatly reduced (Figures IIID-2 and IIID-3). PK99H has been shown previously to block PAK pili binding to asialo-GM_1 (Lee *et al.*, 1994). These anti-adhesin antibodies also inhibited homologous and heterologous whole cell binding to BECs (Figure IIID-5). It is possible that the epitope recognized by these antibodies constitutes

part of the receptor-binding site on the fimbrial adhesin. Alternatively, the epitope is spatially oriented close to the receptor-binding site such that the bound antibody would sterically hinder the interaction between adhesin and receptor. MAb PAK-13 binds to the receptor-binding domain of the PAK pilus adhesin and blocked Bt-fimbriae binding to asialo-GM_1. Synthetic peptides with sequence corresponding to the disulfide-loop region of PAK pilin were shown to inhibit Bt-fimbriae binding asialo-GM_1 and to BECs in competitive binding assays (Chapter IIIE). These observations confirmed our hypothesis that the conserved epitope on the pilus and fimbrial adhesins contains the receptor-binding domain. This conserved structural motif has been termed the 'adhesintope'. The protein sequence of the fimbrial subunit has yet to be determined. Preliminary sequence of the N-terminal region of the fimbrial subunit has recently been obtained and isolation and characterization of the fimbrial gene is in progress.

Krivan *et al.* (1988) have demonstrated that a number of pulmonary pathogens are able to utilize the βGalNAc(1-4)βGal sequence as the receptor to which the micro-organisms can attach to host tissues. The binding of *P. aeruginosa* pilus and *C. albicans* fimbrial adhesins to this disaccharide sequence has been demonstrated (Sheth *et al.*, 1994; Chapter IIIB). The evidence obtained from our present studies with *P. aeruginosa* and *C. albicans* show that a conserved adhesintope that is present on microbial adhesins may account for the recognition of the βGalNAc(1-4)βGal receptors. We have immunological data that demonstrate that the adhesintope is also present on *Bordetella pertussis* (unpublished data). Hence, a conserved adhesintope may be present on adhesins of evolutionarily distinct and distant microorganisms that enable them to interact, with different degrees of avidity, with the same or similar receptors on host cells. The data in Figure IIID-4 showed that *C. albicans* fimbriae have a higher avidity for BEC receptors than *P. aeruginosa* pili. If the adhesintope is indeed present on all microorganisms that utilize asialo-GM_1-like receptors, anti-adhesive antibodies could be developed that could be

efficacious against a wide spectrum of pathogens. The data shown in Figure IIID-5 where PK99H and Fm16 inhibited homologous and heterologous whole cell binding to BECs suggested that these antibodies could be effective agents to block the first stage of the infection. Furthermore, administration of PK99H to mice protected the animals against challenges with lethal doses of live *P. aeruginosa* (Sheth *et al.*, 1995).

Host defense systems are important in control of *Candida* infections. It is generally accepted that classical T-cell mediated immunity is of dominant importance in limiting *C. albicans* infection. However, it is noted that animal models with defective T-cell function (athymic mice, SCID mice and $CD4^+$-depleted mice) do not develop hematogenously disseminated candidiasis (Helstrom and Balish, 1979; Balish *et al.*, 1990; Cantorna and Balish, 1991; Narayanan *et al.*, 1991). Similarly, patients with defects in CMI rarely develop hematogenously disseminated candidiasis (Greenfield, 1992). Although the role of B-cells and antibody has been studied, understanding of its relative importance has not ensued. Virtually all individuals have antibodies to surface mannan or mannoprotein adhesins, most likely a consequence of mucocutaneous colonization (Greenfield *et al.*, 1988). Passive transfer of immune serum has been shown to confer resistance to intravenous challenging by *C. albicans* in mice and baboons (Mourad and Friedman, 1968; Pecyk *et al.*, 1989; Al-Doory, 1970).

Inhibition of *C. albicans* attachment to host cells is a potential mechanism for protection of host from *C. albicans* infection and disseminated diseases (Vudhichamnong *et al.*, 1982; Epstein *et al.*, 1982; Scheld *et al.*, 1983; Cassone *et al.*, 1995; Han and Cutler, 1995). There is a growing body of evidence suggesting that anti-*Candida* adhesin antibodies could protect mice from colonization (Han and Cutler, 1995) and this protection can be transferred to naive mice to resist *Candida* infection (Han and Cutler, 1995; Cassone *et al.*, 1995).

An ultimate goal in the study of microbial adherence mechanisms is the development of measures to prevent adhesion of pathogenic organisms to mucosal surfaces and thus limiting the *Candida* infections. The previous studies indicate that the *C. albicans* adherence to human buccal epithelial cells is primarily mediated by the fimbrial adhesins on *C. albicans* and the asialo-GM_1 like receptors on BECs (Chapter IIIB). The interaction is specific and it can be blocked by both adhesin analoges and receptor analoges (Chapter IIIB, IIIC). MAb Fm16 is a mouse anti-*C. albicans* fimbrial antibody. MAb Fm16 were produced with a hybridoma technique described previously (Doig *et al.*, 1990). The effect of mouse anti-*C. albicans* fimbrial adhesin monoclonal antibody, Fm16, on inhibiting *C. albicans* adherence to human BECs has been demonstrated in these studies. The evidence revealed that : (1) anti-*C. albicans* fimbrial antibodies (Fm16) could specifically agglutinate whole yeast cells (Table IIIA-1); (2) MAb Fm 16 was able to bind to the purified *C. albicans* fimbriae with high affinity (the titer of dilution endpoint is 10^6) in direct binding assays (data not shown) and immunoblots (Figure IIID-5); (3) MAb Fm16 could block *C. albicans* fimbriae/*C. albicans* whole cells binding to their receptors (asialo-GM_1 like receptors) on BECs (Figure IIID-2, IIID-5). Using synthetic peptides, PAK(128-144) spanning the disulfide-bonded C-terminal region of the *Pseudomonas* PAK pilin and PAK(134-140) corresponding to the epitope of PK99H, the binding epitopes of MAb Fm16 have been revealed. Both peptides, PAK(128-144) and PAK(134-140) could block MAb Fm16 binding to immobilized *C. albicans* fimbriae by 80% and 78% respectively (Figure IIIC-3). These data indicate that MAb Fm16 can specifically and significantly block *C. albicans* adherence to BECs *in vitro* and that it seems to be a promising antibody for limiting *C. albicans* colonization on BECs and subsequently preventing *C. albicans* infections. The detailed role of MAb Fm16 in protection of host against *C. albicans* infections will be further studied *in vivo* animal models in the future.

Figure IIID-1

Inhibition of Bt-adhesins binding to asialo-GM_1. The binding of Bt-PAK pili (A) and Bt-fimbriae (B) to immobilized asialo-GM_1 was assayed in the presence of unbiotinylated *P. aeruginosa* PAK pili (●) and *C. albicans* fimbriae (■). The ability of unbiotinylated adhesins to block biotinylated adhesin binding to asialo-GM_1 is represented as the percent inhibition of binding in the presence of competitor with respect to the binding of the biotinylated adhesin in the absence of competitor.

Figure IIID-1

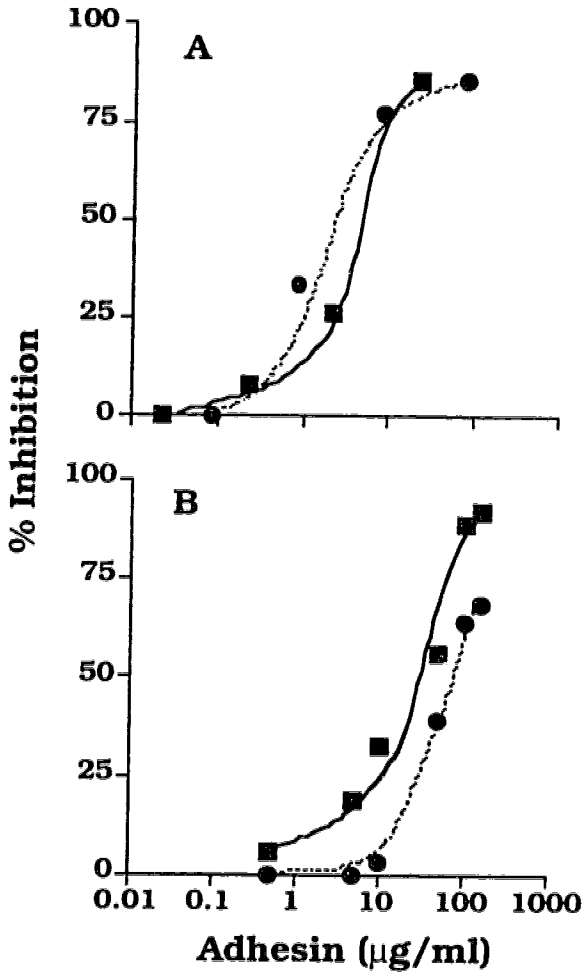


Figure IIID-2

Effect of anti-adhesin monoclonal antibodies on Bt-fimbriae binding to asialo-GM_1. The abilities of anti-adhesin antibodies to block Bt-fimbrial binding to asialo-GM_1 were determined by using Fm16 (■), PK99H (●), PAK-13 (◆), and normal mouse IgG (▲) as a control.

Figure IIID-2

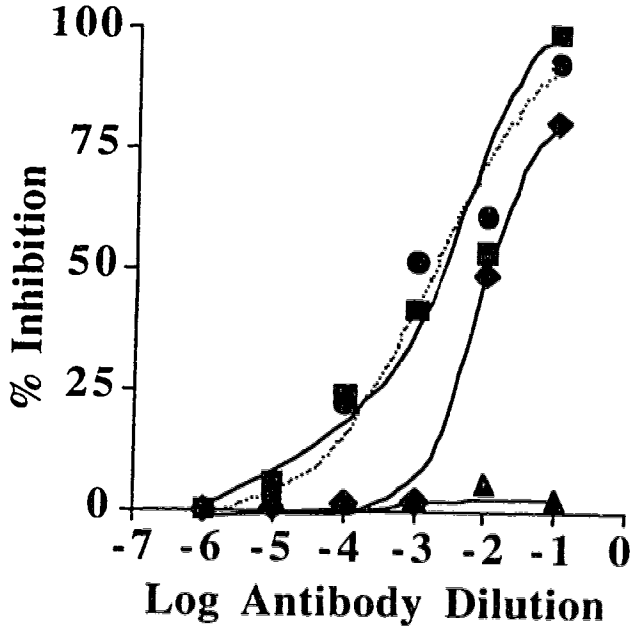


Figure IIID-3

Effect of anti-adhesin monoclonal antibodies on Bt-fimbriae binding to BECs. The abilities of anti-adhesin antibodies to block Bt-fimbrial binding to immobilized BECs were determined by using Fm16 (■), PK99H (●), PAK-13 (▲), and normal mouse IgG (◆) as a control.

Figure IIID-3

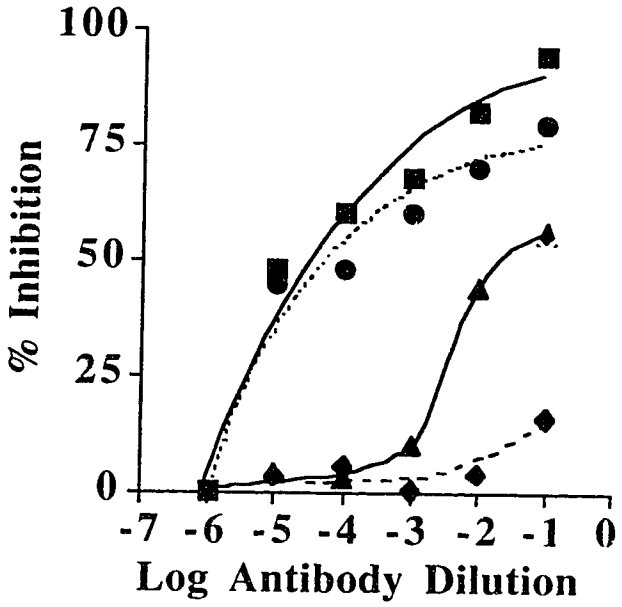


Figure IIID-4

Inhibition of Bt-adhesins binding to BECs. The binding of Bt-PAK pili (A) and Bt-fimbriae (B) to immobilized BECs was assayed in the presence of unbiotinylated *P. aeruginosa* PAK pili (●) and *C. albicans* fimbriae(■). The ability of unbiotinylated adhesins to block biotinylated adhesin binding to BECs is represented as the percent inhibition of binding in the presence of competitor with respect to the binding of the biotinylated adhesin in the absence of competitor.

Figure IIID-4

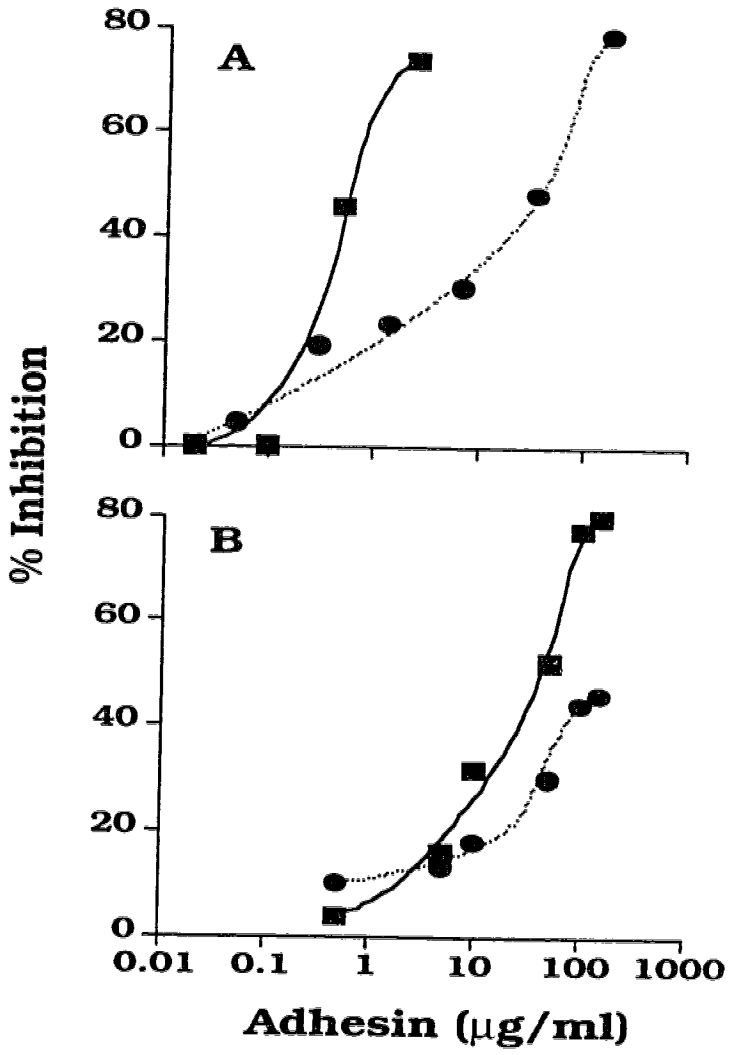
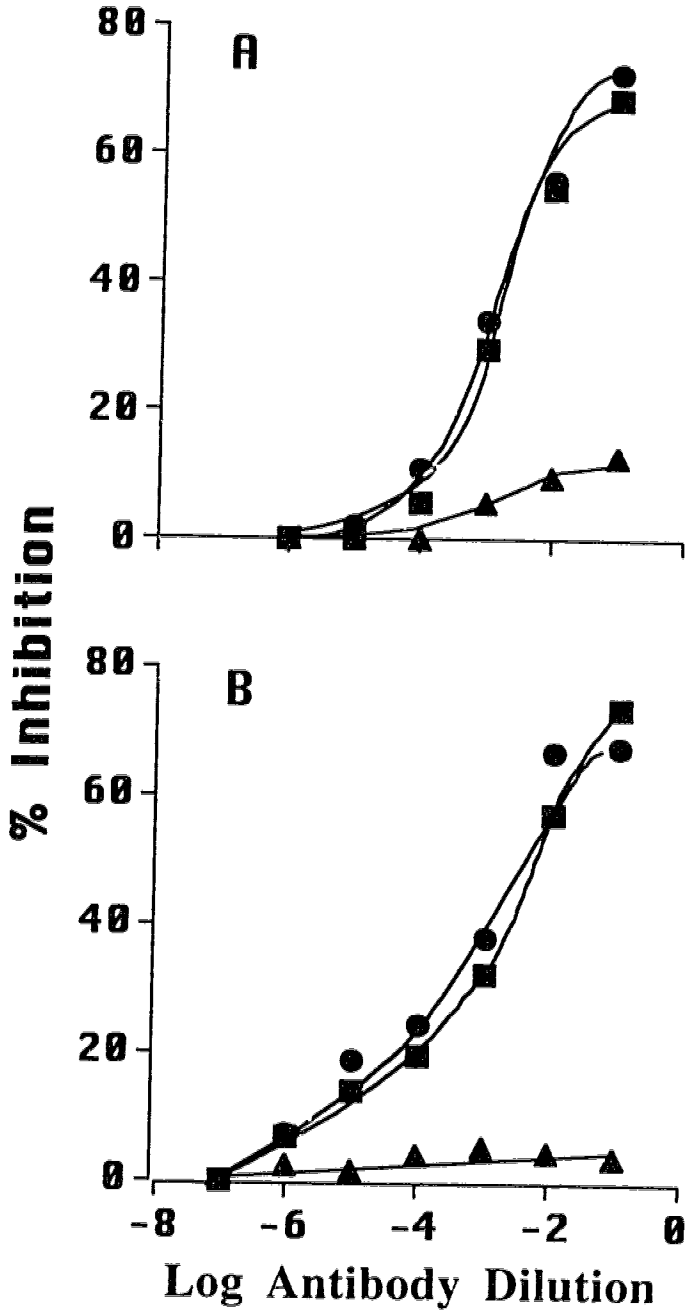


Figure IIID-5

Inhibition of biotinylated *P. aeruginosa* and *C. albicans* whole cell binding to BECs. (A) The binding of Bt-*P. aeruginosa* whole cell to BECs were competed with PK99H (●) and Fm16 (■). Normal mouse IgG (▲) was employed as a control. (B) The binding of Bt-*C. albicans* whole cell to BECs were competed with Fm16 (■) and PK99H (●). Normal mouse IgG (▲) was employed as a control.

Figure IIID-5



E. Use of Synthetic Peptides to Confirm that the *Pseudomonas aeruginosa* PAK Pilus Adhesin and the *Candida albicans* Fimbrial Adhesin Possess a Homologous Receptor-Binding Domain[5]

Results

E-1 Effects of anti-peptide antibodies on Bt-PAK pili and Bt-fimbriae adherence to asialo-GM_1

Anti-PAK(128-144) and anti-PAK(134-140) are rabbit polyclonal monospecific anti-peptide antibodies prepared against the peptides corresponding to the carboxy-terminal region of PAK pilin . The abilities of anti-PAK(128-144) and anti-PAK(134-140) rabbit polyclonal anti-peptide antibodies to recognize the heterologous *C. albicans* fimbriae were demonstrated recently (Chapter IIIC). The effect of these antibodies on the binding of Bt-PAK pili and Bt-*C. albicans* fimbriae to immobilized asialo-GM_1 was demonstrated by inhibition assays. As expected, the binding of PAK pili to immobilized asialo-GM_1 was inhibited by anti-PAK(128-144) and anti-PAK(134-140) peptide antibodies by as much as 82% and 72%, respectively at a dilution of 1/10 (Figure IIIE-1A). The binding of Bt-*C. albicans* fimbriae to immobilized asialo-GM_1 was also greatly reduced by a 1/10 dilution of anti-PAK(128-144) (73.5%) and by anti-PAK(134-140) (64%) (Figure IIIE-1B). Anti-PAK(75-84) peptide antibodies raised against the central region of PAK pilin and normal rabbit whole IgG had no significant effects on the binding of Bt-fimbriae or Bt-PAK pili to asialo-GM_1. In both instances, anti-PAK(128-144) antibodies have slightly higher inhibitory effects on the binding of pilus and fimbrial adhesins to asialo-GM_1 than anti-PAK(134-140) antibodies (Figure IIIE-1).

---

[5] A version of this section of chapter III has been published: Yu, L., Lee, K.K., Paranchych, W., Hodges, R.S., and Irvin, R.T. (1996). Mol. Microbiol. **19**:1107-1116.

E-2 Effect of anti-peptide antibodies on Bt-PAK pilus and Bt-fimbrial adherence to BECs

Anti-PAK(128-144) and anti-PAK(134-140) antibodies were assayed for their ability to inhibit PAK pilus and fimbrial adhesin binding to the receptors present on the surfaces of BECs that were immobilized on the surface of microtiter wells. As shown in Figure IIIE-2A, anti-PAK(128-144) and anti-PAK(134-140) peptide antibodies inhibited Bt-PAK pili binding to BECs by 72% and 65% of control levels, respectively at a dilution of 1/10. Under the same conditions, the binding of Bt-fimbriae to BECs was reduced by 68% and 62% of control levels in the presence of anti-PAK(128-144) and anti-PAK(134-140) peptide antibodies, respectively (Figure IIIE-2B). However, anti-PAK(75-84) peptide antibodies and normal rabbit whole IgG have no effects on Bt-PAK pilus or Bt-fimbrial binding to BECs (Figure IIIE-2). As with the results where immobilized asialo-GM_1 was employed as the receptor, the anti-PAK(128-144) antiserum showed a consistently greater inhibitory effect on Bt-PAK pili and Bt-fimbriae adherence to BECs than anti-PAK(134-140). These data supported our hypothesis that a homologue of the PAK(128-144) peptide sequence of *P. aeruginosa* PAK pilin plays a critical role in the *C. albicans* fimbria binding to asialo-GM_1-like receptors on BEC.

E-3 Effect of anti-peptide antibodies on *P. aeruginosa* and *C. albicans* whole cell binding to BECs

Anti-*P. aeruginosa* PAK pilus MAbs PK99H and PAK13 and anti-*C. albicans* fimbria MAb Fm16 have been shown to block *P. aeruginosa* and *C. albicans* whole cell binding to human BECs (unpublished data). Similar studies were conducted to determine if the anti-peptide antibodies anti-PAK (128-144) and anti-PAK (134-140) peptide antibodies were able to prevent the attachment of *P. aeruginosa* and *C. albicans* to BECs. In competitive binding assays, it was shown that the adherence of *P. aeruginosa* and *C. albicans* to human BECs was reduced to 55.8% and 50.3% of control levels, respectively

in the presence of 1/10 dilutions of anti-PAK (128-144) peptide antibodies (Figure IIIE-3A). Anti-PAK(134-140) antibodies also blocked *P. aeruginosa* and *C. albicans* whole cell binding to BECs by 48% and 43%, respectively (Figure IIIE-3B). Anti-PAK(75-84) anti-peptide antibodies and normal rabbit whole IgG did not significantly inhibit *P. aeruginosa* or *C. albicans* whole cell binding to BECs (Figure IIIE-3).

E-4 Synthetic peptides inhibit Bt-PAK pili and Bt-fimbriae binding to asialo-GM_1

In order to demonstrate that the receptor-binding domain consists of a sequence similar to PAK(128-144), synthetic peptides with this sequence were employed in competitive binding assays. Ac-PAK(128-144)$_{ox}$-OH can specifically bind to asialo-GM_1 in direct binding assays (Lee *et al.*, 1994; Sheth *et al.*, 1994). When synthetic peptides were incubated with Bt-adhesins in competitive binding assays, it was found that Ac-PAK(128-144)$_{ox}$-OH and Ac-PAK(134-140)-NH_2 inhibited Bt-PAK pili binding to immobilized asialo-GM_1 by about 81% and 70% respectively (Figure IIIE-4A). The IC_{50} for Ac-PAK(128-144)$_{ox}$-OH and Ac-PAK(134-140)-NH_2 peptides are approximately 20 μM and 35 μM, respectively. Furthermore, Ac-PAK(128-144)$_{ox}$-OH and Ac-PAK(134-140)-NH_2 peptides also inhibited Bt-fimbrial adhesin binding to asialo-GM_1 by 69.5% and 58.3% and the IC_{50}s were 28 μM and 40 μM, respectively (Figure IIIE-4B). Control peptides, Ac-PAK(75-84)-NH_2 and Ac-TnI(96-139)-NH_2 from rabbit muscle have no effects on the *P. aeruginosa* PAK pilus or *C. albicans* fimbrial adhesin binding to asialo-GM_1 (Figure IIIE-4).

E-5 Synthetic peptides inhibit Bt-PAK pili and Bt-fimbriae binding to human BECs

Synthetic peptides were assayed for their abilities to inhibit the adherence of Bt-PAK pilus and Bt-fimbrial adhesin binding to BECs. As shown in Figure IIIE-5, the binding of

Bt-PAK pili to BECs was reduced by 65% and 58%, respectively, in the presence of Ac-PAK(128-144)$_{ox}$-OH and Ac-PAK(134-140)-NH_2. Under the same conditions, Ac-PAK(128-144)ox-OH and Ac-PAK(134-140)-NH_2 peptides, but not Ac-PAK(75-84)-NH_2 and Ac-TnI(96-139)-NH_2 from rabbit muscle, significantly inhibited the binding of Bt-fimbriae to human BECs by 59% and 48%, respectively.

E-6 Synthetic peptides inhibit Bt-*P. aeruginosa* and Bt-*C. albicans* whole cell binding to BECs

Ac-PAK(128-144)$_{ox}$-OH inhibited the binding of whole cells of *P. aeruginosa* and *C. albicans* to immobilized BECs by 54% and 50%, respectively (Figure IIIE-6). The shorter sequence PAK(134-140) constitute a major portion of the pilus/fimbrial adhesintope as the addition of Ac-PAK(134-140)-NH_2 also resulted in inhibition of binding of whole cell of *P. aeruginosa* and *C. albicans* to immobilized BECs by 49% and 41% respectively (Figure IIIE-6). Ac-PAK(75-84)-NH_2 and Ac-TnI(96-139)-NH_2 from rabbit muscle did not inhibit whole cell binding of either *P. aeruginosa* or *C. albicans* to immobilized BECs.

E-7 Effects of asialo-GM_1 and βGalNAc(1-4)βGal on *C. albicans* whole cell binding to BECs

The abilities of glycosphingolipid (asialo-GM_1) and disaccharide (βGalNAc(1-4)βGal) to inhibit *C.albicans* fimbriae binding to BECs have been described previously (Chapter IIIB). To address whether the binding of whole microorganisms to BECs are mediated mainly by asialo-GM_1 and βGalNAc(1-4)βGal, asialo-GM_1 and βGalNAc(1-4)βGal were used as competitors in the whole cell binding assays. The binding of *C. albicans* to BECs was reduced by 72% with asialo-GM_1 (Figure IIIE-7A) and 65% with βGalNAc(1-4)βGal (Figure IIIE-7B), respectively. The data are quite consistent with the

previous observations that asialo-GM_1 and βGalNAc(1-4)βGal inhibited *C. albicans* fimbriae binding to BECs by 80% and 70% respectively. On the other hand, control glycosphingolipid [ceramide trihexoside (CTH)] and disaccharide (sucrose) showed no effects on *C. albicans* binding to BECs (Figure IIIE-7).

Discussion

Adherence is a critical initial event of pathogenesis (Beachey *et al.*, 1981). Opportunistic pathogens such as *Pseudomonas aeruginosa*, a Gram-negative bacterium, and *Candida albicans*, a dimorphic yeast, employ several distinct adhesins to mediate attachment to host epithelial cells (Prince, 1992; Irvin, 1993; Cutler, 1991; Hostetter, 1994). *P. aeruginosa* and *C. albicans* can utilize long flexible filaments termed pili and fimbriae, respectively, to mediate their adherence to buccal epithelial cell surfaces (Paranchych and Frost, 1979; Chapter IIIB). Pili and fimbriae mediating the attachment of pathogens to the surfaces of the host have been described previously (Irvin, 1990). Although synonymous, the terms pilus and fimbria are used here to differentiate between the two microorganisms. The *P. aeruginosa* PAK pilus subunit, pilin, consists of 144 amino acid residues with an intra-chain disulfide loop at its carboxy-terminus (Sastry *et al.*, 1985) that mediates pilus binding to asialo-GM_1 and BEC receptors (Doig *et al.*, 1990; Irvin *et al.*, 1989; Lee *et al.*, 1989; Paranchych *et al.*, 1986). Although the adhesin is located on the structural subunit of the PAK pilus, it has been shown that the carboxy-terminal region of the PAK pilin is only exposed at the tip of the pilus where receptor binding occurs (Irvin, 1993; Lee *et al.*, 1994). The *C. albicans* fimbrial adhesin was first described by Irvin and co-workers (Chapter IIIC). The major fimbrial subunit of the *C. albicans* fimbria is a 66 kDa protein that is highly glycosylated (85% of the molecular mass consists of carbohydrate, primarily D-mannose residues). The protein sequence of this 66 kDa *C. albicans* fimbrial protein is not yet available.

Recent evidence has shown that : (i) *P. aeruginosa* PAK pilus and *C. albicans* fimbrial adhesins share a similar asialo-GM_1-like receptor on BECs (Chapter IIIA, IIIB; Lee *et al.*, 1994); (ii) the adherence of Bt-PAK pilus adhesin to asialo-GM_1 and human BECs could be competitively inhibited by *C.albicans* fimbriae and *vice versa* (Chapter IIID); and (iii) MAb PK99H which recognize residues 134-140 at the carboxy-terminal

region of PAK pilin (Wong *et al.*, 1992) bound to the 66 kDa *C. albicans* fimbrial subunit (Chapter IIIC). Conversely, anti-*C. albicans* fimbria MAb Fm16 was also cross-reactive and bound to the carboxy-terminal region of the PAK pilin adhesin (Chapter IIIC). These data suggested that the *C. albicans* fimbrial adhesin contains an adhesintope which is structurally similar to the receptor-binding domain on the carboxy-terminal region of the *P. aeruginosa* PAK pilin adhesin.

The presence of a conserved adhesintope was indicated by data that showed that antibodies directed against synthetic peptides corresponding to the carboxy-terminal region of PAK pilin [anti-PAK(128-144) and anti-PAK(134-140)] blocked *P. aeruginosa* PAK pili and *C. albicans* fimbriae adherence to asialo-GM_1 and to human buccal epithelial cells (Figures IIIE-1 and IIIE-2). These results suggested that the interactions between anti-PAK(128-144) and anti-PAK(134-140) antibodies with both PAK pili and fimbriae occurred at or close to the receptor-binding regions on both the pilus and fimbrial adhesins. The interactions occurring between pilus/fimbria and BEC receptors are probably similar to those between the microorganisms and BEC receptors as anti-PAK(128-144) and anti-PAK(134-140) also blocked the *P. aeruginosa* and *C. albicans* whole cells binding to human BECs (Figure IIIE-3). Anti-PAK(75-84) antibodies (control anti-peptide Abs) and normal rabbit whole IgG have no significant effects on either biotinylated adhesin or biotinylated whole cell binding to asialo-GM_1 or BEC receptors.

Anti-peptide antibodies that blocked adhesin binding to asialo-GM_1-like receptors may occur at the receptor-binding domain (adhesintope) or at a site that is sterically close to the adhesintope. To demonstrate that the peptide sequence is responsible for receptor-binding and the inhibitory binding assays is not due to steric interference of the bulky antibodies, synthetic peptides were used in competitive binding assays. The data in Figures IIIE-4, IIIE-5 and IIIE-6 showed that the Ac-PAK(128-144)$_{ox}$-OH and Ac-PAK(134-140)-NH_2 peptides were able to inhibit both the biotinylated adhesins and

biotinylated whole cell binding to asialo-GM_1 and to BECs. This suggested that the Ac-PAK(128-144)ox-OH peptide mimicked the adhesintope of the pilus and fimbrial adhesins. The 7-residue PAK(134-140) peptide contains residues that are important to the structural motif of the adhesintope since this peptide has inhibitory effects that were only slightly lower than the longer PAK(128-144) peptide (Figures IIIE-4, IIIE-5 and IIIE-6). Biotinylated PAK(128-144) and biotinylated PAK(134-140) are also able to bind to asialo-GM_1 (Lee *et al.*, 1994) and BEC receptors (unpublished data). Hence, the peptide sequences are sufficient for binding to asialo-GM_1-like receptors demonstrating that these peptides form a structural motif that constitutes all or part of the receptor-binding epitope of the *P. aeruginosa* PAK pilus adhesins and the *C. albicans* fimbrial adhesins. The synthetic peptides, derived from the PAK pilin sequence, have greater effects on the *P. aeruginosa* PAK pilus adhesin interactions with asialo-GM_1-like receptors than on the *C. albicans* fimbria suggesting that the *C. albicans* likely possesses a homologue of the *P. aeruginosa* sequence. The data from these studies confirmed our hypothesis that an adhesintope similar to that on the *P. aeruginosa* PAK pilus is also present on the *C. albicans* fimbria. It is surprising that organisms as evolutionarily divergent as *P. aeruginosa* and *C. albicans* possess structurally and functionally conserved filamentous adhesins. However, De Mot and Vanderleyden (1994a,b) have observed a conserved surface-exposed domain in major outer membrane proteins of pathogenic *Pseudomonas* and *Branhamella* species. Patel *at el.* (1991) have also demonstrated shared immunogenicity and antigenicity of Type IV pilins expressed by *Pseudomonas aeruginosa, Moraxella bovis, Nesseria gonorrhoeae, Dichelobacter nodosus,* and *Vibrio cholerae.*

It has been demonstrated that one of the mechanisms that *C. albicans* utilizes to adhere to human BECs is mediated by the interaction of *C. albicans* fimbrial adhesins with glycosphingolipid (asialo-GM_1) on the surface of BECs *in vitro*; the interaction within the asialo-GM_1-like receptors is presumably located in a disaccharide [βGalNAc(1-4)βGal]----

the carbohydrate portion of asialo-GM_1, since both asialo-GM_1 and synthetic βGalNAc(1-4)βGal can inhibit the binding of *C. albicans* fimbriae binding to asialo-GM_1 and BECs (Chapter IIIB, IIIC). Asialo-GM_1 and its minimal disaccharide sequence [βGalNAc(1-4)βGal] also can significantly block the *C. albicans* whole cells binding to BECs (Figure IIIE-7). The data are consistent with our previous observations and further confirmed that the fimbriae-βGalNAc(1-4)βGal interaction is the dominant component of the adherence of *C. albicans* to BECs.

Both anti-peptide antibodies and peptides exhibited greater inhibitory effects on the adherence of adhesins to one single receptor than to multiple receptors on BECs (Figures IIIE-1, IIIE-2, IIIE-4 and IIIE-5) and the 'pure receptors', asialo-GM_1 and βGalNAc(1-4)βGal, only inhibited the binding of *C. albicans* whole organisms to BECs by 72 and 75% (Figure IIIE-7) suggesting that other yet undefined factors may contribute to adhesin-BEC receptor interactions. In addition to the pilus/fimbrial mediated interactions with asialo-GM_1-like receptors, other adhesins are probably involved in whole cell binding to BECs as synthetic peptides and anti-peptide antibodies [against PAK(128-144) and PAK(134-140)] could only reduce whole cell binding by about 40 - 50 % of background levels (Figure IIIE-3 and IIIE-6). The use of multiple adhesins by microbial pathogens to mediate their binding to host cells has been reviewed elsewhere (Calderone and Braun, 1991; Hasty *et al.*, 1992; Law, 1994).

Figure IIIE-1

Effect of rabbit polyclonal anti-peptide antibodies on biotinylated adhesins binding to asialo-GM_1. The binding of Bt-PAK pili (A) and Bt-fimbriae (B) to asialo-GM_1 was determined in the presence of rabbit polyclonal anti-peptide antibodies directed against synthetic peptides which corresponded to the carboxy-terminal region of the PAK pilin, anti-PAK(128-144) peptide antibody (■) and anti-PAK(134-140) peptide antibody (●). Anti-PAK(75-84) peptide antibody (▲) and normal rabbit whole IgG (○) were used as controls. Anti-PAK(75-84) peptide antibody is a rabbit polyclonal antibody raised against a peptide sequence which corresponds to central part of the PAK pilin not involved in the pilus adhesin function (Lee *et al.*, 1989a). The error bars in the figure represent ± 1 S.D.

Figure IIIE-1

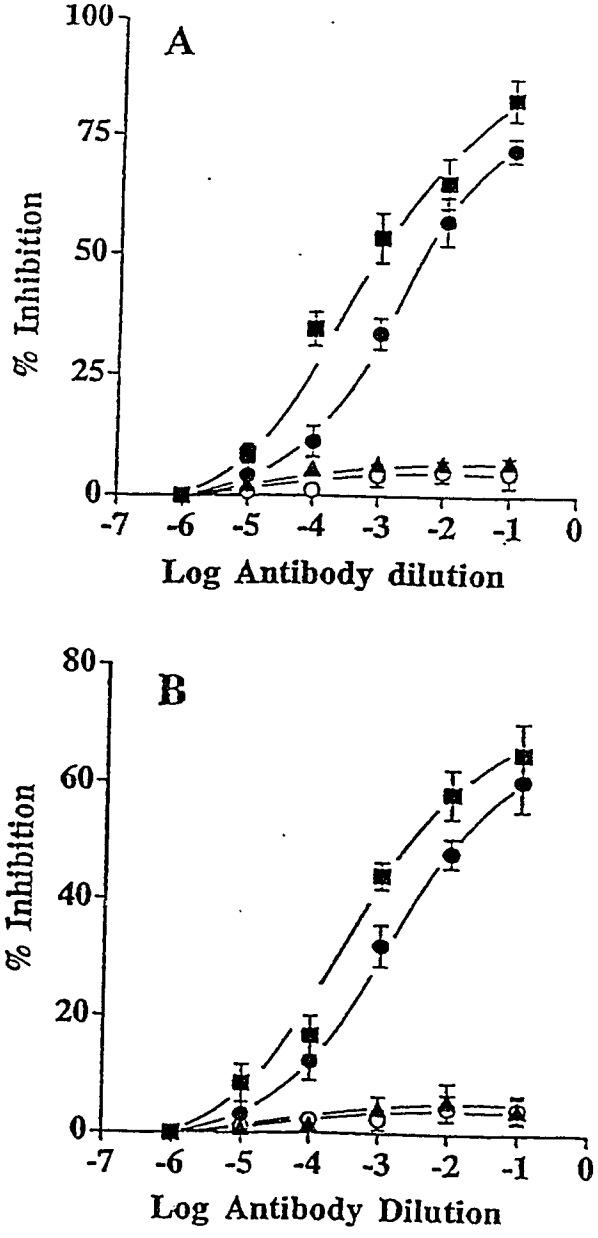


Figure IIIE-2

Effect of rabbit polyclonal anti-peptide antibodies on biotinylated adhesins binding to human BECs. The binding of Bt-PAK pili (A) and Bt-fimbriae (B) to BECs was determined in the presence of polyclonal anti-peptide antibodies directed against synthetic peptides which corresponded to the carboxy-terminal region of the PAK pilin, anti-PAK(128-144) peptide antibody (■), anti-PAK(134-140) peptide antibody (●). Anti-PAK(75-84) peptide antibody (▲) and normal rabbit whole IgG (○)were used as controls. The error bars in the figure represent ± 1 S.D.

Figure IIIE-2

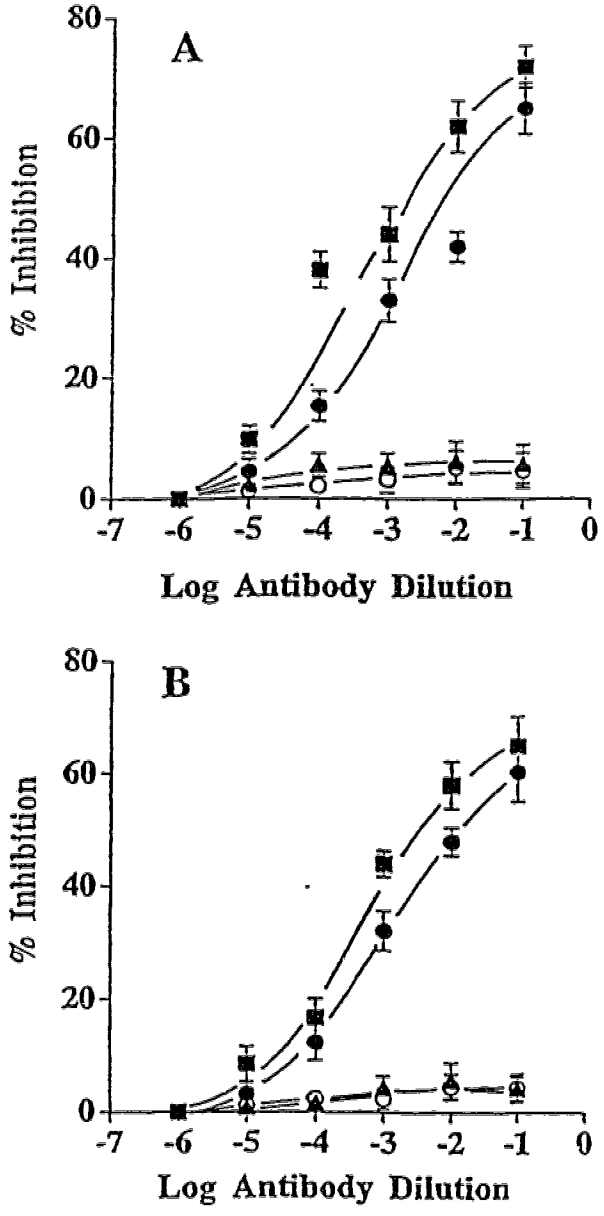


Figure IIIE-3

Effect of rabbit polyclonal anti-peptide antibodies on biotinylated whole cell binding to human BECs. The adherence of Bt-*P. aeruginosa* (A) and Bt-*Candida albicans* (B) to immobilized BECs was determined in the presence of anti-PAK(128-144) peptide antibody (■), anti-PAK(134-140) peptide antibody (●). Anti-PAK(75-84) peptide antibody (▲) and normal rabbit whole IgG (○) were used as controls. The error bars in the figure represent ± 1 S.D.

Figure IIIE-3

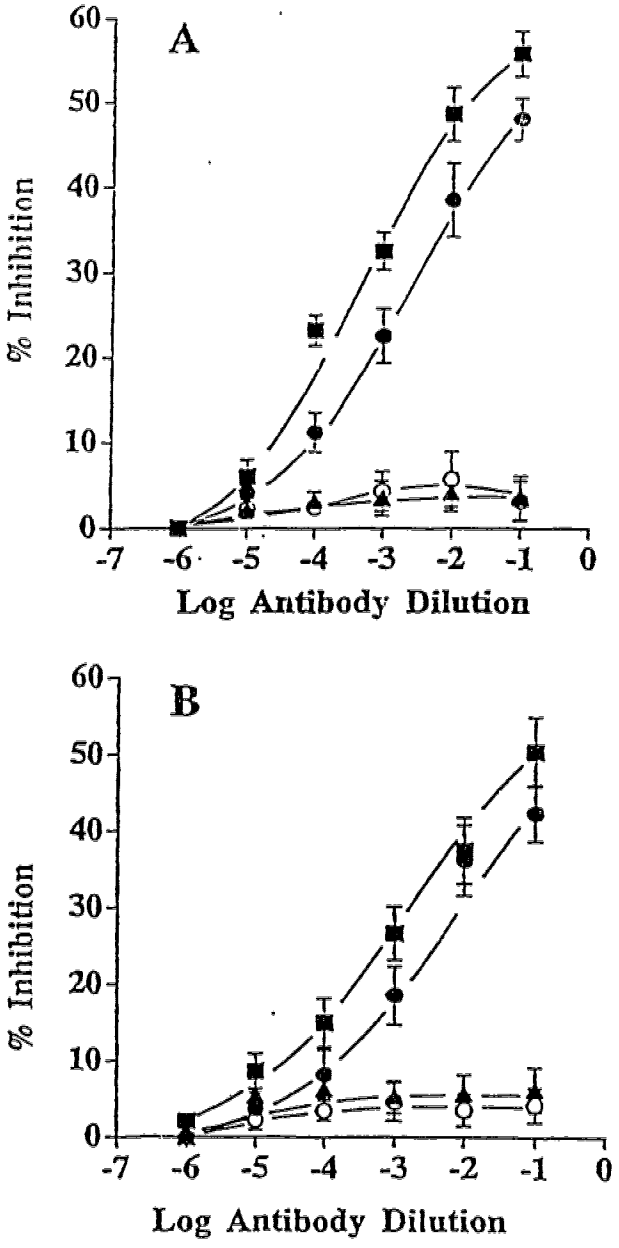


Figure IIIE-4

Inhibition of the binding of biotinylated adhesins to asialo-GM_1. The binding of Bt-PAK pili (A) and Bt-fimbriae (B) to immobilized asialo-GM_1 was determined in the presence of the synthetic peptides Ac-PAK(128-144)ox (■), Ac-PAK(134-140)-NH_2 (●), Ac-PAK(75-84)-NH_2 peptide (▲) and Ac-TnI(96-139)-NH_2 (○)were used as controls. The abilities of synthetic peptides to inhibit biotinylated adhesin binding to asialo-GM_1 are represented as the percent inhibition of the binding of Bt-adhesins to asialo-GM_1 with respect to the level of adhesin binding to asialo-GM_1 in the absence of peptide competitors. The error bars in the figure represent ± 1 S.D.

Figure IIIE-4

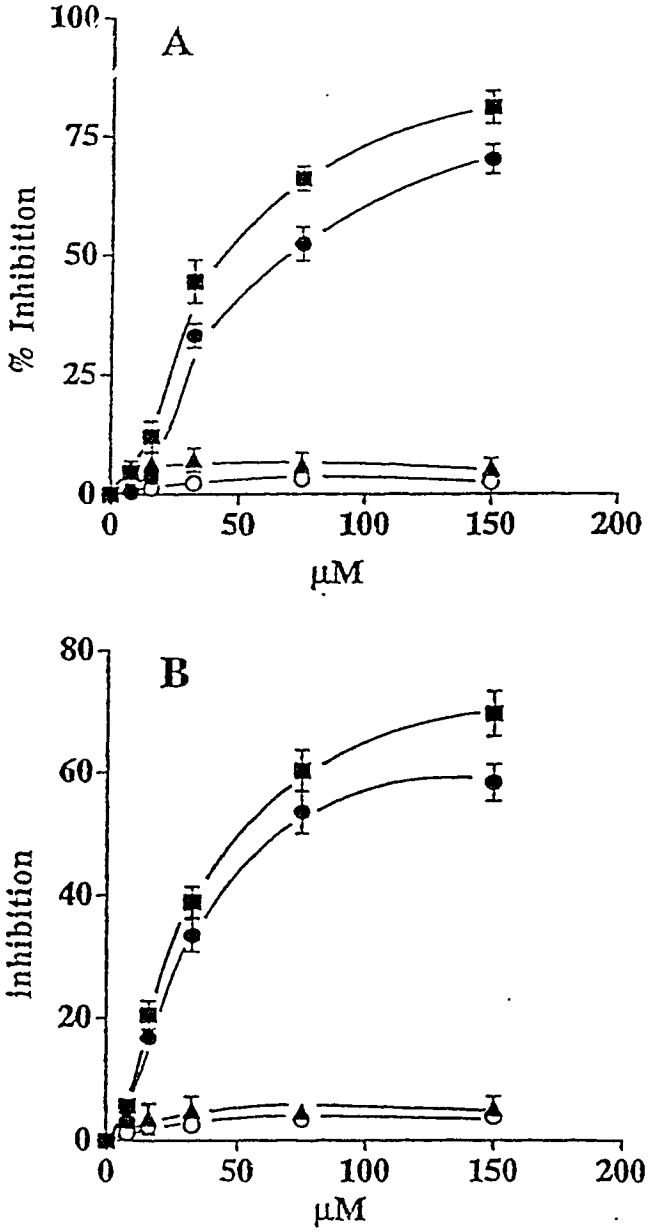


Figure IIIE-5

Inhibition of the binding of biotinylated adhesins to human BECs by C-terminal pilin peptides. The binding of Bt-PAK pili (A) and Bt-fimbriae (B) to immobilized asialo-GM_1 was determined in the presence of the synthetic peptides Ac-PAK(128-144)ox-OH (■), Ac-PAK(134-140)-NH_2 (●). Ac-PAK(75-84)-NH_2 peptide (▲) and Ac-TnI(96-139)-NH_2 (○) were used as controls. The abilities of synthetic peptides to inhibit biotinylated adhesin binding to asialo-GM_1 are presented as the percent inhibition of the binding of the Bt-adhesins to asialo-GM_1 with respect to the level of adhesin binding to asialo-GM_1 in the absence of peptide competitors. The error bars in the figure represent ± 1 S.D.

Figure IIIE-5

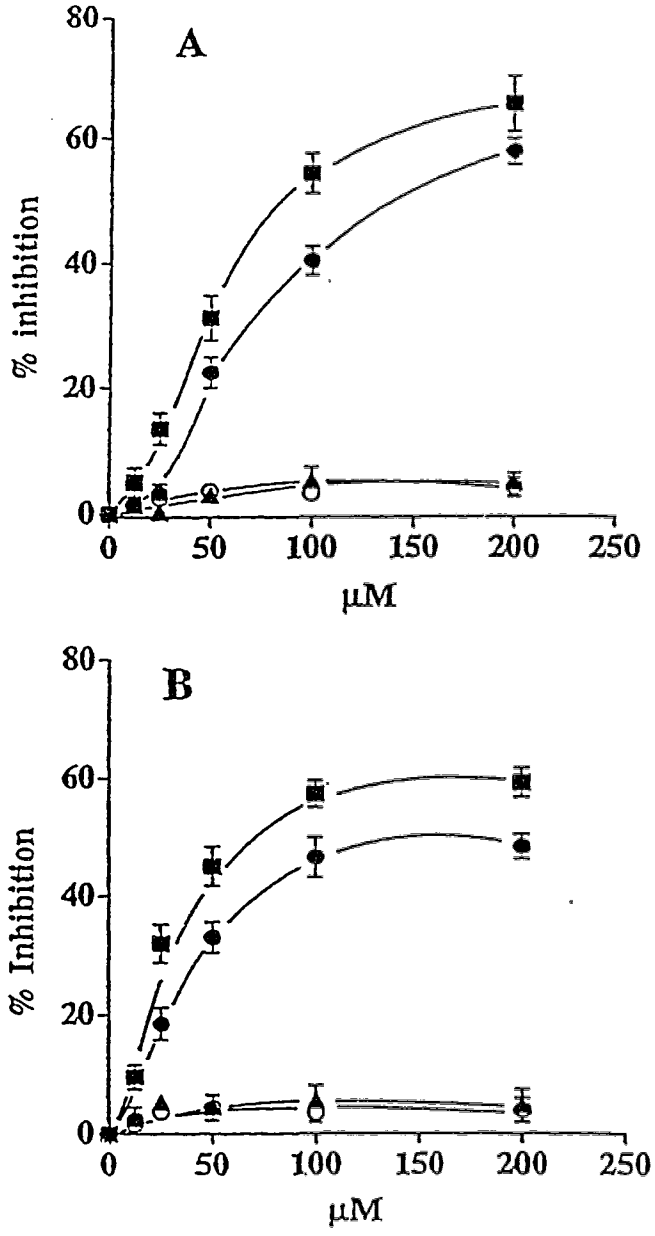


Figure IIIE-6

Inhibition of the binding of biotinylated whole cells to human BECs. The binding of Bt-*P. aeruginosa* (A) and Bt-*Candida albicans* (B) whole cells to immobilized BECs was determined in the presence of the synthetic peptides Ac-PAK(128-144)ox-OH (■), Ac-PAK(134-140)-NH_2 (●), Ac-PAK(75-84)-NH_2 peptide (▲), and Ac-TnI(96-139)-NH_2 (○) were used as controls. The synthetic peptides mediated inhibition of biotinylated *P. aeruginosa* whole cells or biotinylated *C. albicans* whole cells to human BECs is presented as the percent inhibition of whole cell binding to human BECs with respect to the level of whole cell binding to human BECs in the absence of any synthetic peptide. The error bars in the figure represent ± 1 S.D.

Figure IIIE-6

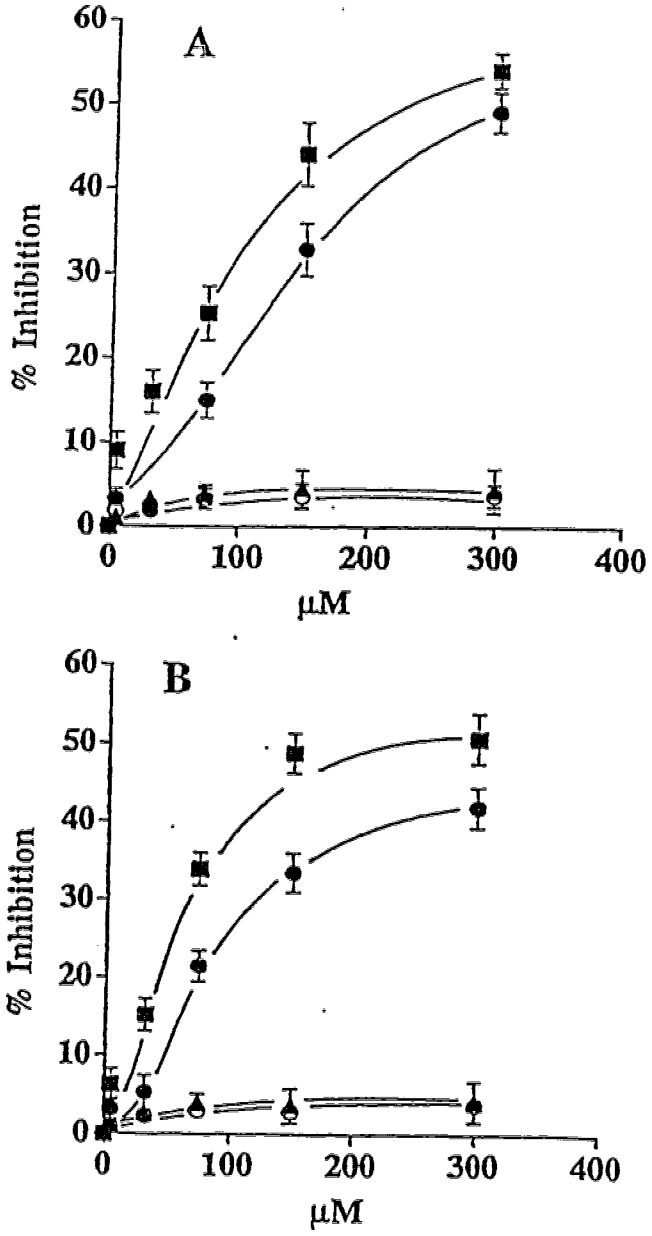
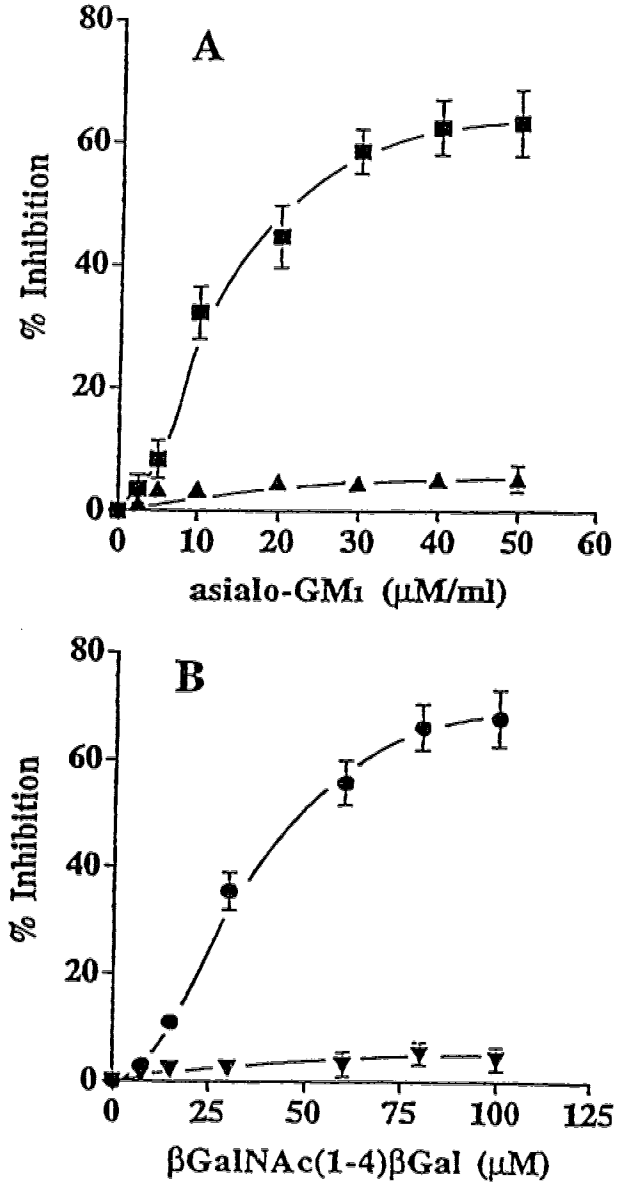


Figure IIIE-7

Inhibition of the binding of *C. albicans* to BECs by asialo-GM_1 (A) and βGalNAc(1-4)βGal (B). *C. albicans* were preincubated with asialo-GM_1 (■). CTH (▲), βGalNAc(1-4)βGal (●), and sucrose(▼). The percent inhibition is the inhibition of binding in the presence of the competitors with respect to the binding in the absence of any competitors. The error bars in the figure represent ± 1 S.D.

Figure IIIE-7



Chapter IV General Discussion

1. *Candida* infection

Candida is unique among opportunistic pathogens because it is a ubiquitous fungus commonly found in human normal flora of the mouth, oropharynx, intestines, vagina, and skin (Odds, 1988). Most *Candida* infections are caused by *C. albicans* (Douglas, 1987a and 1987b; Calderone and Braun, 1991; Cutler, 1991; Kennedy *et al.*, 1992). *Candida* infections emerged as a significant medical problem at the end of the twentieth century, whereas they had been a rarity at the beginning. *Candida* spp. have become common nosocomial pathogens (Bodey, 1993; Odds, 1994). There are several reasons for this dramatic change. Patients with chronic, debilitating illnesses and immunocompromised patients who formerly died of bacterial infections are now surviving due to the availability of more potent antibacterial agents. Unfortunately, these patients are susceptible to superinfections caused by *Candida* spp. Successes with organ transplantation and intensive cancer chemotherapeutic regimens have resulted in an increasing population of immunocompromised hosts (Velegraki, 1995). Indwelling intravascular devices have facilitated the management of many patients, but their use has been associated with candidemia and invasive infection (Witt and Bayer, 1991). *Candida* infections of the oropharynx, esophagus, and vagina are often the first manifestation of AIDS and recurrent *Candida* infections are common in this patient population (Venkateswarlu *et al.*, 1995). The widespread use of broad-spectrum antibiotics also has caused an increase in superficial infection, such as thrush and vaginitis, among normal hosts (Ghannoum, 1992).

2. Pathogenesis

For most infectious diseases, the seriousness of the illness and its eventual outcome are determined by the balance between pathogenicity of the organism and the strength of the host defenses. *C. albicans* are common inhabitants of the respiratory, gastrointestinal, and genitourinary tracts of normal humans (Odds, 1994). In normal hosts, most patients suffer no ill effects as a result of colonization with *C. albicans* because these organisms are usually of low pathogenicity. However, subtle changes in host defenses can alter the host-pathogen balance and allow these organisms to become pathogenic. Superficial candidiasis is generally associated with conditions that alter the hydration, pH, nutrient concentration, or microbial environment of the skin or mucous membranes (Wade, 1993; Hymes and Duvic, 1993; Kirkpatrick, 1993; Sobel, 1993). Primarily, superficial diseases may be seen in patients receiving corticosteroids or who are HIV infected (Odds, 1994). On the other hand, the disseminated form of candidiasis is usually associated with severe defects in the phagocytic cell defense system, or with the multiple deficiencies in host defenses that commonly occur in hospitalized cancer patients receiving antimicrobials and cancer chemotherapy (Karabinis *et al.*, 1988).

2-1 Stages of pathogenesis of epithelial infections by *C. albicans*

Mucosal surfaces are the initial site of infection for most pathogenic *C. albicans*, and the major battle between microbial pathogenic factors and host defenses occurs at this surface (Kennedy *et al.*, 1992). The pathogenesis of infections by *C. albicans* occurs in stages at which different host and fungal factors play a critical role (Odds, 1992). For initial colonization of an epithelial or epidermal site, the surface adhesion components of the fungus will determine whether it succeeds in establishing itself. The host defenses against colonization are other competing microbes and substances at the site capable of inhibiting *C. albicans* growth. Once the fungal cells have begun to embed themselves in epithelial or epidermal surface, they may begin to form hyphae, which, by means of contact sensing,

are guided to penetrate deeper into the epithelia (Soll, 1992). At this stage the host calls a variety of defenses into play, including epidermal and epithelial proliferation (Sobel *et al.*, 1983), direct phagocytosis of *Candida* by keratinocytes, and activation of an inflammatory response (Odds, 1994). Both cell-mediated and humoral-mediated factors may be involved as specific defenses against any or all stages of pathogenesis (Casadevall, 1995).

2-2 Virulence factors of *C. albicans*

Pathogenesis requires differential expression of virulence factors at each new stage of the process. *C. albicans* can express multiple particular virulence factors in achieving individual steps in the infectious process. So far, at least 7 virulence factors of *C. albicans* have been reviewed by Odds (1994) including rapid switching of expressed phenotype, hypha formation, contact sensing, surface hydrophobicity, surface virulence molecules (adhesins and receptors), molecular miniicry, lytic enzymes, growth and undemanding nutrient requirement. However, no single feature has been established on unequivocal scientific grounds as a component essential for pathogenesis (Klotz and Penn, 1987; Calderone and Braun, 1991; Cutler, 1991; Calderone, 1993; Odds, 1994; Hostetter, 1994; Pendrak and Klotz, 1995).

2-3 *Candida*-host interactions

In contrast with the wealth of data now available on bacterial adhesion, relatively little is known about the mechanisms by which yeasts attach to surfaces. Recently, however, there has been increasing interest in adhesion of *C. albicans*. Specific adhesion of certain microorganisms to particular surfaces involves interactions between complementary molecules on the microbial and attachment surfaces. Adhesion of bacteria to animal cells takes place via such a mechanism (Ofek and Beachey, 1980). Bacteria possess binding molecules or adhesins on their surfaces which are capable of interacting stereospecifically with receptors on host-cell membranes in a manner analogous to antigen-antibody interactions. Thus, an initial loose "docking" process is followed by permanent anchoring

which requires formation of many specific glove-and-hand bonds between these complementary molecules.

Colonization of mucosal surfaces by the pathogenic *Candida* species depends on their ability to adhere. Adhesion is the first step in the process leading to colonization and infection, and the ability to adhere constitutes an important determinant of virulence. Adhesive properties may be the least controversial of the putative virulence traits of *C. albicans* (Gardiner *et al.*, 1982; Rotrosen *et al.*, 1986; Kennedy *et al.*, 1987, 1989 and 1991; Calderone and Braun, 1991; Cutler, 1991; Douglas, 1991; Cutler and Kanbe, 1993; Odds, 1994; Pendrak and Klotz, 1995). The adherence of *C. albicans* to mucosal epithelial or endothelial cells represents an important initial step in the process of colonization and invasion (Fukayama and Calderone, 1991). Ability to adhere to mucosal cells is a characteristic of pathogenic *C. albicans*, as compared to some of the less pathogenic *Candida* spp (King *et al.*, 1980; Calderone *et al.*, 1984; Douglas, 1987a and 1987b; Ray *et al.*, 1984; Rotrosen *et al.*, 1986). The most conclusive evidence for adhesion as a determinant of colonization or pathogenesis by *C. albicans* comes from studies showing that spontaneously-derived mutants with reduced ability to adhere to a variety of surfaces *in vitro* are less pathogenic than wild type strains (Calderone *et al.*, 1985; Hoberg *et al.*, 1986; Lehrer *et al.*, 1986).

Adhesion is dependent on surface components of the fungal cells with a specific affinity for epithelial receptors. A great deal of basic information pertaining to the biochemical and molecular basis of adhesion has only become available in recent years due to the complexity of the *C. albicans*-host interaction at both the cellular and molecular levels (Kennedy *et al.*, 1992). Firstly, it is hard to characterize adhesin(s) on the cell surface of *C. albicans* because the cell wall of *C. albicans* is a complex structure in which at least five to eight distinct layers have been identified. Secondly, in order to bind to some tissues, or different tissues within the same host, it may be necessary for *C. albicans* to synthesize one

or more adhesins or switch to alternate phenotypes or morphological forms once it has encountered the tissue (Kennedy, 1988). Modification of the cell wall during active adhesion to epithelial cells has been reported by Tronchin *et al.*, (1984 and 1989), who noted the reorganization and deposition of new cell wall material during adhesion. Thirdly, adherence of *C. albicans* to host cells is a complex process not only because it involves multiple adherence mechanisms but also because it is dependent on the type of host cell and strain of the organism (Douglas, 1987a and 1987b; Kennedy, 1988; Calderone, 1993). *C. albicans* attaches to exfoliated vaginal or buccal cells in much greater numbers than *C. tropicalis* (the next most adherent species) (King *et al.*, 1980). A correlation between adhesion and virulence for different isolates of *C. albicans* has also been reported by Douglas (1987a). The ability of *C. albicans* to synthesize fimbrial adhesins is strain-dependent. Forthly, the interactions are also affected by a number of factors such as concentration and viability of both yeast and host cells, phase and temperature of growth, germ-tube formation and pH (Douglas, 1987a and 1987b).

It is widely accepted that *C. albicans* can utilize multiple adhesion mechanisms to interact with host cells (Staddon *et al.*, 1990; Calderone and Braun, 1991; Cutler, 1991; Hostetter, Chapter IIIB, IIIC; Pendrak and Klotz, 1995). According to the nature of the adhesins and the mechanism of the *C. albicans*-host interaction, at least three types (Calderone and Braun, 1991; Cutler, 1991) or four systems (Calderone, 1993) of surface adhesion molecules plus hydrophobic molecules on the surface of *C. albicans* which contribute non-specific adherence of this fungus to host cells have been described (Cutler, 1991; Hazen and Hazen, 1993; Hostetter, 1994).

3. Adhesins

Most of the major cell wall components, including polysaccharides, proteins and lipids, have been implicated to have at least some adhesive function (Kennedy, 1988, 1991). Cell surface proteins may serve as adhesins in interactions with a variety of blood

proteins. Similarly, a mannose containing moiety, either mannan (Maisch and Calderone, 1981) or possibly a mannoprotein (McCourtie and Douglas, 1985), on the surface of *C. albicans* has also been suggested to mediate the adhesion of *C. albicans* to a number of surfaces (Sandin *et al.*, 1982). Analogous studies have also suggested that cell wall chitin may provide the cell with adhesive activity (Segal *et al.*, 1982; Lee and King, 1983b). Lipids have similarly been implicated to play a role in *C. albicans* adhesion (Ghannoum *et al.*, 1986).

Among all these various putative adhesins, mannoproteins constitute the major antigens and host cell recognition molecules (adhesins). Mannoproteins located on the cell surfaces of *C. albicans* consist of glycoproteins composed of a mannan oligosaccharide covalently linked to protein by both N-(amide) and O-(ether) glycosidic bonds (Shepherd, 1987 and 1991). The mannan oligosaccharide contains the sterospecific epitopes of *C. albicans*. Mannoproteins differ in their degrees of glycosylation and can be variably expressed depending upon cell age and growth form (Calderone, 1993).

4. *C. albicans* fimbrial adhesins

Ultrastructural studies on the adhesion of *C. albicans* indicate that *C. albicans* was able to express a fibrillar structure on the surface, also called fimbriae (Gardiner *et al.*, 1982), which can be seen on the surface of *C. albicans* as thin filamentous structures arranged perpendicularly to the cell surface and evenly distributed around the entire cell (Scherwitz *et al.*, 1978; Jones and Isaacson, 1983). It has been suggested that for *C. albicans*, the outermost layer, which is the one most likely to be involved in adhesion, is fibrillar or fimbriae (Cassone *et al.*, 1973). A fibrillar-floccular layer has been demonstrated on the surface of *C. albicans* growing *in vivo* by electron microscopy of scrapings taken from the tongue and buccal mucosa of patients with oral candidiasis (Montes and Wilborn, 1968; Mohamed, 1975; Marrie and Costerton, 1981).

In my studies, *C. albicans* fimbrial adhesins were first more extensively characterized and identified biochemically and immunologically. The structure-function of *C. albicans* fimbriae was well studied and the interactions of *C. albicans* fimbriae with human buccal epithelial cells were clearly elucidated.

4-1 Components and N-terminal protein sequence of *C. albicans* fimbriae

Purified *C. albicans* fimbriae (Figure IIIA-1) have been demonstrated to contain a major fimbrial subunit of molecular weight of 60 to 66 kDa (Figure IIIA-2) and the protein is highly glycosylated (85% of the molecular mass consists of D-mannose residues). The filamentous structures were observable by electron microscopy (Figure IIIA-4) and the purity was assessed by sodium dodecylsulfate polyacrylamide gel electrophoresis (Figure IIIA-2). The molecular weight of the deglycosylated fimbrial subunit is approximately 8.7 kDa on SDS-PAGE (Figure IIIA-3). The N-terminal protein sequence was recently examined and determined to be NMETPASST/DG; this sequence is not homologous to any fimbriae/pili sequences that have been reported. These N-terminal amino acid residues could be translated to a set of degenerate DNA oligos to be used as probes to clone the DNA sequence of the fimbrial subunit from a *C. albicans* cDNA library.

4-2 The role of *C. albicans* fimbriae in adherence

It is known that fimbriated microorganisms attach more readily than their nonfimbriated counterparts to their host cells. The fibrils of *C. albicans* may represent appendages analogous to bacterial fimbriae, whose role in adhesion is well established. There is now both ultrastructural (Figure IIIA-4) and serological (Figure IIIA-6) evidence for the existence of fimbriae on *C. albicans*.

Because of the common occurrence of *Candida* infections of the mouth and vagina, particular attention has been given to the ability of the most pathogenic species of *C. albicans* to adhere to human buccal and vaginal epithelial cells. In present studies, the role

of *C. albicans* fimbriae in the adherence of *C. albicans* to BECs was thoroughly examined. *C. albicans* fimbriae have been identified as major adhesins which could competitively inhibit yeast cell adherence to human buccal epithelial cells (Figure IIIA-7, IIIA-8 and IIIA-9). This is consistent with previous studies by other groups showing that surface fibrils of *C. albicans* could mediate adhesion of *C. albicans* to exfoliated buccal epithelial cells (Tronchin *et al.*, 1984), vaginal epithelial cells (Lee and King, 1983a; Persi *et al.*, 1985) and renal endothelium (Lee and King, 1983b; Barnes *et al.*, 1983). Infection of the mouth is one of the most common forms of superficial candidiasis. Buccal cells are obtained very easily by swabbing the mouths of volunteers. For these two reason, human buccal epithelial cells have been most frequently used in adherence studies.

4-3 *C. albicans* fimbrial Receptors

If yeasts, like bacteria, attach to mucosal surfaces via specific binding molecules, elucidation of adhesion mechanisms will involve identification of both the yeast adhesin and the epithelial cell receptor. Most known receptors for microorganisms on the surface of animal cells are carbohydrate components of membrane glycoproteins or glycolipids. Glycosides are able to present a greater range of recognition sites than can peptides. This is because the number of permutations possible with glycosidic bonding allows the formation of far more specific structures from a few monosaccharide units than can be produced from the same number of amino acids (Ofek *et al.*, 1985).

Sugar inhibition assays are widely employed to characterize epithelial receptors for microorganisms. Several sugars were proposed to be the putative receptors for *C. albicans* (Table I-5). Fucose inhibited *C. albicans* adherence to vaginal (Sobel *et al.*, 1981) and buccal epithelial cells (Tosh and Douglas, 1992). N-acetyl-D-glucosamine (Douglas, 1987a and 1987b) or D-mannose (Centeno *et al.*, 1983) were also proposed to function as epithelial receptors for *C. albicans*. Recently, Jimenez-Lucho *et al.* (1990) suggested that *C. albicans* and other fungi bound specifically to the glycosphingolipid (lactosylceramide).

In this study, we extended the findings of Jimenez-Lucho *et al.* (1990) and show that *C. albicans* binds to other glycosphingolipids (GSLs). *C. albicans* fimbriae have been demonstrated to bind to BECs (Figure IIIA-7) and to the gangliosides asialo-GM_1 (gangliotetraosyl ceramide) and asialo-GM_2 functioning as receptors (Figure IIIB-1 and IIIB-3) and asialo-GM_1 was able to inhibit fimbriae and yeast cells binding to BECs (Figure IIIB-5, IIIE-7). The fimbriae interact with the glycosphingolipid receptors via the carbohydrate portion of the receptors, since fimbriae were observed to bind to synthetic βGalNAc(1-4)βGal-protein conjugates (Figure IIIB-4) and the disaccharide was able to inhibit binding of fimbriae (Figure IIIB-5) and *C. albicans* whole cells (Figure IIIE-7) to BECs *in vitro* binding assays. Asialo-GM_1 and asialo-GM_2 are important constituent glycosphingolipids of epithelial cell membranes and are known to function as receptor determinants for certain bacteria, but their interactions with *C. albicans* have not been reported before. These data were further supported by the evidence that both asialo-GM_1 and asialo-GM_2 have been found in the lipid extracts from human BECs on TLC assays (Figure IIIB-2).

Addition of asialo-GM_1 to assay mixtures caused only partial inhibition of adhesion with the sensitive strains (Figure IIIE-7) suggesting that the natural mucosal receptors are larger than an asialo-GM_1 and/or that a particular stereochemical configuration is required. Alternatively, additional adhesion mechanisms may operate. These observations further confirm that *C. albicans* may rely on more than one predominant mechanism to adhere to epithelial surfaces (Kennedy *et al.*, 1988, 1989; Staddon *et al.*, 1990; Calderone and Braun, 1991; Cutler, 1991; Calderone, 1993; Odds, 1994; Pendrak and Klotz, 1995).

4-4 *C. albicans* fimbriae differing from other mannoprotein adhesins

C. albicans fimbrial adhesin is a glycoprotein which is similar to many of the other putative *C. albicans* adhesins. Douglas and colleagues (Tosh and Douglas, 1992) have

isolated a mannoprotein enriched fraction from cultures of *C. albicans* which apparently mediates adhesion to buccal epithelial cells. This putative adhesin has lectin-like properties recognizing either fucosyl- or N-acetylglucosamine-containing glycosides of human or rabbit vaginal epithelial cells. The adhesin is associated with the outer fibrillar surface of blastoconidia. The partially purified deglycosylated protein portion is approximately 200-fold more active in blocking adherence of the yeast that the crude extract. However, no protein profile and protein molecular weight have been reported yet. In our hands, the purified fibrillar materials isolated by mechanical shearing has a fimbrial structure when examined by negative staining (Figure IIIA-3). The glycoprotein profile can be consistently recorded on SDS-PAGE and the molecular weight of the fimbria is 60 to 66 kDa (Figure IIIA-2). The putative receptors of *C. albicans* fimbriae on BECs are asialo-GM_1 and asialo-GM_2 (Figure IIIB-1 to Figure IIIB-5; Figure IIIE-7), which are different from those described by the Douglas group. The fimbrial adhesin does not appear to be the same adhesin described by Douglas and coworkers because the amino acid compositions of these two adhesins are different (Chapter IIIA, Douglas, 1993). This could result from the different isolation and purification procedures employed in the different groups or the different strains of *C. albicans* used in the experiments. The fibrillar structural adhesins were recovered directly from the culture media while *C. albicans* fimbrial adhesins were isolated by the mechanical shearing. It has been reported that the fibrillar adhesin adherence to BECs is strain-dependent (Douglas, 1987a; Tosh and Douglas, 1992). So it is not suprising that the different results were obtained by different groups, and this also supports the idea that the interaction of *C. albicans* with host cells is more complex than expected in a traditional view.

C. albicans fimbrial adhesins are different not only from the fibrillar adhesins of *C. albicans* reported by Douglas group (Douglas, 1987; Critchley and Douglas, 1987a and 1987b; Tosh and Douglas, 1992), but also from other mannoprotein adhesins described previously by other groups (Ahrens *et al.*, 1983; Sundstrom and Kenny, 1984 and 1985,

Ponton and Jones, 1986a and 1986b; Sundstrom *et al.*, 1987 and 1988; Casanova *et al.*, 1989 and 1992a; Ollert and Calderone, 1990; Brassart *et al.*, 1991). The integrin-like proteins on *C. albicans* are mannoproteins which are enriched in the hyphal extracts and from culture filtrates of hyphal-grown cells. These proteins have been reported to have binding activity for iC3b and C3d on mammalian cells (Calderone *et al.*, 1988; Gilmore *et al.*, 1988; Gustafson *et al.*, 1991; Alaei *et al.*, 1993). A series of mannoproteins with various molecular weights have also been reported by several groups. These mannoproteins could recognize laminin (Bouchara *et al.*, 1990; Lopez-Ribot *et al.*, 1994), fibrinogen (Casanova *et al.*, 1992b; Martinez *et al.*, 1994; Sepulveda *et al.*, 1995), fibronectin (Klotz and Smith, 1991; Klotz *et al.*, 1992; Klotz *et al.*, 1994), entactin (Lopez-Ribot and Chaffin, 1994), and collagen (Klotz, 1990).

4-5 *C. albicans* fimbriae and *P. aeruginosa* strain K pili share the same receptors on BECs

A number of pathogens utilize the same or very similar receptors (Karlsson, 1989; Klavin *et al.*, 1988). *P. aeruginosa* PAK pili and *C. albicans* fimbriae have been identified as adhesins which mediate the adherence of *P. aeruginosa* and *C. albicans* to human buccal epithelial cells (Doig *et al.*, 1988; Paranchych *et al.*, 1986; Woods *et al.*, 1980; Chapter IIIA, IIIB). The binding of PAK pili and *C. albicans* fimbriae to BECs could be competitively inhibited by asialo-GM_1. The synthetic βGalNAc(1-4)βGal disaccharide, a minimal carbohydrate sequence common to asialo-GM_1 and asialo-GM_2, has also been shown to competitively inhibit the *C. albicans* adherence to asialo-GM_1 and BECs and to significantly inhibit the *P. aeruginosa* PAK pili binding to asialo-GM_1 and asialo-GM_2 (Chapter IIIB; Sheth, *et al.*, 1994).

4-6 *C. albicans* fimbriae and *P. aeruginosa* strain K pili share a conserved receptor-binding domain

Pseudomonas aeruginosa, a Gram-negative bacterium, and *Candida albicans*, a dimorphic yeast, are evolutionarily distant microorganisms that can utilize filamentous structures termed pili and fimbriae, respectively, to mediate adherence to glycosphingolipids (asialo-GM_1 and asialo-GM_2) receptors. Both the *P. aeruginosa* pilus and *C. albicans* fimbria are long, thin filamentous proteinaceous cell surface appendages. The pilus consists of a polymer of a single protein subunit termed pilin (Irvin, 1990). 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 (termed 'adhesintope') of the *P. aeruginosa* PAK pilin adhesin has been shown to reside in the carboxy-terminal disulfide-looped region [PAK(128-144)] of the PAK pilin (Irvin *et al.*, 1989; Lee *et al.*, 1994; Sheth *et al.*, 1994). We have also demonstrated that *Pseudomonas aeruginosa* pili and synthetic C-terminal pilin peptides containing the pilus adhesintope bind to asialo-GM_1 (Lee *et al.*, 1994; Sheth *et al.*, 1994). While the *C. albicans* fimbriae have not been completely characterized, the major subunit of the fimbria is a 66 kDa glycoprotein (Chapter IIIA).

Using monoclonal antibody (MAb, PK99H) against purified pili of *P. aeruginosa* strain PAK 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 (Table III-1 and Table III-2) and crossreacted with *C. albicans* fimbriae in immunoblots (Figure IIIC-1). A control MAb, PKL1, and anti-PAK(75-84) peptide antibodies failed to agglutinate *C. albicans* whole cells or crossreact with the fimbrial proteins (Table IIIC-1, Figure IIIC-1). Conversely, the anti-*C. albicans* fimbriae MAb, Fm16, but not Fm34, agglutinated *P. aeruginosa* PAK whole cells (Table

IIIC-2) and crossreact in Western blots (Figure IIIC-1). The interactions of PK99H and Fm16 with their respective homologous antigens were competitively inhibited by heterologous antigens: this suggested that both adhesins share a common antigenic determinant. The immunocrossreactivity between Fm16 and *P. aeruginosa* PAK pilin is localized onto the PAK134-140 region as shown by competitive ELISA. 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 (Doig *et al*, 1990; Wong *et al*., 1992). The monoclonal antibody PK99H was produced against the *P. aeruginosa* strain K pilus, and inhibits the pilus adhesin (Doig *et al*, 1990). PK99H has been extensively characterized and its native antigenic epitope has been identified as the sequence DEQFIPK (residues 134-140 of the PAK pilin structural subunit) (Wong *et al*., 1995). These observations suggested that *P. aeruginosa* pili and *C. albicans* fimbriae possess adhesins that contain a structurally constrained motif associated with their adhesintope. These results also further supported 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. *P. aeruginosa* PAK pili and *C. albicans* fimbriae have been identified as adhesins which mediate the adherence of *P. aeruginosa* and *C. albicans* to human buccal epithelial cells (Doig *et al*., 1988; Paranchych *et al*., 1986; Woods *et al*., 1980; Chapter IIIA and IIIB).

To further prove that the pilus and fimbrial adhesins express a structurally conserved binding domain that enables them to bind to the same cell surface receptors on human respiratory epithelial cells, PAK pili and *C. albicans* fimbriae were biotinylated. Both the unbiotinylated PAK pilus and fimbrial adhesins inhibited biotinylated pili from *P. aeruginosa* strain PAK and biotinylated *C. albicans* fimbriae binding to asialo-GM_1 (Figure IIID-1) and to receptors present on human buccal epithelial cells (BECs) (Figure IIID-4) which suggested that the same receptor sites were recognized by the two adhesins.

The delineation of the *C. albicans* fimbrial adhesintope was further systematically investigated by using synthetic peptides which correspond to the whole [PAK(128-144)] or part of [PAK(134-140)] adhesintope of the PAK pilus and their respective anti-peptide antisera. Both synthetic peptides, PAK(128-144) and PAK(134-140), were able to inhibit Bt-*C. albicans* fimbriae/Bt-*C. albicans* whole cells and Bt-PAK pili/Bt-*P. aeruginosa* whole cells adherence to asialo-GM_1 (Figure IIIE-1, IIIE-2 and IIIE-3) and BECs (Figure IIIE-4, IIIE-5 and IIIE-6). However, control synthetic peptide [PAK(75-84)] and control antibody [anti-PAK(75-84)] failed to interfere with the adhesins and whole cell binding. The results from these studies confirmed that a structurally conserved motif akin to the PAK(128-144) peptide sequence is present in *C. albicans* fimbrial adhesin and that the 7-amino acid residue PAK(134-140) sequence may play an important role in forming the adhesintope for both *P. aeruginosa* PAK pilus and *C. albicans* fimbrial adhesins.

Taken together, all these data presented in these studies demonstrated that i) *C. albicans* fimbriae function as adhesins to mediate the adherence of *C. albicans* to BECs; ii) the interaction of *C. albicans* fimbriae to BECs is mediated by asialo-GM_1-like receptors (asialo-GM_1 and asialo-GM_2) on the surfaces of human BECs via βGalNAc(1-4)βGal disaccharide, a minimal structural carbohydrate receptor sequence of asialo-GM_1 and asialo-GM_2; iii) the mechanism (nature) of the interaction is protein-carbohydrate interaction; and iv) the binding to these glyco-receptors is mediated by a conserved epitope (DEQFIPK) or homologue of that epitope which has receptor-binding (asialo-GM_1 like) properties.

Bibliography:

Ahrens, J.C., Daneo-Moore, L. and Buckley, H.R. (1983). Differential protein synthesis in *Candida albicans* during blastospore formation at 24.5 degrees C and during germ tube formation at 37 degrees C. J Gen Microbiol **129:**1133-9.

Alaei, S., Larcher, C., Ebenbichler, C., Prodinger, W.M., Janatova, J., and Dierich, M.P. (1993). Isolation and biochemical characterization of the iC3b receptor of *Candida albicans*. Infect Immun **61:**1395-9.

Al-Doory, Y. (1970). An immune factor in baboon anti-*Candida* serum. Saboraudia **8:**41-47.

Antley, P.P. and Hazen, K.C. (1988). Role of yeast cell growth temperature on *Candida albicans* virulence in mice. Infect Immun **56:**2884-90.

Anttila, V.J., Ruutu, P., Bondestam, S., Jansson, S.E., Nordling, S., Farkkila, M., Sivonen, A., Castren, M., and Ruutu, T. (1994). Hepatosplenic yeast infection in patients with acute leukemia: a diagnostic problem. Clin Infecti Dis **18:**979-81.

Ashman, R.B. and Papadimitriou, J.M. (1993). Strain dependence of antibody-mediated protection in murine systemic candidiasis [letter]. J Infect Dis **168:**511-3.

Baker, N.R., Hansson, G.C., Leffler, H., Riise, G., and Svanborg-Eden, C. (1990). Glycosphingolipid receptors for *Pseudomonas aeruginosa*.. Infect Immun **58**:2361-2366.

Baker, N.R., Minor, V., Deal, C., Shahrabadi, M.S., Simpson, D.A., and Woods., D.E. (1991). *Pseudomonas aeruginosa* exoenzyme S is an adhesin. Infect Immun **59:**2859-2863.

Balish, E., Filutowicz, H., and Oberley, T.D. (1990). Correlates of cell-mediated immunity in *Candida albicans*-colonized gnotobiotic mice. Infect Immun **58:**107-13.

Barbour, A. (1989). Antigenic variation in relapsing fever *Borrelia* species: genetic aspects, p783-790. *In* D.E. Berg and M.M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.

Barnes, J.L., Osgood, R.W., Lee, L.C., King, R.D., and Stein, J.H. (1983). Host-parasite interactions in the pathogenesis of experimental renal candidiasis. Lab Invest **49:**460-467.

Beachey, E.H. (1981). Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surface. J Infect Dis **143:**325-45.

Bendel, C.M. and Hostetter, M.K. (1993). Distinct mechanisms of epithelial adhesion for *Candida albicans* and *Candida tropicalis*. Identification of the participating ligands and development of inhibitory peptides. J Clin Invest **92:**1840-9.

Bendel, C.M., St Sauver, J., Carlson, S., and Hostetter, M.K. (1995). Epithelial adhesion in yeast species: correlation with surface expression of the integrin analog. J Infect Dis **171:**1660-3.

Berenguer, J., Buck, M., Witebsky, F., Stock, F., Pizzo, P.A., and Walsh, T.J. (1993). Lysis-centrifugation blood cultures in the detection of tissue-proven invasive candidiasis. Disseminated versus single-organ infection. Diagn Microbiol Infect Dis **17:**103-9.

Bliska, J.B., Galan, J.E., and falkow, S. (1993). Singal transduction in the mammalian cell during bacterial attachment and entry. Cell **73:**903-920.

Bodey, G.P, (1993). Antifungal Agents, p371-406. *In*: Bodey, G.P. (eds), *Candidiasis:* Pathogeniesis, Diagnosis, and Treatment 2nd. Raven Press, New York.

Bodey, G.P. and Sobel, J.D. (1993). Lower gastrointestinal Candidiasis. p205-224. *In*: Bodey, G.P. (eds), *Candidiasis:* Pathogeniesis, Diagnosis, and Treatment 2nd. Raven Press, New York.

Bodey, G.P., Bolivar, R., Fainstein, V., and Jadeja, L. (1983). Infections caused by *Pseudomonas aeruginosa*. Rev Infect Dis **5:**279-313.

Borg, M. and Ruchel, R. (1988). Expression of extracellular acid proteinase by proteolytic *Candida* spp. during experimental infection of oral mucosa. Infect Immun **56:**626-31.

Bouchara, J.P., Tronchin, G., Annaix, V., Robert, R., and Senet, J.M. (1990). Laminin receptors on *Candida albicans* germ tubes. Infect Immun **58:**48-54.

Bradley, D.E. (1974). The adsorption of *Pseudomonas aeruginosa* pilus-dependent bacteriophages to a host mutant with nonretractile pili. Virology **58:**149-63.

Bradley, D.E. (1980). Mobilization of chromosomal determinants for the polar pili *of Pseudomonas aeruginosa* PAO by FP plasmids. Can J Microbiol **26:**155-60.

Brassart, D., Woltz, A., Golliard, M., and Neeser, J. R. (1991). *In vitro* inhibition of adhesion of *Candida albicans* clinical isolates to human buccal epithelial cells by Fuc alpha 1----2Gal beta-bearing complex carbohydrates. Infect Immun **59:**1605-13.

Brunham, R.C., Plummer, F.A., and Stephens, R.S. (1993). Bacterial antigenic variation, host immune response, and pathogen-host coevolution. Infect Immun **61:**2273-6.

Bryn, K. and Jantzen, E. (1982). Analysis of lipopolysaccharides by methanolysis, trifluoroacetylation, and gas chromatography on a fused-silica capillary column. Chromatog **240**:405-413.

Bryn, K. and Jantzen, E. (1986). Quantification of 2-keto-3-deoxyoctonate in (LIPO) polysaccharides by methanolytic release, trifluoroacetylation, and capillary gas chromatography. J Chromatog **370**:103-112.

Calderone, R.A. (1993). Molecular interactions at the interface of *Candida albicans* and host cells. Arch Med Res **24:**275-9.

Calderone, R.A. and Braun, P.C. (1991). Adherence and receptor relationships of *Candida albicans*. Microbiol Rev **55:**1-20.

Cantorna, M.T. and Balish, E. (1991). Role of CD4+ lymphocytes in resistance to mucosal candidiasis. Infect Immun **59:**2447-55.

Carrow, E.W. and Domer, J.E. (1985). Immunoregulation in experimental murine candidiasis: specific suppression induced by *Candida albicans* cell wall glycoprotein. Infect Immun **49:**172-81.

Casadevall, A. (1995). Antibody immunity and invasive fungal infections. Infect Immun **63:**4211-4218.

Casanova, M., Martinez, J.P., and Chaffin, W.L. (1990). Fab fragments from a monoclonal antibody against a germ tube mannoprotein block the yeast-to-mycelium transition in *Candida albicans*. Infect Immun **58:**3810-2.

Casanova, M., Gil, M.L., Cardenoso, L., Martinez, J.P., and Sentandreu, R. (1989). Identification of wall-specific antigens synthesized during germ tube formation by *Candida albicans*. Infect Immun **57:**262-71.

Casanova, M., Lopez-Ribot, J.L., Martinez, J.P., and Sentandreu, R. (1992a). Characterization of cell wall proteins from yeast and mycelial cells of *Candida albicans* by labelling with biotin: comparison with other techniques. Infect Immun **60:**4898-906.

Casanova, M., Lopez-Ribot, J.L., Monteagudo, C., Llombart-Bosch, A., Sentandreu, R., and Martinez, J.P. (1992b). Identification of a 58-kilodalton cell surface fibrinogen-binding mannoprotein from *Candida albicans*. Infect Immun **60:**4221-9.

Cassone, A. and Torosantucci, A. (1991). Immunological moieties of the cell wall. p89-107. In R. Prasad (ed.), *Candida albicans*: Cellular and Molecular Biology. Springer-Verlag.

Cassone, A., Boccanera, M., Adriani, D., Santoni, G., and De Bernardis, F. (1995). Rats clearing a vaginal infection by *Candida albicans* acquire specific, antibody-mediated resistance to vaginal reinfection. Infect Immun **63:**2619-2624.

Castle, A.J., Boulianne, R., Xu, J., andDay, A.W. (1992). Post-translational modification of fimbrial protein from *Ustilago violacea*. Can J Microbiol **38**: 1144-1149.

Centeno, A., Davis, C.P., Cohen, M.S., and Warren, M.M. (1983). Modulation of *Candida albicans* attachment to human epithelial cells by bacteria and carbohydrates. Infect Immun **39:**1354-60.

Chaffin, W.L., Ringler, L., and Larsen, H.S. (1988). Interactions of monospecific antisera with cell surface determinants of *Candida albicans*. Infect Immun **56:**3294-6.

Chaffin, W.L., Collins, B., Marx, J.N., Cole, G.T., and Morrow, K.J., Jr. (1993). Characterization of mutant strains of *Candida albicans* deficient in expression of a surface determinant. Infect Immun **61:**3449-58.

Chilgren, R.A., Hong, R., and Quie, P.G. (1968). Human serum interactions with *Candida albicans*. J Immunol **101:**128-32.

Collins, L.C., Kalbfleisch, J.H., Franson, T.R., and Sohnle, P.G. (1984). Inhibition by surgars of *Candida albicans* adherence to human buccal mucosal cells and corneocytes *in vitro*. Infect. Immun. **46:**831-834.

Cox, F. (1983). Adherence of *Candida albicans* to buccal epithelial cells in children and adults. J Lab Clin Med **102:**960-72.

Critchley, I.A. and Douglas, L.J. (1987a). Isolation and partial characterization of an adhesin from *Candida albicans*. J Gen Microbiol **133:**629-36.

Critchley, I.A. and Douglas, L.J. (1987b). Role of glycosides as epithelial cell receptors for *Candida albicans*. J Gen Microbiol **133:**637-43.

Cutler, J.E. (1991). Putative virulence factors of *Candida albicans*. Annu Rev Microbiol **45:**187-218.

Cutler, J.E. and Kanbe, T. (1993). Antigenic variability of *Candida albicans* cell surface. Curr Top Med Mycol **5:**27-47.

Cutler, J.E., Friedman, L., and Milner, K.C. (1972). Biological and chemical characterization of toxic substances from *Candida albicans*. Infect Immun **6:**616-27.

Day, A.W. and Cummins, J.E. (1981). The genetics and cellular biology of sexual development in *Ustilago violacea*, p. 379-402. *In* O'Day, D.H., and Horgen, P.A., (ed.), Sexual Interactions in eukaryotic microbes. Academic Press. New York.

Day, A.W. and Poon, N.H. (1975). Fungal fimbriae. II. Their role in conjugation in *Ustilago violacea*. Can J Microbiol **21:**547-57.

De Bernardis, F., Chiani, P., Ciccozzi, M., Pellegrini, G., Ceddia, T., D'Offizzi, G., Quinti, I., Sullivan, P.A., and Cassone, A. (1996). Elevated aspartic proteinase secretion and experimental pathogenicity of *Candida albicans* isolates from oral cavities of subjects infected with human immunodeficiency virus. Infect Immun **64:**466-471.

De Mot, R. and Vanderleyden, J. (1994a). The C-terminal sequence conservation between OmpA-related outer membrane proteins and MotB suggests a common function in both gram-positive and gram-negative bacteria, possibly in the interaction of these domains with peptidoglycan [letter]. Mol Microbiol **12:**333-334.

De Mot, R. and Vanderleyden, J. (1994b). A conserved surface-exposed domain in major outer membrane proteins of pathogenic *Pseudomonas* and *Branhamella* species shares sequence homology with the calcium-binding repeats of the eukaryotic extracellular matrix protein thrombospondin [letter]. Mol Microbiol **13:**379-380.

DeMuri, G.P. and Hostetter, M.K. (1995). Resistance to antifungal agents. Pediatr Clin North Am **4285:**665-685.

Doig, P., Sastry, P.A., Hodges, R.S., Lee, K.K., Paranchych, W., and Irvin, R.T. (1990). Inhibition of pilus-mediated adhesion of *Pseudomonas aeruginosa* to human buccal epithelial cells by monoclonal antibodies directed against pili. Infect Immun **58:**124-30.

Doig, P., Smith, N.R., Todd, T., and Irvin, R.T. (1987). Characterization of the binding of *Pseudomonas aeruginosa* alginate to human epithelial cells. Infect Immun **55:**1517-22.

Doig, P., Todd, T., Sastry, P.A., Lee, K.K., Hodges, R.S., Paranchych, W., and Irvin, R.T. (1988). Role of pili in adhesion of *Pseudomonas aeruginosa* to human respiratory epithelial cells. Infect Immun **56:**1641-6.

Donelson, J.E. (1988). A summary of the molecular biology of antigenic variation in African *trypanosomes*. Mem Inst Oswaldo Cruz **83 Suppl 1:**244-8.

Douglas, L.J. (1993). Identification and characterization of *Candida albicans* extracellular polymeric material adhesins. 3th ASM Conference on *Candida* and candidiasis: Biology, Pathogenesis, and Management. 1993; Baltimore, Maryland.

Douglas, L.J. (1985). Surface composition and adhesion of *Candida albicans*. Biochem Soc Trans **13:**982-4.

Douglas, L.J. (1987a). Adhesion to surfaces. p.239-280. *In* A. H. Rose, and J. S. Harriison (ed.), The yeasts, vol. 2. Academic Press, Inc. (London), Ltd., London.

Douglas, L.J. (1987b). Adhesion of *Candida* species to epithelial surfaces. Crit. Rev. Microbiol. **15**:27-43.

Douglas, L.j. (1991). Mannoprotein adhesins of *C. albicans* , p34-50. *In* J.E. Bennett, R.J. Hay, and P.K. Peterson (ed.), New strategies in fungal disease. Churchill Livingstone, London.

Douglas, L.J., Houston, J.G., and McCourtie, J. (1981). Adherence of *Candida albicans* to human buccal epithelial cells after growth on different carbon sources. FEMS Microbiol Lett **12:**241-243.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Anal Chem **28:**350-356.

Edwards, J.E., Jr., Mayer, C.L., Filler, S.G., Wadsworth, E., and Calderone, R.A. (1992). Cell extracts of *Candida albicans* block adherence of the organisms to endothelial cells. Infect Immun **60:**3087-91.

Edwards, J.E., Jr., and. Mayer, C.L (1990). Adherence of *Candida albicans* to mammalian cells, p.179-194. *In* Ayoub, E.M., Cassell, G.H., Branche, W.C., Jr., and Henry, T.J., (ed.), Microbial determinants of virulence and host response. American Society for Microbiology, Washington, D.C.

Edwards, J.E., Jr. and Mayer, C.L. (1990). Adherence of *Candida albicans* to Mammalian Cells, p179-194. In: Ayoub, E.M., Casell, G.H., Branche, W.C., Jr., and Henry, T.J. (eds.), Microbial Determinants of Virulence and Host Response

Eigentler, A., Schulz, T.F., Larcher, C., Breitwieser, E.M., Myones, B.L., Petzer, A.L., and Dierich, M.P. (1989). C3bi-binding protein on *Candida albicans*: temperature-dependent expression and relationship to human complement receptor type 3. Infect Immun **57:**616-22.

Elleman, T.C. (1988). Pilins of Bacteroides nodosus: molecular basis of serotypic variation and relationships to other bacterial pilins. Microbiol Rev **52:**233-47.

Elleman, T.C. and Peterson, J.E. (1987). Expression of multiple types of N-methyl Phe pili in Pseudomonas aeruginosa. Mol Microbiol **1:**377-80.

Elmets, C.A. (1994). Management of common superficial fungal infections in patients with AIDS. JAAD **31**(3 Pt 2):S60-63.

Ener, B. and Douglas, L.J. (1992). Correlation between cell-surface hydrophobicity of *Candida albicans* and adhesion to buccal epithelial cells. FEMS Microbiol Lett **78:**37-42.

Epstein, J.B., Kimura, L.H., Menard, T.W., Truelove, E.L., and Pearsall, N.N. (1982). Effects of specific antibodies on the interaction between the fungus *Candida albicans* and human oral mucosa. Arch Oral Biol **27:**469-74.

Falkow, S., Isberg, R.R., and Portnoy, D.A. (1992). The interaction of bacteria with mammalian cells. Annu Rev Cell Biol **8:**333-363.

Farinha, M.A., Conway, B.D., Glasier, L.M., Ellert, N.W., Irvin, R.T., Sherburne, R., and Paranchych, W. (1994). Alteration of the pilin adhesin of *Pseudomonas aeruginosa* PAO results in normal pilus biogenesis but a loss of adherence to human pneumocyte cells and decreased virulence in mice. Infect Immun **62:**4118-4123.

Fasoli, M.O., Kerridge, D., and Ryley, J.F. (1990). Pathogenicity of 5-fluorocytosine resistant strains of *Candida albicans*. J MedVet Mycol **28:**27-34.

Finlay, B.B. (1990). Cell adhesion and invasion mechanisms in microbial pathogenesis. Curr Opin Cell Biol **2:**815-20.

Finlay, B.B. and S. Falkow. (1989). Common themes in microbial pathogenicity. Microbiol Rev **53**:210-230.

Fischer, A., Ballet, J.J., and Griscelli, C. (1978). Specific inhibition of *in vitro Candida*-induced lymphocyte proliferation by polysaccharide antigens present in the serum of patients with chronic mucoculaneous candidiasis. J clin Invest **62:**1005-1013.

Folkhard, W., Marvin, D.A., Watts, T.H., and Paranchych, W. (1981). Structure of polar pili from *Pseudomonas aeruginosa* strains K and O. J Mol Biol **149:**79-93.

Franzke, S., Calderone, R.A., and Schaller, K. (1993). Isolation of avirulent clones of *Candida albicans* with reduced ability to recognize the CR2 ligand C3d. Infect Immun **61:**2662-9.

Frost, L.S. and Paranchych, W. (1977). Composition and molecular weight of pili purified from Pseudomonas aeruginosa K. J Bacteriol **131:**259-69.

Fukayama, M. and Calderone, R.A. (1991). Adherence of cell surface mutants of *Candida albicans* to buccal epithelial cells and analyses of the cell surface proteins of the mutants. Infect Immun **59:**1341-5.

Fukazawa, Y., Nishikawa, M., Suzuki, M., and Shinoda, T. (1980). Immunochemical basis of the serological specificity of the yeast: Immunochemical determinants of several antigenic factors of yeast. Zentralbl. Bakteriol. Parasitenkd. Infection-skr. Hyg Abt 1: Otih **8:**127-35.

Gardiner, R.B. Canton, M., and Day, A.W. (1982). Serological studies on the fimbriae of yeasts and yeast-like species. Bot Gaz **143:**534-541.

Gardiner, R.B. and Day, A.W. (1985). Fungal fimbriae. IV. Composition and Properties of fimbriae from *Ustilago violacea*. Exp Mycology **9:**334-350.

Ghannoum, M.A. (1992). *Candida albicans* antifungal-resistant strains: studies on adherence and other pathogenicity related characteristics. Mycoses **35:**131-9.

Ghannoum, M.A. and Elteen, K.A. (1990). Pathogenicity determinants of *Candida*. Mycoses **33:**265-82.

Ghannoum, M.A., Elteen, K.A., and Radwan, S.S. (1987). Blocking adherence of *Candida albicans* to buccal epithelial cells by yeast glycolipids, yeast wall lipids and lipids from epithelial cells. Mykosen **30:**371-8.

Ghannoum, M.A., Burns, G.R., Elteen, K.A., and Radwan, S.S. (1986). Experimental evidence for the role of lipids in adherence of *Candida* spp. to human buccal epithelial cells. Infect Immun **54:**189-93.

Gilmore, B.J., Retsinas, E.M., Lorenz, J.S., and Hostetter, M.K. (1988). An iC3b receptor on *Candida albicans*: structure, function, and correlates for pathogenicity. J Infect Dis **157:**38-46.

Giron, J.A., Ho, A.S., and Schoolnik, G.K. (1991). An inducible bundle-forming pilus of enteropathogenic Escherichia coli. Science **254:**710-3.

Glasgwo, A.C., Hughes, K.T., and Simon, M.I. (1989). Bacterial DNA inversion systems, p.637-660. In Berg, D.E., and Howe, M.M. (ed.), Mobile DBA. American Society for Microbiology, Washington, D.C.

Glee, P.M., Sundstrom, P., and Hazen, K.C. (Apr). (1995). Expression of surface hydrophobic proteins by *Candida albicans in vivo*. Infect Immun **63:**1373-9.

Goodman, N.L. and Roberts, G.D. (1993). General Mycology. *In*: Sarosi GA and Davies, SF (eds.) Fungal Diseases of the Lung Raven Press, Ltd., New York.

Greenfield, R.A. (1992). Host defense system interations with *Candida albicans*. J Med Vet Mycol **30:**89-104.

Greenfield, R.A., Troutt, D.L., Rickard, R.C., altmiller, D.H. (1988). Comparison of antibody, antigen, and metabolite assays in rat models of systemic and gastrointestinal candidiasis. J Clin Microbiol **26:**409-417.

Gupta, S.K., Berk, R.S., Masinick, S., and Hazlett, L.D. (1994). Pili and lipopolysaccharide of *Pseudomonas aeruginosa* bind to the glycolipid asialo-GM_1. Infect Immun **62:**4572-9.

Gustafson, K.S., Vercellotti, G.M., Bendel, C.M., and Hostetter, M.K. (1991). Molecular mimicry in *Candida albicans*. Role of an integrin analogue in adhesion of the yeast to human endothelium. J Clin Invest **87:**1896-902.

Han, Y. and Cutler, J.E. (1995). Antibody response that protects against disseminated candidiasis. Infect Immun **63:**2714-2719.

Han, Y., Van Rooijen, N., and Cutler, J.E. (1993). Binding of *Candida albicans* yeast cells to mouse popliteal lymph node tissue is mediated by macrophages. Infect Immun **61:**3244-9.

Hasty, D.L., Ofek, I., Courtney, H.S., and Doyle, R.J. (1992). Multiple adhesins of *streptococci*. Infect Immun **60:**2147-52.

Hazen, K.C. (1989). Participation of yeast cell surface hydrophobicity in adherence of *Candida albicans* to human epithelial cells. Infect Immun **57:**1894-900.

Hazen, K.C. (1990). Cell surface hydrophobicity of medically important fungi, especially *Candida* species, p.249-295. *In* R. J. Doyle, M. Rosenberg (ed.), Microbial cell surface hydrophobicity. American Society for Microbiology, Washington, DC.

Hazen, K.C. and Glee, P.M. (1995). Adhesion of fungi. Methods Enzymol **253:**414-424.

Hazen, K.C. and Hazen, B.W. (1987). Temperature-modulated physiological characteristics of *Candida albicans*. Microbiol Immunol **31:**497-508.

Hazen, K.C. and Hazen, B.W. (1992). Hydrophobic surface protein masking by the opportunistic fungal pathogen *Candida albicans*. Infect Immun **60:**1499-508.

Hazen, K.C. and Hazen, B.W. (1993). Surface hydrophobic and hydrophilic protein alterations in *Candida albicans*. FEMS Microbiol Lett **107:**83-7.

Hazen, K.C., Brawner, D.L., Riesselman, M.H., Jutila, M.A., and Cutler, J.E. (1991). Differential adherence of hydrophobic and hydrophilic *Candida albicans* yeast cells to mouse tissues. Infect Immun **59:**907-12.

Hazlett, L.D., Masinick, S., Barrett, R., and Rosol, K. (1993). Evidence for asialo GM1 as a corneal glycolipid receptor for *Pseudomonas aeruginosa* adhesion. Infect Immun **61:**5164-73.

Helstrom, P.B. and Balish, E. (1979). Effect of oral tetracycline, the microbial flora, and the athymic state on gastrointestinal colonization and infection of BALB/c mice with *Candida albicans*. Infect Immun **23:**764-74.

Hoberg, K.A., Cihlar, R.L., and Calderone, R.A. (1986). Characterization of cerulenin-resistant mutants of *Candida albicans*. Infect Immun **51:**102-9.

Hoepelman, A.I. and Tuomanen, E.I. (1992). Consequences of microbial attachment: directing host cell functions with adhesins. Infect Immun **60:**1729-33.

Hostetter, M.K. (1994). Adhesins and ligands involved in the interaction of *Candida* spp. with epithelial and endothelial surfaces. Clin Microbiol Rev **7:**29-42.

Hostetter, M.K., Lorenz, J.S., Preus, L., and Kendrick, K.E. (1990). The iC3b receptor on *Candida albicans:* subcellular localization and modulation of receptor expression by glucose. J. Infect. Dis.**161:**761-768.

Hymes, S.R. and Duvic, M. (1993). Cutaneous candidiasis, p159-166. *In*: Bodey, G.P. (eds), Candidiasis*: Pathogeniesis, Diagnosis, and Treatment* 2nd. Raven Press, New YorkKohler, R.B., and White, A. 1979. Miscellaneous *Pseudomonas* disease, p446-494. *In* Doggett, R.G. (ed.), *Pserdomonas aeruginosa*: clinical manifestations of infection and current therapy. Academic Press, New York.

Ibrahim, A.S., Mirbod, F., Filler, S.G., Banno, Y., Cole, G.T., Kitajima, Y., Edwards, J.E., Jr., Nozawa, Y., and Ghannoum, M.A. (1995). Evidence implicating phospholipase as a virulence factor of *Candida albicans*. Infect Immun **63:**1993-8.

Irvin, R.T. (1993). Attachment and colonization of *Pseudomonas aeruginosa*: role of surface structures. p.19-42. *In* M. Campas et al. (eds.), *Pseudomonas aeruginosa* as an opportunistic pathogen. Plenum Press, New York.

Irvin, R.T. and Ceri, H. (1984). Immunochemical examination of the *Pseudomonas aeruginosa* glycocalyx: a monoclonal antibody which recognizes L-guluronic acid residues of alginic acid. Can J Microbiol **31:**268-75.

Irvin, R.T., Doig, P., Lee, K.K., Sastry, P.A., Paranchych, W., Todd, T., and Hodges, R.S. (1989). Characterization of the *Pseudomonas aeruginosa* pilus adhesin: confirmation that the pilin structural protein subunit contains a human epithelial cell-binding domain. Infect Immun **57:**3720-6.

Jakab, E., Paulsson, M., Ascencio, F., and Ljungh, A. (1993). Expression of vitronectin and fibronectin binding by *Candida albicans* yeast cells. APMIS. **101:**187-193.

Jimenez-Lucho, V., Ginsburg, V., and Krivan, H.C. (1990). *Cryptococcus neoformans*, *Candida albicans*, and other fungi bind specifically to the glycosphingolipid lactosylceramide (Gal beta 1-4Glc beta 1-1Cer), a possible adhesion receptor for yeasts. Infect Immun **58:**2085-90.

Jones, G.W. and Isaacson, R.E. (1983). Proteinaceous bacterial adhesins and their receptors. Crit Rev Microbiol **10:**229-60.

Jose, J., Jahnig, F., and Meyer, T.F. (1995). Common structural features of IgA1 protease-like outer membrane protein autotransporters. Mol Microbiol **18:**378-379.

Joshi, K.R. and Gavin, J.B. (1975). The morphology of colony variants of three species of *Candida*. Sabouraudia **13:**274-9.

Joshi, K.R., Wheeler, E.E., and Gavin, J.B. (1973). Scanning electron microscopy of colonies of six species of *Candida*. J Bacteriol **115:**341-8.

Kagaya, K., Shinoda, T., and Fukazawa, Y. (1981). Murine defense mechanism against *Candida albicans* infection. I. Collaboration of cell-mediated and humoral immunities in protection against systemic *C. albicans* infection. Microbiol Immunol **25:**647-54.

Kahana, M., Segal, E., Schewach Millet, M., and Gov, Y. (1988). *In vitro* adherence of *Candida albicans* to human corneocytes. Inhibition by chitin-soluble extract. Acta Dermato-Venereologica. **68**(2):98-101.

Kalo, A., Segal, E., Sahar, E., and Dayan, D. (1988). Interaction of *Candida albicans* with genital mucosal surfaces: involvement of fibronectin in adherence. J Infect Dis **157:**1253-1256.

Kaminishi, H., Miyaguchi, H., Tamaki, T., Suenaga, N., Hisamatsu, M., Mihashi, I., Matsumoto, H., Maeda, H., and Hagihara, Y. (1995). Degradation of humoral host defense by *Candida albicans* proteinase. Infect Immun **63:**984-8.

Kanbe, T., Han, Y., Redgrave, B., Riesselman, M.H., and Cutler, J.E. (1993). Evidence that mannans of *Candida albicans* are responsible for adherence of yeast forms to spleen and lymph node tissue. Infect Immun **61:**2578-84.

Karlsson, K.A. (1989). Animal glycosphingolipids as membrane attachment sites for bacteria. Annu Rev Biochem **58:**309-50.

Karlsson, K.A. and Stromberg, N. (1987). Overlay and solid-phase analysis of glycolipid receptors for bacteria and viruses. Methods Enzymol **138:**220-32.

Karlsson, K.A., Angstrom, J., Bergstrom, J., and Lanne, B. (1992). Microbial interaction with animal cell surface carbohydrates. APMIS Suppl **27:**71-83.

Kearns, M.J., Davies, P., and Smith, H. (1983). Variability of the adherence of *Candida albicans* strains to human buccal epithelial cells: inconsistency of differences between strains related to virulence. Sabouraudia **21:**93-8.

Keinanen, K.P. (1988) Effect of deglycosylation on the structure and hormone-binding activity of the lutropin receptor. Biochem J **256:**719-24.

Kennedy, M.J. (1988). Adhesion and association mechanisms of *Candida albicans.* Curr Top Med Mycol **2:**73-169.

Kennedy, M.J. (1990). Models for studying the role of fungal attachment in colonization and pathogenesis. Mycopathologia **109:**123-37.

Kennedy, M.J. and Volz, P.A. (1985). Ecology of *Candida albicans* gut colonization: inhibition of *Candida* adhesion, colonization, and dissemination from the gastrointestinal tract by bacterial antagonism. Infect Immun **49:**654-63.

Kennedy, M.J., Calderone, R.A., Cutler, J.E., Kanabe, T., Riesselman, M.H., Robert, R., Senet, J.M., Annaix, V., Bouali, A., and Mahaza, C. (1992). Molecular basis of *Candida albicans* adhesion. J Med Vet Mycol **30:**95-122.

Kennedy, M.J., Rogers, A.L., Hanselmen, L.R., Soll, D.R., and Yancey, R.J., Jr. (1988). Variation in adhesion and cell surface hydrophobicity in *Candida albicans* white and opaque phenotypes. Mycopathologia **102:**149-56.

Kennedy, M.J., Rogers, A.L., and Yancey, R.J., Jr. (1989). Environmental alteration and phenotypic regulation of *Candida albicans* adhesion to plastic. Infect Immun **57:**3876-81.

Kennett, R.H. (1980a). Fusion of cells suspended in polyethyleneglycol, p. 365-367. *In* Kennett, R.H., Mckearn, J.J., and Bechtol, K.B. (ed.). Monoclonal antibodies. Hybridomas: a new dimension in biological analyses. Plenum Publishing Corp., New York.

Kennett, R.H. (1980b). Freezing of hybridoma cells, p. 375. *In* Kennett, R.H., Mckearn, J.J., and Bechtol, K.B. (ed.), Monoclonal antibodies. Hybridomas: a new dimension in biological analyses. Plenum Publishing Corp., New York.

Kimura, L.H. and Pearsall, N.N. (1978). Adherence of *Candida albicans* to human buccal epithelial cells. Infect Immun **21:**64-8.

King, R.D., Lee, J.C., and Morris, A.L. (1980). Adherence of *Candida albicans* and other Candida species to mucosal epithelial cells. Infect Immun **27:**667-74.

Kirkpatrick, C.H. (1993). Chronic mucocutaneous candidiasis, p167-184. In: *In*: Bodey, G.P. (ed), *Candidiasis:* Pathogeniesis, Diagnosis, and Treatment 2nd. Raven Press, New York

Klotz, S.A. (1990). Adherence of *Candida albicans* to components of the subendothelial extracellular matrix. FEMS Microbiol Lett **56:**249-54.

Klotz, S.A. (1994). Adherence of *Candida albicans* to endothelial cells is inhibited by prostaglandin I2. Infect Immun **62:**1497-500.

Klotz, S.A., and Penn, R.L. (1987). Multiple mechanisms may contribute to the adherence of *Candida* yeasts to lining cells. Curr Microbiol **16:**119-122.

Klotz, S.A. and Smith, R.L. (1991). A fibronectin receptor on *Candida albicans* mediates adherence of the fungus to extracellular matrix. J Infect Dis **163:**604-10.

Klotz, S.A. and Smith, R.L. (1992). Glycosaminoglycans inhibit *Candida albicans* adherence to extracellular matrix proteins. FEMS Microbiol Lett **78:**205-8.

Klotz, S.A. and Smith, R.L. (1995). Gelatin fragments block adherence of *Candida albicans* to extracellular matrix proteins. Microbiology **141:**2681-2684.

Klotz, S.A., Drutz, D.J., and Zajic, J.E. (1985). Factors governing adherence of *Candida* species to plastic surfaces. Infect Immun **50:**97-101.

Klotz, S.A., Hein, R.C., Smith, R.L., and Rouse, J.B. (1994). The fibronectin adhesin of *Candida albicans*. Infect Immun **62:**4679-81.

Klotz, S.A., Rutten, M.J., Smith, R.L., Babcock, S.R., and Cunningham, M.D. (1993). Adherence of *Candida albicans* to immobilized extracellular matrix proteins is mediated by calcium-dependent surface glycoproteins. Microb Pathog **14:**133-47.

Kohler, R.B. and White, A. (1979). Miscellaneous Pseudomonas disease. p446-494. *In* Doggett, R.G. (ed.), *Pseduomonas aeruginosa*: clinical manifestations of infection and current therapy. academic Press. New York.

Komshian, S.V., Uwaydah, A.K., Sobel, J.D., and Crane, L.R. (1989). Fungemia caused by *Candida* species and Torulopsis glabrata in the hospitalized patient: frequency, characteristics, and evaluation of factors influencing outcome. Rev Infect Dis **11:**379-90.

Kroprowski, H., Gerhard,W., and Croce, C.M. (1977). Production of antibodies against influenza virus by somatic cell hybrids between mouse myeloma and primed spleen cells. Proc Natl Acad Sci U S A **74:**2985-2988.

Krivan, H.C., Ginsburg,V., and Roberts, D.D. (1988a). *Pseudomonas aeruginosa* and *Pseudomonas cepacia* isolated from cystic fibrosis patients bind specifically to gangliotetraosylceramide (asialo-GM1) and gangliotriaosylceramide (asialo-GM2). Arch Biochem Biophys **260:**493-6.

Krivan, H.C., Roberts, D.D., and Ginsburg, V. (1988b). Many pulmonary pathogenic bacteria bind specifically to the carbohydrate sequence GalNAcβ(1-4)Gal found in some glycolipids. Proc Natl Acad Sci USA **85:**6157-61.

Krivan, H.C., Nilsson, B., Lingwood, C.A., and Ryu, H. (1991). *Chlamydia trachomatis* and *Chlamydia pneumoniae* bind specifically to phosphatidylethanol-amine in HeLa cells and to GalNAc beta 1-4Gal beta 1-4GLC sequences-found in asialo-GM_1 and asialo-GM_2. Biochem Biophys Res Commun **175:**1082-9.

Kuruganti, U., Henderson, L.A., Garner, R.E., Asofsky, R., Baker, P.J. and Domer, J.E. (1988). Nonspecific and *Candida*-specific immune responses in mice suppressed by chronic administration of anti-mu. J Leuk Biol **44:**422-33.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:**680-5.

Laemmli, U.K. and Favre, M. (1973). Maturation of the head of bacteriophage T4. I. DNA packaging events. J Mol Biol **80:**575-99.

Law, D. (1994). Adhesion and its role in the virulence of enteropathogenic *Escherichia coli*. Clin Microbiol Rev **7:**152-73.

Law, D., Moore, C.B., Wardle, H.M., Ganguli, L.A., Keaney, M.G., and Denning, D. W. (1994). High prevalence of antifungal resistance in *Candida* spp. from patients with AIDS. J Antimicrob Chemother **34:**659-668.

Lee, J.C. and King, R.D. (1983a). Characterization of *Candida albicans* adherence to human vaginal epithelial cells *in vitro*. Infect Immun **41:**1024-30.

Lee, J.C., and King, R.D. (1983b). Adherence mechanisms of *Candida albicans*. p269-272. *In*: Microbiology. D. Schlessinger (ed.), American Society for Microbiology Washington, D.C.

Lee, K.K., Doig, P., Irvin, R.T., Paranchych, W., and Hodges, R.S. (1989). Mapping the surface regions of *Pseudomonas aeruginosa* PAK pilin: the importance of the C-terminal region for adherence to human buccal epithelial cells. Mol Microbiol **3:**1493-9.

Lee, K.K., Paranchych, W., and Hodges, R.S. (1990). Cross-reactive and strain-specific antipeptide antibodies to *Pseudomonas aeruginosa* PAK and PAO pili. Infect. Immun. **58:**2727-32.

Lee, K.K., Sheth, H.B., Wong, W.Y., Sherburne, R., Paranchych, W., Hodges, R.S., Lingwood, C.A., Krivan, H., and Irvin, R.T. (1994). The binding of *Pseudomonas aeruginosa* pili to glycosphingolipids is a tip-associated event involving the C-terminal region of the structural pilin subunit. Mol Microbiol **11:**705-13.

Lee, K.K., Wong, W.Y., Sheth, H.B., Hodges, R.S., Paranchych, W., and Irvin, R.T. (1995). Use of synthetic peptides in characterization of microbial adhesins. Methods Enzymol **253:**115-131.

Leffler, H. and Svanborg-Eden, C. (1986). Glycolipids as receptors for *Escherichia coli* lectins or adhesins, p.83-109. *In* D. Mirelman (ed.), Microbial lectins and agglutinins: properties and biological activity. Wiley-Interscience Pub., New York.

Lehrer, N., Segal, E., Cihlar, R.L., and Calderone, R.A. (1986). Pathogenesis of vaginal candidiasis: studies with a mutant which has reduced ability to adhere in vitro. J Med Vet Mycol **24:**127-31.

Lehrer, N., Segal, E., Lis, H., and Gov, Y. (1988). Effect of *Candida albicans* cell wall components on the adhesion of the fungus to human and murine vaginal mucosa. Mycopathologia. **102:**115-21.

Leusch, H.G. (1989). Detection and characterization of two antigens specific for cell wall of *Candida albicans* mycelial growth phase. Curr Microbiol **19:**193-198.

Levitz, S.M. (1992). Overview of host defenses in fungal infections. Clin Infect Dis **14(suppl. 1):**S37-S42.

Li, R.K. and Cutler, J.E. (1993). Chemical definition of an epitope/adhesin molecule on *Candida albicans*. J Biol Chem **268:**18293-9.

Liljemark, W.F. and Gibbons, R.J. (1973). Suppression of *Candida albicans* by human oral streptococci in gnotobiotic mice. Infect Immun **8:**846-9.

Lingwood, C.A., Cheng, M., Krivan, H.C., and Woods, D. (1991). Glycolipid receptor binding specificity of exoenzyme S from *Pseudomonas aeruginosa* [published erratum appears in Biochem Biophys Res Commun 1992 Jun 15;186(1):601]. Biochem Biophys Res Commun **175:**1076-81.

Lopez-Ribot, J.L., Casanova, M., Martinez, J.P., and Sentandreu, R. (1991). Characterization of cell wall proteins of yeast and hydrophobic mycelial cells of *Candida albicans*. Infect Immun **59:**2324-32.

Lopez-Ribot, J.L., Casanova, M., Monteagudo, C., Sepulveda, P., and Martinez, J.P. (1994). Evidence for the presence of a high-affinity laminin receptor-like molecule on the surface of *Candida albicans* yeast cells. Infect Immun **62:**742-6.

Lopez-Ribot, J.L. and Chaffin, W.L. (1994). Binding of the extracellular matrix component entactin to *Candida albicans*. Infect Immun **62:**4564-71.

Lopez-Ribot, J.L., Martinez, J.P., and Chaffin, W.L. (1995). Comparative study of the C3d receptor and 58-kilodalton fibrinogen- binding mannoproteins of *Candida albicans*. Infect Immun **63:**2126-2132.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. J Biol Chem **193**:265-275.

Macdonald, D.L. (1994). Structure and function of *P. aeruginosa* pili. (thesis).

Macdonald, F. and Odds, F.C. (1983). Virulence for mice of a proteinase-secreting strain of *Candida albicans* and a proteinase-deficient mutant. J Gen Microbiol **129:**431-8.

Mackenzie, D.W.R. (1965). Studies on the morphogenesis of *C. albicans*. II. Growth in organ extract. Sabouraudia **4:**126-130.

Maisch, P.A. and Calderone, R.A. (1980). Adherence of *Candida albicans* to a fibrin-platelet matrix formed in vitro. Infect Immun **27:**650-6.

Maisch, P.A. and Calderone, R.A. (1981). Role of surface mannan in the adherence of *Candida albicans* to fibrin-platelet clots formed in vitro. Infect Immun **32:**92-7.

Makrides, H.C. and MacFarlane, T.W. (1982). Effects of commensal bacteria on the adherence of *Candida albicans* to epithelial cells *in vitro*. Microbios Lett **21**:55-61.

Marcantonio, E.E. and Hynes, R.O. (1988). Antibodies to the conserved cytoplasmic domain of the integrin beta 1 subunit react with proteins in vertebrates, invertebrates, and fungi. J Cell Biol **106:**1765-72.

Marrie, T.J. and Costerton, J.W. (1981). The ultrastructure of *Candida albicans* infections. Can J Microbiol **27:**1156-1164.

Martinez, J.P., Lopez-Ribot, J.L., and Chaffin, W.L. (1994). Heterogeneous surface distribution of the fibrinogen-binding protein on *Candida albicans*. Infect Immun **62:**709-12.

Matthews, R. and Burnie, J. (1992). The role of hsp90 in fungal infection. Immunol Today **13:**345-8.

Matthews, R., Burnie, J., Smith, D., Clark, I., Midgley, J., Conolly, M., and Gazzard, B. (1988). *Candida* and AIDS: evidence for protective antibody. Lancet **2:**263-6.

Mayer, C.L., Diamond, R.D., and Edwards, J.E., Jr. (1990). Recognition of binding sites on *Candida albicans* by monoclonal antibodies to human leukocyte antigens. Infect Immun **58:**3765-9.

Mayer, C.L., Filler, S.G., and Edwards, J.E., Jr. (1992). *Candida albicans* adherence to endothelial cells. Microvasc Res **43:**218-226.

McCourtie, J. and Douglas, L.J. (1981). Relationship between cell surface composition of *Candida albicans* and adherence to acrylic after growth on different carbon sources. Infect Immun **32:**1234-41.

McCourtie, J. and Douglas, L.J. (1984). Relationship between cell surface composition, adherence, and virulence of *Candida albicans*. Infect Immun **45:**6-12.

McCourtie, J. and Douglas, L.J. (1985). Extracellular polymer of *Candida albicans*: isolation, analysis and role in adhesion. J Gen Microbiol **131:**495-503.

McEachran, D.W. and Irvin, R.T. (1985). Adhesion of *Pseudomonas aeruginosa* to human buccal epithelial cells: evidence for two classes of receptors. Can J Microbiol **31:**563-9.

Merril, C.R. (1990a). Gel-staining techniques. Methods Enzymol **182:**477-88.

Merril, C.R. (1990b). Silver staining of proteins and DNA. Nature **343:**779-80.

Miyakawa, Y., Kuribayashi, T., Kagaya, K., Suzuki, M., Nakase, T., and Fukazawa, Y. (1992). Role of specific determinants in mannan of *Candida albicans* serotype A in adherence to human buccal epithelial cells. Infect Immun **60:**2493-9.

Mohamed. A.M.H. (1975). Ultrastructural aspects of chronic oral candidiasis. J Oral Pathol **4:**180-194.

Monod, M., Togni, G., Hube, B., and Sanglard, D. (1994). Multiplicity of genes encoding secreted aspartic proteinases in *Candida* species. Mol Microbiol **13:**357-68.

Montes, L.F. and Wilborn, W.H. (1968). Ultrastructural features of host-parasite relationship in oral candidiasis. J Bacteriol **96:**1349-56.

Moser, S.A. and Domer, J.E. (1980). Effects of cyclophosphamide on murine candidiasis. Infect Immun **27:**376-86.

Mourad, S. and Friedman, L. (1968). Passive immunization of mice against *Candida albicans*. sabouraudia **6**:103-105.

Narayanan, R., Joyce, W.A., and Greenfield, R.A. (1991). Gastrointestinal candidiasis in a murine model of severe combined immunodeficiency syndrome. Infect Immun **59:**2116-9.

Negre, E., Vogel, T., Levanon, A., Guy, R., Walsh, T.J., and Roberts, D.D. (1994). The collagen binding domain of fibronectin contains a high affinity binding site for *Candida albicans*. J Biol Chem **269:**22039-22045.

Nikawa, H. and Hamada, T. (1990). Binding of salivary or serum proteins to *Candida albicans* in vitro. Arch Oral Biol **35:**571-3.

Odds, F.C. (1988). *Candida* and candidosis. 2nd ed. London: Bailliere Tindall.

Odds, F.C. (1992). Antifungal susceptibility testing of *Candida* spp. by relative growth measurement at single concentrations of antifungal agents. Antimicrob Agents Chemother **36:**1727-37.

Odds, F.C. (1994). Pathogenesis of *Candida infections*. JAAD **31:**S2-5.

Ofek, I., Courtney, H.S., Schifferli, D.M., and Beachey, E.H. (1986). Enzyme-linked immunosorbent assay for adherence of bacteria to animal cells. J Clin Microbiol **24:**512-6.

Ofek, I and Beachey, E.H. (1980). Receptors and Recoginition, series B, vol. 6, p1-29. *In* E.H. Beachey, (ed.), Bacterial adherence. Chapman and Hall, London.

Ollert, M.W. and Calderone, R.A. (1990). A monoclonal antibody that defines a surface antigen on *Candida albicans* hyphae cross-reacts with yeast cell protoplasts. Infect Immun **58:**625-31.

Ollert, M.W., Wadsworth, E., and Calderone, R.A. (1990). Reduced expression of the functionally active complement receptor for iC3b but not for C3d on an avirulent mutant of *Candida albicans*. Infect Immun **58:**909-13.

Page, S. and Odds, F.C. (1988). Binding of plasma proteins to *Candida* species *in vitro*. J Gen Microbiol **134:**2693-702.

Parge, H.E., Forest, K.T., Hickey, M.J., Christensen, D.A., Getzoff, E.D., and Tainer, J.A. (1995). Structure of the fibre-forming protein pilin at 2.6 A resolution. Nature **378**:32-8.

Paranchych, W. and Frost, L.S. (1988). The physiology and biochemistry of pili. Adv Microb Physiol **29**:53-114.

Paranchych, W., Sastry, P.A., Frost, L.S., Carpenter, M., Armstrong, G.D., and Watts, T. H. (1979). Biochemical studies on pili isolated from *Pseudomonas aeruginosa* strain PAO. Can J Microbiol **25**:1175-81.

Paranchych, W., Sastry, P.A., Volpel, K., Loh, B.A., and Speert, D.P. (1986). Fimbriae (pili): molecular basis of *Pseudomonas aeruginosa* adherence. Clin Invest Med **9**:113-8.

Parker, J.M.R. and Hodges, R.S. (1985). Photoaffinity probes provide a general method to prepare synthtic peptide conjugates. J Protein Chem **3**:465-478.

Patel, P., Marrs, C.F., Mattick, J.S., Ruehl, W.W., Taylor, R.K., and Koomey, M. (1991). Shared antigenicity and immunogenicity of type 4 pilins expressed by *Pseudomonas aeruginosa, Moraxella bovis, Neisseria gonorrhoaea, Dichelobacter nodosus,* and *Vibrio cholerae*. Infect Immun **59**:4674-6.

Pearsall, N.N., Adams, B.L., and Bunni,R. (1978). Immunologic responses to *Candida albicans*. III. Effects of passive transfer of lymphoid cells or serum on murine candidiasis. J Immunol **120**:1176-80.

Pecyk, R.A., Fraser-Smith, E.B., and Matthews, T.R. (1989). Efficacy of interleukin-1 beta against systemic *Candida albicans* infections in normal and immunosuppressed mice [published erratum appears in Infect Immun 1990 Apr;58(4):1132]. Infect Immun **57**:3257-8.

Pendrak, M.L. and Klotz, S.A. (1995). Adherence of *Candida albicans* to host cells. FEMS Microbiol Lett **129**:103-113.

Persi, M.A. and Burnham, J.C. (1981). Use of tannic acid as a fixative-mordant to improve the ultrastructural appearance of *Candida albicans* blastospores. Sabouraudia **19**:1-8.

Persi, M.A., Burnham, J.C., and Duhring, J.L. (1985). Effects of carbon dioxide and pH on adhesion of *Candida albicans* to vaginal epithelial cells. Infect Immun **50:**82-90.

Polonelli, L., De Bernardis, F., Conti, S., Boccanera, M., Gerloni, M., Morace, G., Magliani, W., Chezzi, C., and Cassone, A. (1994a). Idiotypic intravaginal vaccination to protect against candidal vaginitis by secretory, yeast killer toxin-like anti-idiotypic antibodies. J Immunol **152:**3175-82.

Polonelli, L., Poulain, D., Cole, G.T., Conti, S., Elad, D., Gerloni, M., Kirkland, T., Matthews, R., Segal, E., and Wyckoff, E. (1994b). New strategies in vaccination against fungal infections. J Med Vet Mycol **32:**105-112.

Ponton, J. and Jones, J.M. (1986a). Analysis of cell wall extracts of *Candida albicans* by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blot techniques. Infect Immun **53:**565-72.

Ponton, J. and Jones, J.M. (1986b). Identification of two germ-tube-specific cell wall antigens of *Candida albicans*. Infect Immun **54:**864-8.

Poon, N.H. and Day, A.W. (1975a). Fungal fimbriae. I. Structure, origin, and synthesis. Can J Microbiol **21:**537-46.

Poon, N.H., and Day, A.W. (1975b). Fungal fimbriae II. Their role in conjugation in *Ustilago violacea*. Can J Microbiol **21**:547-557.

Prince, A. (1992). Adhesins and receptors of *Pseudomonas aeruginosa* associated with infection of the respiratory tract. Microb Pathog **13:**251-60.

Pugh, D. and Cawson, R.A. (1978). The surface layer of *Candida albicans*. Microbios **23:**19-23.

Ramphal, R. and Pier, G.B. (1985). Role of *Pseudomonas aeruginosa* mucoid exopolysaccharide in adherence to tracheal cells. Infect Immun **47:**1-4.

Ramphal, R. and Shands, J.W., Jr. (1984). The therapy for infective endocarditis. Cardiovasc Clin **14:**285-98.

Ray, T.L., and Payne, C.D. (1990). *Candida albicans* acid Proteinase: A role in virulence, p.160-194. *In* Ayoub, E.M.,. Cassell, G.H., Branche, W.C., Jr., and Henry, T.J. (ed.), Microbial determinants of virulence and host response. American Society for Microbiology, Washington, D.C.

Reboli, A.C. (1993). Diagnosis of invasive candidiasis by a dot immunobinding assay for *Candida antigen* detection. J Clin Microbiol **31:**518-23.

Reef, S.E. (1993). Epidemiology of Nosocomial Candidiasis, abstr. p. 12. Abstr. 3th ASM Conference on *Candida* and candidiasis: Biology, Pathogenesis, and Management. 1993; Baltimore, Maryland.

Rinaldi, M.G. (1993). Biology and Pathogenicity of *Candida* Species, p1-20. *In*: Bodey, G.P. (eds), *Candidiasis*: Pathogeniesis, Diagnosis, and Treatment 2nd. Raven Press, New York.

Roseff, S.A. and Sugar, A.M. (1993). Oral and Esophageal Candidiasis, p185-204. *In*: Bodey, G.P. (eds), *Candidiasis*: Pathogeniesis, Diagnosis, and Treatment 2nd. Raven Press, New York.

Rotrosen, D., Calderone, R.A., and Edwards, J.E., Jr. (1986). Adherence of *Candida* species to host tissues and plastic surfaces. Rev Infect Dis **8:**73-85.

Rotrosen, D., Edwards, J.E., Jr., Gibson, T.R., Moore, J.C., Cohen, A.H., and Green, I. (1985). Adherence of *Candida* to cultured vascular endothelial cells: mechanisms of attachment and endothelial cell penetration. J Infect Dis **152:**1264-74.

Ruchel, R. (1981). Properties of a purified proteinase from the yeast *Candida albicans*. Biochim Biophys Acta **659:**99-113.

Ruchel, R. (1986). Cleavage of immunoglobulins by pathogenic yeasts of the genus *Candida*. Microbiol Sci **3:**316-319.

Ruchel, R., Ritter, B., and Schaffrinski, M. (1990). Modulation of experimental systemic murine candidosis by intravenous pepstatin. Inter J Med Microbiol **273:**391-403.

Saiman, L., Cacalano, G., and Prince, A. (1990). *Pseudomonas cepacia* adherence to respiratory epithelial cells is enhanced by *Pseudomonas aeruginosa*. Infect Immun **58:**2578-84.

Saltarelli, C.G. (1989). *Candida albicans*: The pathogenic fungus. Hemisphere Publishing Corporation, New York.

Samaranayake, L.P. and MacFarlane, T.W. (1981). The adhesion of the yeast *Candida albicans* to epithelial cells of human origin *in vitro*. Arch Oral Biol **26:**815-20.

Sandin, R.L. (1987a). The attachment to human buccal epithelial cells by *Candida albicans*: an *in vitro* kinetic study using concanavalin A. Mycopathologia **98:**179-84.

Sandin, R.L. (1987b). Studies on cell adhesion and concanavalin A-induced agglutination of *Candida albicans* after mannan extraction. J Med Microbiol **24:**145-50.

Sandin, R.L., Rogers, A.L., Beneke, E.S., and Fernandez, M.I. (1987a). Influence of mucosal cell origin on the in vitro adherence of *Candida albicans*: are mucosal cells from different sources equivalent? Mycopathologia **98:**111-9.

Sandin, R.L., Rogers, A.L., Fernandez, M.I., and Beneke, E.S. (1987b). Variations in affinity to *Candida albicans* in vitro among human buccal epithelial cells. J Med Microbiol **24:**151-5.

Sandin, R.L., Rogers, A.L., Patterson, R.J., and Beneke, E.S. (1982). Evidence for mannose-mediated adherence of *Candida albicans* to human buccal cells in vitro. Infect Immun **35:**79-85.

Sastry, P.A., Finlay, B.B., Pasloske, B L., Paranchych, W., Pearlstone, J.R., and Smillie, L.B. (1985). Comparative studies of the amino acid and nucleotide sequences of pilin derived from *Pseudomonas aeruginosa* PAK and PAO. J Bacteriol **164:**571-7.

Sato, H. and Okinaga, K. (1987). Role of pili in the adherence of *Pseudomonas aeruginosa* to mouse epidermal cells. Infect Immun **55:**1774-8.

Sato, Y., Arai, H., and Suzuki, K. (1974). Leukocytosis-promoting factor of *Bordetella pertussis*. 3. Its identity with protective antigen. Infect Immun **9:**801-10.

Schagger, H. and Von Jagow, G. (1987). Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Analyt Biochem **166:**368-79.

Scheld, W.M., Calderone, R.A., Brodeur, J.P., and Sande, M.A. (1983). Influence of preformed antibody on the pathogenesis of experimental *Candida albicans* endocarditis. Infect. Immun. **40:**950-955.

Scherwitz, C., Martin, R., and Ueberberg, H. (1978). Ultrastructural investigations of the formation of *Candida albicans* germ tubes and septa. Sabouraudia. **16:**115-24.

Segal, E. and Savage, D. (1986). Adhesion of *Candida albicans* to mouse intestinal mucosa *in vitro*: development of the assay and test of inhibitors. J Med Vet Mycol **24**:477-479.

Segal, E., Gottfied, L., and Lehrer, N. (1988). *Candidal* vaginitis in hormone-treated mice: prevention by a chitin extract. Mycopathologia **102:**157-163.

Sepulveda, P., Murgui, A., Lopez-Ribot, J.L., Casanova, M., Timoneda, J., and Martinez, J.P. (1995). Evidence for the presence of collagenous domains in *Candida albicans* cell surface proteins. Infect Immun **63:**2173-2179.

Sexton, M. and Reen, D.J. (1992). Characterization of antibody-mediated inhibition of *Pseudomonas aeruginosa* adhesion to epithelial cells. Infect Immun **60:**3332-8.

Sharon, N. (1986). Bacterial lectins, p68-97. *In* Liener, I.E. (ed.), The lectins: properties, functions, and applications in biology and medicine. Acad. Press, Inc.

Shaw, C.E. and Taylor, R.K. (1990). *Vibrio cholerae* O395 tcpA pilin gene sequence and comparison of predicted protein structural features to those of type 4 pilins. Infect Immun **58:**3042-9.

Shepard, M.G. (1990). Biology of *Candida* Species. p10-20. In Samaranayake LP., MacFarlane TW (eds.): Oral Candidiasis. London: Wright.

Shepherd, M.G. (1987). Cell envelope of *Candida albicans*. Crit Rev Microbiol **15:**7-25.

Shepherd, M.G. (1991). Morphogenesis in *Candida albicans*. *In*: Prasad, R (ed.) *Candida albicans*: Cellular and Molecular Biology. Springer-Verlag

Shepherd, M.G., Poutter, R.I.M., and Sullivan, P.A. (1985). *Candida albicans:* Biology, Genetics, and Pathogenicity. Annu Rev Microbiol **39:**579-614.

Sherwood, J., Gow, N.A., Gooday, G.W., Gregory, D.W., and Marshall, D. (1992). Contact sensing in *Candida albicans*: a possible aid to epithelial penetration. J Med Vet Mycol **30:**461-9.

Sheth, H.B., Lee, K.K., Wong, W.Y., Srivastava, G., Hindsgaul, O., Hodges, R.S., Paranchych, W., and Irvin, R.T. (1994). The pili of *Pseudomonas aeruginosa* strains PAK and PAO bind specifically to the carbohydrate sequence beta GalNAc(1-4)beta Gal found in glycosphingolipids asialo-GM1 and asialo-GM2. Mol Microbiol **11:**715-23.

Sheth, H.B., Glasier, L.M.G., Ellert, N.W., Cachia, P., Kohn, W., Lee, K.K., Paranchych, W., Hodges, R.S., and Irvin, R.T. (1995). Development of an anti-adhesive vaccine for *Pseudomonas aeruginosa* targeting the C-terminal region of the pilin structural protein. Biomedical Peptides, Proteins & Nucleic Acids **1**:141-148.

Skerl, K.G., Calderone, R.A., Segal, E., Sreevalsan, T., and Scheld, W.M. (1984). *In vitro* binding of *Candida albicans* yeast cells to human fibronectin. Can J Microbiol **30**:221-7.

Slutsky, B., Buffo, J., and Soll, D.R. (1985). High-frequency switching of colony morphology in *Candida albicans*. Science **230**:666-9.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., and Klenk, D.C. (1985). Measurement of protein using bicinchoninic acid [published erratum appears in Anal Biochem 1987 May 15;163(1):279]. Analyt Biochem **150**:76-85.

Sobel, J.D. (1993). Genital Candidiasis, p225-248. *In*: Bodey, G.P. (eds), *Candidiasis*: Pathogeniesis, Diagnosis, and Treatment 2nd. Raven Press, New York.

Sobel, J.D., Muller, G., and Buckley, H.R. (1984). Critical role of germ tube formation in the pathogenesis of candidal vaginitis. Infect Immun **44**:576-80.

Sobel, J.D., Myers, P., Levison, M.E., and Kaye, D. (1982). Comparison of bacterial and fungal adherence to vaginal exfoliated epithelial cells and human vaginal epithelial tissue culture cells. Infect Immun **35**:697-701.

Sobel, J.D., Schneider, J., Kaye, D., and Levison, M.E. (1981). Adherence of bacteria to vaginal epithelial cells at various times in the menstrual cycle. Infect Immun **32**:194-7.

Soll, D.R. (1988). High-frequency switching in *Candida albicans* and its relations to vaginal candidiasis. Am J Obstet Gynecol **158**:997-1001.

Soll, D.R. (1992). High-frequency switching in *Candida albicans*. Clin Microbiol Rev **5**:183-203.

Soll, D.R., Langtimm, C.J., McDowell, J., Hicks, J., and Galask, R. (1987). High-frequency switching in *Candida* strains isolated from vaginitis patients. J Clin Microbiol **25**:1611-22.

Soll, D.R., Morrow, B., and Srikantha, T. (1993). High-frequency phenotypic switching in *Candida albicans*. Trends Genet **9:**61-5.

Soll, D.R., Morrow, B., Srikantha, T., Vargas, K., and Wertz, P. (1994). Developmental and molecular biology of switching in *Candida albicans*. Oral Surg Oral Med Oral Pathol **78:**194-201.

Staddon, W., Todd, T., and Irvin, R.T. (1990). Equilibrium analysis of binding of *Candida albicans* to human buccal epithelial cells. Can J Microbiol **36:**336-40.

Stewart, T.S. and Ballou, C.E. (1968). A comparison of yeast mannans and phosphomannans by acetolysis. Biochemistry. **7:**1855-1863.

Stromberg, N., Deal, C., Nyberg, G., Normark. S., So, M., and Karlsson, K.A. (1988a). Identification of carbohydrate structures that are possible receptors for *Neisseria gonorrhoeae*. Proc Natl Acad Sci U S A **85:**4902-6.

Stromberg, N., Ryd, M., Lindberg, A.A., and Karlsson, K.A. (1988b). Studies on the binding of bacteria to glycolipids. Two species of *Propioni bacterium* apparently recognize separate epitopes on lactose of lactosylceramide. FEBS Lett **232:**193-8.

Stromberg, N., Marklund, B.I., Lund, B., Ilver, D., Hamers, A., Gaastra, W., Karlsson, K.A., and Normark, S. (1990). Host-specificity of uropathogenic *Escherichia coli* depends on differences in binding specificity to Gal alpha 1-4Gal-containing isoreceptors. Embo J **9:**2001-10.

Sundstrom, P.M. and Kenny, G.E. (1984). Characterization of antigens specific to the surface of germ tubes of *Candida albicans* by immunofluorescence. Infect Immun **43:**850-5.

Sundstrom, P.M. and Kenny, G.E. (1985). Enzymatic release of germ tube-specific antigens from cell walls of *Candida albicans*. Infect Immun **49:**609-14.

Sundstrom, P.M., Nichols, E.J., and Kenny, G.E. (1987). Antigenic differences between mannoproteins of germ tubes and blastospores of *Candida albicans*. Infect Immun **55:**616-20.

Sundstrom, P.M., Tam, M.R., Nichols, E.J., and Kenny, G.E. (1988). Antigenic differences in the surface mannoproteins of *Candida albicans* as revealed by monoclonal antibodies. Infect Immun **56:**601-6.

Suzuki, K., Okawa, Y., Hashimoto, K., Suzuki, S., and Suzuki, M. (1984). Protecting effect of chitin and chitosan on experimentally induced murine candidiasis. Microbiol Immunol **28:**903-12.

Swanson, J. and Koomey, J.M. (1989). Mechanisms for variation of pili and outer membrane protein II in *Neisseria gonorrhoea*, p743-762. in d.e. Berg and M.M. Howe (ed.), Mobile DNA. American society for Microbiology, Washington, D.C.

Tosh, F.D. and Douglas, L.J. (1992). Characterization of a fucoside-binding adhesin of *Candida albicans*. Infect Immun **60:**4734-9.

Towbin, H., Staehelin, T., and Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA **76:**50-59.

Tronchin, G., Bouchara, J.P., and Robert, R. (1989). Dynamic changes of the cell wall surface of *Candida albicans* associated with germination and adherence. Eur J Cell Biol **50:**285-90.

Tronchin, G., Poulain, D., Herbaut, J., and Biguet, J. (1981). Localization of chitin in the cell wall of *Candida albicans* by means of germ agglutinin. Fluorescence and ultrastructural studies. Eur J Cell Biol **26:**121-128.

Tronchin, G., Poulain, D., and Vernes, A. (1984). Cytochemical and ultrastructural studies of *Candida albicans*. III. Evidence for modifications of the cell wall coat during adherence to human buccal epithelial cells. Arch Microbiol **139:**221-4.

Tronchin, C., Bouchara, J.P., Annaix, V., Robert, R., and Senet, J.M. (1991). Fungal cell adhesion molecules in *Candida albicans*. Eru J Infect Epidemiol **7:**23-33.

Tsai, C.M. and Frasch, C.E. (1982). A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Analyt Biochem **119:**115-9.

Tsushima, H., Mine, H., Kawakami, Y., Hyodoh, F., and Ueki, A. (1994). *Candida albicans* aspartic proteinase cleaves and inactivates human epidermal cysteine proteinase inhibitor, cystatin A. Microbiology **140:**167-71.

Umazume, M., Ueta, E., and Osaki, T. (1995). Reduced inhibition of *Candida albicans* adhesion by saliva from patients receiving oral cancer therapy. J Clin Microbiol **33:**432-9.

Vecchiarelli, A., Mazzolla, R., Farinelli, S., Cassone, A., and Bistoni, F. (1988). Immunomodulation by *Candida albicans*: crucial role of organ colonization and chronic infection with an attenuated agerminative strain of *C. albicans* for establishment of anti-infectious protection. J Gen Microbiol **134:**2583-92.

Velegraki, A. (1995). *In vitro* susceptibility to itraconazole and fluconazole of switch phenotypes of *Candida albicans* serotypes A and B, isolated from immuno-compromised hosts. J Med Vet Mycol **33**(1):83-5.

Venkateswarlu, K., Denning, D.W., Manning, N.J., and Kelly, S.L. (1995). Resistance to fluconazole in *Candida albicans* from AIDS patients correlated with reduced intracellular accumulation of drug. FEMS Microbiol Lett **131**(3):337-41.

Vudhichamnong, K., Walker, D.M., and Ryley, H.C. (1982). The effect of secretory immunoglobulin A on the in-vitro adherence of the yeast *Candida albicans* to human oral epithelial cells. Arch Oral Biol **27:**617-21.

Wade, J.C. (1993). Epidemiology of *Canidda* Infections, p85-108. *In*: Bodey, G.P. (eds). *Candidiasis*: Pathogeniesis, Diagnosis, and Treatment 2nd. Raven Press, New York.

Watts, T.H., Kay, C.M., and Paranchych, W. (1983). Spectral properties of three quaternary arrangements of Pseudomonas pilin. Biochemistry **22**(15):3640-6.

Westerlund, B. and Korhonen, T.K. (1993). Bacterial proteins binding to the mammalian extracellular matrix. Mol Microbiol **9:**687-94.

Wong, W.Y., Campbell, A.P., McInnes, C., Sykes, B.D., Paranchych, W., Irvin, R.T., and Hodges, R. S. (1995). Structure-function analysis of the adherence-binding domain on the pilin of *Pseudomonas aeruginosa* strains PAK and KB7. Biochemistry **34:**12963-12972.

Wong, W.Y., Irvin, R.T., Paranchych, W., and Hodges, R.S. (1992). Antigen-antibody interactions: elucidation of the epitope and strain- specificity of a monoclonal antibody directed against the pilin protein adherence binding domain of *Pseudomonas aeruginosa* strain K. Protein Sci **1:**1308-18.

Woods, D.E., Bass, J.A., Johanson, W.G., Jr., and Straus, D.C. (1980). Role of adherence in the pathogenesis of *Pseudomonas aeruginosa* lung infection in cystic fibrosis patients. Infect Immun **30:**694-9.

Yamakawa, T. and Nagai, U. (1978). Glycolipids at the cell surface and their biological functions. TIBS. pp.128-131.